République Algérienne Démocratique et Populaire

Université Kasdi Merbah Ouargla

Faculté des Sciences de la Nature et de la Vie

Département des Sciences Biologiques

N° d'enregistrement : /......../......../......../......../

Thèse Articles

En vue de l'obtention du diplôme de Doctorat Ès-Sciences

Spécialité : Microbiologie

Présentée Par :

Mr. Mohamed Amine GACEM

Screening phytochimique et activité antifongique et antibactérienne des substances bioactives de plantes médicinales et d'actinomycètes prélevées des sols sahariens.

Soutenue publiquement le : **.../.../2021**

Devant le jury composé de :

Président :	OULD EL HADJ Mohamed Didi	Professeur	Univ. Ouargla
Directrice de thèse :	OULD EL HADJ-KHELIL Aminata	Professeur	Univ. Ouargla
Examinateur :	KEMASSI Abdellah	Professeur	Univ. Ouargla
Examinateur :	CHAIBI Rachid	Professeur	Univ. Laghouat
Examinateur :	BOURAS Noureddine	Professeur	Univ. Ghardaia
Examinateur :	BOULENOUAR Noureddine	Professeur	C. Univ. El-Bayadh

Année universitaire : 2020 /2021

« If you think that education is too expensive

... try ignorance, it may be less expensive »

In Loving Memory Of My Dear Grandmother.

Dedicated To :

To My Beloved Parents.

To My Dear Sisters.

To My Friends.

Without Them None Of My Successes Would Be Possible.

Table des matières

Page

Remerciements **I**
Liste des figures **II**
Résumé **III**
Abstract **IV**
ملخص **V**

Introduction **01**

Partie bibliographique.

I. Occurrence, toxicité et méthodes de détection des mycotoxines. **07**

II. Toxicités et maladies induites par les mycotoxines. **54**

III. Décontamination et méthodes de contrôle des mycotoxines. **97**

IV. Phytochimie, toxicité et pharmacologie de *Pistacia lentiscus, Artemisia herba-alba* et *Citrullus colocynthis*. **138**

V. Actinobactéries des niches microbiennes étonnantes et non exploitables du désert algérien : Importance biotechnologique et environnementale. **179**

Partie expérimentale

I. Plan expérimental **183**

Résultats et discussion

II.1. Criblage phytochimique, activités antifongiques et antioxydantes de trois plantes médicinales de la steppe et du Sahara algériens (études préliminaires de criblage). **197**

II.2. Effets antimicrobiens et antioxydants d'une actinobactérie forestière V_{002} en tant que nouvelle productrice de spectinabiline, undécylprodigiosine et métacycloprodigiosine. **213**

II.3. Activités antimycotoxinogène et antifongique des graines de *Citrullus colocynthis* contre *Aspergillus flavus* et *Aspergillus ochraceus* contaminant le blé tendre stocké. **231**

Conclusion générale et perspectives **244**
Références bibliographiques **247**
Annexe **252**

Remerciements

Le présent travail est réalisé au laboratoire de Protection Des Ecosystèmes En Zones Arides Et Semi-Arides de l'université de Ouargla et au laboratoire de Collection de souches microbiennes, Helmholtz Centre for Infection Research (HZI), Inhoffenstrasse, Braunschweig, Allemagne.

Avant toute chose, je remercie Dieu, le tout puissant, de m'avoir donné la force et la patience pour achever ce travail.

J'exprime d'abord mes profonds remerciements et ma vive reconnaissance à Mme OULD EL HADJ-KHELIL Aminata, Professeure à la faculté des Sciences de la Nature et de la Vie, Université Kasdi Merbah-Ouargla pour avoir encadré et dirigé ce travail avec une grande rigueur scientifique. Sa disponibilité, ses conseils et la confiance qu'elle m'a accordé m'ont permis de réaliser ce travail.

J'exprime mes profonds remerciements et ma vive reconnaissance à Mr. Joachim WINK et Mr. MARC Stadler qui m'ont accueilli au sein de leurs laboratoires au HZI et qui ont mis à ma disposition les conditions et le matériel nécessaire pour la réalisation de ce travail tout au long de la période de recherche. Qu'ils trouvent ici mon respect et ma reconnaissance.

Je tiens à exprimer ma profonde reconnaissance à Melle. Alia TELLI Maître de conférences à la faculté des Sciences de la Nature et de la Vie, Université Kasdi Merbah-Ouargla pour sa contribution à la réalisation de ce travail et ses précieux conseils qu'elle trouve ici mes sincères remerciements.

J'adresse mes sincères remerciements à Mr. OULD EL HADJ Mohamed Didi, Professeur à la faculté des Sciences de la Nature et de la Vie, Université Kasdi Merbah-Ouargla, d'avoir accepté de présider le jury de ma soutenance.

J'exprime mes vifs remerciements à Mr. KEMASSI Abdellah Professeur à la faculté des Sciences de la Nature et de la Vie, Université Kasdi Merbah-Ouargla, Mr. CHAIBI Rachid Professeur à l'Université de Laghouat, Mr. BOURAS Noureddine Professeur à l'Université de Ghardaïa et Mr. BOULENOUAR Noureddine Professeur au Centre Universitaire d'El-Bayadh pour l'honneur qu'ils nous ont fait en acceptant d'examiner cette thèse.

Mes vifs remercîments s'adressent également à Melle. Romy SCHADE, Melle. Kathrin MOHR, Mr. Simon NWAKOR, Melle. Nasim SAFEI, Mr. Chandra et Iniyan.

Mohamed Amine GACEM

Liste des figures

N°	Titre de la figure	Page
01	Localisation géographique des stations de récolte de plantes médicinales et des échantillons de sol.	**185**
02	Préparation des extraits organiques et étude phytochimique.	**186**
03	Techniques d'isolement et d'identification des genres fongiques. .	**187**
04	Détermination des espèces fongiques et identification des espèces productrices de mycotoxine.	**188**
05	Technique d'isolement et d'identification phénotypique des actinobactéries.	**189**
06	Technique d'extraction, d'amplification d'ADN 16S et d'identification phylogénétique des espèces d'actinobactéries.	**190**
07	Technique d'extraction des métabolites secondaires à partir des espèces d'actinobactéries cultivées dans les milieux de cultures liquides.	**191**
08	Détermination de l'activité antifongique et antimycotoxinogène des extraits organiques.	**192**
09	Détermination de l'activité antibactérienne et antioxydante des extraits organiques.	**193**
10	Détermination de l'activité antioxydante des extraits organiques.	**194**

Screening phytochimique et activités antifongique et antibactérienne des substances bioactives de plantes médicinales et d'actinomycètes prélevées des sols sahariens.

Résumé

L'objectif de ce travail est de mettre en évidence l'intérêt biologique de trois plantes médicinales et d'un isolat d'actinobactérie dans le contrôle de quelques souches bactériennes et fongiques ainsi que l'inhibition de la synthèse de quelques mycotoxines.

Pistacia lentiscus, Citrullus colocynthis, Artemisia herba-alba et un isolat d'actinobactérie forestière ont été sélectionnés pour évaluer leur activités antibactérienne, antifongique, antimycotoxinogène et antioxydante. Les trois plantes sont prélevées des régions steppique et saharienne de l'Algérie. Les résultats du screening phytochimique ont révélé la richesse des trois espèces végétales en substances bioactives. Les résultats des tests biologiques suggèrent que les extraits méthanolique et aqueux d'*A. herba alba* sont dotés d'une excellente activité antifongique contre *F. graminirium* CECT 2150, *A. ochraceus* NRRL 3174 et *P. expansum* CECT 2278. La synthèse d'ochratoxine est significativement inhibée par les extraits méthanoliques d'*A. herba alba* et des graines de *C. colocynthis*. L'activité antioxydante des extraits organiques des trois plantes est démontrée.

L'actinobactérie est isolée d'un sol forestier de la région de Saida. L'identification moléculaire à base d'ARNr 16S a révélé que l'isolat d'actinobactérie désigné V_{002} appartient au genre *Streptomyces*. Cette souche est considérée comme nouvelle espèce productrice de spectinabiline, d'undecylprodigiosine et de metacycloprodigiosine. La souche *Streptomyces sp.* V_{002} est considérée comme un producteur prometteur de prodigiosine. Elle est également considérée comme agent antimicrobien efficace en raison de son rôle important dans l'inhibition des bactéries à Gram positif. Les métabolites secondaires de cette souche ont une puissante capacité antioxydante dans l'inhibition d'ABTS et le piégeage de DPPH.

Les métabolites secondaires de *P. lentiscus, C. colocynthis, A. herba alba* et de la souche V_{002} représentent une alternative prometteuse pour éliminer certains microbes pathogènes. Ils sont aussi considérés comme agents détoxifiant permettant d'éliminer certaines mycotoxines. L'excellente activité antioxydante des extraits leur confère une place importante dans le traitement des maladies causées par le stress oxydatif.

Mots-clés : *Pistacia lentiscus*; *Citrullus colocynthis*; *Artemisia herba-alba*; Actinobactérie; Activité antioxydante; Activité antibactérienne; Activité antifongique; Spectinabiline. Undecylprodigiosine. Metacycloprodigiosine.

Phytochemical screening and antifungal and antibacterial activity of bioactive substances from medicinal plants and actinomycetes collected from Saharan soils.

Abstract

The aim of this work is to demonstrate the biological interest of three medicinal plants and an isolate of actinobacteria in the control of certain bacterial and fungal strains as well as the inhibition of the synthesis of certain mycotoxins.

Pistacia lentiscus, Citrullus colocynthis, Artemisia herba-alba and a forest actinobacterium were selected to assess their antibacterial, antifungal, antimycotoxin and antioxidant activities. The three plants are collected from the steppes and the Saharan regions of Algeria. The results of the phytochemical screening revealed the richness of the three medicinal plant species in bioactive substances. The results of biological tests suggest that the methanolic and aqueous extracts of *A. herba-alba* have a significant antifungal activity against *F. graminirium* CECT 2150, *A. ochraceus* NRRL 3174 and *P. expansum* CECT 2278. Ochratoxin synthesis is significantly inhibited by methanolic extracts from *A. herba-alba* and *C. colocynthis* seeds. The antioxidant activity of organic extracts from the three plants has been demonstrated.

The actinobacterium is isolated from forest soil in the Saida region. Molecular identification using 16S rRNA revealed that the actinobacterium isolate designated V_{002} belongs to the genus *Streptomyces*. This strain is considered to be a new species producing spectinabilin, undecylprodigiosin and metacycloprodigiosin. The strain *Streptomyces sp*. V_{002} is considered as a promising producer of prodigiosin. It is also considered to be an effective antimicrobial agent due to its important role in inhibiting Gram-positive bacteria. The secondary metabolites of this strain have a potent antioxidant capacity to inhibit ABTS and scavenge DPPH.

The secondary metabolites of *P. lentiscus, C. colocynthis, A. herba alba* and strain V_{002} represent a promising alternative to eliminate certain pathogenic microbes. They are also considered to be detoxifying agents which eliminate certain mycotoxins. The excellent antioxidant activity of the extracts gives them an important place in the treatment of diseases caused by oxidative stress.

Keywords: *Pistacia lentiscus; Citrullus colocynthis; Artemisia herba-alba;* Actinobacterium; Antioxidant activity; Antibacterial activity; Antifungal activity; Spectinabilin; Undecylprodigiosin; Metacycloprodigiosin.

تشخيص المكونات الكيمائية النباتية والنشاطات الحيوية المضادة للفطريات والبكتيريا لبعض المواد الحيوية المستخلصة من النباتات الطبية ونوع من بكتيريا التربة (الأكتينوبكتيريا) المأخوذة من تربة الصحراء.

الملخص

الهدف من إنجاز هذا العمل هو إظهار الفوائد البيولوجية لثلاث أنواع من النباتات الطبية وكذا عزل الأكتينوبكتيريا وذلك من اجل مكافحة بعض السلالات البكتيرية والفطرية الضارة وكذلك تثبيط بعض السموم الفطرية.

تم اختيار شجرة الضرو، فاكهة الحنظل وعشبة الشيح إضافة الى عزل الأكتينوبكتيريا وذلك من اجل دراسة وتقييم أنشطتها المضادة للبكتيريا، للفطريات، لسموم بعض الفطريات وكذلك لتقيم نشاطها الحيوي المضاد للأكسدة. تم جمع النباتات الطبية الثلاث من مناطق السهوب والصحراء الجزائرية. أظهرت نتائج الفحص الكيميائي النباتي ثراء النباتية الطبية بالمواد البيوكيميائية النشطة، كما تشير نتائج الاختبارات البيولوجية إلى أن المستخلصات الميثانولية والمائية لنبات الشيح ان لها نشاط بيولوجي كبير مضاد للفطر *P. expansum CECT 2278, A. ochraceus NRRL 3174, F. graminirium CECT 2150*

تشير نتائج الاختبارات البيولوجية أيضا ان المستخلصات الميثانولية لبذور الحنظل وعشبة الشيح لها القدرة على تثبيط السموم الفطرية (الأكراتوكسين)، كما تم إثبات النشاطات المضادة للأكسدة للمستخلصات العضوية للنباتات الطبية الثلاثة.

تم عزل الأكتينوبكتيريا من تربة غابة العقبان لولاية سعيدة حيث كشف التحديد الجيني باستخدام ARNr 16s الى ان الأكتينوبكتيريا V_{002} المعزولة تنتمي الى سلالة *Streptomyces*. تعتبر هذه البكتيريا كمنتج جديد لسبيكتينابلين ، وندسيل بروديجيوسين وميتاسيكلوبروديجيوسين. كما تعتبر أيضا عاملًا فعالًا مضادًا للميكروبات نظرًا لدورها المهم في تثبيط البكتيريا Gram+. تحتوي المستقلبات الثانوية لهذه البكتيريا مضادات للأكسدة.

تمثل المستقلبات الثانوية للنباتات الطبية الثلاث وكذا المستقلبات الثانوية للأكتينوبكتيريا المعزولة بديلاً واعدًا للقضاء على بعض الميكروبات المسببة للأمراض، كما تعتبر أيضًا عوامل محفزة للقضاء على الفطريات او تثبيط سمومها. يمنح النشاط الممتاز المضاد للأكسدة للمستخلصات مكانًا مهمًا في علاج الأمراض الناتجة عن الإجهاد التأكسدي.

الكلمات المفتاحية: شجرة الضرو، فاكهة الحنظل، عشبة الشيح، الأكتينوبكتيريا، مضاد للفطر، مضادة للبكتيريا، مضاد للأكسدة، سبيكتينابلين، ندسيل بروديجيوسين، ميتاسيكلوبروديجيوسين.

Introduction

Introduction

Les mycotoxines sont des métabolites secondaires fongiques très toxique pour l'Homme. Certains de ces métabolites fongiques possèdent des activités pharmacologiques, ils sont utilisés dans la production d'antibiotiques, d'autres sont introduits dans le développement des armes biologiques et chimiques (**Sava et al. 2006**).

La plupart des mycotoxines sont d'origine polycétide (**Huffman et al. 2010**). Elles sont produites par six genres fongiques à savoir *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium* et *Stachybotrys* (**Greco et al. 2012**). Ces métabolites se produisent couramment dans les denrées alimentaires et induisent des pathologies chez les consommateurs. Ils sont détectés dans une large variété d'aliments et sont repérées dans les champs agricoles avant et après la récolte, et même durant les longues périodes de stockage ou pendant la transformation industrielle des aliments (**Almeida-Ferreira et al. 2013 ; Perši et al. 2014 ; Pizzolato Montanha et al. 2018**). Leurs détections est aussi possible dans l'eau et les boissons (**De Jesus et al. 2018 ; Mata et al. 2015**).

Les mycotoxines constituent un groupe très diversifié de composés chimiques. Leur toxicité est alarmante pour l'Homme, les animaux et les végétaux (**Peraica et al. 1999**). Elles sont capables d'induire une néphrotoxicité (**Palabiyik et al. 2013 ; Limonciel et Jennings 2014**), une génotoxicité (**Adgigitov et al. 1984 ; Bárta et al. 1984 ; Theumer et al. 2018**), une tératogénicité (**Celik et al. 2000 ; Sur et Celik 2003**), une neurotoxicité (**Ikegwuonu 1983 ; Mehrzad et al. 2017**), une hépatotoxicité (**Gagliano et al. 2006 ; Gayathri et al. 2015**), une immunotoxicité (**Al-Anati et Petzinger 2006 : Thuvander et al. 1995**), une altération des membranes (**Ciacci-Zanella et al. 1998**), une toxicité gastrointestinal (**Bouhet et al. 2004 ; Loiseau et al. 2007**), une cardiotoxicité (**Constable et al. 2000**), une toxicité pulmonaire (**Smith et al. 1999**), et conduisant parfois au développement des cancers (**Barrett et al. 2005 ; Wu et Santella 2012 ; Magnussen et Parsi 2013**).

Afin de limiter la diffusion et le développement des moisissures toxinogènes et leurs mycotoxicoses dans les denrées alimentaires, une politique de contrôle est appliquée ces dernières années. Cette politique est basée sur la prévention de la contamination, la décontamination et la protection contre les toxicités des mycotoxines par application de plusieurs techniques physiques, chimiques et biologiques (**Varga et al., 2010**). Cependant, il est très difficile de détruire les mycotoxines par les techniques physiques sans altéré la qualité des aliments (**Calado et al. 2018**). Par ailleurs, l'utilisation des procédés chimiques ont aussi des effets indésirables, car elles

participent dans la génération des radicaux libres et par conséquent, une induction d'un stress oxydatif (**Štikarová et al., 2013 ; Fatokun et al., 2006**).

Malgré l'utilisation contrôlée des antibiotiques, et la mise en place de plusieurs plans correctifs par les services de santé, l'antibiorésistance continue de se développer et de se disséminer en installant des impacts négatifs chez les patients et le système de santé (**Friedman et al. 2016**).

Devant l'impuissance des plans correctifs établis par les organisations de santé dans le but de stopper l'antibiorésistance, les chercheurs ont quitté les plateformes classiques vers la recherche de nouvelles stratégies de lutte et de prévention. L'une des plateformes suivies par les scientifiques est l'extraction des métabolites secondaires des plantes, leur caractérisation et la recherche de leurs activités biologiques (**Njoki et al., 2017**).

Les actinobactérie sont des aérobies, sporogènes, Gram positif et produisant un mycélium aérien. Dans le sol, elles jouent un rôle dans le cyclisme de la matière organique et la dégradation des débris organiques, inhibent les agents pathogènes par production des métabolites secondaire et participent à la bio-dépollution des sols pollué par les hydrocarbures (**Bhatti et al. 2017**).

La recherche de nouvelles souches d'actinobactéries productrices de nouveaux antibiotiques est considérée comme une autre stratégie suivie par les chercheurs pour combattre les germes pathogènes (**Mahajan et Balachandran 2012**).

Les actinobactéries extrêmophile isolées du désert correspondent à des bactéries alcalithermophiles, thermophiles, thermoacidophiles, thermophiles radiotolerantes, thermophiles alcalitolerantes, halophiles et haloalcalophiles (**Kurapova et al. 2012**). Les polyextrémophiles et les polyextrémotolérantes existent également dans les environnements avec des conditions extrêmes et peuvent s'adapter à des environnements à contraintes multiples (**Gupta et al. 2014**).

Plusieurs recherches ont révélé le rôle, l'importance écologique et l'intérêt médical des actinobactéries (**Goudjal et al. 2013 ; Shirokikh et Shirokikh 2017 ; Baoune et al. 2018**), mais peu d'études se sont intéressées au rôle et l'importance des actinobactéries isolées des environnements extrêmes (**Mohammadipanah et Wink 2015**).

L'utilisation des substances naturelles dans la lutte contre les microbes pathogènes et toxinogènes, et la détermination de leur activité antiradicalaire est à l'origine du choix de notre thème qui consiste en l'extraction de quelques métabolites de *Pistacia. lentiscus, Artemisia herba-alba,* et les graines de *Citrullus colocynthis.* Un isolat d'actinobactérie a aussi fait l'objet de cette

étude. Les métabolites secondaires des plantes et de l'isolat d'actinobactérie sont soumis à une série de tests afin de déterminer leurs activités biologiques.

Le manuscrit de la thèse est structuré de huit chapitres et d'une annexe. Il est ainsi organisé comme suit :

Introduction générale ; qui consiste en une brève présentation de la thèse. Les objectifs et les contributions originales de la thèse sont aussi précisés dans cette introduction.

Le manuscrit est composé d'une partie bibliographique qui traite essentiellement cinq chapitres publiés sous forme d'articles ou de chapitres dans différents livres à savoir :

Chapitre I : dans lequel l'occurrence des mycotoxines dans les denrées alimentaires et les liquides biologiques humain est dévoilée, les facteurs qui contrôle leurs biosynthèses sont aussi discutés. Le chapitre s'achève par un aperçu général sur méthodes de détection des mycotoxines.

Chapitre II : consacré à discuter les toxicités de quatre mycotoxines très abondantes dans les denrées alimentaires. La génotoxicité, la tératogénicité, l'immunotoxicité, la néphrotoxicité, l'hépatotoxicité et la neurotoxicité de l'aflatoxine, l'ochratoxine, la fumonisine et la zéaralénone sont expliquées d'une manière approfondie.

Chapitre III : restitue les techniques appliquées dans les champs afin de prévenir la contamination des cultures agricoles par les moisissures et/ou leurs mycotoxines. Les méthodes de décontamination et de détoxification biologiques, chimique et physiques sont discuter dans cette partie. Le chapitre s'achève par une présentation de la nouvelle approche nanométriques et son mode d'action dans le contrôle des mycotoxines et/ou leurs moisissures.

Chapitre IV : rapporte la composition phytochimique et les activités pharmacologiques de trois plantes médicinales à savoir *Artemisia herba-alba*, *Citrullus colocynthis*, et *Pistacia lentiscus*. La toxicité des plantes est également discutée.

Chapitre V : se concentre principalement sur certains sites inexploités du désert algérien. Il divulgue les principaux sites géologiques jamais étudiés par les microbiologistes. Le chapitre discute d'une manière approfondie les mécanismes d'adaptation des actinobactéries des sols désertiques et les facteurs biotiques et abiotiques impliqués dans cette adaptation. Le chapitre se termine par des arguments garantissant que ces niches hébergent de nouvelles souches d'actinobactéries capables de synthétiser de nouveaux métabolites secondaires.

La seconde partie est consacrée à l'expérimentation et la discussion des résultats obtenues durant la réalisation de l'étude, elle est structurée en deux parties :

I. Plan expérimental : présente les différents dispositifs expérimentaux adoptés lors de la réalisation des différentes parties de cette étude.

lI. Résultats et discussion : organisée en trois chapitres :

Chapitre VI : dans lequel sont présentés les résultats et les discussions des travaux de recherche déterminant la composition phytochimique, l'activité antimicrobienne et antioxydante des trois plantes médicinales récoltées dans la steppe et le Sahara algérienne.

Chapitre VII : consacré à l'identification et à l'évaluation de la capacité antimicrobienne et antioxydante de l'isolat d'actinobactérie forestière V_{002}. Les métabolites secondaire produits par l'isolat V_{002} sont aussi déterminés

Chapitre VIII : rapporte les résultats et les discussions de l'efficacité des extraits de plantes et de l'isolat V_{002} à inhiber la biosynthèse de l'aflatoxine et de l'ochratoxine.

Conclusion générale et perspective ; permettant de clôturer le travail réalisé et annoncer les perspectives envisagées à l'échelle du laboratoire et à l'échelle du terrain pour une meilleure compréhension de la thématique entreprise.

Il est à noter que la présente thèse est sous forme de thèse-Articles. Elle commence par une introduction en Français suivie d'une partie bibliographique et une partie expérimentale. Chaque partie est représentée par des chapitres ou des articles parus dans des livres ou dans des revues scientifiques. Une conclusion générale des travaux réalisés est également présentée en Français.

Partie bibliographique

I. Occurrence, toxicité et méthodes de détection des mycotoxines.

I. Occurrence, toxicité et méthodes de détection des mycotoxines.

«Mycotoxins occurrence, toxicity and detection methods»

(Chapitre publié en Février 2020 dans la série de livre : Sustainable Agriculture Reviews-- Springer Nature).

Les mycotoxines sont des métabolites secondaires toxiques d'origine polykétides synthétisées par des microorganismes filamenteux appartenant à six genres fongiques. Malgré que certaines d'entre elles possèdent des aptitudes pharmaceutiques, la majorité des mycotoxines sont considérées comme des poisons.

Ces composés très diversifiés dans leur nature, leur structure, et leur toxicité sont abondants dans une large gamme de produits alimentaires d'origines végétale et animale tels que le blé, le maïs, le riz, les légumes, les fruits, le lait, les viandes, les poissons et les œufs.

Les mycotoxines sont produites durant les différents stades de la croissance d'une plante. Elles sont élaborées au niveau du champs, durant la récolte, au-cours du séchage, pendant les longues périodes de stockage, pendant le transport, en particulier le transport maritime et durant les processus de transformation. Ces métabolites peuvent aussi être élaborés au cours des procédures de fermentation alimentaires, notamment, la production des boissons alcoolisés et les légumes fermentés.

L'occurrence des mycotoxines ou de leurs sous-produits dans les produits carnés et/ou le lait résulte d'une alimentation animale contaminée. Leur détection dans les liquides et/ou les tissus biologiques est le résultat d'une bio-détoxification incomplète établie dans les cellules hépatiques et/ou dans les glomérules des reins. Les mycotoxines sont aussi détectées dans l'eau potable résultant de l'endommagement des conduites, ces atteintes négligées forment une véritable source de contamination fongique.

La synthèse des mycotoxines est sous le contrôle de plusieurs facteurs tels que les conditions biotique et abiotique adéquates qui sont la force motrice recommandée pour la biosynthèse. En effet, lorsque les conditions sont favorables, la production des mycotoxines est exponentielle. La connaissance de ces facteurs est un point crucial dans la suppression ou l'expression des gènes requis dans la biosynthèse des toxines. L'état de la grappe des gènes, la

présence d'une source de carbone et d'azote, la température et l'humidité optimales sont parmi les plus importants facteurs impliqués dans la biosynthèse des mycotoxines.

Par leur richesse en azote, les acides aminés jouent un rôle primordial dans l'élaboration des aflatoxines, alors que chez d'autres espèces de moisissure, la présence du tryptophane bloque la synthèse des toxines. Par ailleurs, les acides aminés, la présence des nitrites et/ou des nitrates peut aussi bloquer la grappe de gènes de biosynthèse par la liaison de ces composés au régulateur de l'expression des gènes (aflR).

Chez certaines espèces fongiques, l'humidité n'est pas un facteur limitant, vu que la synthèse des mycotoxines est possible dans des atmosphères peu humides. Quant au pH, l'environnement acide est favorable pour la biosynthèse des mycotoxines. Chez certaines espèces fongiques, l'obscurité et la luminosité influencent considérablement le taux de production des mycotoxines.

Le mécanisme par lequel les conditions environnementales contrôlent l'expression ou la répression de la grappe des gènes de biosynthèse des mycotoxines est très compliqué. Les facteurs abiotiques sont convertis sous forme de signaux chimiques puis transmis à l'intérieur de la cellule fongique, où ils sont reçus par les gènes régulateurs portés par le génome. La modification de la transcription et/ou la traduction des gènes impliqués dans la réception et la transmission des signaux réceptionnés du milieu extracellulaire, ou les gènes impliqués dans le métabolisme affecte négativement la biosynthèse des mycotoxines. Par ailleurs, les mutations au niveau de la grappe des gènes de biosynthèse des mycotoxines conduisent à la baisse de leur synthèse.

L'exposition permanente des organismes vivants supérieurs à de fortes concentrations de mycotoxines supérieures à l'apport quotidien tolérable cause l'apparition de plusieurs mycotoxicoses caractérisées dans la majorité des cas par des mutations génomiques irréversibles et de sérieux dommages cellulaires. Les pathologies résultantes sont principalement dues au stress oxydatif imposé par les toxines franchissant les cellules des tissus cibles. Les mycotoxicoses causées sont très nombreuses, elles dépendent d'une part des types de mycotoxines, et d'autre part, de leurs cibles tissulaires.

Afin d'empêcher la persistance de la toxicité menant au développement d'une maladie, la détection précoce des toxines dans les aliments, les liquides biologiques, et même dans les tissus est une approche recommandée. Cette voie permet de limiter les dommages cellulaires et permet également de gérer les situations avant l'induction de la maladie. L'occurrence des mycotoxines

ou de leurs résidus dans le sang et les tissus biologiques est due à la décomposition incomplète des toxines lors des processus de détoxification. En outre, l'abondance des toxines dans le sang dépend fortement de leur demi-vie biologique et de leur affinité aux composants sanguins. Les mycotoxines peuvent se lier aux récepteurs cellulaires membranaires avec des affinités variables. Plusieurs types de mycotoxines ou de leurs sous-produits se lient à l'albumine sérique formant un complexe caractérisé par une faible toxicité et une incapacité d'être absorbés par les cellules.

Suite aux appels alarmants des organisations mondiales, en particulier l'OMS et la FAO signalant les risques et les maladies induites par les toxines, la détection des mycotoxines dans les produits destinés à l'alimentation de l'Homme et l'animal est devenue une nécessité. Cependant, le contrôle de ces poisons est loin d'être appliqué dans les pays sous-développés, alors que pour d'autre pays, il est complètement négligé.

Les méthodes analytiques de contrôle et de détection ont subi une importante innovation et progression ces dernières années. Ces méthodes sont actuellement divisées en deux classes à savoir les méthodes conventionnelles et les méthodes de détection basées sur les nanoparticules. La différence entre les deux réside dans le temps de réponse, la limite de détection, la qualification du personnel, le cout de la détection et la préparation des échantillons qui précède l'analyse.

La chromatographie est l'une des techniques les plus utilisées dans la révélation et la quantification des mycotoxines après leur extraction des matrices alimentaires. C'est une technique de séparation des molécules basée sur leur poids. Afin d'éviter les interférences avec d'autres composés organiques, la chromatographie nécessite une bonne préparation des échantillons. Parmi les chromatographies utilisées pour la détection des mycotoxines, on cite la chromatographie sur couche mince (CCM) et chromatographie liquide à haute performance (HPLC).

Les puces à ADN sont une autre approche capable de révéler directement les espèces fongiques productrices de mycotoxines. La technique nécessite tout d'abord une amplification de l'ADN fongique par PCR. Les produits de l'amplification obtenus subissent par la suite une hybridation avec les oligonucléotides immobilisés sur la puce.

La technique ELISA est aussi une technique recommandée dans les tests analytiques qualitatif et/ou quantitatif. Elle est basée sur l'immobilisation des anticorps ou des antigènes spécifiques sur les surfaces des microplaques. Les mycotoxines sont détectées avec une sensibilité

élevée après une seconde réaction avec des anticorps marqués par une enzyme chromogène. Les résultats sont révélés par le lecteur de microplaque.

La Radio immuno assay (RIA) est une nouvelle approche qui permet de détecter les mycotoxines dans les tissus biologiques, elle permet la détection de certains types de mycotoxines sans passer par l'étape d'extraction.

La deuxième classe des méthodes de détection des mycotoxines est basée sur les nanoparticules. Ces nanostructures sont devenues une technologie à part, franchissant presque tous les domaines. Les nanoparticules inorganiques à base d'or, d'argent, de cuivre, de carbone et de zinc sont les plus utilisées dans la conception des nanocomposites. Les techniques basées sur les nanoparticules sont capables de détecter et de quantifier plusieurs mycotoxines en même temps avec une sélectivité et une sensibilité très élevée.

Chapter 1
Mycotoxins Occurrence, Toxicity and Detection Methods

Mohamed Amine Gacem, Aminata Ould El Hadj-Khelil, Badreddine Boudjemaa, and Hiba Gacem

Abstract Mycotoxins and their derivatives constitute a toxic group of bioproducts for human and animals' health, they induce economic losses in cereals and stored food products. The knowledge of the biosynthetic mechanisms of mycotoxins are important to improve food quality. Polyketides are the first precursors, they are synthesized by a variety of multifunctional enzymes named polyketide synthases. This review discusses the occurrence of mycotoxin in food and human biological fluids, the toxicities caused *in vitro* and *in vivo* in human and animals' organs, and finally, some conventional and recent detection methods for their detection and quantification.

Keywords Mycotoxins · Occurrence of mycotoxin · Toxicities · Detection methods

M. A. Gacem (✉)
Laboratory of Ecosystems Protection in Arid and Semi-Arid Area,
University of Kasdi Merbah, Ouargla, Algeria

Department of Biology, Faculty of science, University of Amar Tlidji, Laghouat, Algeria

A. Ould El Hadj-Khelil
Laboratory of Ecosystems Protection in Arid and Semi-Arid Area,
University of Kasdi Merbah, Ouargla, Algeria

B. Boudjemaa
Department of Biology, Faculty of science, University of Amar Tlidji, Laghouat, Algeria

H. Gacem
Epidemiology Service and Preventive Medicine, Faculty of Medicine,
University of Djillali Liabes, Sidi-Bel-Abbes, Algeria

© Springer Nature Switzerland AG 2020 1
E. Lichtfouse (ed.), *Sustainable Agriculture Reviews 40*, Sustainable Agriculture Reviews 40, https://doi.org/10.1007/978-3-030-33281-5_1

1.1 Introduction

Mycotoxins are fungal secondary metabolites, some of which have pharmacological activities and they are used in antibiotics production, growth promoters, or in other classes of drugs (Sava et al. 2006). The majority of mycotoxins are polyketide origin (Huffman et al. 2010), polyketides (PK) are natural metabolites found in several compounds and sometimes have important biological activities (Gomes et al. 2013), their enzymatic mechanisms of biosynthesis is similar to fatty acids synthesis (Huffman et al. 2010).

Mycotoxins are produced by several fungal genera (Greco et al. 2012) and are present in a wide variety of foods, either before harvest or after industrial processing (Almeida-Ferreira et al. 2013; Schaafsma and Hooker 2007; Perši et al. 2014; Pizzolato Montanha et al. 2018). Their detections are also possible in water, drinks and wine (De Jesus et al. 2018; Mata et al. 2015). They are a very diverse chemicals group with a high toxicity for humans, animals and even plants (Peraica et al. 1999). Their toxicities may be manifested in all vital human or animal organs inducing nephrotoxicity (Palabiyik et al. 2013; Limonciel and Jennings 2014), genotoxicity (Adgigitov et al. 1984; Bárta et al. 1984; Theumer et al. 2018), teratogenicity (Celik et al. 2000; Sur and Celik 2003), neurotoxicity (Ikegwuonu 1983; Mehrzad et al. 2017), hepatotoxicity (Gagliano et al. 2006; Gayathri et al. 2015), immunotoxicity (Al-Anati and Petzinger 2006; Thuvander et al. 1995), membrane damage (Ciacci-Zanella et al. 1998), gastrointestinal toxicity (Bouhet et al. 2004; Loiseau et al. 2007), cardiotoxicity (Constable et al. 2000), pulmonary toxicity (Smith et al. 1999), and sometimes leading to cancer development (Barrett 2005; Wu and Santella 2012; Magnussen and Parsi 2013).

The destruction of these mycotoxins is very difficult because of their crystallization state or their high resistance under gamma radiation and high temperatures (Calado et al. 2018), whereas, chemical products detoxifying cause other health problems. The researchers have left the classic platforms of struggle, towards a new strategy using lactic acid bacteria (Khanafari et al. 2007), yeasts (Armando et al. 2012), actinobacteria (El Khoury et al. 2017, 2018), medicinal plants (Brinda et al. 2013). New research in progress aims to develop a new nanoparticles and nanomaterials to fight against these mycotoxins and their production sources (Hernández-Meléndez et al. 2018).

This review provides an overview of mycotoxins occurrence in food and biological fluids, the toxicities of some mycotoxins studied *in vivo* and *in vitro*, and the last section deals with the conventional and nanotechnological methods applied in the detection.

1.2 Occurrence of Mycotoxin in Food

Ochratoxin, aflatoxin, fumonisin, zearalenone and other mycotoxins are present in wheat and maize (Taheri et al. 2012; Almeida-Ferreira et al. 2013; Schaafsma and Hooker 2007; Shephard et al. 2005), they are also present in fruit, oil seeds and animal feeds that subsequently render them contaminated (Minervini and Dell'Aquila 2008), the biotransformation of mycotoxins in the animal body makes them present in cow's milk (Dehghan et al. 2014; Flores-Flores et al. 2015), meat (Perši et al. 2014; Pizzolato Montanha et al. 2018) and even eggs (Giancarlo et al. 2011).

Many drinks like wine and beer (De Jesus et al. 2018; Mateo et al. 2007), coffee and tea (García-Moraleja et al. 2015; Malir et al. 2014; Haas et al. 2013) are contaminated with mycotoxins. They are also present in bread produced from corn and wheat (Juan et al. 2008), and fermented food products (Kaymak et al. 2018; Adekoya et al. 2017). A recent study done on 84 samples identified the presence of mycotoxins in 99% of medicinal and aromatic plants, the percentage of damage depends on the toxin itself and the plant species (Santos et al. 2009). Mycotoxins are also present in herbal medicaments (Zhang et al. 2018). In bottle water, aflatoxin B2 was the most frequently detected mycotoxin with a concentration followed by aflatoxin B1, aflatoxin G1 and ochratoxin A (Mata et al. 2015). Table 1.1 shows some variety of food contaminated by mycotoxins in different countries.

1.3 Factors Controlling Mycotoxins Genesis

Mycotoxins require factors and precursors stimulating their biosynthesis, aflatoxin biosynthesis factors include nutritional factors such as nitrogen and carbon sources, and environmental factors such as pH, temperature, water activity, osmotic pressure … etc. the knowledge of these factors is very important in order to combat these poisons and their production sources. In culture medium, carbon and nitrogen are the most prominent nutritional factors fot the synthesis of aflatoxin or one of their metabolites, carbon is abundant in sugars such as glucose, sucrose, maltose, fructose, amino acids and proteins (Bennett et al. 1979). Plants, cereals and meats are also rich in these compounds in a complex form and favouring the biosynthesis of aflatoxins (Woloshuk et al. 1997). In *A. parasiticus,* researchers identified a group of genes near to aflatoxin synthesis genes, and these genes able to degrade and use sugars (Yu et al. 2000). Another gene named lipA produces substrates for aflatoxin biosynthesis, to determine it activity, this gene is cloned in *A. parasiticus* and *A. flavus*, the expression of this gene is induced by lipid substrates (Yu et al. 2003).

Table 1.1 Examples of food contamination by mycotoxins in the world

Food category	Feed type	Country	Mycotoxins	Limits of the legislation (µg.kg^{-1})	Range or mean	References
Milk and dairy products	Cattle milk	Japan	AFM_1		0.005 to 0.011 ng.g^{-1}	Sugiyama et al. (2008)
	Cow milk	Iran		500 ng.l^{-1}	60.1 ± 57.4 ng.l^{-1}	Rahimi et al. (2010)
	Camel milk				19.0 ± 7.5 ng.l^{-1}	
	Sheep milk				28.1 ± 13.7 ng.l^{-1}	
	Goat milk				30.1 ± 18.3 ng.l^{-1}	
		Cameroon		0,05 µg.kg^{-1}	0.006 to 0.527 µg.l^{-1}	Tchana et al. (2010)
	Milk from traditional dairies	Morocco		0,05 µg.kg^{-1}	10 and 100 ng.l^{-1}	El Marnissi et al. (2012)
	Milk	Algeria		0,05 µg.kg^{-1}	9 à 103 ng.l^{-1}	Redouane-Salah et al. (2015)
	Milk	Brazil			50–240 ng.l^{-1}	Garrido et al. (2003)
	Milk	Jordan			9,71 à 288,68 ng.kg^{-1}	Omar (2016)
	Fresh cheese	Costa Rica			31 to 276 ng.l^{-1}	Chavarría et al. (2015)
	Raw bulk milk	France		50 ng.l^{-1}	26 ng.l^{-1} or less	Boudra et al. (2007)
	Milk	Taiwan			1.17 to 54.7 ng.l^{-1}	Peng and Chen (2009)
	Milk	Pakistan		50 ng.l^{-1}	20 to 3090 ng.l^{-1}	Asghar et al. (2018)
Alcoholic beverages and juice	Apple juice	South Africa	PAT	50 ng.ml^{-1}	<10 to 75.2 ng.ml^{-1}	Katerere et al. (2007)
	Apple juice	Turkey			7 to 375 µg.l^{-1}	Gökmen and Acar (1998)
	Apple juice	Iran		50 µg.ml^{-1}	>3 µg.l^{-1}	Poostforoushfard et al. (2017)
	Maize based opaque beers	Malawi	FB_1		1522 µg.kg^{-1}	Matumba et al. (2014)
			FB_2		251 µg/kg^{-1}	
			FB_3		229 µg/kg^{-1}	

			AFB_1		36 µg/kg^{-1}	
			AFB_2		4 µg/kg^{-1}	
			AFG_1		55 µg/kg^{-1}	
			AFG_2		8 µg/kg^{-1}	
		South African	Deoxynivalenol (mean)		47 µg.l^{-1}	Adekoya et al. (2018)
			Neosolaniol (mean)		21	
			Fusarenon-X (mean)		167	
			AFB1 (mean)		6	
			AFG2 (mean)		5	
			Alternariol (mean)		47	
			Alternariol methyl ether (mean)		41	
			FB1 (mean)		151	
			FB2 (mean)		96	
			FB3 (mean)		36	
			Sterigmatocystin (mean)		18	
			Roquefortine C (mean)		3	
			Enniatin B (mean)		17	
	Traditional beer	Botswana	Zearalenone		20–201 µg.l^{-1}	Nkwe et al. (2005)
Meat, egg and fish	Egg	Cameroon	Aflatoxine B_1, B_2, B_{2a}, G_1 and M_1		0.002 to 7.604 ppb	Tchana et al. (2010)
	Chicken livers	Mozambique	AFB_1		1.73 µg.kg^{-1}	Sineque et al. (2017)
	Gizzards				1.07 µg.kg^{-1}	

(continued)

Table 1.1 (continued)

Food category	Feed type	Country	Mycotoxins	Limits of the legislation (µg. kg^{-1})	Range or mean	References
	Fermented sausage 'slavonski kulen	Croatia	AFB_1		Nd to 14.46 µg.kg^{-1}	Pleadin et al. (2017)
			OTA		Nd to 19.84 µg.kg^{-1}	
	Artisanal and industrial dry sausages	Italy	OTA		3 to 18 µg.kg^{-1}	Iacumin et al. (2009)
	Dried insects	Zambia	AFS	10 µg.kg^{-1}	2.9 to 36.8 µg.kg^{-1}	Kachapulula et al. (2018)
	Fish				0 to 20.4 µg.kg^{-1}	
	Fish	Argentina	T-2 toxin		Median 70.08 ppb	Greco et al. (2015)
			ZEA		Median 87.97 ppb	
			Aflatoxins		Median 2.82 ppb	
Fruits and vegetables fresh and processed	Date palm fruits	Egypt	AFB_1		14.4 µg.kg^{-1}	Abdallah et al. (2018)
			AFB_2		2.44 µg.kg^{-1}	
			OTA		1.48 to 6070 µg.kg^{-1}	
			OTB		0.28 to 692 µg.kg^{-1}	
			FB_1		4.99 to 16.2 µg.kg^{-1}	
	Dried vine fruits	Greek	AFB_1	2 µg.kg^{-1}	0.05 to 0.1 µg.kg^{-1}	Kollia et al. (2014)
			OTA	10 µg.kg^{-1}	5.5 to 101.5 µg.kg^{-1}	
	Dried vine fruits	China	OTA	10 µg.kg^{-1}	<0.07 to 12.83 µg.kg^{-1}	Zhang et al. (2014)
	Raisins and currants	Italy	OTA	10 µg.kg^{-1}	Nd to 15.99 µg.kg^{-1}	Fanelli et al. (2017)
	Dried fruits	United States	OTA	10 ng.g^{-1}	0.28 to 15.34 ng.g^{-1}	
	Dried fruits	Tunisia	AFB_1		45 ng.g^{-1}	Ghali et al. (2010)
			AFB_2		40 ng.g^{-1}	
			AFG_2		5 ng.g^{-1}	

	Peanut butter	Zambia	AFB_1 (2012, 2013 and 2014)		>20 to 130 µg.kg^{-1} >20 to 10,740 µg.kg^{-1} >20 to 1000 µg.kg^{-1}	Njoroge et al. (2016)
		Zimbabwe	AFS		6.1 to 247 ng.g^{-1}	Mupunga et al. (2014)
Cereals and cereal-based products	Maize	India	AFB_1		48 to 8 µg.kg^{-1}	Mudili et al. (2014)
			FB1		76 to 123 µg.kg^{-1}	
			T-2		38 to 50 µg.kg^{-1}	
			DON		72 to 94 µg.kg^{-1}	
		Croatia	FB_1		196.8 to 1377.6 µg. kg^{-1}	Domijan et al. (2005)
			FB2		68.4 to 3084 µg.kg^{-1}	
			ZEA		0.62 to 3.22 µg.kg^{-1}	
			OTA		0.73–2.54 µg.kg^{-1}	
		Pakistan	AFB_1		0 to 30.92 µg.kg^{-1}	Shah et al. (2010)
			OTA		<0.001 to 7.32 µg.kg^{-1}	
		Brazil	AFB_1		0.2 to 129 µg.kg^{-1}	Vargas et al. (2001)
			ZEA		36.8 to 719 µg.kg^{-1}	
			FB_1		200 to 6100 µg.kg^{-1}	
	Rice	Pakistan	AFB_1		1.07 to 24.65 µg.kg^{-1}	Asghar et al. (2014)
			AFB_2		0.52 to 2.62 µg.kg^{-1}	
		Nigeria	AF		28–372 µg.kg^{-1}	Makun et al. (2011)
			OTA		134–341 µg.kg^{-1}	
		Côte d'Ivoire	AFB_1		<1.5 to 10 µg.kg^{-1}	Sangare-Tigori et al. (2006)
			OTA		0.16 to 0.92 µg.kg^{-1}	
			ZEA		20 to 200 µg.kg^{-1}	
		Sweden	AFB_1		Nd to 46.2 µg.kg^{-1}	Fredlund et al. (2009)
			AFB_2		Nd to 4.5 µg.kg^{-1}	

(continued)

Table 1.1 (continued)

Food category	Feed type	Country	Mycotoxins	Limits of the legislation (µg. kg^{-1})	Range or mean	References
	Wheat and by-product wheat	Romania	OTA		3.88 to 11.3 ppb	Alexa et al. (2013)
			DON		294 to 3390 ppb	
			FB		960 to 1180 ppb	
		Morocco (Wheat semolina couscous)	DON		20.6 to 106.6 ng.g^{-1}	Zinedine et al. (2017)
			AFG$_1$		1.0 to 2.5 ng.g^{-1}	
			AFG$_2$		1.6 to 5.5 ng.g^{-1}	
			ENA		2.6 to 651.7 ng.g^{-1}	
		Thailand (Noodles and breads)	DON		0.14 to 1.13 µg.g^{-1}	Poapolathep et al. (2008)
	Barley	Swiss (2013)	DON		239.8 ± 56.2 µg.kg^{-1}	Schöneberg et al. (2016)
			NIV		12.5 ± 4.3 µg.kg^{-1}	
			ZEA		3.7 ± 1.0 µg.kg^{-1}	
	Sorghum	Ethiopia	AFB$_1$		<LOD to 33.10 µg kg^{-1}	Taye et al. (2016)
		Uruguay	AFB1		1 to 14 µg kg^{-1}	Del Palacio et al. (2016)
			FB		533 to 933 µg kg^{-1}	

Nd not determined

AFB$_1$ Aflatoxin B$_1$, *AFB$_2$* Aflatoxin B$_2$, *OTA* Ochratoxin A, *AFG$_1$* Aflatoxin G$_1$, *OTB* Ochratoxin B, *AFG2* Aflatoxin G2, *OTC* Ochratoxin C, *AFM* Aflatoxin M, *AFs* Aflatoxin, *FB* Fumonisin, *FB$_1$* Fumonisin B$_1$, *FB$_2$* Fumonisin B$_2$, *FB$_3$* Fumonisin B$_3$, *ZEA* Zearalenone, *FB$_4$* Fumonisin B$_4$

The synthesis of aflatoxins is related also to the presence of nitrogen, this compound is present in a wide variety of compounds such as aspartate, alanine, ammonium sulfate, glutamate, glutamine, asparagine, yeast extract, casitone, peptone, ammonium nitrate, ammonium nitrite (Reddy et al. 1971, 1979). Despite the presence of nitrogen in nitrates, their presence suppresses the biosynthesis of aflatoxins (Niehaus and Jiang 1989) by increasing the expression of a protein encoded by aflR gene which subsequently binds to DNA and inhibits the biosynthesis (Chang et al. 1995), tryptophan also inhibits aflatoxin formation in *A. flavus* (Wilkinson et al. 2007). Some nitrogen regulatory genes are known such as nitrate reductase gene niaD, niiA gene for nitrite reductase, and areA gene responsible for assimilation (Chang et al. 1996).

It is well known that aflatoxin biosynthesis occurs in high water activities, but the biosynthesis is also possible during drought or in the areas with low water activity (Cotty and Jaime-Garcia 2007). In the biosynthetic mechanism, temperature has a direct influence, optimal production is localized between 28 °C and 30 °C, above 37 °C, the biosynthesis is almost inhibited (O'Brian et al. 2007), the increase in the temperature affects negatively the production by refusing the transcription of transcriptional regulator gene (aflR) (Yu et al. 2011). Oxidative stress is another factor that induces the formation and production of aflatoxin in *A. parasitcus* (Jayashree and Subramanyam 2000). Regarding pH, the production of aflatoxin is optimal in acidic environments whereas in alkaline media, the biosynthesis is inhibited. The pacC gene is a factor responsible for pH homeostasis and transcription (Tilburn et al. 1995), in alkaline media, it is possible that this pacC gene binds to aflatoxin transcriptional genes and inhibits the synthesis (Espeso and Arst 2000).

It should be noted that ochratoxins biosynthesis is linked to biotic and abiotic stress. The production of these mycotoxins is sensitive to environmental factors, such as carbon and nitrogen levels (Medina et al. 2008; Hashem et al. 2015), in *A. nidulans,* the AreA factor is necessary for permeases expression and catabolic enzymes of nitrogen (Wong et al. 2007). Nutrients can also stimulate Ochratoxin A (OTA) production such as bee pollen (Medina et al. 2004). Temperature, water activity and pH also affect ochratoxin synthesis, for example, OTA production by *A. ochraceus* and *A. carbonarius* in culture medium, is affected by water activity and not by changes in pH (Kapetanakou et al. 2009), for *A. niger*, the aggregate of cells allows them to grow over a wide range of pH (Esteban et al. 2006), another study confirms that optimal conditions for OTA production differ between strains (Passamani et al. 2014).

Few studies described the role of regulators in the transmission of environmental signals to the genome in order to activate or inhibit the expression of mycotoxin biosynthesis genes. In some fungal strains such as *P. verrucosum, P. nordicum*, and *A. carbonarius*, difficult osmotic conditions activate the signal cascade of HOG MAP kinase, which in turn activates various osmoregulatory genes (Stoll et al. 2013). OTA biosynthesis is also affected by oxidative stress (Moye-Rowley 2003), under conditions of high oxidative stress, evoked by increasing concentrations of Cu^{2+} in the medium; *P. verrucosum* produces citrinin, the latter normalizes the oxi-

dative status of fungal cells leading to an adaptation in these environmental conditions, the biosynthesis of citrinin is regulated by a cAMP/PKA signalling pathway (Schmidt-Heydt et al. 2015).

The suppression of hdaA, encoding a histone deacetylase in *A. nidulans* (HDAC), causes a subsequent increase in toxin production levels (Shwab et al. 2007). The LaeA, VeA and VelB complex is studied in fungal strains to clarify the relationship between light-dependent fungal morphology and sexual development for secondary metabolism production, the complex is functional in the dark, in addition, the deletion of laeA and veA in *A. carbonarius* caused a sharp reduction in conidial production and a decrease in OTA production, correlated with a downregulation of PKS-AcOTAnrps gene (Crespo-Sempere et al. 2013). In *A. niger*, OTA production was strongly inhibited by red and blue light compared with dark incubation, with an average reduction about 40-fold (Fanelli et al. 2012).

In addition to the proteins regulating genes transcription, other regulators are involved in the regulation of secondary metabolism, among these, the most studied are; pacC, the key factor for pH regulation in *A. nidulans* (Peñalva et al. 2008), deletion of the entire pacC coding region leads to poor growth and conidiation (Tilburn et al. 1995); CBC, which regulates the response to oxidation-reduction and iron stress; CreA, involved in the regulation of carbon metabolism; AreA and AreB, involved in nitrogen metabolism (Gallo et al. 2017).

Carbohydrates are an important source of carbon required for fumonisins biosynthesis In *F. verticillioides*, the ZFR1 gene controls the biosynthesis of FB_1 by regulating the genes involved in the perception or absorption of carbohydrates (Bluhm et al. 2008), nitrogen is also necessary for the growth of *F. verticillioides* to synthesize proteins and other compounds, the SGE1 gene increases and decreases the expression of certain genes including many effector genes encoding cell surface and transcription activator of fumonisin genes group (Brown et al. 2014).

Fumonisin production by *F. verticillioides* is dependent on water activity in the substrate (Medina et al. 2013). The optimum temperature for fumonisin production is 30 °C (Samapundo et al. 2005), but this temperature depends essentially on the species and can change (Mogensen et al. 2009) Oxidative stress also plays an important role in the modulation of fumonisin biosynthesis (Ferrigo et al. 2015). The pH significantly influences the production of FB_1, at a pH above 5, *F. proliferatum* develops normally but FB_1 is little produced. At a pH below 5, there is less growth but much more FB_1 produced. Below pH 2.5, growth and metabolism are slower with very little FB_1 produced (Keller and Hohn 1997).

Some genes are involved in the regulation of fumonisin biosynthesis and they are affected by environmental conditions. In *F. verticillioides*, the FST1 gene seems to be linked to the expression of several gene networks, particularly those involved in secondary metabolism, cell wall structure, production of conidia, virulence and ROS resistance (Niu et al. 2015), the FUG1 gene represents a new class of fungal transcription factors or genes that are otherwise involved in signal transduction, and play a role in the pathogenicity and fumonisins biosynthesis (Ridenour and Bluhm 2017).

Carbon and nitrogen are essential for the biosynthesis of zearalenone, in *G. zeae*, GzSNF1 gene is responsible for the use of carbon, it grants other biological functions in the sexual and asexual development of the species (Lee et al. 2009), while areA gene is a factor of nitrogen use in the same species (Min et al. 2012). Some mutant strain do not have Nit gene to assimilate nitrate but they are able to use nitrite (Robert et al. 1992). The temperature and water activity affect directly the synthesis of zearalenone, a large production by *F. graminearum* is observed at 25 °C with a water activity equal to 0.97 (Montani et al. 1988), above 37 °C the production of zearalenone is weakened (Jiménez et al. 1996). Under laboratory conditions, the desirable yield of mycotoxins from *F. graminearum* is obtained at an average pH of 6.86 and an incubation temperature of 17.76 °C during 28 days (Wu et al. 2017). In *F. graminearum*, the pac1 gene is responsible for the regulation of metabolite synthesis in the case of pH variations (Merhej et al. 2011), FgLaeA gene is a factor controlling metabolism, virulence and reproduction (Kim et al. 2013), FgVELB gene is a factor developing resistance under osmotic stress conditions (Jiang et al. 2012a).

1.4 Occurrence of Mycotoxins in Humans and Associated Diseases

Mycotoxin-producing fungi are almost ubiquitous in agricultural products before, during and/or after harvest, and may even be present during food processing processes. Various diets taken by humans can be contaminated by a wide range of mycotoxins, and following multiple studies in different countries of the world, these mycotoxins are the cause of several diseases, other studies have even demonstrated, *in vitro* and *in vivo*, the cytotoxicity mechanisms of these poisons.

The frequency and levels exposure of human to mycotoxins may be estimated by measuring the levels of biomarker in urine (Solfrizzo et al. 2011). During the realization of the Swedish National Nutrition Survey, the analysis done in 252 adult participants, simultaneously exposed to mycotoxins, using LC-MS/MS-based multibiomarker approaches, demonstrated that 69% of studied population was exposed to more than one toxin (Wallin et al. 2015). The detection of mycotoxins in biological fluids and human tissues is possible, a study done in patients exposed to mycotoxins with chronic fatigue syndrome (CFS) demonstrated that from 104 urine samples taken from patients, 93% were positive for at least one mycotoxin. The most frequently detected mycotoxin was OTA (83%), followed by macrocyclic trichothecenes. The results of this study demonstrated that 90% of patients were exposed to water-damaged buildings (WDB) (Brewer et al. 2013). Another study done in patients exposed to mycotoxin-producing fungi in their environment demonstrated that the levels of trichothecene in urine, sputum, biological tissues of the lung, liver and brain, after biopsy, varies from undetectable to 18 ppb. In the same tissue, aflatoxin and ochratoxin ranged from 1 to 5 ppb and from 2 to >10 ppb respectively (Hooper et al. 2009).

Application of multi-mycotoxin LC-MS/MS method for human urine analysis in the study conducted in Transkei region in South Africa, known by the high rate of esophageal cancer, demonstrated that morning urine of farmers participating in this survey contained fumonisin B_1, deoxynivalenol-15-glucuronide, zearalenone, α-zearalenol, deoxynivaleno, β-zearalenol and OTA (Shephard et al. 2013), another study with the same objective, carried out in a German population, was able to determine six mycotoxins in their urine, namely; deoxynivalenol, DON-3-glucuronide, zearalenone-14-O-glucuronide, toxin T-2, enniatin B and dihydrocitrinone, with an average daily intake of 0.52 $\mu g.kg^{-1}$ (bw) for DON, which is greater than the tolerable daily intake (Gerding et al. 2014). The study conducted by Gerding and his team in 2015 approved the previous result, this latest study also demonstrated that AFM_1 is detected in urine samples in a population of Bangladesh and Haiti with a higher rate of OTA and dihydrocitrinone in Bangladesh samples (Gerding et al. 2015). In Belgium, biomarker analysis showed a clear exposure of a large segment of the Belgian population to DON, OTA and CIT (Heyndrickx et al. 2015). A more serious situation is observed, the detection of multiple mycotoxins is recorded among HIV-positive people in Cameroon, demonstrating that this situation is very serious for cases of very low immunity (Abia et al. 2013). The detection of mycotoxins or their metabolites in the blood or blood serum is also possible by the fast-multi-mycotoxin approach, the study of Osteresch and its collaborators detected enniatin B, OTA and 2'R-OTA in dried blood and serum spots (Osteresch et al. 2017).

The detection of OTA and 2'R-OTA in the blood of drinkers and non-coffee drinkers demonstrated the presence of OTA in both groups with an average concentration of 0.21 $\mu g.l^{-1}$, whereas, 2'R-OTA was present only in coffee drinkers with a maximum concentration of 0.414 $\mu g.l^{-1}$, this result is explained by the formation of 2'R-OTA from OTA during roasting process (Cramer et al. 2015), in human blood, 2'R-OTA has a biological half-life seven times higher in comparison with OTA (Sueck et al. 2018a), this long period of life in human blood is explained by the affinity of both toxins to human serum albumin (HSA), which attenuates or even suppresses the acute cellular toxicity of mycotoxins (Faisal et al. 2018a), however, another study demonstrated that OTA has more binding affinity to ASH than 2'R-OTA with binding percentages of 99.6% and 97.2% respectively, suggesting that another human blood protein with a very high affinity for 2'R-OTA is responsible for its accumulation in the blood, or the high bioavailability of 2'R-OTA compared to OTA, or differences in the metabolism of this metabolite and its transport in tissues (Sueck et al. 2018b).

Citrinin and its metabolite dihydrocitrinone are also detected in human urine, a study done in a German population showed that the levels of these two metabolites varied between 0.02 to 0.08 $ng.ml^{-1}$ and 0.05 to 0.51 $ng.ml^{-1}$ respectively (Ali et al. 2015a), the both mycotoxins are also detected in two populations in Bangladesh (Ali et al. 2015b). Citrinin can also bind to human serum albumin, it main binding site is in sub-domain IIA (Sudlow's Site I), this binding forming the CIT-albumin complex, it has beneficial effects by decreasing the cellular absorption of CIT and consequently, the decrease of its toxicity (Poór et al. 2015).

Zearalenone can also form with human serum albumin a complex of great biological interest (Poór et al. 2017a), the both metabolites α-zearalenol and β-zearalenol can also bind to ASH but with a lower affinity in comparison to zearalenone, this low affinity may be due to the difference in position or binding site (Faisal et al. 2018b). Citreoviridine can also bind with ASH through hydrophobic bonds (Hou et al. 2015), AFB_1 also forms stable bonds with ASH, the high affinity binding site is Sudlow's Site I of subdomain IIA, furthermore, the reactive epoxide metabolite of AFB_1 forms covalent adducts with ASH (Poór et al. 2017b), a recent study done in calf using multispectroscopic techniques, DNA fusion, viscosity measurements, and molecular Docking techniques demonstrated that AFB_1 and AFG_1 can bind with thymic DNA (Ma et al. 2017). A study done in Malawi in 230 persons from a rural population living in three districts (Kasungu, Mchinji, and Nkhotakota), receiving a diet rich in groundnuts and maize, detected AFB_1-lys adducts in 67% of blood, with an average concentration of 20.5 ± 23.4 pg.mg^{-1} of albumin (Seetha et al. 2018).

This bioaccumulation in liquids, organs and tissues is reported worldwide, and most mycotoxins have toxic effects at low concentrations (Escrivá et al. 2017), mycotoxicosis is among the adverse effects of mycotoxins, the exposure to these fungal metabolites is mainly by ingestion, or also dermal and inhalation pathway (Peraica et al. 1999), the diseases caused by mycotoxins are varied and affect a wide range of animal species and human (Richard 2007), among proven diseases *in vitro*, caused by exposure to mycotoxins; cutaneous toxicity and skin tumorigenesis in several rodent models induced by oxidative stress (Doi and Uetsuka 2014). Fumonisin also causes several diseases with variable toxicities by interference with the sphingolipids biosynthesis in several organs (Bucci et al. 1998). Aflatoxin induces hepatotoxicity, in addition, acute exposure to AFB_1 increases plasma and hepatic cholesterol, triglyceride and phospholipid levels (Rotimi et al. 2017). The Table 1.2 below shows the different toxicities caused by mycotoxins, its target organs and the mode of action.

1.5 Conventional Methods for Mycotoxins Detection

Mycotoxin detection techniques classified as conventional techniques are very expensive analytical methods and require qualified personnel for each method to achieve the analyses, analytical instruments and products used in these techniques are also expensive and very complicated without neglected their detection limits, the samples analysed must be prepared beforehand in order to extract their contents in mycotoxins. Food safety is a challenge which is not yet achieved in the world, and following this crucial point, several official methods used in laboratories are validated by the Association of Official Agricultural Chemists (AOAC) to improve detection techniques. Protect the consumer and to ensure a good quality of food.

Table 1.2 Examples of mycotoxin toxicity

Toxicity	Mycotoxin	Target organ or cells lines	Mode of action	References
Hepato-gastrointestinal toxicity	ZEA	Intestines of pregnant sows	It caused changes in the structure of jejunum with oxidative stress and an inflammatory response.	Liu et al. (2018)
			Alterations of the bacterial numbers in cecal digesta are noted.	
	ZEA	Intestine of pregnant Sprague-Dawley.	It induced oxidative stress, affected the villous structure and reduced the expression of junction proteins claudin-4, occludin and connexin43 (Cx43)	Liu et al. (2014b)
	ZEA	Rats liver	It induced an increase on the transcription of cytochrome P450 2E1 (CYP2E1).	Zhou et al. (2015) and Jiang et al. (2012b)
			It induced an increase of liver enzymes.	
	T-2 toxin	Hela, Bel-7402, and Chang liver cells	It induced apoptosis.	Zhuang et al. (2013)
		Porcine stomach and duodenum	It caused an increase of VIP-LI nerve cells and nerve fibers in both fragments of gastrointestinal tract.	Makowska et al. (2018)
		Female genital organs (animals).	Decreased in follicle integrity.	Schoevers et al. (2012)
			Reduced in the quantity of healthy follicles, which may lead to premature oocyte depletion in adulthood.	
	FA	Oesophageal SNO cells	It induced an increase in cells apoptosis.	Devnarain et al. (2017)
	HT-2 toxin	Liver	It induced oxidative stress and cell damage.	Yang et al. (2016, 2017, 2019)
			It induced changes in mRNA, protein expression and apoptosis.	
	FB	Intestine	It caused disturbance of the sphingolipid biosynthesis pathway and conducted to an alteration in intestinal epithelial cell viability and proliferation.	Bouhet and Oswald (2007)
	ENN	Intestinal cell line IPEC1 and jejunal explants	It caused a decrease in cell proliferation.	Kolf-Clauw et al. (2013)
		IPEC-J2 cells	It caused a complete disruption with declined in cells viability.	Fraeyman et al. (2018)

	CB	Rat liver	It caused a decrease in albumin synthesis, glucosamine, and minimally affected glycoprotein secretion	Kaufman et al. (1988)
	MPA	LS180 cell	It affected intracellular nucleotide levels, expression of structural proteins, fatty acid and lipid metabolism.	Heischmann et al. (2017)
	RB	Mouse hepatic microsomes	It inhibited an enzyme activity located in the endoplasmic reticulum.	Watson and Hayes (1981)
		Dog	It is suggested as a primary etiological factor forhepatitis X.	Hayes and Williams (1978)
	VIM	Mice liver	It caused necrosis, periductal fibrosis, and hypertrophy and hyperplasia of biliary epithelium.	Carlton et al. (1976)
	CPA	Rat liver	It caused cells lysis and affected the endoplasmic reticulum.	Hinton et al. (1985)
		Monkey liver	It caused mild pathological changes in hepatocyte rough endoplasmic reticulum, small vessels and myocardium	Jaskiewicz et al. (1988)
	AF	Rat liver	It caused an increase levels of plasma and liver cholesterol, triglycerides and phospholipids.	Rotimi et al. (2017)
		Male broiler chicks	It caused an alteration in gene expression.	Yarru et al. (2009)
		Hy-Line W36 hens	It affected the functionality of the gastrointestinal tract.	Applegate et al. (2009)
Reproductive and developmental toxicity		Female genital organs (animals).	20 mg.kg^{-1} ZEA diets increased follicle-stimulating hormone concentrations and decreased oestradiol.	Gao et al. (2017)
		Male genital organs (animals).	20 mg.kg^{-1} ZEA caused a follicular atresia and a thinning uterine layer.	
			It inhibited mRNA and protein levels of oestrogen receptor-alpha (Esr1) and 3β-hydroxysteroid dehydrogenase (HSD).	
			Neonatal exposure to ZEA could negatively influence male fertility and his spermatogenesis by the exposure of testis to ABC transporter substrates.	Koraïchi et al. (2013)

(continued)

Table 1.2 (continued)

Toxicity	Mycotoxin	Target organ or cells lines	Mode of action	References
	ZEA	Mouse TM4 Sertoli cells	It caused damage to the cytoskeletal by autophagy stimulated through PI3K-AKT-mTOR and MAPK signaling pathways via elevated oxidative stress.	Zheng et al. (2018a)
	ZEA	Murine ES cells cardiac differentiation	It caused an inhibition of cardiac differentiation with ROS accumulated in murine ES cells.	Fang et al. (2016)
	T-2 toxin	Embryonic stem cells (ES cells D3) and fibroblast 3 T3 cells,	It induced cell cycle arrest and apoptosis.	Fang et al. (2012)
			It caused the release of cytochrome C from mitochondria with the upregulation of p53, caspase-9, caspase-3 expression.	
			It induced oxidative damage and apoptosis in the cells.	
		Zebrafish embryos.	It increased the mortality and malformation rate in early developmental stages of zebrafish.	Yuan et al. (2014)
		Sertoli cells	It inhibited the cell proliferation and through ATP/AMPK pathway induced by ROS generation, the toxin induced cell apoptosis.	Zheng et al. (2018b)
	DAS	Mouse	It induced a reduction in fetal body weight and a variety of fetal malformations.	Mayura et al. (1987)
		Pig	It induced a development of multifocal, proliferative, gingival, buccal and lingual lesions.	Weaver et al. (1981)
	FB	Chicken embryos	It induced embryonic mortality.	Henry and Wyatt (2001)
	VIM	Chicken embryos	It was mildly toxic and teratogenic	Anthony et al. (1982)
	CTN		It is embryocidal and fetotoxic	Flajs and Peraica (2009)
	PAT	Growing male rats	It caused an increase in testosterone levels and a decrease in T4 levels.	Selmanoglu and Koçkaya (2004)
			It caused an increase of TLR4 protein expression and inflammatory cytokines.	
	AF	Rabbits	AFB_1 was found teratogenic for fetal development in rabbits.	Wangikar et al. (2005)

Renal toxicity	OTA		It caused a developmental and reproductive toxicity	Malir et al. (2014)
	ZEA	Kidney and other organs (Spleen (rate), thymus, bursa of Fabricius)	It caused a histopathological damage in the spleen and a decrease viability of splenocytes and T-cell proliferation.	Yin et al. (2014)
			It reduced the level of anti-Newcastle disease virus antibody.	Xue et al. (2010)
			It reduced the concentrations of interleukin-2 and interferon-gamma in serum.	
			It caused a histological abnormality.	
	AF	HEK 293 cells model and CD-1 mice model	It caused kidney toxicity through oxidative stress and induced downstream apoptosis.	Li et al. (2018)
	OTA	Pig kidney	It affected genes expression in kidney.	Marin et al. (2017)
	MPA	Renal transplant	Toxicity is caused by an increase of free MPA concentration.	Mudge et al. (2004)
Immunomodulatory and immunopathological effects	OTA+ T-2 toxin	Spleen, thymus, and bursa of Fabricius (yellow-feathered broiler chickens)	It caused a decrease of spleen, thymus, and bursa of Fabricius weight.	Wang et al. (2009)
			It caused a diminution of the mitogenic responses of peripheral blood lymphocytes.	
		Lymphoid organs of mice	It caused a decrease in number of lymphocytes in the thymic cortex and splenic follicles.	Shinozuka et al. (1997)
			It caused a pyknosis or karyorrhexis in lymphocytes nuclei.	
	BT-2 toxin	Bone marrow and splenic red pulp of mice	It caused a hypocellularity with a decrease in the number of myelocytes due to the loss of immature granulocytes, erythroblasts, and lymphocytes.	Shinozuka et al. (1998)
		TM3 cells	It induced an apoptosis in TM3 cells by inhibiting mTORC2/AKT and promoting Ca2+ production.	Wang et al. (2018)

(continued)

Table 1.2 (continued)

Toxicity	Mycotoxin	Target organ or cells lines	Mode of action	References
	GT	Immune cells	It removed immune cells responsible for tissue rejection.	Waring and Beaver (1996)
			It induced degranulation, leukotriene C4 secretion, and TNF-alpha and IL-13 production.	Niide et al. (2006)
			It induced intracellular production of superoxide.	
		Immune organ	It induced apoptosis in cells.	Sutton et al. (1994)
	ENN	Jurkat T-cell	It caused a mitochondrial alteration.	Manyes et al. (2018)
		RAW 267.4 murine macrophages	It caused an accumulation of cells in the G0/G1-phase.	Gammelsrud et al. (2012)
			It caused lysosomal damage.	
			It caused an apoptotic and necrotic cell.	
	MPA	T-lymphoblast cell line	It can modulate Rho GDI 2 levels in T lymphocytes and disrupt cell signalling pathways.	Heller et al. (2009)
	PNT	Human neutrophil granulocytes	It caused an increase in ROS production by the activation of several MAPK-signalling pathways.	Berntsen et al. (2017)
	CPA	CD34+, monocytes, THP-1 and Caco-2)	It disturbed human monocytes differentiation into macrophages.	Hymery et al. (2014)
	PA	Rat alveolar macrophages	It caused an inhibition of phagocytosis	Sorenson and Simpson (1986)
	PAT	Thymus of growing male rats	The thymus showed haemorrhage, and alteration of tissue.	Arzu Koçkaya et al. (2009)
		Dendritic cells (IDCs) of rat thymus	It caused cell apoptosis.	Ozsoy et al. (2008)

Cytotoxicity	T-2 toxin	HepG2 and HEK293T cells	It induced an increase in the levels of mitochondrial biogenesis and ROS, by upregulation of SIRT1, which is controlled by miR-449a, whose expression was inhibited by toxin.	Ma et al. (2018)
		HeLa cells	It caused generation of ROS and increased lipid peroxidation. An apoptotic morphology with condensed chromatin and nuclear fragmentation are also noted.	Chaudhari et al. (2009)
		Human chondrocytes	It caused a decrease in chondrocytes viabilities.	Liu et al. (2014a)
	FA	Hepatocellular carcinoma (HepG2) cells	It induced apoptosis and necrosis by increasing activity of caspases.	Sheik Abdul et al. (2016)
			It induced mitochondrial stress.	
			It caused DNA damage and post-translational modifications of p53.	Ghazi et al. (2017)
			It induced NRF2 as a cytoprotective response to prevent NLRP3 (activator protein inflammasome).	Sheik Abdul et al. (2019)
	FB	U-118MG glioblastoma cells	It induced an oxidative stress, apoptosis and internucleosomal DNA fragmentation.	Stockmann-Juvala et al. (2004a)
	BEA	Chinese hamster ovary (CHO-K1) cells	It induced a disruption in mitochondrial enzymatic activity and cell proliferation and lead to the cell death.	Mallebrera et al. (2016)
		Caco-2 cells	It caused cytotoxicity and apoptosis via ROS production and mitochondrial damage	Prosperini et al. (2013)
	ENN	Balb 3 T3 and HepG2 cells	It altered cellular energy metabolism and reduced cell proliferation.	Jonsson et al. (2016)
			It caused necrotic cell death.	
		Caco-2 cells	It caused a lysosomal membrane permeabilization	Ivanova et al. (2012)
			It caused cell cycle arrest in the G2/M phase leading to the apoptosis or necrosis.	
		HepG2	It caused an apoptosis and necrosis.	Juan-García et al. (2013 2015)

(continued)

Table 1.2 (continued)

Toxicity	Mycotoxin	Target organ or cells lines	Mode of action	References
	NIV	Human erythroleukemia cell line	It caused DNA damage and apoptosis.	Minervini et al. (2004)
		HepaRG human hepatocyte cell line	It caused hepatotoxicity.	Smith et al. (2017)
	CB	Cells lines	It induced cellular DNA fragmentation.	Kolber et al. (1990)
	MPA	Human umbilical vein endothelial cells	It inhibited the lymphocyte proliferation and decreased the expression of adhesion molecules on endothelial cells.	Raab et al. (2001)
		Human peripheral blood mononuclear cells	It induced a decrease in GTP levels and an increase of UTP.	Daxecker et al. (2001)
	CTN	Porcine kidney PK15 cells	It stimulated Hsps expressions.	Segvić Klarić et al. (2014)
			It caused alterations in cellular redox status.	
		HepG2 cell	It caused a production of ROS, DNA damage and mitochondria-mediated intrinsic apoptosis.	Gayathri et al. (2015)
			It caused a disturbance of Ca^{2+} homeostasis, cell genotoxicity and death.	Klarić et al. (2012)
	OTA	HepG2 and KK-1 cells	It caused a production of ROS.	Li et al. (2014)
		HT-29-D4 and Caco-2-14 cells	It caused an alteration of the barrier and absorption functions of the intestinal epithelium	Maresca et al. (2001)
	ZEA	Destruction of cytoskeletal structure in kidney	It caused kidney dysfunction and damage.	Jia et al. (2014)
		Cell cycle arrest and apoptosis in kidney and spleen (rate)	It induced a degenerative change in the kidney and an increase of the biochemical parameters and inflammatory cytokines.	Jia et al. (2015)
Neurotoxicity	FA	Swine	It caused vomiting and neurochemical changes.	Smith and MacDonald (1991)
		Brain and pineal gland of rats.	It caused an increase in brain serotonin, 5-hydroxyindoleacetic acid, tyrosine and dopamine, and a decrease in norepinephrine.	Porter et al. (1995)
		Pineal cell cultures	It caused an increase in melatonin levels.	Rimando and Porter (1997)

	FB	Human SH-SY5Y neuroblastoma, rat C6 glioblastoma and mouse GT1-7 hypothalamic cells	It induced an oxidative stress characterised by the production of reactive oxygen species, lipid peroxidation and necrotic cell death.	Stockmann-Juvala et al. (2004b)
	RB	Mouse brain	It induced a toxicity in specific brain regions by altering SOD and OGG1 activities.	Sava et al. (2018)
	PNT	Rat cerebellar granule neurons	ROS production and the GABAA receptor are the causes of neuronal death.	Berntsen et al. (2013)
	CPA	Rabbit	10 mg CPA.kg^{-1} (bw) produced tachycardia, tachypnoea and sedation.	Nishie et al. (1985)
	AF	Zebrafish embryos and larvae	It affected the behaviors and neurodevelopment	Wu et al. (2019)
	OTA	Mouse brain	It altered the proliferation and differentiation in subventricular zone.	Paradells et al. (2015)
	CIV	Rat brain	It inhibited brain synaptosomal and altered the enzymatic activities.	Datta and Ghosh (1981)
Pulmonary toxicity	FB	Swine	It caused pulmonary edema and death.	Osweiler et al. (1992)
	RQF	Dog	It caused a development of aspiration pneumonia	Young et al. (2003)
	OTA	RAT	It caused a damage in tissue like alveolar congestion, alveolar cell hyperplasia, and respiratory epithelial proliferation.	Okutan et al. (2004)
	AF	Mousse lung	It caused a development of tumorigenesis.	Massey et al. (2000)
	CIV	Rabbit	It caused respiratory arrest and death.	Nishie et al. (1988)

Some toxicity induced (in vivo and in vitro) by the different mycotoxins
AF aflatoxin, *BEA* beauvericin, *CIV* citreoviridin, *CTN* citrinin, *CPA* cyclopiazonic acid, *CB* cytochalasin, *DAS* diacetoxyscirpenol, *ENN* enniatin, *FRE* frequentin, *FB* fumonisins, *FA* fusaric acid, *GT* gliotoxin, *HT-2 toxin*, *T-2 Toxin*, *MPA* mycophenolic acid, *NIV* nivalenol, *OTA* ochratoxin A, *PAT* patulin, *PA* penicillic acid, *PNT* penitrem A, *RQF* roquefortine, *RB* rubratoxin B, *VIM* viomellein, *ZEA* zearalenone
bw body weigh

1.5.1 Chromatographic-Based Techniques

1.5.1.1 Thin-Layer Chromatography

Thin layer chromatography can detect a wide range of mycotoxins after extraction with different solvents and at different pH, it is applicable in different types of food, after mycotoxin extraction (Gimeno 1979). In order to limit interference with lipids, pigments and other food compound, this chromatographic screening technique has undergone improvements, by using membrane cleaning (Roberts and Patterson 1975). Toluene, ethyl acetate and formic acid are the three solvents used for the separation. The revelation of the chromatogram is carried out by exposing the plate to pyridine or acetic anhydride vapors, or a mixture of both, the spots of the mycotoxins appear fluorescent under UV light at 365 nm (Goliński and Grabarkiewicz-Szczesna 1984). AFB_1 is detected at concentrations ranging from 0.1 to 0.3 $\mu g.kg^{-1}$ (Patterson and Roberts 1979), while for others, such as diacetoxyscirpenol, ochratoxin A, patulin, penitrem A, sterigmatocystin, toxin T-2 and zearalenone are detected in a less reliable manner (Roberts and Patterson 1975).

1.5.1.2 High-Performance Thin-Layer Chromatography

After determining the role and toxicity of mycotoxins in human and animal organism, the chromatography technique has developed with more performance, high-performance thin-layer chromatography (HPLC) and Reversed-phase liquid chromatography (RPLC) coupled with a fluorescence detector are the two most used instruments for mycotoxins detection (Orti et al. 1986), other machines are currently running with wavelength variation, a fluorescence detector, and require an extraction and immunoaffinity column (IAC) for clean-up (Pena et al. 2006; Chen et al. 2017). Liquid chromatographic-tandem mass spectrometric method (LC-MS/MS) is another instrument developed for the determination of mycotoxins biomarkers in human or animal urine, the sample preparation requires several steps of clean-up (Solfrizzo et al. 2011), the latter technique is recommended for mycotoxin biomarkers analysis in biological fluids (Gerding et al. 2014). Other technique are developed such as liquid chromatography combined with heated electrospray ionization triple quadrupole tandem mass spectrometry (LC-h-ESI-MS/MS) which has been developed for the multiple detection of mycotoxin in plasma with LOD ranging from <0.01 to 0.5 $ng.ml^{-1}$ (Devreese et al. 2012) and et Ultrasound-assisted solid-liquid extraction and immunoaffinity column clean-up coupled with high performance liquid chromatography and on-line post-column photochemical derivatization-fluorescence detection (USLE-IAC-HPLC-PCD-FLD) (Kong et al. 2013).

1.5.2 Microarrays for Mycotoxin Detection

Bio-analytical technology based on DNA microarrays are used for the detection and selection of fungal mycotoxin-producing strains, this transcriptomic technique based on hybridization is very effective for the identification of mycotoxin expression genes in complete sequences of fungal DNA (Emri et al. 2017), Several microarray oligonucleotides are developed to determine the pathogens in a single reaction, the fungal DNA must be amplified by universal primers and the PCR product after clean-up will undergo hybridization with the microarray oligonucleotide (Huang et al. 2006), there are microarray containing oligonucleotides of the biosynthetic pathways of fumonisin, aflatoxin, ochratoxin and others, even recently identified biosynthetic genes can be added to these chips (Schmidt-Heydt and Geisen 2007). These DNA microarrays reveal high specificity and stability for pathogen detection (Bouchara et al. 2009).

1.5.3 Enzyme-Linked Immunosorbent

This technique is based on the antigen-antibody immune reaction, the ELISA technique allows a highly sensitive and selective quantitative and/or qualitative analysis. For the assay, an antigen or antibody is labelled with an enzyme, hence his name comes from. The antigen is immobilized in a solid phase such as a microplate. In the immunological reaction, the antigen reacted with the antibody which will subsequently be detected by a secondary antibody labelled with an enzyme developing a color (chromogenic), the reaction time is estimated between 30 min and 1 h, at the end of the reaction, the colored products are detected using a microplate reader (Sakamoto et al. 2017). This assay technique has proved its effectiveness in detecting and measuring mycotoxins even in foods (Liu et al. 1985; Gao et al. 2012).

1.5.4 Radioimmunoassay

This technique is applicable for the detection of mycotoxins such as toxin T-2 in the liver, spleen and kidney (Hewetson et al. 1987), it can also detect T-2 toxin in biological fluids such as serum and urine with elimination of the extraction step that preceded the analysis, the sensitivity of the technique was 1 ng per analysis or 10 $ng.ml^{-1}$ with a significant reaction (10.3%) of the toxin (Fontelo et al. 1983). RIA can also be applied for the detection of T-2 toxin in milk with a sensitivity of 2.5 ppb (Lee and Chu 1981). This radioimmunoassay (RIA), with the tracer [3H] AFB_1 has proven effective for detecting AFB_1, AFM_1 and a major urinary metabolite of AFB_1 in several species (Sizaret et al. 1982).

1.6 Nanotechnology-Based Techniques for Mycotoxin Detection

The manufacture of nanomaterials based on carbon or gold or other materials has recognized in recent years an exponential progress, by the technological development known in these nanomaterials, and their applications in different fields. Developed nanoparticles can bind to different antibodies types, which are considered as molecular recognition receptors and can trap mycotoxins due to their specificity and sensitivity. Aptamers based on synthetic oligonucleotides also have a specific affinity for mycotoxins detection. Some new methods are demonstrated in the following paragraphs.

1.6.1 Quartz Crystal Microbalance Immunosensor (QCMI)

Quartz Crystal Microbalance Immunosensor (QCMI) are widely used in the biological field, the principle of the method is based on the piezoelectric properties of quartz crystals that are used in their manufacture. These AT-cut crystals used in the QCM transducers resonate at a fixed frequency when applying an electric current, this frequency is relative to changes in the contact conditions with the surface of the crystals due to an increase in mass during the immunoreaction with anti-mycotoxin antibodies. This technique is able to detect mycotoxins by their direct immobilizations on sensor surfaces containing, for example -ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/N-Hydroxysuccinimide (NHS), this surface is able of be regenerated 13 times after each analysis (Pirinçci et al. 2018), quartz crystals can also be covered by gold nanoparticles (Vidal et al. 2009).

1.6.2 Surface Plasmon Resonance (SPR)

Surface plasmon resonance is one of the methods that provide rapid quantitative and qualitative detection of mycotoxins with a detection limit of about 200 Da, this method became popular in the 1990s following the commercialization of biosensors. The principle of the method is based on the phenomenon of surface plasmon resonance, the latter occurs between a very thin metal film (formed in gold) deposited between two transparent support (prism) having a different refractive index, a polarized incident light undergoes internal refraction in the prism which induces the components of the electromagnetic field of light to penetrate the gold film, the interaction of this field with free oscillating electrons on the gold film will cause the excitation of the plasmons and will cause the decrease of the intensity of the light received by an optical detector, this system detects the changes in the refraction of the surface layer in contact with the solution which is related to the molecules

binding to the film (Hodnik and Anderluh 2009). The thin layer may contain gold nanoparticles, as demonstrated in the study of Hossain and his group, in the latter, the team was able to determine the T-2 toxin and T-2 toxin-3-glucoside in wheat after their extractions, on a device containing antibodies conjugated with gold nanoparticles (Hossain et al. 2018). SPR containing nanostructured films are called imaging nanoplasmonics, they can even detect several mycotoxins in same times, with regeneration surfaces after analysis (Joshi et al. 2016; Hossain and Maragos 2018). The technique undergone modifications and perfections such as indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) which demonstrated its reliability (Dong et al. 2018).

1.6.3 Electrochemical Immuno-Sensor Based on Nanoparticle

Different Electrochemical Immunosensors are developed to determine a type of mycotoxin, such as electrochemical Immunosensor based on polythionine/gold nanoparticles for the determination of AFB_1 (Owino et al. 2008), but following the multi-presence of mycotoxins in foods, a design of an electrochemical immunosensor is carried out for the simultaneous detection, during a single test, of two mycotoxins namely; fumonisin B_1 (FB_1) and deoxynivalenol (DON), two electrodes were functionalized with gold nanoparticles and anti-FB_1 and anti-DON antibodies. After incubation of the electrodes in the sample solution, the immunocomplex formation generates an electrochemical signal which is then compared with the control signal to quantify and calculate the concentration of mycotoxins, the LODs were 97 pg.ml^{-1} and 35 pg.ml^{-1}, respectively for FB_1 and DON. The stability of the electrodes is marked good (Lu and Gunasekaran 2019). Another indirect competition enzyme immunoassay technique is developed to determine the presence of AFB_1 in Palm kernel cake, modified electrodes prepared from multi-walled carbon nanotubes and chitosan are prepared, the attachment of AFB_1-BSA antigens on the composites surface is activated by N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS). A competitive reaction takes place between AFB_1 and the fixation site with producing a signal, the detection range of the electrochemical immunosensor was between 0.0001 and 10 ng.ml^{-1} with an LOD of 0.1 pg.ml^{-1} (Azri et al. 2017). The application of these ultrasensitive methods requires improvement and optimization of several parameters such as antigens concentration, blocking agents, incubation time, temperature and reagents pH, this optimization is performed on the microplates by ELISA test (Azri et al. 2018). This detection technique is a promising approach for mycotoxin screening because it is simple, fast, extremely sensitive, specific and does not require pre-concentration of sample, it can be applied for the detection of mycotoxins in a wide variety of foods (Tan et al. 2009; Wang et al. 2017).

1.6.4 Optical Waveguide Lightmode Spectroscopy

The OWLS technique is based on the precise calculation of the resonance angle of a polarized laser light beam (632.8 nm), diffracted by a grating and coupled to a thin waveguide. Determination of the thickness of the adsorbed or bonded material layer is performed with great sensitivity from the effective refractive index determined from the coupling angle of the resonance (Székács et al. 2009). This technique is also applied for mycotoxins detection in a competitive and direct immunoassay format. The improvement of the detection signal is relative to the surface of the immune sensor formed by immobilization of gold nanoparticles (AuNPs) containing the conjugated antibodies or antigens, after fixation of the mycotoxins, an immunodetection method has been developed for the analysis (Adányi et al. 2018). Comparison of the detection results of this method with the ELISA and HPLC test demonstrated an excellent correlation indicating that the OWLS immunosensor has a good potential for the rapid determination of mycotoxins (Majer-Baranyi et al. 2016).

References

Abdallah MF, Krska R, Sulyok M (2018) Occurrence of ochratoxins, fumonisin B2, aflatoxins (B1 and B2), and other secondary fungal metabolites in dried date palm fruits from Egypt: a mini-survey. J Food Sci 83:559–564. https://doi.org/10.1111/1750-3841.14046

Abia WA, Warth B, Sulyok M et al (2013) Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. Food Chem Toxicol 62:927–934. https://doi.org/10.1016/j.fct.2013.10.003

Adányi N, Nagy ÁG, Takács B et al (2018) Sensitivity enhancement for mycotoxin determination by optical waveguide lightmode spectroscopy using gold nanoparticles of different size and origin. Food Chem 267:10–14. https://doi.org/10.1016/j.foodchem.2018.04.089

Adekoya I, Njobeh P, Obadina A, Chilaka C, Okoth S, De Boevre M, De Saeger S (2017) Awareness and prevalence of mycotoxin contamination in selected nigerian fermented foods. Toxins 9:363. https://doi.org/10.3390/toxins9110363

Adekoya I, Obadina A, Adaku CC, De Boevre M, Okoth S, De Saeger S, Njobeh P (2018) Mycobiota and co-occurrence of mycotoxins in South African maize-based opaque beer. Int J Food Microbiol 270:22–30. https://doi.org/10.1016/j.ijfoodmicro.2018.02.001

Adgigitov F, Kosichenko LP, Popandopulo PG et al (1984) Frequency of chromosome aberrations in bone marrow of monkeys and their F1 after aflatoxin B1 injection. Exp Pathol 26:163–169

Al-Anati L, Petzinger E (2006) Immunotoxic activity of ochratoxin A. J Vet Pharmacol Ther 29:79–90. https://doi.org/10.1111/j.1365-2885.2006.00718.x

Alexa E, Dehelean CA, Poiana MA et al (2013) The occurrence of mycotoxins in wheat from western Romania and histopathological impact as effect of feed intake. Chem Cent J 7:99. https://doi.org/10.1186/1752-153X-7-99

Ali N, Blaszkewicz M, Degen GH (2015a) Occurrence of the mycotoxin citrinin and its metabolite dihydrocitrinone in urines of German adults. Arch Toxicol 89:573–578. https://doi.org/10.1007/s00204-014-1363-y

Ali N, Blaszkewicz M, Mohanto NC et al (2015b) First results on citrinin biomarkers in urines from rural and urban cohorts in Bangladesh. Mycotoxin Res 31:9–16. https://doi.org/10.1007/s12550-014-0217-z

Almeida-Ferreira GC, Barbosa-Tessmann IP, Sega R et al (2013) Occurrence of zearalenone in wheat- and corn-based products commercialized in the State of Paraná, Brazil. Braz J Microbiol 44:371–375. https://doi.org/10.1590/S1517-83822013005000037

Anthony J, Delucca JJ, Dunn LS et al (1982) Toxicity, mutagenicity and teratogenicity of brevianamide, viomellein and xanthomegnin; secondary metabolites of penicillium viridicatum. J Food Saf 4:165–168. https://doi.org/10.1111/j.1745-4565.1982.tb00440.x

Applegate TJ, Schatzmayr G, Prickel K et al (2009) Effect of aflatoxin culture on intestinal function and nutrient loss in laying hens. Poult Sci 88:1235–1241. https://doi.org/10.3382/ps.2008-00494

Armando MR, Pizzolitto RP, Dogi CA et al (2012) Adsorption of ochratoxin A and zearalenone by potential probiotic *Saccharomyces cerevisiae* strains and its relation with cell wall thickness. J Appl Microbiol 113:256–264. https://doi.org/10.1111/j.1365-2672.2012.05331.x

Arzu Koçkaya E, Selmanoğlu G, Ozsoy N et al (2009) Evaluation of patulin toxicity in the thymus of growing male rats. Arh Hig Rada Toksikol 60:411–418. https://doi.org/10.2478/10004-1254-60-2009-1973

Asghar MA, Iqbal J, Ahmed A et al (2014) Occurrence of aflatoxins contamination in brown rice from Pakistan. Iran J Public Health 43:291–299

Asghar MA, Ahmed A, Asghar MA (2018) Aflatoxin M1 in fresh milk collected from local markets of Karachi, Pakistan. Food Addit Contam Part B Surveill 11:167–174. https://doi.org/10.1080/19393210.2018.1446459

Azri FA, Selamat J, Sukor R (2017) Electrochemical immunosensor for the detection of aflatoxin B_1 in palm kernel cake and feed samples. Sensors 17:2776. https://doi.org/10.3390/s17122776

Azri FA, Sukor R, Selamat J et al (2018) Electrochemical immunosensor for detection of aflatoxin B_1 based on indirect competitive ELISA. Toxins 10:196. https://doi.org/10.3390/toxins10050196

Barrett JR (2005) Liver cancer and aflatoxin: New information from the kenyan outbreak. Environ Health Perspect 113: A837–A838. https://doi.org/10.1289/ehp.113-a837

Bárta I, Adámková M, Markarjan D et al (1984) The mutagenic activity of aflatoxin B1 in the *Cricetulus griseus* hamster and *Macaca mulatta* monkey. J Hyg Epidemiol Microbiol Immunol 28:149–159

Bennett JW, Rubin PL, Lee LS et al (1979) Influence of trace elements and nitrogen sources on versicolorin production by a mutant strain of *Aspergillus parasiticus*. Mycopathologia 69:161–166. https://doi.org/10.1007/BF00452829

Berntsen HF, Wigestrand MB, Bogen IL et al (2013) Mechanisms of penitrem-induced cerebellar granule neuron death in vitro: possible involvement of GABAA receptors and oxidative processes. Neurotoxicology 35:129–136. https://doi.org/10.1016/j.neuro.2013.01.004

Berntsen HF, Bogen IL, Wigestrand MB et al (2017) The fungal neurotoxin penitrem A induces the production of reactive oxygen species in human neutrophils at submicromolar concentrations. Toxicology 392:64–70. https://doi.org/10.1016/j.tox.2017.10.008

Bluhm BH, Kim H, Butchko RA et al (2008) Involvement of ZFR1 of *Fusarium verticillioides* in kernel colonization and the regulation of FST1, a putative sugar transporter gene required for fumonisin biosynthesis on maize kernels. Mol Plant Pathol 9:203–211. https://doi.org/10.1111/j.1364-3703.2007.00458.x

Bouchara JP, Hsieh HY, Croquefer S et al (2009) Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. J Clin Microbiol 47:142–152. https://doi.org/10.1128/JCeM.01668-08

Boudra H, Barnouin J, Dragacci S et al (2007) Aflatoxin M1 and ochratoxin A in raw bulk milk from French dairy herds. J Dairy Sci 90:3197–3201. https://doi.org/10.3168/jds.2006-565

Bouhet S, Oswald IP (2007) The intestine as a possible target for fumonisin toxicity. Mol Nutr Food Res 51:925–931. https://doi.org/10.1002/mnfr.200600266

Bouhet S, Hourcade E, Loiseau N et al (2004) The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. Toxicol Sci 77:165–171. https://doi.org/10.1093/toxsci/kfh006

Brewer JH, Thrasher JD, Straus DC et al (2013) Detection of mycotoxins in patients with chronic fatigue syndrome. Toxins 5:605–617. https://doi.org/10.3390/toxins5040605

Brinda R, Vijayanandraj S, Uma D et al (2013) Role of *Adhatoda vasica* (L.) *Nees* leaf extract in the prevention of aflatoxin-induced toxicity in Wistar rats. J Sci Food Agric 30:2743–2748. https://doi.org/10.1002/jsfa.6093

Brown DW, Busman M, Proctor RH (2014) *Fusarium verticillioides* SGE1 is required for full virulence and regulates expression of protein effector and secondary metabolite biosynthetic genes. Mol Plant-Microbe Interact 27:809–823. https://doi.org/10.1094/MPMI-09-13-0281-R

Bucci TJ, Howard PC, Tolleson WH et al (1998) Renal effects of fumonisin mycotoxins in animals. Toxicol Pathol 26:160–164. https://doi.org/10.1177/019262339802600119

Calado T, Fernández-Cruz ML, Cabo Verde S et al (2018) Gamma irradiation effects on ochratoxin A: degradation, cytotoxicity and application in food. Food Chem 240:463–471. https://doi.org/10.1016/j.foodchem.2017.07.136

Carlton WW, Stack ME, Eppley RM (1976) Hepatic alterations produced in mice by xanthomegnin and viomellein, metabolites of Penicillium viridicatum. Toxicol Appl Pharmacol 38:455–459. https://doi.org/10.1016/0041-008X(76)90151-4

Celik I, Oğuz H, Demet O et al (2000) Embryotoxicity assay of aflatoxin produced by *Aspergillus parasiticus* NRRL 2999. Br Poult Sci 41:401–409. https://doi.org/10.1080/713654961

Chang PK, Ehrlich KC, Yu J et al (1995) Increased expression of *Aspergillus parasiticus* aflR, encoding a sequence-specific DNA-binding protein, relieves nitrate inhibition of aflatoxin biosynthesis. Appl Environ Microbiol 61:2372–2377

Chang PK, Ehrlich KC, Linz JE et al (1996) Characterization of the *Aspergillus parasiticus* niaD and niiA gene cluster. Curr Genet 30:68–75

Chaudhari M, Jayaraj R, Bhaskar AS et al (2009) Oxidative stress induction by T-2 toxin causes DNA damage and triggers apoptosis via caspase pathway in human cervical cancer cells. Toxicology 262:153–161. https://doi.org/10.1016/j.tox.2009.06.002

Chavarría G, Granados-Chinchilla F, Alfaro-Cascante M et al (2015) Detection of aflatoxin M1 in milk, cheese and sour cream samples from Costa Rica using enzyme-assisted extraction and HPLC. Food Addit Contam Part B Surveill 8:128–135. https://doi.org/10.1080/19393210.2015.1015176

Chen F, Luan C, Wang L et al (2017) Simultaneous determination of six mycotoxins in peanut by high-performance liquid chromatography with a fluorescence detector. J Sci Food Agric 97:1805–1810. https://doi.org/10.1002/jsfa.7978

Ciacci-Zanella JR, Merrill AH Jr, Wang E et al (1998) Characterization of cell-cycle arrest by fumonisin B1 in CV-1 cells. Food Chem Toxicol 36:791–804. https://doi.org/10.1016/S0278-6915(98)00034-9

Constable PD, Smith GW, Rottinghaus GE et al (2000) Ingestion of fumonisin B1-containing culture material decreases cardiac contractility and mechanical efficiency in swine. Toxicol Appl Pharmacol 162:151–160. https://doi.org/10.1006/taap.1999.8831

Cotty PJ, Jaime-Garcia R (2007) Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int J Food Microbiol 119:109–115. https://doi.org/10.1016/j.ijfoodmicro.2007.07.060

Cramer B, Osteresch B, Muñoz KA et al (2015) Biomonitoring using dried blood spots: detection of ochratoxin A and its degradation product 2'R-ochratoxin A in blood from coffee drinkers. Mol Nutr Food Res 59:1837–1843. https://doi.org/10.1002/mnfr.201500220

Crespo-Sempere A, Marín S, Sanchis V, Ramos AJ (2013) VeA and LaeA transcriptional factors regulate ochratoxin aA biosynthesis in *Aspergillus carbonarius*. Int J Food Microbiol 166:479–486. https://doi.org/10.1016/j.ijfoodmicro.2013.07.027

Datta SC, Ghosh JJ (1981) Effect of citreoviridin, a mycotoxin from *Penicillium citreoviride*, on kinetic constants of acetylcholinesterase and ATPase in synaptosomes and microsomes from rat brain. Toxicon 19:555–562. https://doi.org/10.1016/0041-0101(81)90014-3

Daxecker H, Raab M, Cichna M et al (2001) Determination of the effects of mycophenolic acid on the nucleotide pool of human peripheral blood mononuclear cells in vitro by high-

performance liquid chromatography. Clin Chim Acta 310:81–87. https://doi.org/10.1016/S0009-8981(01)00526-5

De Jesus CL, Bartley A, Welch AZ et al (2018) High incidence and levels of ochratoxin A in wines sourced from the United States. Toxins 10:1. https://doi.org/10.3390/toxins10010001

Dehghan P, Pakshir K, Rafiei H et al (2014) Prevalence of ochratoxin a in human milk in the Khorrambid Town, Fars Province, South of Iran. Jundishapur J Microbiol 7:e11220. https://doi.org/10.5812/jjm.11220

Del Palacio A, Mionetto A, Bettucci L et al (2016) Evolution of fungal population and mycotoxins in sorghum silage. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 33:1864–1872. https://doi.org/10.1080/19440049.2016.1244732

Devnarain N, Tiloke C, Nagiah S et al (2017) Fusaric acid induces oxidative stress and apoptosis in human cancerous oesophageal SNO cells. Toxicon 126:4–11. https://doi.org/10.1016/j.toxicon.2016.12.006

Devreese M, De Baere S, De Backer P et al (2012) Quantitative determination of several toxicological important mycotoxins in pig plasma using multi-mycotoxin and analyte-specific high performance liquid chromatography-tandem mass spectrometric methods. J Chromatogr A 1257:74–80. https://doi.org/10.1016/j.chroma.2012.08.008

Doi K, Uetsuka K (2014) Mechanisms of mycotoxin-induced dermal toxicity and tumorigenesis through oxidative stress-related pathways. J Toxicol Pathol 27:1–10. https://doi.org/10.1293/tox.2013-0062

Domijan AM, Peraica M, Cvjetković B et al (2005) Mould contamination and co-occurrence of mycotoxins in maize grain in Croatia. Acta Pharma 55:349–356

Dong G, Pan Y, Wang Y et al (2018) Preparation of a broad-spectrum anti-zearalenone and its primary analogues antibody and its application in an indirect competitive enzyme-linked immunosorbent assay. Food Chem 247:8–15. https://doi.org/10.1016/j.foodchem.2017.12.016

El Khoury R, Mathieu F, Atoui A et al (2017) Ability of soil isolated actinobacterial strains to prevent, bind and biodegrade ochratoxin A. Toxins 9:pii: E222. https://doi.org/10.3390/toxins9070222

El Khoury R, Choque E, El Khoury A et al (2018) OTA prevention and detoxification by actinobacterial strains and activated carbon fibers: preliminary results. Toxins 24:pii: E137. https://doi.org/10.3390/toxins10040137

El Marnissi B, Belkhou R, Morgavi DP et al (2012) Occurrence of aflatoxin M1 in raw milk collected from traditional dairies in Morocco. Food Chem Toxicol 50:2819–2821. https://doi.org/10.1016/j.fct.2012.05.031

Emri T, Zalka A, Pócsi I (2017) Detection of transcriptionally active mycotoxin gene clusters: DNA microarray. Methods Mol Biol 1542:345–365. https://doi.org/10.1007/978-1-4939-6707-0_23

Escrivá L, Font G, Manyes L et al (2017) Studies on the presence of mycotoxins in biological samples: an overview. Toxins 9:251. https://doi.org/10.3390/toxins9080251

Espeso EA, Arst HN Jr (2000) On the mechanism by which alkaline pH prevents expression of an acid-expressed gene. Mol Cell Biol 20:3355–3363

Esteban A, Abarca ML, Bragulat MR et al (2006) Effect of pH on ochratoxin A production by *Aspergillus niger* aggregate species. Food Addit Contam 23:616–622. https://doi.org/10.1080/02652030600599124

Faisal Z, Derdák D, Lemli B et al (2018a) Interaction of 2'R-ochratoxin A with serum albumins: binding site, effects of site markers, thermodynamics, species differences of albumin-binding, and influence of albumin on its toxicity in MDCK cells. Toxins 10:pii: E353. https://doi.org/10.3390/toxins10090353

Faisal Z, Lemli B, Szerencsés D et al (2018b) Interactions of zearalenone and its reduced metabolites α-zearalenol and β-zearalenol with serum albumins: species differences, binding sites, and thermodynamics. Mycotoxin Res 34:269–278. https://doi.org/10.1007/s12550-018-0321-6

Fanelli F, Schmidt-Heydt M, Haidukowski M et al (2012) Influence of light on growth, conidiation and the mutual regulation of fumonisin B-2 and ochratoxin A biosynthesis by *Aspergillus niger*. World Mycotoxin J 5:169–176. https://doi.org/10.3920/WMJ2011.1364

Fanelli F, Cozzi G, Raiola A et al (2017) Raisins and currants as conventional nutraceuticals in Italian market: natural occurrence of ochratoxin A. J Food Sci 82:2306–2312. https://doi.org/10.1111/1750-3841.13854

Fang H, Wu Y, Guo J et al (2012) T-2 toxin induces apoptosis in differentiated murine embryonic stem cells through reactive oxygen species-mediated mitochondrial pathway. Apoptosis 17:895–907. https://doi.org/10.1007/s10495-012-0724-3

Fang H, Cong L, Zhi Y et al (2016) T-2 toxin inhibits murine ES cells cardiac differentiation and mitochondrial biogenesis by ROS and p-38 MAPK-mediated pathway. Toxicol Lett 258:259–266. https://doi.org/10.1016/j.toxlet.2016.06.2103

Ferrigo D, Raiola A, Bogialli S et al (2015) *In vitro* production of fumonisins by *Fusarium verticillioides* under oxidative stress induced by H_2O_2. J Agric Food Chem 63:4879–4885. https://doi.org/10.1021/acs.jafc.5b00113

Flajs D, Peraica M (2009) Toxicological properties of citrinin. Arh Hig Rada Toksikol 60:457–464. https://doi.org/10.2478/10004-1254-60-2009-1992

Flores-Flores ME, Lizarraga E, López de Cerain A et al (2015) Presence of mycotoxins in animal milk: a review. Food Control 53:163–176. https://doi.org/10.1016/j.foodcont.2015.01.020

Fontelo PA, Beheler J, Bunner DL et al (1983) Detection of T-2 toxin by an improved radioimmunoassay. Appl Environ Microbiol 45:640–643

Fraeyman S, Meyer E, Devreese M et al (2018) Comparative in vitro cytotoxicity of the emerging Fusarium mycotoxins beauvericin and enniatins to porcine intestinal epithelial cells. Food Chem Toxicol 121:566–572. https://doi.org/10.1016/j.fct.2018.09.053

Fredlund E, Thim AM, Gidlund A et al (2009) Moulds and mycotoxins in rice from the Swedish retail market. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 26:527–533. https://doi.org/10.1080/02652030802562912

Gagliano N, Donne ID, Torri C et al (2006) Early cytotoxic effects of ochratoxin A in rat liver: a morphological, biochemical and molecular study. Toxicology 15:214–224. https://doi.org/10.1016/j.tox.2006.06.004

Gallo A, Ferrara M, Perrone G (2017) Recent advances on the molecular aspects of ochratoxin A biosynthesis. Curr Opin Food Sci 17:49–56. https://doi.org/10.1016/j.cofs.2017.09.011

Gammelsrud A, Solhaug A, Dendelé B et al (2012) Enniatin B-induced cell death and inflammatory responses in RAW 267.4 murine macrophages. Toxicol Appl Pharmacol 261:74–87. https://doi.org/10.1016/j.taap.2012.03.014

Gao Y, Yang M, Peng C et al (2012) Preparation of highly specific anti-zearalenone antibodies by using the cationic protein conjugate and development of an indirect competitive enzyme-linked immunosorbent assay. Analyst 137:229–236. https://doi.org/10.1039/c1an15487g

Gao X, Sun L, Zhang N et al (2017) Gestational zearalenone exposure causes reproductive and developmental toxicity in pregnant rats and female offspring. Toxins 9:21. https://doi.org/10.3390/toxins9010021

García-Moraleja A, Font G, Mañes J et al (2015) Analysis of mycotoxins in coffee and risk assessment in Spanish adolescents and adults. Food Chem Toxicol 86:225–233. https://doi.org/10.1016/j.fct.2015.10.014

Garrido NS, Iha MH, Santos Ortolani MR et al (2003) Occurrence of aflatoxins M(1) and M(2) in milk commercialized in Ribeirão Preto-SP, Brazil. Food Addit Contam 20:70–73. https://doi.org/10.1080/0265203021000035371

Gayathri L, Dhivya R, Dhanasekaran D et al (2015) Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: in vitro study in HepG2 cell. Food Chem Toxicol 83:151–163. https://doi.org/10.1016/j.fct.2015.06.009

Gerding J, Cramer B, Humpf HU (2014) Determination of mycotoxin exposure in Germany using an LC-MS/MS multibiomarker approach. Mol Nutr Food Res 58:2358–2368. https://doi.org/10.1002/mnfr.201400406

Gerding J, Ali N, Schwartzbord J et al (2015) A comparative study of the human urinary mycotoxin excretion patterns in Bangladesh, Germany, and Haiti using a rapid and sensitive LC-MS/MS approach. Mycotoxin Res 31:127–136. https://doi.org/10.1007/s12550-015-0223-9

Ghali R, Khlifa KH, Ghorbel H et al (2010) Aflatoxin determination in commonly consumed foods in Tunisia. J Sci Food Agric 90:2347–2351. https://doi.org/10.1002/jsfa.4069
Ghazi T, Nagiah S, Tiloke C et al (2017) Fusaric acid induces DNA damage and post-translational modifications of p53 in human hepatocellular carcinoma (HepG2) cells. J Cell Biochem 118:3866–3874. https://doi.org/10.1002/jcb.26037
Giancarlo B, Elisabetta B, Edmondo C et al (2011) Determination of ochratoxin A in eggs and target tissues of experimentally drugged hens using HPLC–FLD. Food Chem 126:1278–1282. https://doi.org/10.1016/j.foodchem.2010.11.070
Gimeno A (1979) Thin layer chromatographic determination of aflatoxins, ochratoxins, sterigmatocystin, zearalenone, citrinin, T-2 toxin, diacetoxyscirpenol, penicillic acid, patulin, and penitrem A. J Assoc Off Anal Chem 62:579–585
Gökmen V, Acar J (1998) Incidence of patulin in apple juice concentrates produced in Turkey. J Chromatogr A 815:99–102. https://doi.org/10.1016/S0021-9673(97)01280-6
Goliński P, Grabarkiewicz-Szczesna J (1984) Chemical confirmatory tests for ochratoxin A, citrinin, penicillic acid, sterigmatocystin, and zearalenone performed directly on thin layer chromatographic plates. J Assoc Off Anal Chem 67:1108–1110
Gomes ES, Schuch V, de Macedo Lemos EG (2013) Biotechnology of polyketides: new breath of life for the novel antibiotic genetic pathways discovery through metagenomics. Braz J Microbiol 44:1007–1034
Greco MV, Pardo AG, Ludemann V et al (2012) Mycoflora and natural incidence of selected mycotoxins in rabbit and chinchilla feeds. Sci World J 2012:956056. https://doi.org/10.1100/2012/956056
Greco M, Pardo A, Pose G (2015) Mycotoxigenic fungi and natural co-occurrence of mycotoxins in rainbow trout (Oncorhynchus mykiss) feeds. Toxins 7:4595–4609. https://doi.org/10.3390/toxins7114595
Haas D, Pfeifer B, Reiterich C et al (2013) Identification and quantification of fungi and mycotoxins from Pu-erh tea. Int J Food Microbiol 166:316–322. https://doi.org/10.1016/j.ijfoodmicro.2013.07.024
Hashem A, Fathi Abd-Allah E, Sultan Al-Obeed R et al (2015) Effect of carbon, nitrogen sources and water activity on growth and ochratoxin production of *Aspergillus carbonarius* (Bainier) Thom. Jundishapur J Microbiol 23:e17569. https://doi.org/10.5812/jjm.17569
Hayes AW, Williams WL (1978) Acute toxicity of aflatoxin B1 and rubratoxin B in dogs. J Environ Pathol Toxicol 1:59–70
Heischmann S, Dzieciatkowska M, Hansen K et al (2017) The immunosuppressant mycophenolic acid alters nucleotide and lipid metabolism in an intestinal cell model. Sci Rep 7:45088. https://doi.org/10.1038/srep45088
Heller T, Asif AR, Petrova DT et al (2009) Differential proteomic analysis of lymphocytes treated with mycophenolic acid reveals caspase 3-induced cleavage of rho GDP dissociation inhibitor 2. Ther Drug Monit 31:211–217. https://doi.org/10.1097/FTD.0b013e318196fb73
Henry MH, Wyatt R (2001) The toxicity of fumonisin B1, B2, and B3, individually and in combination, in chicken embryos. Poult Sci 80:401–407. https://doi.org/10.1093/ps/80.4.401
Hernández-Meléndez D, Salas-Téllez E, Zavala-Franco A et al (2018) Inhibition effect of flower-shaped zinc oxide nanostructures on growth and aflatoxin production of a highly toxigenic strain of *Aspergillus flavus* link. Materials 11:1–13. https://doi.org/10.3390/ma11081265
Hewetson JF, Pace JG, Beheler JE (1987) Detection and quantitation of T-2 mycotoxin in rat organs by radioimmunoassay. J Assoc Off Anal Chem 70:654–657
Heyndrickx E, Sioen I, Huybrechts B et al (2015) Human biomonitoring of multiple mycotoxins in the Belgian population: results of the BIOMYCO study. Environ Int 84:82–89. https://doi.org/10.1016/j.envint.2015.06.011
Hinton DM, Morrissey RE, Norred WP et al (1985) Effects of cyclopiazonic acid on the ultrastructure of rat liver. Toxicol Lett 25:211–218. https://doi.org/10.1016/0378-4274(85)90084-0
Hodnik V, Anderluh G (2009) Toxin detection by surface plasmon resonance. Sensors 9:1339–1354. https://doi.org/10.3390/s9031339

Hooper DG, Bolton VE, Guilford FT et al (2009) Mycotoxin detection in human samples from patients exposed to environmental molds. Int J Mol Sci 10:1465–1475. https://doi.org/10.3390/ijms10041465

Hossain MZ, Maragos CM (2018) Gold nanoparticle-enhanced multiplexed imaging surface plasmon resonance (iSPR) detection of Fusarium mycotoxins in wheat. Biosens Bioelectron 101:245–252. https://doi.org/10.1016/j.bios.2017.10.033

Hossain MZ, McCormick SP, Maragos CM (2018) An imaging surface plasmon resonance biosensor assay for the detection of T-2 toxin and masked T-2 toxin-3-glucoside in wheat. Toxins 10:119. https://doi.org/10.3390/toxins10030119

Hou H, Qu X, Li Y et al (2015) Binding of citreoviridin to human serum albumin: multispectroscopic and molecular docking. Biomed Res Int 2015:162391. https://doi.org/10.1155/2015/162391

Huang A, Li JW, Shen ZQ et al (2006) High-throughput identification of clinical pathogenic fungi by hybridization to an oligonucleotide microarray. J Clin Microbiol 44:3299–3305. https://doi.org/10.1128/JCM.00417-06

Huffman J, Gerber R, Du L (2010) Recent advancements in the biosynthetic mechanisms for polyketide-derived mycotoxins. Biopolymers 93:764–776. https://doi.org/10.1002/bip.21483

Hymery N, Masson F, Barbier G et al (2014) Cytotoxicity and immunotoxicity of cyclopiazonic acid on human cells. Toxicol In Vitro 28:940–947. https://doi.org/10.1016/j.tiv.2014.04.003

Iacumin L, Chiesa L, Boscolo D et al (2009) Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages. Food Microbiol 26:65–70. https://doi.org/10.1016/j.fm.2008.07.006

Ikegwuonu FI (1983) The neurotoxicity of aflatoxin B1 in the rat. Toxicology 28:247–259. https://doi.org/10.1016/0300-483X(83)90121-X

Ivanova L, Egge-Jacobsen WM, Solhaug A et al (2012) Lysosomes as a possible target of enniatin B-induced toxicity in Caco-2 cells. Chem Res Toxicol 25:1662–1674. https://doi.org/10.1021/tx300114x

Jaskiewicz K, Close PM, Thiel PG et al (1988) Preliminary studies on toxic effects of cyclopiazonic acid alone and in combination with aflatoxin B1 in non-human primates. Toxicology 52:297–307. https://doi.org/10.1016/0300-483X(88)90134-5

Jayashree T, Subramanyam C (2000) Oxidative stress as a prerequisite for aflatoxin production by *Aspergillus parasiticus*. Free Radic Biol Med 29:981–985. https://doi.org/10.1016/S0891-5849(00)00398-1

Jia Z, Liu M, Qu Z et al (2014) Toxic effects of zearalenone on oxidative stress, inflammatory cytokines, biochemical and pathological changes induced by this toxin in the kidney of pregnant rats. Environ Toxicol Pharmacol 37:580–591. https://doi.org/10.1016/j.etap.2014.01.010

Jia Z, Yin S, Liu M et al (2015) Modified halloysite nanotubes and the alleviation of kidney damage induced by dietary zearalenone in swine. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 32:1312–1321. https://doi.org/10.1080/19440049.2015.1048748

Jiang J, Yun Y, Liu Y et al (2012a) FgVELB is associated with vegetative differentiation, secondary metabolism and virulence in *Fusarium graminearum*. Fungal Genet Biol 49:653–662. https://doi.org/10.1016/j.fgb.2012.06.005

Jiang SZ, Yang ZB, Yang WR et al (2012b) Effect on hepatonephric organs, serum metabolites and oxidative stress in post-weaning piglets fed purified zearalenone-contaminated diets with or without Calibrin-Z. J Anim Physiol Anim Nutr 96:1147–1156. https://doi.org/10.1111/j.1439-0396.2011.01233.x

Jiménez M, Máñez M, Hernández E (1996) Influence of water activity and temperature on the production of zearalenone in corn by three *Fusarium* species. Int J Food Microbiol 29:417–421. https://doi.org/10.1016/0168-1605(95)00073-9

Jonsson M, Jestoi M, Anthoni M et al (2016) Fusarium mycotoxin enniatin B: cytotoxic effects and changes in gene expression profile. Toxicol In Vitro 34:309–320. https://doi.org/10.1016/j.tiv.2016.04.017

Joshi S, Segarra-Fas A, Peters J et al (2016) Multiplex surface plasmon resonance biosensing and its transferability towards imaging nanoplasmonics for detection of mycotoxins in barley. Analyst 141:1307–1318. https://doi.org/10.1039/C5AN02512E

Juan C, Pena A, Lino C et al (2008) Levels of ochratoxin A in wheat and maize bread from the central zone of Portugal. Int J Food Microbiol 127:284–289. https://doi.org/10.1016/j.ijfoodmicro.2008.07.018

Juan-García A, Manyes L, Ruiz MJ et al (2013) Involvement of enniatins-induced cytotoxicity in human HepG2 cells. Toxicol Lett 218:166–173. https://doi.org/10.1016/j.toxlet.2013.01.014

Juan-García A, Ruiz MJ, Font G et al (2015) Enniatin A1, enniatin B1 and beauvericin on HepG2: evaluation of toxic effects. Food Chem Toxicol 84:188–196. https://doi.org/10.1016/j.fct.2015.08.030

Kachapulula PW, Akello J, Bandyopadhyay R et al (2018) Aflatoxin contamination of dried insects and fish in Zambia. J Food Prot 81:1508–1518. https://doi.org/10.4315/0362-028X.JFP-17-527

Kapetanakou AE, Panagou EZ, Gialitaki M et al (2009) Evaluating the combined effect of water activity, pH and temperature on ochratoxin A production by *Aspergillus ochraceus* and *Aspergillus carbonarius* on culture medium and Corinth raisins. Food Control 20:725–732. https://doi.org/10.1016/j.foodcont.2008.09.008

Katerere DR, Stockenström S, Thembo KM et al (2007) Investigation of patulin contamination in apple juice sold in retail outlets in Italy and South Africa. Food Addit Contam 24:630–634. https://doi.org/10.1080/02652030601137668

Kaufman SS, Tuma DJ, Park JH et al (1988) Effects of cytochalasin B on the synthesis and secretion of plasma proteins by developing rat liver. J Pediatr Gastroenterol Nutr 7:107–114

Kaymak T, Koca E, Atak M et al (2018) Determination of aflatoxins and ochratoxin A in traditional turkish cereal-based fermented food by multi-affinity column cleanup and LC fluorescence detection: single-laboratory validation. J AOAC Int 102(1):156–163. https://doi.org/10.5740/jaoacint.17-0490

Keller NP, Hohn TM (1997) Metabolic pathway gene clusters in filamentous fungi. Fungal Genet Biol 21:17–29. https://doi.org/10.1006/fgbi.1997.0970

Khanafari A, Soudi H, Miraboulfathi M et al (2007) An *in vitro* investigation of aflatoxin B1 biological control by *Lactobacillus plantarum*. Pak J Biol Sci 10:2553–2556. https://doi.org/10.3923/pjbs.2007.2553.255

Kim HK, Lee S, Jo SM et al (2013) Functional roles of FgLaeA in controlling secondary metabolism, sexual development, and virulence in *Fusarium graminearum*. PLoS One 8:e68441. https://doi.org/10.1371/journal.pone.0068441

Klarić MS, Zelježić D, Rumora L et al (2012) A potential role of calcium in apoptosis and aberrant chromatin forms in porcine kidney PK15 cells induced by individual and combined ochratoxin a and citrinin. Arch Toxicol 86:97–107. https://doi.org/10.1007/s00204-011-0735-9

Kolber MA, Broschat KO, Landa-Gonzalez B (1990) Cytochalasin B induces cellular DNA fragmentation. FASEB J 4:3021–3027. https://doi.org/10.1096/fasebj.4.12.2394319

Kolf-Clauw M, Sassahara M, Lucioli J et al (2013) The emerging mycotoxin, enniatin B1, down-modulates the gastrointestinal toxicity of T-2 toxin in vitro on intestinal epithelial cells and ex vivo on intestinal explants. Arch Toxicol 87:2233–2241. https://doi.org/10.1007/s00204-013-1067-8

Kollia E, Kanapitsas A, Markaki P (2014) Occurrence of aflatoxin B1 and ochratoxin A in dried vine fruits from Greek market. Food Addit Contam Part B Surveill 7:11–16. https://doi.org/10.1080/19393210.2013.825647

Kong WJ, Liu SY, Qiu F et al (2013) Simultaneous multi-mycotoxin determination in nutmeg by ultrasound-assisted solid-liquid extraction and immunoaffinity column clean-up coupled with liquid chromatography and on-line post-column photochemical derivatization-fluorescence detection. Analyst 138:2729–2739. https://doi.org/10.1039/C3AN00059A

Koraïchi F, Inoubli L, Lakhdari N et al (2013) Neonatal exposure to zearalenone induces long term modulation of ABC transporter expression in testis. Toxicology 310:29–38. https://doi.org/10.1016/j.tox.2013.05.002

Lee S, Chu FS (1981) Radioimmunoassay of T-2 toxin in biological fluids. J Assoc Off Anal Chem 64:684–688

Lee SH, Lee J, Lee S et al (2009) GzSNF1 is required for normal sexual and asexual development in the ascomycete *Gibberella zeae*. Eukaryot Cell 8:116–127. https://doi.org/10.1128/EC.00176-08

Li Y, Zhang B, He X et al (2014) Analysis of individual and combined effects of ochratoxin A and zearalenone on HepG2 and KK-1 cells with mathematical models. Toxins 6:1177–1192. https://doi.org/10.3390/toxins6041177

Li H, Xing L, Zhang M et al (2018) The toxic effects of aflatoxin B1 and aflatoxin M1 on kidney through regulating L-proline and downstream apoptosis. Biomed Res Int 2018:9074861. https://doi.org/10.1155/2018/9074861

Limonciel A, Jennings P (2014) A review of the evidence that ochratoxin A is an Nrf2 inhibitor: implications for nephrotoxicity and renal carcinogenicity. Toxins 6:371–379. https://doi.org/10.3390/toxins6010371

Liu MT, Ram BP, Hart LP et al (1985) Indirect enzyme-linked immunosorbent assay for the mycotoxin zearalenone. Appl Environ Microbiol 50:332–336

Liu J, Wang L, Guo X et al (2014a) The role of mitochondria in T-2 toxin-induced human chondrocytes apoptosis. PLoS One 9:e108394. https://doi.org/10.1371/journal.pone.0108394

Liu M, Gao R, Meng Q et al (2014b) Toxic effects of maternal zearalenone exposure on intestinal oxidative stress, barrier function, immunological and morphological changes in rats. PLoS One 9:e106412. https://doi.org/10.1371/journal.pone.0106412

Liu M, Zhu D, Guo T et al (2018) Toxicity of zearalenone on the intestines of pregnant sows and their offspring and alleviation with modified halloysite nanotubes. J Sci Food Agric 98:698–706. https://doi.org/10.1002/jsfa.8517

Loiseau N, Debrauwer L, Sambou T et al (2007) Fumonisin B1 exposure and its selective effect on porcine jejunal segment: sphingolipids, glycolipids and trans-epithelial passage disturbance. Biochem Pharmacol 74:144–152. https://doi.org/10.1016/j.bcp.2007.03.031

Lu L, Gunasekaran S (2019) Dual-channel ITO-microfluidic electrochemical immunosensor for simultaneous detection of two mycotoxins. Talanta 194:709–716. https://doi.org/10.1016/j.talanta.2018.10.091

Ma L, Wang J, Zhang Y (2017) Probing the characterization of the interaction of aflatoxins B1 and G1 with calf thymus DNA *in vitro*. Toxins 9:pii: E209. https://doi.org/10.3390/toxins9070209

Ma S, Zhao Y, Sun J et al (2018) miR449a/SIRT1/PGC-1α is necessary for mitochondrial biogenesis induced by T-2 toxin. Front Pharmacol 8:954. https://doi.org/10.3389/fphar.2017.00954

Magnussen A, Parsi MA (2013) Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol 19:1508–1512. https://doi.org/10.3748/wjg.v19.i10.1508

Majer-Baranyi K, Zalán Z, Mörtl M et al (2016) Optical waveguide lightmode spectroscopy technique-based immunosensor development for aflatoxin B1 determination in spice paprika samples. Food Chem 211:972–977. https://doi.org/10.1016/j.foodchem.2016.05.089

Makowska K, Obremski K, Gonkowski S (2018) The impact of T-2 toxin on vasoactive intestinal polypeptide-like immunoreactive (VIP-LI) nerve structures in the wall of the porcine stomach and duodenum. Toxins 10:138. https://doi.org/10.3390/toxins10040138

Makun HA, Dutton MF, Njobeh PB et al (2011) Natural multi-occurrence of mycotoxins in rice from Niger State, Nigeria. Mycotoxin Res 27:97–104. https://doi.org/10.1007/s12550-010-0080-5

Malir F, Ostry V, Pfohl-Leszkowicz A et al (2014) Transfer of ochratoxin A into tea and coffee beverages. Toxins 6:3438–3453. https://doi.org/10.3390/toxins6123438

Mallebrera B, Juan-Garcia A, Font G et al (2016) Mechanisms of beauvericin toxicity and antioxidant cellular defense. Toxicol Lett 246:28–34. https://doi.org/10.1016/j.toxlet.2016.01.013

Manyes L, Escrivá L, Ruiz MJ et al (2018) Beauvericin and enniatin B effects on a human lymphoblastoid Jurkat T-cell model. Food Chem Toxicol 115:127–135. https://doi.org/10.1016/j.fct.2018.03.008

Maresca M, Mahfoud R, Pfohl-Leszkowicz A et al (2001) The mycotoxin ochratoxin A alters intestinal barrier and absorption functions but has no effect on chloride secretion. Toxicol Appl Pharmacol 176:54–63. https://doi.org/10.1006/taap.2001.9254

Marin DE, Braicu C, Gras MA et al (2017) Low level of ochratoxin A affects genome-wide expression in kidney of pig. Toxicon 136:67–77. https://doi.org/10.1016/j.toxicon.2017.07.004

Massey TE, Smith GB, Tam AS (2000) Mechanisms of aflatoxin B1 lung tumorigenesis. Exp Lung Res 26:673–683. https://doi.org/10.1080/01902140150216756

Mata AT, Ferreira JP, Oliveira BR et al (2015) Bottled water: analysis of mycotoxins by LC-MS/MS. Food Chem 176:455–464. https://doi.org/10.1016/j.foodchem.2014.12.088

Mateo R, Medina A, Mateo EM et al (2007) An overview of ochratoxin A in beer and wine. Int J Food Microbiol 20:79–83. https://doi.org/10.1016/j.ijfoodmicro.2007.07.029

Matumba LU, Van Poucke CU, Biswick T et al (2014) A limited survey of mycotoxins in traditional maize based opaque beers in Malawi. Food Control 36:253–256. https://doi.org/10.1016/j.foodcont.2013.08.032

Mayura K, Smith EE, Clement BA et al (1987) Developmental toxicity of diacetoxyscirpenol in the mouse. Toxicology 45:245–255. https://doi.org/10.1016/0300-483X(87)90016-3

Medina A, González G, Sáez JM et al (2004) Bee pollen, a substrate that stimulates ochratoxin A production by *Aspergillus ochraceus* Wilh. Syst Appl Microbiol 27:261–267. https://doi.org/10.1078/072320204322881880

Medina A, Mateo EM, Valle-Algarra FM et al (2008) Influence of nitrogen and carbon sources on the production of ochratoxin A by ochratoxigenic strains of *Aspergillus spp*. isolated from grapes. Int J Food Microbiol 122:93–99. https://doi.org/10.1016/j.ijfoodmicro.2007.11.055

Medina A, Schmidt-Heydt M, Cárdenas-Chávez DL et al (2013) Integrating toxin gene expression, growth and fumonisin B1 and B2 production by a strain of *Fusarium verticillioides* under different environmental factors. J R Soc Interface 10:20130320. https://doi.org/10.1098/rsif.2013.0320

Mehrzad J, Malvandi AM, Alipour M et al (2017) Environmentally relevant level of aflatoxin B1 elicits toxic pro-inflammatory response in murine CNS-derived cells. Toxicol Lett 279:96–106. https://doi.org/10.1016/j.toxlet.2017.07.902

Merhej J, Richard-Forget F, Barreau C (2011) The pH regulatory factor Pac1 regulates Tri gene expression and trichothecene production in *Fusarium graminearum*. Fungal Genet Biol 48:275–284. https://doi.org/10.1016/j.fgb.2010.11.008

Min K, Shin Y, Son H et al (2012) Functional analyses of the nitrogen regulatory gene areA in *Gibberella zeae*. FEMS Microbiol Lett 334:66–73. https://doi.org/10.1111/j.1574-6968.2012.02620.x

Minervini F, Dell'Aquila ME (2008) Zearalenone and reproductive function in farm animals. Int J Mol Sci 9:2570–2584. https://doi.org/10.3390/ijms9122570

Minervini F, Fornelli F, Flynn KM (2004) Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicol In Vitro 18:21–28. https://doi.org/10.1016/S0887-2333(03)00130-9

Mogensen JM, Nielsen KF, Samson RA et al (2009) Effect of temperature and water activity on the production of fumonisins by *Aspergillus niger* and different *Fusarium* species. BMC Microbiol 9:281. https://doi.org/10.1186/1471-2180-9-281

Montani ML, Vaamonde G, Resnik SL et al (1988) Influence of water activity and temperature on the accumulation of zearalenone in corn. Int J Food Microbiol 6:1–8. https://doi.org/10.1016/0168-1605(88)90078-5

Moye-Rowley WS (2003) Regulation of the transcriptional response to oxidative stress in fungi: similarities and differences. Eukaryot Cell 2:381–389. https://doi.org/10.1128/EC.2.3.381-389.2003

Mudge DW, Atcheson BA, Taylor PJ et al (2004) Severe toxicity associated with a markedly elevated mycophenolic acid free fraction in a renal transplant recipient. Ther Drug Monit 26:453–455

Mudili V, Siddaih CN, Nagesh M et al (2014) Mould incidence and mycotoxin contamination in freshly harvested maize kernels originated from India. J Sci Food Agric 94:2674–2683. https://doi.org/10.1002/jsfa.6608

Mupunga I, Lebelo SL, Mngqawa P et al (2014) Natural occurrence of aflatoxins in peanuts and peanut butter from Bulawayo, Zimbabwe. J Food Prot 77:1814–1818. https://doi.org/10.4315/0362-028X.JFP-14-129

Niehaus WG Jr, Jiang WP (1989) Nitrate induces enzymes of the mannitol cycle and suppresses versicolorin synthesis in *Aspergillus parasiticus*. Mycopathologia 107:131–137. https://doi.org/10.1007/BF00707550

Niide O, Suzuki Y, Yoshimaru T et al (2006) Fungal metabolite gliotoxin blocks mast cell activation by a calcium- and superoxide-dependent mechanism: implications for immunosuppressive activities. Clin Immunol 118:108–116. https://doi.org/10.1016/j.clim.2005.08.012

Nishie K, Cole RJ, Dorner JW (1985) Toxicity and neuropharmacology of cyclopiazonic acid. Food Chem Toxicol 23:831–839. https://doi.org/10.1016/0278-6915(85)90284-4

Nishie K, Cole RJ, Dorner JW (1988) Toxicity of citreoviridin. Res Commun Chem Pathol Pharmacol 59:31–52

Niu C, Payne GA, Woloshuk CP (2015) Transcriptome changes in *Fusarium verticillioides* caused by mutation in the transporter-like gene FST1. BMC Microbiol 15:90. https://doi.org/10.1186/s12866-015-0427-3

Njoroge SM, Matumba L, Kanenga K et al (2016) A case for regular aflatoxin monitoring in peanut butter in sub-Saharan Africa: lessons from a 3-year survey in Zambia. J Food Prot 79:795–800. https://doi.org/10.4315/0362-028X.JFP-15-542

Nkwe DO, Taylor JE, Siame BA (2005) Fungi, aflatoxins, fumonisin Bl and zearalenone contaminating sorghum-based traditional malt, wort and beer in Botswana. Mycopathologia 160:177–186. https://doi.org/10.1007/s11046-005-6867-9

O'Brian GR, Georgianna DR, Wilkinson JR et al (2007) The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. Mycologia 99:232–239

Okutan H, Aydin G, Ozcelik N (2004) Protective role of melatonin in ochratoxin a toxicity in rat heart and lung. J Appl Toxicol 24:505–512. https://doi.org/10.1002/jat.1010

Omar SS (2016) Aflatoxin M1 levels in raw milk, pasteurised milk and infant formula. Ital J Food Saf 5:5788. https://doi.org/10.4081/ijfs.2016.5788

Orti DL, Hill RH Jr, Liddle JA et al (1986) High performance liquid chromatography of mycotoxin metabolites in human urine. J Anal Toxicol 100:41–45

Osteresch B, Viegas S, Cramer B et al (2017) Multi-mycotoxin analysis using dried blood spots and dried serum spots. Anal Bioanal Chem 409:3369–3382. https://doi.org/10.1007/s00216-017-0279-9

Osweiler GD, Ross PF, Wilson TM et al (1992) Characterization of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings. J Vet Diagn Investig 4:53–59. https://doi.org/10.1177/104063879200400112

Owino JH, Arotiba OA, Hendricks N et al (2008) Electrochemical immunosensor based on polythionine/gold nanoparticles for the determination of aflatoxin B_1. Sensors 8:8262–8274. https://doi.org/10.3390/s8128262

Ozsoy N, Selmanoğlu G, Koçkaya EA et al (2008) Effect of patulin on the interdigitating dendritic cells (IDCs) of rat thymus. Cell Biochem Funct 26:192–196. https://doi.org/10.1002/cbf.1431

Palabiyik SS, Erkekoglu P, Zeybek ND et al (2013) Protective effect of lycopene against ochratoxin A induced renal oxidative stress and apoptosis in rats. Exp Toxicol Pathol 65:853–861. https://doi.org/10.1016/j.etp.2012.12.004

Paradells S, Rocamonde B, Llinares C et al (2015) Neurotoxic effects of ochratoxin A on the subventricular zone of adult mouse brain. J Appl Toxicol 35:737–751. https://doi.org/10.1002/jat.3061

Passamani FR, Hernandes T, Lopes NA et al (2014) Effect of temperature, water activity, and pH on growth and production of ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* from Brazilian grapes. J Food Prot 77:1947–1952. https://doi.org/10.4315/0362-028X.JFP-13-495

Patterson DS, Roberts BA (1979) Mycotoxins in animal feedstuffs: sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone, and T-2 toxin. J Assoc Off Anal Chem 62:1265–1267

Pena A, Seifrtová M, Lino C et al (2006) Estimation of ochratoxin A in portuguese population: new data on the occurrence in human urine by high performance liquid chromatography with fluorescence detection. Food Chem Toxicol 44:1449–1454. https://doi.org/10.1016/j.fct.2006.04.017

Peñalva MA, Tilburn J, Bignell E et al (2008) Ambient pH gene regulation in fungi: making connections. Trends Microbiol 16:291–300. https://doi.org/10.1016/j.tim.2008.03.006

Peng KY, Chen CY (2009) Prevalence of aflatoxin M1 in milk and its potential liver cancer risk in Taiwan. J Food Prot 72:1025–1029. https://doi.org/10.4315/0362-028X-72.5.1025

Peraica M, Radić B, Lucić A et al (1999) Toxic effects of mycotoxins in humans. Bull World Health Organ 77:754–766. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2557730/pdf/10534900.pdf

Perši N, Pleadin J, Kovačević D et al (2014) Ochratoxin A in raw materials and cooked meat products made from OTA-treated pigs. Meat Sci 96:203–210. https://doi.org/10.1016/j.meatsci.2013.07.005

Pirinçci ŞŞ, Ertekin Ö, Laguna DE et al (2018) Label-free QCM immunosensor for the detection of ochratoxin A. Sensors 18:1161. https://doi.org/10.3390/s18041161

Pizzolato Montanha F, Anater A, Burchard JF et al (2018) Mycotoxins in dry-cured meats: a review. Food Chem Toxicol 111:494–502. https://doi.org/10.1016/j.fct.2017.12.008

Pleadin J, Zadravec M, Brnić D et al (2017) Moulds and mycotoxins detected in the regional speciality fermented sausage 'slavonski kulen' during a 1-year production period. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 34:282–290. https://doi.org/10.1080/19440049.2016.1266395

Poapolathep A, Poapolathep S, Klangkaew N et al (2008) Detection of deoxynivalenol contamination in wheat products in Thailand. J Food Prot 71:1931–1933. https://doi.org/10.4315/0362-028X-71.9.1931

Poór M, Lemli B, Bálint M et al (2015) Interaction of citrinin with human serum albumin. Toxins 7:5155–5166. https://doi.org/10.3390/toxins7124871

Poór M, Kunsági-Máté S, Bálint M et al (2017a) Interaction of mycotoxin zearalenone with human serum albumin. J Photochem Photobiol B 170:16–24. https://doi.org/10.1016/j.jphotobiol.2017.03.016

Poór M, Bálint M, Hetényi C et al (2017b) Investigation of non-covalent interactions of aflatoxins (B1, B2, G1, G2, and M1) with serum albumin. Toxins 9:339. https://doi.org/10.3390/toxins9110339

Poostforoushfard A, Pishgar AR, Berizi E et al (2017) Patulin contamination in apple products marketed in Shiraz, Southern Iran. Curr Med Mycol 3:32–35. https://doi.org/10.29252/cmm.3.4.32

Porter JK, Bacon CW, Wray EM et al (1995) Fusaric acid in *Fusarium moniliforme* cultures, corn, and feeds toxic to livestock and the neurochemical effects in the brain and pineal gland of rats. Nat Toxins 3:91–100. https://doi.org/10.1002/nt.2620030206

Prosperini A, Juan-García A, Font G et al (2013) Beauvericin-induced cytotoxicity via ROS production and mitochondrial damage in Caco-2 cells. Toxicol Lett 222:204–211. https://doi.org/10.1016/j.toxlet.2013.07.005

Raab M, Daxecker H, Karimi A et al (2001) In vitro effects of mycophenolic acid on the nucleotide pool and on the expression of adhesion molecules of human umbilical vein endothelial cells. Clin Chim Acta 310:89–98. https://doi.org/10.1016/S0009-8981(01)00527-7

Rahimi E, Bonyadian M, Rafei M et al (2010) Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. Food Chem Toxicol 48:129–131. https://doi.org/10.1016/j.fct.2009.09.028

Reddy TV, Viswanathan L, Venkitasubramanian TA (1971) High aflatoxin production on a chemically defined medium. Appl Microbiol 22:393–396

Reddy TV, Viswanathan L, Venkitasubramanian TA (1979) Factors affecting aflatoxin production by *Aspergillus parasiticus* in a chemically defined medium. J Gen Microbiol 114:409–413. https://doi.org/10.1099/00221287-114-2-409

Redouane-Salah S, Morgavi DP, Arhab R et al (2015) Presence of aflatoxin M1 in raw, reconstituted, and powdered milk samples collected in Algeria. Environ Monit Assess 187:375. https://doi.org/10.1007/s10661-015-4627-y

Richard JL (2007) Some major mycotoxins and their mycotoxicoses–an overview. Int J Food Microbiol 20:3–10. https://doi.org/10.1016/j.ijfoodmicro.2007.07.019

Ridenour JB, Bluhm BH (2017) The novel fungal-specific gene FUG1 has a role in pathogenicity and fumonisin biosynthesis in *Fusarium verticillioides*. Mol Plant Pathol 18:513–528. https://doi.org/10.1111/mpp.12414

Rimando AM, Porter JK (1997) Fusaric acid increases melatonin levels in the weanling rat and in pineal cell cultures. J Toxicol Environ Health 50:275–284. https://doi.org/10.1080/009841097160483

Robert L, Bowden F, John FL (1992) Nitrate-nonutilizing mutants of *Gibberella zeae* (*Fusarium graminearum*) and their use in determining vegetative compatibility. Exp Mycol 16:308–315. https://doi.org/10.1016/0147-5975(92)90007-E

Roberts BA, Patterson DS (1975) Detection of twelve mycotoxins in mixed animal feedstuffs, using a novel membrane cleanup procedure. J Assoc Off Anal Chem 58:1178–1181

Rotimi OA, Rotimi SO, Duru CU et al (2017) Acute aflatoxin B1 – induced hepatotoxicity alters gene expression and disrupts lipid and lipoprotein metabolism in rats. Toxicol Rep 4:408–414. https://doi.org/10.1016/j.toxrep.2017.07.006

Sakamoto S, Putalun W, Vimolmangkang S et al (2017) Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. J Nat Med 72:32–42. https://doi.org/10.1007/s11418-017-1144-z

Samapundo S, Devliehgere F, De Meulenaer B et al (2005) Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on corn. J Food Prot 68:1054–1059. https://doi.org/10.4315/0362-028X-68.5.1054

Sangare-Tigori B, Moukha S, Kouadio HJ et al (2006) Co-occurrence of aflatoxin B1, fumonisin B1, ochratoxin A and zearalenone in cereals and peanuts from Côte d'Ivoire. Food Addit Contam 23:1000–1007

Santos L, Marín S, Sanchis V et al (2009) Screening of mycotoxin multicontamination in medicinal and aromatic herbs sampled in Spain. J Sci Food Agric 89:1802–1807. https://doi.org/10.1002/jsfa.3647

Sava V, Reunova O, Velasquez A et al (2006) Can low level exposure to ochratoxin-A cause parkinsonism? J Neurol Sci 249:68–75. https://doi.org/10.1016/j.jns.2006.06.006

Sava V, Mosquera D, Song S et al (2018) Rubratoxin B elicits antioxidative and DNA repair responses in mouse brain. Gene Expr 11:211–219. https://doi.org/10.3727/000000003783992261

Schaafsma AW, Hooker DC (2007) Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. Int J Food Microbiol 119:116–125. https://doi.org/10.1016/j.ijfoodmicro.2007.08.006

Schmidt-Heydt M, Geisen R (2007) A microarray for monitoring the production of mycotoxins in food. Int J Food Microbiol 117:131–140. https://doi.org/10.1016/j.ijfoodmicro.2007.01.014

Schmidt-Heydt M, Stoll D, Schütz P et al (2015) Oxidative stress induces the biosynthesis of citrinin by *Penicillium verrucosum* at the expense of ochratoxin. Int J Food Microbiol 192(192):1–6. https://doi.org/10.1016/j.ijfoodmicro.2014.09.008

Schoevers EJ, Santos RR, Colenbrander B et al (2012) Transgenerational toxicity of Zearalenone in pigs. Reprod Toxicol 34:110–119. https://doi.org/10.1016/j.reprotox.2012.03.004

Schöneberg T, Martin C, Wettstein FE et al (2016) Fusarium and mycotoxin spectra in Swiss barley are affected by various cropping techniques. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 33:1608–1619. https://doi.org/10.1080/19440049.2016.1219071

Seetha A, Monyo ES, Tsusaka TW et al (2018) Aflatoxin-lysine adducts in blood serum of the Malawian rural population and aflatoxin contamination in foods (groundnuts, maize) in the corresponding areas. Mycotoxin Res 34:195–204. https://doi.org/10.1007/s12550-018-0314-5
Segvić Klarić M, Medić N, Hulina A et al (2014) Disturbed Hsp70 and Hsp27 expression and thiol redox status in porcine kidney PK15 cells provoked by individual and combined ochratoxin a and citrinin treatments. Food Chem Toxicol 71:97–105. https://doi.org/10.1016/j.fct.2014.06.002
Selmanoglu G, Koçkaya EA (2004) Investigation of the effects of patulin on thyroid and testis, and hormone levels in growing male rats. Food Chem Toxicol 42:721–727. https://doi.org/10.1016/j.fct.2003.12.007
Shah HU, Simpson TJ, Alam S et al (2010) Mould incidence and mycotoxin contamination in maize kernels from Swat Valley, North West Frontier Province of Pakistan. Food Chem Toxicol 48:1111–1116. https://doi.org/10.1016/j.fct.2010.02.004
Sheik Abdul N, Nagiah S, Chuturgoon AA (2016) Fusaric acid induces mitochondrial stress in human hepatocellular carcinoma (HepG2) cells. Toxicon 119:336–344. https://doi.org/10.1016/j.toxicon.2016.07.002
Sheik Abdul N, Nagiah S, Chuturgoon AA (2019) Fusaric acid induces NRF2 as a cytoprotective response to prevent NLRP3 activation in the liver derived HepG2 cell line. Toxicol In Vitro 55:151–159. https://doi.org/10.1016/j.tiv.2018.12.008
Shephard GS, van der Westhuizen L, Gatyeni PM et al (2005) Do fumonisin mycotoxins occur in wheat? J Agric Food Chem 53:9293–9296. https://doi.org/10.1021/jf052101s
Shephard GS, Burger HM, Gambacorta L et al (2013) Multiple mycotoxin exposure determined by urinary biomarkers in rural subsistence farmers in the former Transkei, South Africa. Food Chem Toxicol 62:217–225. https://doi.org/10.1016/j.fct.2013.08.040
Shinozuka J, Li G, Kiatipattanasakul W et al (1997) T-2 toxin-induced apoptosis in lymphoid organs of mice. Exp Toxicol Pathol 49:387–392. https://doi.org/10.1016/S0940-2993(97)80124-8
Shinozuka J, Suzuki M, Noguchi N et al (1998) T-2 toxin-induced apoptosis in hematopoietic tissues of mice. Toxicol Pathol 26:674–681. https://doi.org/10.1177/019262339802600512
Shwab EK, Bok JW, Tribus M et al (2007) Histone deacetylase activity regulates chemical diversity in *Aspergillus*. Eukaryot Cell 6:1656–1664. https://doi.org/10.1128/EC.00186-07
Sineque AR, Macuamule CL, Dos Anjos FR (2017) Aflatoxin B1 contamination in chicken livers and gizzards from industrial and small abattoirs, measured by ELISA technique in Maputo, Mozambique. Int J Environ Res Public Health 14:951. https://doi.org/10.3390/ijerph14090951
Sizaret P, Malaveille C, Montesano R et al (1982) Detection of aflatoxins and related metabolites by radioimmunoassay. J Natl Cancer Inst 69:1375–1381
Smith TK, MacDonald EJ (1991) Effect of fusaric acid on brain regional neurochemistry and vomiting behavior in swine. J Anim Sci 69:2044–2049. https://doi.org/10.2527/1991.6952044x
Smith GW, Constable PD, Tumbleson ME et al (1999) Sequence of cardiovascular changes leading to pulmonary edema in swine fed culture material containing fumonisin. Am J Vet Res 60:1292–1300
Smith MC, Hymery N, Troadec S et al (2017) Hepatotoxicity of fusariotoxins, alone and in combination, towards the HepaRG human hepatocyte cell line. Food Chem Toxicol 109:439–451. https://doi.org/10.1016/j.fct.2017.09.022
Solfrizzo M, Gambacorta L, Lattanzio VM et al (2011) Simultaneous LC-MS/MS determination of aflatoxin M1, ochratoxin A, deoxynivalenol, de-epoxydeoxynivalenol, α and β-zearalenols and fumonisin B1 in urine as a multi-biomarker method to assess exposure to mycotoxins. Anal Bioanal Chem 401:2831–2841. https://doi.org/10.1007/s00216-011-5354-z
Sorenson WG, Simpson J (1986) Toxicity of penicillic acid for rat alveolar macrophages in vitro. Environ Res 41:505–513. https://doi.org/10.1016/S0013-9351(86)80145-1
Stockmann-Juvala H, Mikkola J, Naarala J et al (2004a) Fumonisin B1-induced toxicity and oxidative damage in U-118MG glioblastoma cells. Toxicology 202:173–183. https://doi.org/10.1016/j.tox.2004.05.002

Stockmann-Juvala H, Mikkola J, Naarala J et al (2004b) Oxidative stress induced by fumonisin B1 in continuous human and rodent neural cell cultures. Free Radic Res 38:933–942. https://doi.org/10.1080/10715760412331273205

Stoll D, Schmidt-Heydt M, Geisen R (2013) Differences in the regulation of ochratoxin A by the HOG pathway in *Penicillium* and *Aspergillus* in response to high osmolar environments. Toxins 5:1282–1298. https://doi.org/10.3390/toxins5071282

Sueck F, Cramer B, Czeschinski P et al (2018a) Human study on the kinetics of 2'R-ochratoxin A in the blood of coffee drinkers. Mol Nutr Food Res 63(4):e1801026. https://doi.org/10.1002/mnfr.201801026

Sueck F, Poór M, Faisal Z et al (2018b) Interaction of ochratoxin A and

Fig. 3.6 Main isolated and identified compounds from *Pistacia lentiscus*. Chemical studies of *P. lentiscus* revealed diverse secondary metabolites and high levels of vitamins and minerals. Monoterpenoids, sesquiterpenoids, and essential oil are the main compounds reported in different parts of *P. lentiscus*. α-pinene are reported as the main compound following by limonene, myrcene, sabinene, and teroinen-4-ol. The plant contains phenolic compounds like gallic acid, digallic acid, catechin, epicatechin, and gallic acid methyl. The leaf is a rich with galloyl derivatives like mono, di, and tri-O-galloyl quinic acid and monogalloyl glucose. Quercetin-3-glucoside are reported as the most abundant flavonoid compound. Oleic acid and linoleic acid are detected in the fruit of this plant. (The structures of the compounds are download from https://pubchem.ncbi.nlm.nih.gov)

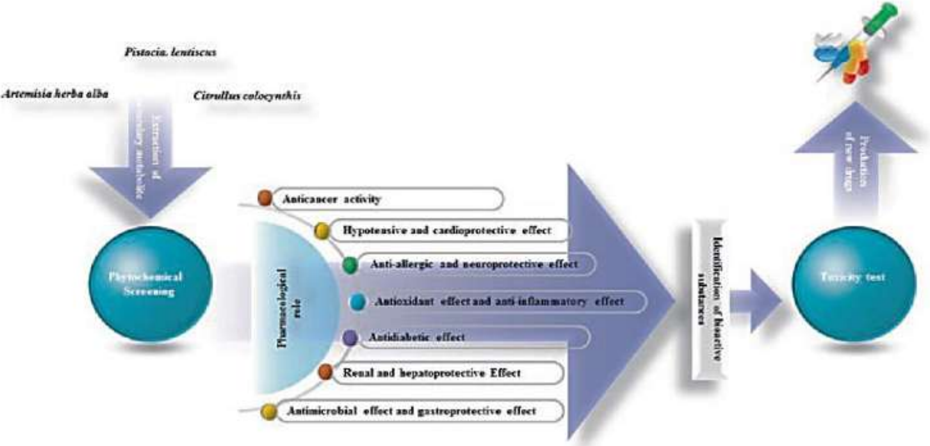


Fig. 3.7 Pharmacological role of the extracts and essential oils obtained from *A. herba-alba*, *C. colocynthis* and *P. lentiscus*. Selected described Plants are recommended in traditional medicine as a cure for various diseases, current researches try through different technique and methods to prove these biological activities and its origins. All medicinal plants must undergo a chemical extraction or a hydro-distillation to obtain an organic extract or an essential oil, these latter are tested in order to check its anti-cancer, hypotensive, cardioprotective, neuroprotective, antiallergic, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, gastroprotective and antimicrobial activities. When the biological activities are proven, the fractionation, isolation and identification of bioactive compounds are performed to obtain pure compounds. If the latter are biologically active (in vitro and in vivo), the toxicity must be determined before any pharmaceutical reproduction

pressure and heart rate, as well as an increase in sodium and potassium levels in urine (Zeggwagh et al. 2014). Evaluation of the vasorelaxant effect of the aqueous extract in the rat aorta, demonstrated that this plant records another important effect, the result revealed that *A. herba-alba* extract produced an endothelium-dependent relaxation in the rat aorta (Skiker et al. 2010). Table 3.3 presents some cardioprotective and hypotensive effects of some substances isolate from these plants.

3.6.3 Anti-allergic and Neuroprotective Effect

The use *P. lentiscus* essential oils increased the protection of brain tissue against ischemia, which causes inflammation and an oxidative stress that strongly damages membranes and polyunsaturated fatty acids, and finally inducing a neuronal death (Quartu et al. 2012). Some studies have indicated an allergic effect of *P. lentiscus* gum, contact dermatitis was observed following the use of an orthopaedic solution containing in its composition a quantity of gum (Lee and Lam 1993; Wakelin 2001). In the mouse ear, a concentration of 100 and 125 $mg.kg^{-1}$, cucurbitacin E 2-O-b-D-glucopyranoside and cucurbitacin E from *C. colocynthis* exhibited an antiallergic activity against passive cutaneous anaphylactic reactions (Yoshikawa et al. 2007).

Table 3.2 Anticancer activity of *P. lentiscus*, *C. colocynthis*, and *A. herba-alba*

Plant	Type of extract	Type of cancer	Dose	Molecule	References
P. lentiscus	Gum mastic	Prostate cancer cell line LNCaP[a].	6–12 μg/mL		He et al. (2006)
		Androgen-independent prostate cancer PC-3 cells[b].	20–60 μg/mL	Masticadienonic acid	He et al. (2007)
		Promyelocytic leukemia HL-60 cells.	0–200 μg/mL (solid mastic) or 0–2 (v/v)% of liquid mastic	Masticadienonic acid, oleanonic acid	Sakagami et al. (2009), Chávez et al. (2005) and Nguyen et al. (2005)
		Mmyeloblastic leukemia (ML-1, KG-1) cells.			
		Erythroleukemia K-562.			
		Oral squamous cell carcinoma (HSC-2, HSC-3, HSC-4).			
		Hepatocellular carcinoma (HepG2) cells.			
		Glioblastoma (T98G, U87MG) cells [c].			
	Gemcitabine in combination with gum mastic	Pancreatic cancer cells (BxPC-3 and COLO 357)[d].	Gemcitabine (10 μg/mL) and gum mastic (40 μg/mL)		Huang et al. (2010)
	Mastic oil	Lewis Lung Carcinoma (LLC) cells[e].	45 mg/kg 0.01–0.02% v/v		Magkouta et al. (2009) and Moulos et al. (2009)
	Hexane extracts of mastic gum	Human HCT116 colon cancer cells[f].	100–220 mg/kg		Dimas et al. (2009)

(continued)

Table 3.2 (continued)

Plant	Type of extract	Type of cancer	Dose	Molecule	References
C. colocynthis		SH-SY5Y human neuroblastoma[g].	0–128 μM	Cucurbitacin B	Zheng et al. (2014)
		A549 lung cancer cell line[h].	0.02–62.5 μmol/L		Zhang et al. (2014)
		Human pancreatic cancer cells PANC-1[i].	0.1–10-Kmol/L		Zhang et al. (2010)
		Human laryngeal cancer Hep-2 cells[j].			Liu et al. (2008, 2010)
		BEL-7402 human hepatocellular carcinoma cells[k].	0.01–1000 μM		Chan et al. (2010)
		Osteosarcoma cells[l].	20–100 μM		Zhang et al. (2017)
		HT-29 and HCT-116 cell lines[m].	CuB (0.0125–80 μM) and Gef (0.1–80 μM)	Cucurbitacin B in combination with Gefitinib	Yar Saglam et al. (2016)
		Human pancreatic cancer cells PANC-1[n].	0.01–100 μM	Cucurbitacin E	Sun et al. (2010)
		Bcap37 and MDA-MB-231 cancer cells[o].	0.1–100 μM		Lan et al. (2013)
		MCF7/ADR breast cancer cells[p].	0.125–16 μg/mL	Cucurbitacin D	Ku et al. (2015)
	Essential Oils	P815 and BSR cancer cell lines[q].		Sesquiterpene compounds	Tilaoui et al. (2015)

The table below illustrates the anticancer activity and lethal doses of the main bioactive compound of *P. lentiscus, C. colocyntis* and *A. herba-alba* in several cell lines. [a]Mastic gum tested in LNCaP prostate cancer cell line demonstrated that the mastic gum inhibits the transcriptional AR expression and reduces the NKX3.1 and PSA expression. [b]In PC-3 cells, mastic gum inhibits the cell growth, blocks the cell cycle in G1 phase and suppresses the activity of NF-kappaB. [c]Promyelocytic leukemia HL-60 was the most sensitive to the cytotoxicity of mastic, followed by myeloblastic leukemia (ML-1, KG-1), erythroleukemia (K-562), oral squamous cell carcinoma (HSC-2, HSC-3, HSC-4, hepatocellular carcinoma (HepG2) and glioblastoma (T98G, U87MG). [d]In pancreatic cancer cells, the combination of gemcitabine and mastic gum demonstrated an increase of the

(continued)

Table 3.2 (continued)

antiproliferative and apoptotic effects, an increase in the level of IκBα and Bax protein, and an inhibition of NF-κB activation. [e]In lewis lung carcinoma (LLC) cells, mastic oil demonstrated an increase of apoptosis, a decrease of neovascularization, and an inhibition of chemokine expression. [f]Hexane extracts of mastic demonstrated a decrease of tumor growth with approximately 35% of inhibition. [g]In SH-SY5Y human neuroblastoma cells, CuB induced cell cycle arrest at G2/M phase followed by apoptosis, the same compound altered the expression of gene products that mediated cell proliferation (Cyclin B1 and cyclin-dependent kinase 1), cell survival (B-cell lymphoma 2, Bcl2-associated X protein) and increased the expression of p53 and p21. [h]CuB induced an apoptosis in A549 cells and inhibited lung cancer cell proliferation. [i]CuB inhibited the growth of PANC-1 cells, through the accumulation of G2/M phase cells and apoptosis. [j]In Hep-2 cells, combination of CuB and cisplatin displayed synergistic effects of growth inhibition. [k]in BEL-7402 human hepatocellular carcinoma cells, treatment with CuB induced S phase arrest. [l]CuB reduced cell viability in osteosarcoma cells, and induced an effective inhibition of MAPK signalling and JAK2/STAT3 cascades. [m]CuB with gefitinib treatment caused a growth inhibition, induced apoptosis in HT-29 and HCT-116 cell lines and decreased the expression levels of B-Cell CLL/Lymphoma 2 (Bcl-2), BCL2-like 1 (BCL2L1), cyclin D1, pSTAT3, and pEGFR. [n]CuE inhibited the growth of PANC-1 cells and caused accumulation of cells at the G(2)/M phase. [o]CuE inhibited the growth in human breast cancer cells and induced G2/M phase arrest. [p]CuD caused more than 60% cell death in MCF7/ADR breast cancer cells, the combination of CuD and doxorubicin induced apoptosis and cell cycle arrest. [q]Essential oil tested for its antitumor activity in P815 and BSR cancer cell lines showed that P815 cells are the most sensitive to the cytotoxic effect

Table 3.3 Hypotensive and cardioprotective activity of *P. lentiscus* and *C. colocynthis*

Plant	Activity	Molecule	References
P. lentiscus	Hypotensive activity [(1)]	Polymeric procyanidin fraction α-pinene, β-pinene	Sanz et al. (1992) and Menezes et al. (2010)
C. colocynthis	The vasodilator responses, protecting cardiomyocytes and protective effects against chronic DOX cardiotoxicity [(2)]	Quercetin kaempférol	Pérez-Vizcaíno et al. (2002), Chen et al. (2013), and Matouk et al. (2013)

(1): α-pinene and β-pinene induced an hypotension. (2): Quercetin and kaempférol showed vasodilator effects with selectivity toward the resistance vessels. In H9C2 cells, quercetin blocks the H_2O_2-induced inflammatory response and contributes to prevent ischemia/reperfusion injury in cardiomyocytes. Quercetin can also increase the cardioprotective effect of losartan against chronic cardiotoxicity induced by doxorubicin which produced leukocyte infiltration and myocardial lesions

3.6.4 Antioxidant Effect

P. lentiscus oil attenuated a lipid peroxidation, superoxide dismutase, and catalase in the liver (Saidi et al. 2017), rat lungs (Abidi et al. 2017), and human skin (Ben Khedir et al. 2016), it showed a good antioxidant effect (Barra et al. 2007), with a radical scavenging activity between 21% and 35% against DPPH radicals (Negro et al. 2015). Ethyl acetate and methanolic leaves extract showed a high antioxidant

capacity against DPPH radicals with IC_{50} = 0.0068 $mg.ml^{-1}$ for the first extract (Yemmen et al. 2017). A high scavenging activity are also noted for ethanolic extract against DPPH and ABTS radicals (Belhachat et al. 2017; Beghlal et al. 2016). The polar extract inhibited the release of nitric oxide and reactive oxygen species in RAW 264.7 macrophage cells (Grace et al. 2016). Pre-treatment of THP-1 cells with 100 $\mu g.ml^{-1}$ of *P. lentiscus* extract caused an inhibition of H_2O_2 cytotoxicity through increasing cell viability to 108.25% (Remila et al. 2015). Methanolic extracts of the leaves and fruits added to pork sausage at 300 $mg.kg^{-1}$ significantly inhibited the oxidation (Botsaris et al. 2015). Oleanolic acid and ursolic acid showed a high antioxidant activity (Assimopoulou et al. 2015).

Organic extracts of *C. colocynthis* leaves demonstrated that methanolic extract exerts a high antioxidant activity followed in descending order by hydro-methanol, chloroformic and hexanoic extract (Nessa and Khan 2014). In another study, ethyl acetate seed extract recorded a percentage reduction of 88.8% against DPPH radicals followed by hydro-methanolic extract with a percentage reduction of 74.5% (Benariba et al. 2013a, b). Butanolic fruits extract exercised a high antioxidant activity with an IC_{50} = 61 $\mu g.ml^{-1}$ in comparison with aqueous extract which demonstrated an IC_{50} = 241.25 $\mu g.ml^{-1}$ (Chekroun et al. 2015). At a concentration of 800 $\mu g.ml^{-1}$, methanolic fruits extract showed a percentage inhibition of 76% against DPPH radicals (Vakiloddin et al. 2015). Oil extracted from mature seeds reduced cadmium-induced damage with an increase of activity levels of antioxidant enzymes (Amamou et al. 2015). In rat testes, *C. colocynthis* pulp increased catalase and peroxidase activities, and decreased malondialdehyde (Ostovan et al. 2017).

Essential oil of *A. herba-alba* tested with various methods demonstrated a remarkable antioxidant activity (Mighri et al. 2010; Rafiq et al. 2016), another study demonstrated that the essential oil had a high potency to reduce and eliminate radicals in comparison to rosemary essential oil (Aouadi et al. 2014), it recorded an IC_{50} of 9.1 $mg.ml^{-1}$ and 2.97 mg eq $EDTA.g^{-1}$ respectively with DPPH and FRAP test (Aloui et al. 2016). In rats, an oral administration of 50 $mg.kg^{-1}$ (bw) of essential oil can overcome the deleterious effects of Malathion (neurotoxic pesticide) by reducing lipid peroxidation and hydrogen peroxide (Selmi et al. 2016), in the blood of rats, tea and *A. herba-alba* decoction demonstrated an increase of the antioxidant status and activities of glutathione peroxidase with levels ranging from 83.5–111% to 23–38%, respectively (Abid et al. 2007). Table 3.4 presents some antioxidant substances isolate from these plants with their IC_{50}.

3.6.5 Antidiabetic Effect

In healthy individuals, *P. lentiscus* caused a remarkable decrease in blood glucose (Kartalis et al. 2016), in the diabetic rat induced by streptozotocin, *P. lentiscus* has a very good antidiabetic effect (Mehenni et al. 2016), another study confirmed that

Table 3.4 Antioxidant activity of *P. lentiscus* and *C. colocynthis* and *A. herba-alba*

Plant		Measurement method	Molecule or plant organ with it IC[50]	References
P. lentiscus		DPPH[a]	Gallic acid IC_{50} = 2µg/ml. 1,2,3,4,6-pentagalloylglucose withIC_{50} = 1µg/ml	Abdelwahed et al. (2007)
		DPPH[b]	5-O-galloyl IC_{50} = 18.7µM 3,5-di-O-galloyl IC_{50} = 7.1 µM 3,4,5-tri-O-galloyl IC_{50} = 3.9 µM	Baratto et al. (2003)
		O_2^- Radical (KOH/ Acetone) [b]	5-O-galloyl IC_{50} = 25.4 µM 3,5-di-O-galloyl IC_{50} = 22.6 µM 3,4,5-tri-O-galloyl with IC_{50} = 11.5 µM	
		OH Radical[b]	5-O-galloyl IC_{50} = 71.3 µM 3,5-di-O-galloyl IC_{50} = 16.0 µM 3,4,5-tri-O-galloyl IC_{50} = 26.6 µM	
C. colocynthis		ABTS[c]	Cucurbitacin B glucoside and cucurbitacin E glucoside.	Tannin-Spitz et al. (2007)
	Ethanol, hexane and methanol extract of the root, leave and fruit.	DPPH[d]	Ferulic acid, vanillic acid, p-coumeric acid, gallic acid, p-hydroxy benzoic acid, chlorogenic acid, quercetin, myricetin and catechin.	Hussain et al. (2013)
	Methanol extract (70%)		Seeds	Yasir et al. (2016)
A. herba alba.	*Water extract*	DPPH[e]	10 mg/ml of Artemisinin with 91% radical scavenging activity	Kim et al. (2015)

[a]1,2,3,4,6-pentagalloylglucose is effective to scavenge DPPH∗ radical and protect against lipid peroxidation. [b]The scavenger activities of gallic acid, 5-O-galloyl, 3,5-O-digalloyl, 3,4,5-O-trigalloyl, estimated against DPPH, superoxide radical, and hydroxyl (OH) radical, demonstrated a good antioxidant potential. [c]The combination of cucurbitacin B+E demonstrated an antioxidant activity, probably through the scavenging of several free radicals. [d] Ethanolic extract of the leaves possesses a radical scavenging activity against DPPH followed by ethanolic extracts root and fruit. [e]Antioxidant properties of aqueous extract demonstrated an activity of 91.0 ± 3.2%, which is similar to α-tocopherol (92.6%)

100 mg.kg^{-1} of crude gum of *P. lentiscus* reduced blood glucose (Rehman et al. 2015), the contribution of the antidiabetic activity of the gum is suggested to the inhibition of 11β-hydroxysteroid dehydrogenase, exercised by two compound, those are masticadienonic acid and isomasticadienonic acid with IC_{50} = 2.51 μM and IC_{50} = 1.94 μM, respectively (Vuorinen et al. 2015).

According to an ethnopharmacological survey carried out in the region of Ouargla (Algeria), *A. Herba-alba* and *C. colocynthis* are the most common herbs used to fight diabetes (Telli et al. 2016). All studies performed on different parts of *C. colocynth* confirmed that ethanolic, methanolic and aqueous extracts of the seeds, roots, fruits, and leaves have an hypoglycemic activity at a dose ranging from 10 to 500 mg.kg-1 (bw) (Shi et al. 2014; Chekroun et al. 2017). Root aqueous extract showed a percentage reduction of 58.7% in blood glucose in comparison to chloroformic and ethanolic extract with improvement of biochemical blood parameters (Agarwal et al. 2012). Aqueous extracts and other bioactive substances like saponins and alkaloids from the seeds showed a good hypoglycemic effect (Lahfa et al. 2017; Amin et al. 2017). in vitro study performed in pancreatic cells isolated from rat demonstrated that seed extracts obtained by various solvents exert a positive insulinotropic action in the presence of 8.3 mM of D-glucose (Nmila et al. 2000; Benariba et al. 2012, 2013a, b). In rats, the use of 300 mg.kg^{-1} of water-ethanolic extract of seeds for 3 days demonstrated a decrease in alloxan-induced blood glucose (Oryan et al. 2014). The hydroalcoholic extract of *C. colocynthis* leaves showed a potential effect in insulin secretion from islets of Langerhans (Ebrahimi et al. 2016). In streptozotocin-induced diabetic rats, other relevant results are obtained; colocynth oil demonstrated a beneficial effect through tretaining or restoring pancreatic beta-cell mass (Sebbagh et al. 2009). Another study, carried out in diabetic patients receiving a treatment of 100 mg of *C. colocynthis* over a period of 2 months, showed a positive effect on glycosylated hemoglobin (HbA1c) and fasting blood glucose, leading to their decrease without any change in other blood parameters (Huseini et al. 2009).

In diabetic rat induced by alloxan, an oral administration of 0.39 g.kg^{-1} (bw) of aqueous solution of *A. Herba-alba* showed a decrease in blood glucose (Twaij and Al-Badr 1988; Al-Shamaony et al. 1994; Taştekin et al. 2006). Another study confirmed the previous result demonstrating that 0.3 g.kg^{-1} (bw) prepared by aqueous infusion, reduces the blood glucose more effectively in comparison to glibenclamide (an antidiabetic drug used against type 2 diabetes), due to the presence of mono- and di-cinnamoylquinic acids, 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, and vicenin-2 (apigenin 6,8-di-C-glucoside) (Boudjelal et al. 2015). Hepatic steatosis caused by a high-fat diet leading to diabetes is weakly reduced by the extract of this plant (Hamza et al. 2015).

3.6.6 Renal and Hepatoprotective Effect

Medicinal plants with a hepatoprotective effect are accompanied with a protective effect for the stomach; *P. lentiscus* is among these plants (Shamsi-Baghbanan et al. 2014). Arsenic is a highly hepatotoxic metal, however, administration of *P. lentiscus* oil with sodium arsenite decreased the damage and disruption of the pro-oxidant/antioxidant status in the liver (Klibet et al. 2016), it reduces liver damage caused by intestinal ischemia-reperfusion–induced in rats (Saidi et al. 2017). Following the presence of phenolic compounds, *P. lentiscus* possessed a high effect against hepatic necrosis resulting an overdose of paracetamol (Mehenni et al. 2016), its aqueous extract showed an anti-hepatotoxic effect against carbon tetrachloride by reducing bilirubin, SGTO, SGPT and ALP levels (Janakat and Al-Merie 2002), otherwise, in human kidney cells (HK-cells), its fruit ethanolic extract incubated in vitro with calcium oxalate monohydrate crystals was effective in reducing the size, concentration and adherence of crystals, which makes this plant effective in preventing kidney stones (Cheraft-Bahloul et al. 2017).

Methanolic extract of *C. colocynthis* demonstrated its protective effect against toxicity following an overdose of paracetamol, pre-treatment of rats with 300 mg. kg^{-1} of this extract reduces hepatic enzymes, and total bilirubin levels (Vakiloddin et al. 2015), ethanolic extract from fruit demonstrated a high hepatoprotective and nephroprotective activities in hepatic and renal organs against damage caused by cisplatin in cancer therapy (Adeyemi et al. 2017). The co-treatment of colocynth extract and the gentamicin against the nephrotoxicity of the antibiotic did not succeed to protect the kidneys against the lesions (Ullah et al. 2013). The use of colocynth oil can reduce the harmful effect of Cadmium in the liver by reducing oxidative stress and increasing antioxidant activity (Amamou et al. 2015).

The use of *A. herba alba* extract reduced hepatic steatosis induced experimentally in mice by high-fat food and characterized by the accumulation of fat in the liver cells (Hamza et al. 2015).

3.6.7 Anti-inflammatory Effect

In laboratory experiments, the organic extracts of *P. lentiscus* are known by their anti-inflammatory activity against carrageenan induced inflammation (Dellai et al. 2013; Remila et al. 2015), oil extracted from fruit demonstrated also a high anti-inflammatory activity against carrageenan induced inflammation, the percentage inhibition was 70% in comparison to Inflocin activity, which had only 51.5% (Ben Khedir et al. 2016). Another study done on the resin revealed an inflammation inhibition of 100% at 800 $mg.kg^{-1}$ (bw) without any toxicity recorded until 3 $g.kg^{-1}$ (Mahmoudi et al. 2010). First study done on the oil against the experimental inflammations in Wistar rats, showed after 8 days of therapy that the oil has a high healing effect against CO_2 laser induced burns (Khedir et al. 2017), another study showed

an excellent healing of virgin fatty oil from *P. lentiscus* tested for its anti-inflammatory effect on rabbits burned on the skin by metal immersed in boiling water and held on the skin, after 28 days of therapy in comparison to Madicassol and Vaseline (Djerrou et al. 2010). *P. lentiscus* essential oil decreased carrageenan-induced paw edema, inhibited cotton pellet-induced granuloma and also reduced serum tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) (Maxia et al. 2011), in normal tissues, IL-6 and TNF-α can cause damage, *P. lentiscus* ethanolic extract with LPS (inducing the synthesis of IL-6 and TNF-α), tested on polymorphonuclear cells obtained from fresh blood and cultured in RPMI medium supplemented with streptomycin, penicillin, and bovine serum, showed that gallic acid and p-coumaric acid present in plant extract reduced the expression of IL-6 and TNF-α (Qabaha et al. 2016).

Organic extracts of *C. colocynthis* seeds and mature fruits revealed a high anti-inflammatory activity in rats (Marzouk et al. 2010a, b, 2011), among its active compounds, cucurbitacins triterpenes are known for its anti-inflammatory activities (Kaushik et al. 2015), in mice macrophage, cucurbitacin B attained the expression of CD40, CD54, and CD80, blocked the release of LPS-activated pro-inflammatory mediators, and reduce the expression levels of pro-inflammatory enzymes induced by LPS (Kim et al. 2015), another study done in LPS-induced RAW 264.7 cells reported that cucurbitacin E inhibits the production of TNF-α and IL-1β, probably mediated by the suppression of NF-jB nuclear translocation (Qiao et al. 2013). A new cucurbitacin, namely; 11-deoxocucurbitacin-I-2-O-β-d-glucoside isolated from *C. colocynthis* seeds showed a remarkable anti-inflammatory activity (Marzouk et al. 2013).

At doses of 400, 200 and 100 mg.kg^{-1}, *A. Herba-alba* ethanolic extract showed edema percentage inhibition of 46.8%, 35.0% and 62.5%, respectively (Jaleel et al. 2016), astragalin and eupatilin are among compounds that revealed a high effect against carrageenan inflammation (Qnais et al. 2014). in male mice, hydro-ethanolic extract of *A. herba-alba* demonstrated a low activity against hepatic steatosis induced by a diet rich in fat (Hamza et al. 2015). In macrophages and in microglia, at concentrations of 1.25 µl.ml^{-1} and 0.32 µl.ml^{-1}, respectively, essential oil inhibited nitric oxide production without cytotoxicity (Abu-Darwish et al. 2015).

3.6.8 Antimicrobial Effect

Since a long time, *P. lentiscus* is known by its antimicrobial effects (Iauk et al. 1996), *H. pylori* infection became a serious illness because of its complications leading to gastric cancer, a literature review indicates that *P. lentiscus* decreases the growth capacity of this pathogen (Bonifácio et al. 2014; Sharif Sharifi and Hazell 2009), at a concentration of 0.75 mg per day during 3 months, tested in vivo, total mastic extract showed approximately 30-fold reduction in *H. pylori* colonization, isomasticadienolic acid was the most active compound with MBC of 0.202 mg.ml^{-1} (Paraschos et al. 2007), α-terpineol and (E)-methyl isoeugenol isolated from mastic

gum essential oil showed also a high anti-*H. pylori* activity (Miyamoto et al. 2014). Essential oil tested against *L. monocytogenes serovar* 4b CECT 935 presents a less effect (Djenane et al. 2011). Oil extracted from *P. lentiscus* fruits exerts an average antimicrobial activity against *A. niger* and *S. aureus* (Mezni et al. 2015). Mastic gum methanolic extract showed a moderate antimicrobial activity against *Porphyromonas gingivalis* with inhibition zones of 10.5 to 13.7 mm at 0.5% to 4% (wt/vol) (Sterer 2006). Methanolic extract from fruits and leaves used as ingredient in pork sausage reduce microbial spoilage (Botsaris et al. 2015).

Some studies state that the organic extract from *C. colocynthis* is more active in comparison to the aqueous extract (Benariba et al. 2013a, b), the hydromethanolic extract was less toxic against *F. oxysporum* and its enzymes (CMCase, pectinase, and protease) (Mohamed et al. 2017), hydroethanolic extract revealed a moderate antifungal activity against *A. fumigatus* DSM_{790} and *A. niger* DSM_{1988} (Eidi et al. 2015), the crude acetone extract of the leaves showed an antibacterial activity against *P. aeruginosa* with an inhibition diameter of 14 mm (Gowri et al. 2009), hydroacetonic extract from seeds demonstrated its effects against several *Candida* species (*C. albicans* $ATCC_{90028}$, *C. glabrata* $ATCC_{90030}$, *C. krusei* $ATCC_{6258}$ and *C. parapsilosis* $ATCC_{22019}$) (Marzouk et al. 2010a, b). Otherwise, ursolic acid and cucurbitacin E 2-0-β-d-glucopyranoside obtained from seeds methanolic extract are identified as the main active biomarkers against *Mycobacterium tuberculosis* H37Rv with MIC of 50 and 25 $\mu g.ml^{-1}$ respectively (Mehta et al. 2013). The lectin extracted from *C. colocynthis* affects the digestive enzymes activities and the intermediate metabolism of *E. ceratoniae* larvae through the reduction of the α-amylase, protease and perturbation of hepatic transaminase activities (Ramzi et al. 2014). The oil of this plant has medium antifungal activity (Sahua et al. 2017).

Essential oil of *A. herba alba* showed a good antimicrobial activity against several fungi and bacteria (Younsi et al. 2016; Chaftar et al. 2016), the most sensitive unveiled microorganisms are *S aureus* $ATCC_{25923}$, *M luteus* $NCIMB_{8166}$, *B cereus* $ATCC_{11778}$, *E faecalis* $ATCC_{29212}$, *E coli* $ATCC_{35218}$, *S typhimirium* $NRLB_{4420}$ (Mighri et al. 2010), *Trichophyton rubrum*, *Epidermophyton floccosum* and *Cryptococcus neoformans* (Abu-Darwish et al. 2015). The essential oil tested with an efflux pump inhibitor (PaβN: phenylalanine arginyl ß-naphthylamide) against several microorganisms revealed that MIC enhanced in the presence of PAßN (Fadli et al. 2016).

3.6.9 Gastroprotective Effect

Gastric ulcer also called stomach ulcer, if located in the stomach, and called duodenal ulcer when formed in the duodenum. Erosion can form many wounds; these wounds can penetrate deeply in the walls and the membrane of the digestive tissues. *P. lentiscus* has a good effect against gastric and duodenal ulcer and also against gastrointestinal disorders (Paraschos et al. 2012), for functional dyspepsia, a good result is observed in patients received an oral dose of 350 mg for three times per day during 3 weeks, the percentage of therapy was significant in 77% of patients with a

decrease in burns and stomach pain (Dabos et al. 2010), otherwise, organic extracts of *P. lentiscus* leaves inhibit gastric lesions caused by HCl (Dellai et al. 2013). *P. lentiscus* oil possesses a protective effect against colitis and intestinal inflammation (Naouar et al. 2016). In rats, *A. Herba-alba* extract showed its effectiveness to be a gastro-protective agent against alcohol-induced gastric ulcers (Jaleel et al. 2016).

3.7 Plant Toxicity

The toxicity of plants to human body should be studied before any consumption or use, according to their composition in chemical and bioactive substances, which can cause irreversible reactions in human organs. A study done in rabbits showed that the rectally use of *P. lentiscus* oil has no adverse effect on hepatic and renal function (Djerrou et al. 2011), another study done in mice showed that the oral administration of the oil at doses varying between 10 and 100 $ml.kg^{-1}$ causes death with LD_{50} of 37 ± 1 $ml.kg^{-1}$ (Boukeloua et al. 2012). *P. lentiscus* oil targets the hepatic cytochrome P450 activity with a decrease activity of 42% for CYP2E1, and 40–80% for CYP3A4, CYP1A1, and CYP1A2, which requires vigilance when this oil is used in combination with other drugs (Attoub et al. 2014). Otherwise, the measurements made from mRNA through RT-PCR demonstrated that an administration of chios mastic gum with recommended doses does not cause any change in CYP1A1 and CYP1A2 (Katsanou et al. 2014). However, a long-term administration of aqueous extract in healthy rats induces inflammation with appearance of liver fibrosis and mild cholestasis (Ljubuncic et al. 2005). Study conducted in rabbits to determine the irritating effect of oil on skin and eyes showed that *P. lentiscus* oil is slightly irritating to skin (PII = 1.037) and eyes (OII = 5.33), prolonged use may cause irritant contact dermatitis (Djerrou et al. 2013).

C. colocynthis seeds must be controlled before any use, according to recent study showing the presence of mycotoxins (aflatoxin, ochratoxin, and citrinin) known by their toxicities (Somorin et al. 2016). A study performed on pulp methanolic extract demonstrated its lethal effect in mice, accompanied by histological changes in liver and kidneys (Shaikh et al. 2016), in mice, another study demonstrated that severe histopathological changes assessment of *C. colocynthis* in small intestine, liver, and kidney revealed a median lethal dose (LD50) of saponin equal to 200 $mg.kg^{-1}$ (Diwan et al. 2000).

Little studies revealed the toxicity of *A. Herba-alba*. Treatment at a doses of 375 and 500 $\mu g.ml^{-1}$ of *A. herba-alba* extract induced a reduction in bone marrow cells division with induction of chromatid exchanges and micronucleus formation (Abderrahman and Shbailat 2014), other recent survey reported that *A. herba-alba* causes acute renal failure (Brown 2017). In female rats, prolonged exposure to *A. herba-alba* causes an adverse effect on sexual function and fertility (Almasad et al. 2007).

3.8 Discussion

Medicinal plants have been described for a very long time, according to the symptoms of patient as remedies against many infections and pathologies. The current development of advanced isolation and identification techniques explained and argued the importance of these plants compared to others in the ancient populations, and despite the evolution and discovery of new antibiotics and bioactive molecules from various origins, current research does not stop to explore medicinal plants following to their richness in bioactive substances.

These miracles remedies described in this review by three medicinal plants don't mean that the plants are safe and without side effects, this last point is the crucial point neglected by consumers and herbalists as a result of their abuse during therapy or treatment regime (Brown 2017). On the other hand, conditions and duration of medicinal plants storage must be controlled and respected, according to their composition in primary metabolites, which make them easily altered and damaged by mycotoxins, and finally, inedible. Collectors should also consider the harvesting location of these plants as they play a key role in their quality and chemical composition (Nedjimi and Beladel 2015).

The comparison of the composition of each species selected in this review with the same species identified in other regions, shows a slight difference in the percentage of substances, they can also be characterized by one or more unidentified molecules. This difference in the composition of plants in the same species can be explained by the difference in the region and harvest season and even, environmental stress conditions (Cirak et al. 2014; Belhattab et al. 2014; Sivaci and Duman 2014).

Pharmacological information obtained during this deepening reveal that selected medicinal plants have high remedies. These remedies published in various databases are the result of biological effects of bioactive substances, and their synergistic effects that characterize these plants. One of these miracles is their effect against the most monstrous disease in the world as a result of the activities of their extracts or pure compounds tested in different cancer cell lines (Chávez et al. 2005; Zhang et al. 2010, 2014; Tilaoui et al. 2015). Experts should appreciate these results to include these compounds, if possible, in chemotherapy. The plants have also proved their high effects to establish metabolic disorders such as hyperlipidaemia and hyperglycaemia, leading to serious problems with irreversible or even fatal complications affecting the heart and kidneys (Mohana et al. 2012). Tannins, alkaloids, flavonoids, and polyphenols disclose the importance of these plants by their anti-hyperglycaemic action (Kartalis et al. 2016; Lahfa et al. 2017; Boudjelal et al. 2015). The mode of action of these compounds is explained by the increase of insulin secretion and glucose absorption in muscle and adipose tissue, prevention of lipid peroxidation and radicals elimination, prevention of glucose absorption in the intestine and it production in the liver.

Inflammation is a defensive biological response of vascular tissue, this response involves the release of mediators acting as a gravity marker (Bozkurt et al. 2010; Ferrero-Miliani et al. 2007). During this review, selected plants and their extracts

show a high effect inhibition of pro-inflammatory cytokines inducing NF-κB, which is a crucial transcription factor in the orchestration of immune and inflammatory responses (Loizou et al. 2009; Qiao et al. 2013). Cytokines participate in the activation of phospholipase A2, which can cause chronic pain in older Persons. Other anti-inflammatory mechanisms are also possible, and depend on bioactive compounds of plants, for example, linolenic acid may block the pathways of arachidonate metabolism involved in inflammation (Adegbola et al. 2017).

In recent years, research in antioxidant activities progressed more with the appearance of different revelation techniques (Alam et al. 2013; Nile et al. 2012). In this review, the antioxidant activity of selected plants is considered high in comparison to synthetic antioxidants or other plants, following the existence of a high content of non-enzymatic secondary metabolites (Krishnaiah et al. 2011), such as quercetin, kaempferol, myricetin, and ferulic acid (Adegbola et al. 2017), these compounds are formed in response to biotic and abiotic stress conditions (Morkhade 2017). Classification of selected plants according to their antioxidant activities is difficult following to the different experimental tests applied.

3.9 Conclusion

Herbs and medicinal plants are known by their primary metabolites containing proteins, lipids, and carbohydrates, and secondary metabolites covering polyphenols, alkaloids, steroids …etc. In this bibliographical review, we presented the phytochemical composition and the pharmacological role of three medicinal plants (*Citrullus colocynthis, Pistacia lentiscus* and *Artemisia herba-alba*) obtained from results of extensive research recently published. The synthesis of the phytochemical part allowed to know the origin of the biological activities and the remedies concluded after the use of the plants. The different pharmacological role illustrated in the second part have been known in traditional medicine and approve that the extracts and compounds of these plants possess important and valuable biological activities, making these species among the most used medicinal plants in traditional medicine. While for others compounds, they are not yet unveiled by scientists and can be considered as topical research subjects. As future prospects concluded from this review:

- Following long-term treatment exposure for chronic cases; the long-term toxicity is far from being evaluated, which requires a great care and caution. Consequently, the study of the toxicity of these plants should be carried out mainly on the aqueous extracts, herbal teas, oils, essential oils, and infusions because they are the most practiced preparations. Doses described must be relative to body weight and health status of the consumer to avoid any complications, especially in the liver and kidneys.
- Few studies are interested to study the biological actions of the pure compounds isolated from these three selected plants, while the majority focuses to study the

biological activities of crude extracts (organic and aqueous) and whole essential oils. Whereas, now we must think to find a new strategy based on the last results and begin to characterize and study in depth each pure compound alone or in synergy with another compound (Morkhade 2017) and even publish the negative results. The primary objective of this strategy is to build a strong and diverse database for medical research and for the pharmaceutical, chemical, and biotechnological industry.

- Despite the potent antioxidant activity, few studies have published the *in vivo* results, so that these plants can be introduced into food and preventive medicine (Kasote et al. 2015).

Acknowledgments The authors thank the staff of Laboratory for protection of ecosystems in the arid and semi-arid area. Ouargla University. Algeria, the library staff Saida University. Algeria, for their support and for providing valuable information during this work.

Conflict of Interest Statement We declare that we have no conflict of interest.

References

Abad MJ, Bedoya LM, Apaza L et al (2012) The *Artemisia L.* genus: a review of bioactive essential oils. Molecules 17:2542–2566. https://doi.org/10.3390/molecules17032542

Abdelwahed A, Bouhlel I, Skandrani I et al (2007) Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from *Pistacia lentiscus*. Confirmation by microarray expression profiling. Chem Biol Interact 165:1–13. https://doi.org/10.1016/j.cbi.2006.10.003

Abderrahman SM, Shbailat SJ (2014) Genotoxic and cytotoxic effects of *Artemisia herba-alba* on mammalian cells. Caryologia 67:265–272. https://doi.org/10.1080/0144235X.2014.974355

Abid ZB, Feki M, Hédhili A et al (2007) Artemisia herba-alba Asso (Asteraceae) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. Ann Nutr Metab 51:216–222. https://doi.org/10.1159/000104140

Abidi A, Aissani N, Sebai H, Ben Khamsa S et al (2017) Protective effect of *Pistacia lentiscus* oil against Bleomycin-induced lung fibrosis and oxidative stress in rat. Nutr Cancer 69:490–497. https://doi.org/10.1080/01635581.2017.1283423

Abu-Darwish MS, Cabral C, Gonçalves MJ et al (2015) *Artemisia herba-alba* essential oil from Buseirah (South Jordan): chemical characterization and assessment of safe antifungal and anti-inflammatory doses. J Ethnopharmacol 174:153–160. https://doi.org/10.1016/j.jep.2015.08.005

Adegbola P, Aderibigbe I, Hammed W et al (2017) Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. Am J Cardiovasc Dis 7:19–32

Adeyemi OO, Ishola IO, Ajani ID (2017) Citrullus colocynthis Linn. Fruit extract ameliorates cisplatin-induced hepato-renal toxicity in rats. J Complement Integr Med 2017:1–11. https://doi.org/10.1515/jcim-2017-0086

Agarwal V, Sharma AK, Upadhyay A et al (2012) Hypoglycemic effects of *Citrullus colocynthis* roots. Acta Pol Pharm 69:75–79. http://www.ptfarm.pl/pub/File/Acta_Poloniae/2012/1/075.pdf

Alam MN, Bristi NJ, Rafiquzzaman M (2013) Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharm J 21:143–152. https://doi.org/10.1016/j.jsps.2012.05.002

Al-Ghaithi F, El-Ridi MR, Adeghate E et al (2004) Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats. Mol Cell Biochem 261:143–149. https://doi.org/10.1023/B:MCBI.0000028749.63101.cc

Almasad MM, Qazan WS, Daradka H (2007) Reproductive toxic effects of *Artemisia herba alba* ingestion in female Spague-Dawley rats. Pak J Biol Sci 10:3158–3161. https://doi.org/10.3923/pjbs.2007.3158.3161

Aloui Z, Messaoud C, Haoues M et al (2016) *Asteraceae Artemisia campestris* and *Artemisia herba-alba* essential oils trigger apoptosis and cell cycle arrest in *Leishmania infantum* promastigotes. Evid Based Complement Altern Med 2016:9147096. https://doi.org/10.1155/2016/9147096

Al-Shamaony L, al-Khazraji SM, Twaij HA (1994) Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol 43:167–171. https://doi.org/10.1016/0378-8741(94)90038-8

Alves-Silva JM, Romane A, Efferth T, Salgueiro L (2017) North African medicinal plants traditionally used in cancer therapy. Front Pharmacol 26:383. https://doi.org/10.3389/fphar.2017.00383

Amamou F, Nemmiche S, Meziane RK et al (2015) Protective effect of olive oil and colocynth oil against cadmium-induced oxidative stress in the liver of Wistar rats. Food Chem Toxicol 78:177–184. https://doi.org/10.1016/j.fct.2015.01.001

Amin A, Tahir M, Lone KP (2017) Effect of *Citrullus colocynthis* aqueous seed extract on beta cell regeneration and intra-islet vasculature in alloxan induced diabetic male albino rats. J Pak Med Assoc 67:715–721

Aouadi D, Luciano G, Vasta V et al (2014) The antioxidant status and oxidative stability of muscle from lambs receiving oral administration of *Artemisia herba alba* and *Rosmarinus officinalis* essential oils. Meat Sci 97:237–243. https://doi.org/10.1016/j.meatsci.2014.02.005

Armas C, Padilla FM, Pugnaire FI et al (2010) Hydraulic lift and tolerance to salinity of semiarid species: consequences for species interactions. Oecologia 162:11–21. https://doi.org/10.1007/s00442-009-1447-1

Assimopoulou AN, Zlatanos SN, Papageorgiou VP (2015) Antioxidant activity of natural resins and bioactive triterpenes in oil substrates. Food Chem 92:721–727. https://doi.org/10.1016/j.foodchem.2004.08.033

Attoub S, Karam SM, Nemmar A et al (2014) Short-term effects of oral administration of *Pistacia lentiscus* oil on tissue-specific toxicity and drug metabolizing enzymes in mice. Cell Physiol Biochem 33:1400–1410. https://doi.org/10.1159/000358706

Aviara NA, Shittu SK, Haque MA (2007) Physical properties of Guna fruits relevant in bulk handling and mechanical processing. Int Agrophysics 21:7–16

Bacchetta G, Cappai G, Carucci A et al (2015) Use of native plants for the remediation of abandoned mine sites in Mediterranean semiarid environments. Bull Environ Contam Toxicol 94:326–333. https://doi.org/10.1007/s00128-015-1467-y

Balan KV, Prince J, Han Z et al (2007) Antiproliferative activity and induction of apoptosis in human colon cancer cells treated in vitro with constituents of a product derived from *Pistacia lentiscus L. var chia*. Phytomedicine 14:263–272. https://doi.org/10.1016/j.phymed.2006.03.009

Baratto MC, Tattini M, Galardi C et al (2003) Antioxidant activity of galloyl quinic derivatives isolated from *P. lentiscus* leaves. Free Radic Res 37:405–412. https://doi.org/10.1080/1071576031000068618

Barra A, Coroneo V, Dessi S et al (2007) Characterization of the volatile constituents in the essential oil of *Pistacia lentiscus L.* from different origins and its antifungal and antioxidant activity. J Agric Food Chem 55:7093–7098. https://doi.org/10.1021/jf071129w

Beghlal D, El Bairi K, Marmouzi I et al (2016) Phytochemical, organoleptic and ferric reducing properties of essential oil and ethanolic extract from *Pistacia lentiscus (L.)*. Asian Pac J Trop Dis 6:305–310. https://doi.org/10.1016/S2222-1808(15)61035-0

Belhachat D, Aid F, Mekimene L et al (2017) Phytochemical screening and *in vitro* antioxidant activity of *Pistacia lentiscus* berries ethanolic extract growing in Algeria. Med J Nutrit Metab 10:273–285. https://doi.org/10.3233/MNM-17169

Belhattab R, Amor L, Barroso JG et al (2014) Essential oil from *Artemisia herba-alba Asso* grown wild in Algeria: variability assessment and comparison with an updated literature survey. Arab J Chem 7:243–251. https://doi.org/10.1016/j.arabjc.2012.04.042

Ben Khedir S, Mzid M, Bardaa S et al (2016) In Vivo evaluation of the anti-inflammatory effect of *Pistacia lentiscus* fruit oil and its effects on oxidative stress. Evid Based Complement Alternat Med 2016:6108203. https://doi.org/10.1155/2016/6108203

Benarba B (2016) Medicinal plants used by traditional healers from South-West Algeria: an ethnobotanical study. J Intercult Ethnopharmacol 5:320–330. https://doi.org/10.5455/jice.20160814115725

Benariba N, Djaziri R, Zerriouh BH et al (2012) Short- and long-term effects of various *Citrullus colocynthis* seed extracts in normal and streptozotocin-induced diabetic rats. Int J Mol Med 30:1528–1536. https://doi.org/10.3892/ijmm.2012.1127

Benariba N, Djaziri R, Bellakhdar W et al (2013a) Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. Asian Pac J Trop Biomed 3:35–40. https://doi.org/10.1016/S2221-1691(13)60020-9

Benariba N, Djaziri R, Hupkens E et al (2013b) Insulinotropic action of *Citrullus colocynthis* seed extracts in rat pancreatic islets. Mol Med Rep 7:233–236. https://doi.org/10.3892/mmr.2012.1151

Bhandari MM (1995) Flora of the Indian desert. MPS Repros, Jodhpur

Boelens MH, Jimenez R (1991) Chemical composition of the essential oils from the gum and from various parts of *Pistacia lentiscus l.* (mastic gum tree). Flavour Fragr J 6:271–275. https://doi.org/10.1002/ffj.2730060406

Bonifácio BV, dos Santos Ramos MA, da Silva PB et al (2014) Antimicrobial activity of natural products against *Helicobacter pylori*: a review. Ann Clin Microbiol Antimicrob 13:1–10. https://doi.org/10.1186/s12941-014-0054-0

Bora KS, Sharma A (2011) The genus *Artemisia*: a comprehensive review. Pharm Biol 49:101–109. https://doi.org/10.3109/13880209.2010.497815

Botsaris G, Orphanides A, Yiannakou E et al (2015) Antioxidant and antimicrobial effects of *Pistacia lentiscus L.* Extracts in Pork Sausages. Food Technol Biotechnol 53:472–478. https://doi.org/10.17113/ftb.53.04.15.4051

Boudjelal A, Siracusa L, Henchiri C et al (2015) Antidiabetic effects of aqueous infusions of *Artemisia herba-alba* and *Ajuga iva* in Alloxan-induced diabetic rats. Planta Med 81:696–704. https://doi.org/10.1055/s-0035-1546006

Boukeloua A, Belkhiri A, Djerrou Z et al (2012) Acute toxicity of *Opuntia ficus indica* and *Pistacia lentiscus* seed oils in mice. Afr J Tradit Complement Altern Med 9:607–611. https://doi.org/10.4314/ajtcam.v9i4.19

Bozkurt B, Mann DL, Deswal A (2010) Biomarkers of inflammation in heart failure. Heart Fail Rev 15:331–341. https://doi.org/10.1007/s10741-009-9140-3

Bozorgi M, Memariani Z, Mobli M (2013) et al Five *Pistacia* species (*P. vera, P. atlantica, P. terebinthus, P. khinjuk,* and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. Sci World J 219815. https://doi.org/10.1155/2013/219815

Brahmia N, Scognamiglio M, Pacifico S et al (2015) ^{1}H NMR based metabolic profiling of eleven Algerian aromatic plants and evaluation of their antioxidant and cytotoxic properties. Food Res Int 76:334–341. https://doi.org/10.1016/j.foodres.2015.07.005

Brown AC (2017) Kidney toxicity related to herbs and dietary supplements: online table of case reports. Part 3 of 5 series. Food Chem Toxicol 107:502–519. https://doi.org/10.1016/j.fct.2016.07.024

Chaftar N, Girardot M, Labanowski J et al (2016) Comparative evaluation of the antimicrobial activity of 19 essential oils. Adv Exp Med Biol 901:1–15. https://doi.org/10.1007/5584_2015_5011

Chan KT, Meng FY, Li Q et al (2010) Cucurbitacin B induces apoptosis and S phase cell cycle arrest in BEL-7402 human hepatocellular carcinoma cells and is effective via oral administration. Cancer Lett 294:118–124. https://doi.org/10.1016/j.canlet.2010.01.029

Chávez IO, Apan TR, Martínez-Vázquez M (2005) Cytotoxic activity and effect on nitric oxide production of tirucallane-type triterpenes. J Pharm Pharmacol 57:1087–1091. https://doi.org/10.1211/jpp.57.9.0003

Chekroun E, Benariba N, Adida H et al (2015) Antioxidant activity and phytochemical screening of two *Cucurbitaceae*: *Citrullus colocynthis* fruits and *Bryonia dioica* roots. Asian Pac J Trop Dis 5:632–637. https://doi.org/10.1016/S2222-1808(15)60903-3

Chekroun E, Bechiri A, Azzi R et al (2017) Antidiabetic activity of two aqueous extracts of two *Cucurbitaceae*: *Citrullus colocynthis* and *Bryonia dioica*. Phytothérapie 15:57–66. https://doi.org/10.1007/s10298-016-1050-7

Chen YW, Chou HC, Lin ST et al (2013) Cardioprotective effects of quercetin in cardiomyocyte under ischemia/reperfusion injury. Evid Based Complement Alternat Med:364519. https://doi.org/10.1155/2013/364519

Cheraft-Bahloul N, Husson C, Ourtioualous M et al (2017) Protective effects of *Pistacia lentiscus L.* fruit extract against calcium oxalate monohydrate induced proximal tubular injury. J Ethnopharmacol 209:248–254. https://doi.org/10.1016/j.jep.2017.07.018

Cherbal A, Kebieche M, Yilmaz EM et al (2017) Antidiabetic and hypolipidemic activities of Algerian *Pistachia lentiscus L.* leaves extract in alloxan-induced diabetic rats. S Afr J Bot 108:157–162. https://doi.org/10.1016/j.sajb.2016.10.024

Cirak C, Radusiene J, Ivanauskas L et al (2014) Changes in the content of bioactive substances among *Hypericum montbretii* populations from Turkey. Braz J Pharm Sci 24:20–24. https://doi.org/10.1590/0102-695X20142413352

Dabos KJ, Sfika E, Vlatta LJ et al (2010) Is Chios mastic gum effective in the treatment of functional dyspepsia? A prospective randomised double-blind placebo controlled trial. J Ethnopharmacol 127:205–209. https://doi.org/10.1016/j.jep.2009.11.021

Daradka H, Almasad MM, Qazan WSH et al (2007) Hypolipidaemic effects of *Citrullus colocynthis L.* in rabbits. Pak J Biol Sci 10:2768–2771. https://doi.org/10.3923/pjbs.2007.2768.2771

Davis PH (1965) Flora of Turkey and the East Aegean islands. University Press, Edinburgh

Dellai A, Souissi H, Borgi W et al (2013) Antiinflammatory and antiulcerogenic activities of *Pistacia lentiscus L.* leaves extracts. Ind Crop Prod 49:879–882. https://doi.org/10.1016/j.indcrop.2013.07.010

Dimas K, Hatziantoniou S, Wyche JH et al (2009) A mastic gum extract induces suppression of growth of human colorectal tumor xenografts in immunodeficient mice. In Vivo 23(1):63–68

Dimas KS, Pantazis P, Ramanujam R (2012) Review: Chios mastic gum: a plant-produced resin exhibiting numerous diverse pharmaceutical and biomedical properties. In Vivo 26:777–785

Diwan FH, Abdel-Hassan IA, Mohammed ST (2000) Effect of saponin on mortality and histopathological changes in mice. East Mediterr Health J 6:345–351

Djenane D, Yangüela J, Montañés L et al (2011) Antimicrobial activity of *Pistacia lentiscus* and *Satureja montana* essential oils against *Listeria monocytogenes CECT 935* using laboratory media: efficacy and synergistic potential in minced beef. Food Control 22:1046–1053. https://doi.org/10.1016/j.foodcont.2010.12.015

Djerrou Z, Maameri Z, Hamdi-Pacha Y et al (2010) Effect of virgin fatty oil of *Pistacia lentiscus* on experimental burn wound's healing in rabbits. Afr J Tradit Complement Altern Med 7:258–263

Djerrou Z, Hamdi-Pacha Y, Belkhiri AM et al (2011) Evaluation of *Pistacia lentiscus* fatty oil effects on glycemic index, liver functions and kidney functions of New Zealand rabbits. Afr J Tradit Complement Altern Med 8:214–219. https://doi.org/10.4314/ajtcam.v8i5S.27

Djerrou Z, Djaalab H, Riachi F et al (2013) Irritantcy potential and sub acute dermal toxicity study of Pistacia lentiscus fatty oil as a topical traditional remedy. Afr J Tradit Complement Altern Med 10:480–9. https://dx.doi.org/10.4314%2Fajtcam.v10i3.15

Dogan Y, Baslar S, Aydin H et al (2003) A study of the soil-plant interactions of *Pistacia lentiscus L.* distributed in the western Anatolian part of Turkey. Acta Bot Croat 62:73–88. https://hrcak.srce.hr/3544

Ebrahimi E, Bahramzadeh S, Hashemitabar M et al (2016) Effect of hydroalcoholic leaves extract of *Citrullus colocynthis* on induction of insulin secretion from isolated rat islets of Langerhans. Asian Pac J Trop Dis 6:638–641. https://doi.org/10.1016/S2222-1808(16)61101-5

Eddouks M, Maghrani M, Lemhadri A et al (2002) Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). J Ethnopharmacol 82:97–103. https://doi.org/10.1016/S0378-8741(02)00164-2

Eidi S, Azadi HG, Rahbar N et al (2015) Evaluation of antifungal activity of hydroalcoholic extracts of *Citrullus colocynthis* fruit. J Herb Med 5:36–40. https://doi.org/10.1016/j.hermed.2015.01.003

Fadli M, Pagès JM, Mezrioui NE et al (2016) *Artemisia herba-alba Asso* and *Cymbopogon citratus (DC.) Stapf* essential oils and their capability to restore antibiotics efficacy. Ind Crop Prod 89:399–404. https://doi.org/10.1016/j.indcrop.2016.05.039

Ferrero-Miliani L, Nielsen OH, Andersen PS et al (2007) Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. Clin Exp Immunol 147:227–235. https://doi.org/10.1111/j.1365-2249.2006.03261.x

Gardeli C, Papageorgiou V, Mallouchos A et al (2008) Essential oil composition of *Pistacia lentiscus L.* and *Myrtus communis L.*: evaluation of antioxidant capacity of methanolic extracts. Food Chem 107:1120–1130. https://doi.org/10.1016/j.foodchem.2007.09.036

Giaginis C, Theocharis S (2011) Current evidence on the anticancer potential of Chios mastic gum. Nutr Cancer 63:1174–1184. https://doi.org/10.1080/01635581.2011.607546

Gioxari A, Kaliora AC, Papalois A et al (2011) *Pistacia lentiscus* resin regulates intestinal damage and inflammation in trinitrobenzene sulfonic acid-induced colitis. J Med Food 14:1403–1411. https://doi.org/10.1089/jmf.2010.0240

Gowri SS, Priyavardhini S, Vasantha K et al (2009) Antibacterial activity on *Citrullus colocynthis* leaf extract. Anc Sci Life 29:12–13

Grace MH, Esposito D, Timmers MA et al (2016) Chemical composition, antioxidant and anti-inflammatory properties of pistachio hull extracts. Food Chem 210:85–95. https://doi.org/10.1016/j.foodchem.2016.04.088

Hamilton A (2003) Medicinal plants and conservation: issues and approaches. WWF, Surrey

Hamza N, Berke B, Cheze C et al (2015) Effect of *Centaurium erythraea Rafn*, *Artemisia herba-alba Asso* and *Trigonella foenum-graecum L.* on liver fat accumulation in C57BL/6J mice with high-fat diet-induced type 2 diabetes. J Ethnopharmacol 171:4–11. https://doi.org/10.1016/j.jep.2015.05.027

He ML, Yuan HQ, Jiang AL et al (2006) Gum mastic inhibits the expression and function of the androgen receptor in prostate cancer cells. Cancer 106:2547–2555. https://doi.org/10.1002/cncr.21935

He ML, Li A, Xu CS et al (2007) Mechanisms of antiprostate cancer by gum mastic: NF-kappaB signal as target. Acta Pharmacol Sin 28:446–452. https://doi.org/10.1111/j.1745-7254.2007.00536.x

Huang XY, Wang HC, Yuan Z et al (2010) Gemcitabine combined with gum mastic causes potent growth inhibition and apoptosis of pancreatic cancer cells. Acta Pharmacol Sin 31:741–745. https://doi.org/10.1038/aps.2010.54

Huseini HF, Darvishzadeh F, Heshmat R et al (2009) The clinical investigation of *Citrullus colocynthis (L.) schrad* fruit in treatment of Type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. Phytother Res 23:1186–1189. https://doi.org/10.1002/ptr.2754

Hussain AI, Rathore HA, Sattar MZA et al (2013) Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis (L.)* from the Pakistani flora. Ind Crop Prod 45:416–422. https://doi.org/10.1016/j.indcrop.2013.01.002

Hussein MA, El-Gizawy HA, Gobba NAEK et al (2017) Synthesis of cinnamyl and caffeoyl derivatives of Cucurbitacin-E-glycoside isolated from *Citrullus colocynthis* fruits and their structure antioxidant and anti-inflammatory activities relationship. Curr Pharm Biotechnol 18:677–693. https://doi.org/10.2174/1389201018666171004144615

Iauk L, Ragusa S, Rapisarda A et al (1996) *In vitro* antimicrobial activity of *Pistacia lentiscus L.* extracts: preliminary report. J Chemother 8:207–209. https://doi.org/10.1179/joc.1996.8.3.207

Jaleel GARA, Abdallah HMI, Gomaa NELS (2016) Pharmacological effects of ethanol extract of Egyptian *Artemisia herba-alba* in rats and mice. Asian Pac J Trop Biomed 6:44–49. https://doi.org/10.1016/j.apjtb.2015.10.005

Janakat S, Al-Merie H (2002) Evaluation of hepatoprotective effect of *Pistacia lentiscus, Phillyrea latifolia* and *Nicotiana glauca*. J Ethnopharmacol 83:135–138. https://doi.org/10.1016/S0378-8741(02)00241-6

Jouad H, Haloui M, Rhiouani H et al (2001) Ethnobotanical survey of medicinal plants used for the treatment of diabetes, cardiac and renal diseases in the North Centre region of Morocco (Fez-Boulemane). J Ethnopharmacol 77:175–182. https://doi.org/10.1016/S0378-8741(01)00289-6

Kartalis A, Didagelos M, Georgiadis I et al (2016) Effects of Chios mastic gum on cholesterol and glucose levels of healthy volunteers: a prospective, randomized, placebo-controlled, pilot study (CHIOS-MASTIHA). Eur J Prev Cardiol 23:722–729. https://doi.org/10.1177/2047487315603186

Kasote DM, Katyare SS, Hegde MV et al (2015) Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 11:982–991. https://doi.org/10.7150/ijbs.12096

Katsanou ES, Kyriakopoulou K, Emmanouil C et al (2014) Modulation of CYP1A1 and CYP1A2 hepatic enzymes after oral administration of Chios mastic gum to male Wistar rats. PLoS One 9:e100190. https://doi.org/10.1371/journal.pone.0100190

Kaushik U, Aeri V, Mir SR (2015) Cucurbitacins – an insight into medicinal leads from nature. Pharmacogn Rev 9:12–18. https://doi.org/10.4103/0973-7847.156314

Khatib S, Vaya J (2010) Chapter 17–fig, carob, pistachio, and health. Bioact Foods Promot Health Fruits Vegetab:245–263. https://doi.org/10.1016/B978-0-12-374628-3.00017-7

Khedir SB, Bardaa S, Chabchoub N et al (2017) The healing effect of *Pistacia lentiscus* fruit oil on laser burn. Pharm Biol 55:1407–1414. https://doi.org/10.1080/13880209.2016.1233569

Khlifi D, Sghaier RM, Amouri S et al (2013) Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisia herba-alba*, *Ruta chalpensis L.* and *Peganum harmala L.* Food Chem Toxicol 55:202–208. https://doi.org/10.1016/j.fct.2013.01.004

Kim M, Park SY, Jin ML et al (2015) Cucurbitacin B inhibits immunomodulatory function and the inflammatory response in macrophages. Immunopharmacol Immunotoxicol 37:473–480. https://doi.org/10.3109/08923973.2015.1085065

Klibet F, Boumendjel A, Khiari M et al (2016) Oxidative stress-related liver dysfunction by sodium arsenite: alleviation by *Pistacia lentiscus* oil. Pharm Biol 54:354–363. https://doi.org/10.3109/13880209.2015.1043562

Koutsoudaki C, Krsek M, Rodger A (2005) Chemical composition and antibacterial activity of the essential oil and the gum of *Pistacia lentiscus* Var. chia. J Agric Food Chem 53:7681–7685. https://doi.org/10.1021/jf050639s

Krishnaiah D, Sarbatly R, Nithyanandam R (2011) A review of the antioxidant potential of medicinal plant species. Food Bioprod Process 89:217–233. https://doi.org/10.1016/j.fbp.2010.04.008

Ku JM, Kim SR, Hong SH et al (2015) Cucurbitacin D induces cell cycle arrest and apoptosis by inhibiting STAT3 and NF-κB signaling in doxorubicin-resistant human breast carcinoma (MCF7/ADR) cells. Mol Cell Biochem 409:33–43. https://doi.org/10.1007/s11010-015-2509-9

Lahfa FB, Azzi R, Mezouar D et al (2017) Hypoglycemic effect of *Citrullus colocynthis* extracts. Phytothérapie 15:50–56. https://doi.org/10.1007/s10298-015-0997-4

Lan T, Wang L, Xu Q et al (2013) Growth inhibitory effect of Cucurbitacin E on breast cancer cells. Int J Clin Exp Pathol 6:1799–1805

Larcher W (2000) Temperature stress and survival ability of Mediterranean sclerophyllous plants. Plant Biosyst 134:279–295. https://doi.org/10.1080/11263500012331350455

Lee TY, Lam TH (1993) Allergic contact dermatitis due to a Chinese orthopaedic solution tieh ta yao gin. Contact Dermatitis 28:89–90. https://doi.org/10.1111/j.1600-0536.1993.tb03348.x

Li DL, Xing FW (2016) Ethnobotanical study on medicinal plants used by local Hoklos people on Hainan Island. China J Ethnopharmacol 194:358–368. https://doi.org/10.1016/j.jep.2016.07.050

Liu T, Zhang M, Deng Y et al (2008) Effects of cucurbitacin B on cell proliferation and apoptosis in Hep-2 cells. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 22:403–407

Liu T, Peng H, Zhang M et al (2010) Cucurbitacin B, a small molecule inhibitor of the Stat3 signaling pathway, enhances the chemosensitivity of laryngeal squamous cell carcinoma cells to cisplatin. Eur J Pharmacol 641:15–22. https://doi.org/10.1016/j.ejphar.2010.04.062

Ljubuncic P, Song H, Cogan U et al (2005) The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental liver disease. J Ethnopharmacol 100:198–204. https://doi.org/10.1016/j.jep.2005.03.006

Longo L, Scardino A, Vasapollo G (2007) Identification and quantification of anthocyanins in the berries of *Pistacia lentiscus L., Phillyrea latifolia L.* and *Rubia peregrina L.* Innov Food Sci Emerg Technol 8:360–364. https://doi.org/10.1016/j.ifset.2007.03.010

Lopez-Gonzalez G (2001) Los árboles y arbustos de la Península Ibérica e Islas Baleares. Tomo I, II. Mundi-Prensa, Madrid

Magkouta S, Stathopoulos GT, Psallidas I et al (2009) Protective effects of mastic oil from *Pistacia lentiscus* variation chia against experimental growth of Lewis lung carcinoma. Nutr Cancer 61:640–648. https://doi.org/10.1080/01635580902825647

Mahmoudi M, Ebrahimzadeh MA, Nabavi SF et al (2010) Antiinflammatory and antioxidant activities of gum mastic. Eur Rev Med Pharmacol Sci 14:765–769

Margaris NS (1981) Adaptative strategies in plants dominating Mediterranean-type ecosystems. In: Di Castri F, Goodal DW, Specht R (eds) Ecosystems of the world, Mediterranean type Shrublands. Amsterdam, Elsevier, pp 309–315

Marzouk B, Marzouk Z, Décor R et al (2010a) Antibacterial and antifungal activities of several populations of Tunisian *Citrullus colocynthis Schrad.* Immature fruits and seeds. Med Mycol J 20:179–184. https://doi.org/10.1016/j.mycmed.2010.05.006

Marzouk B, Marzouk Z, Haloui E et al (2010b) Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. J Ethnopharmacol 128:15–19. https://doi.org/10.1016/j.jep.2009.11.027

Marzouk B, Marzouk Z, Fenina N et al (2011) Anti-inflammatory and analgesic activities of Tunisian *Citrullus colocynthis Schrad.* Immature fruit and seed organic extracts. Eur Rev Med Pharmacol Sci 15:665–672

Marzouk B, Mahjoub MA, Bouraoui A et al (2013) Anti-inflammatory and analgesic activities of a new cucurbitacin isolated from *Citrullus colocynthis* seeds. Med Chem Res 22:3984–3990. https://doi.org/10.1007/s00044-012-0406-2

Matouk AI, Taye A, Heeba GH et al (2013) Quercetin augments the protective effect of losartan against chronic doxorubicin cardiotoxicity in rats. Environ Toxicol Pharmacol 36:443–450. https://doi.org/10.1016/j.etap.2013.05.006

Maxia A, Sanna C, Frau MA et al (2011) Anti-inflammatory activity of *Pistacia lentiscus* essential oil: involvement of IL-6 and TNF-alpha. Nat Prod Commun 6:1543–1544

Mehenni C, Atmani-Kilani D, Dumarçay S et al (2016) Hepatoprotective and antidiabetic effects of *Pistacia lentiscus* leaf and fruit extracts. J Food Drug Anal 24:653–669. https://doi.org/10.1016/j.jfda.2016.03.002

Mehta A, Srivastva G, Kachhwaha S et al (2013) Antimycobacterial activity of *Citrullus colocynthis (L.) Schrad.* Against drug sensitive and drug resistant *Mycobacterium tuberculosis* and MOTT clinical isolates. J Ethnopharmacol 149:195–200. https://doi.org/10.1016/j.jep.2013.06.022

Menezes IA, Barreto CM, Antoniolli AR et al (2010) Hypotensive activity of terpenes found in essential oils. Z Naturforsch C 65:562–566. https://doi.org/10.1515/znc-2010-9-1005

Messaoudene D, Belguendouz H, Ahmedi ML et al (2011) Ex vivo effects of flavonoïds extracted from *Artemisia herba alba* on cytokines and nitric oxide production in Algerian

patients with Adamantiades-Behçet's disease. J Inflamm (Lond) 8(35). https://doi.org/10.1186/1476-9255-8-35

Mezni F, Aouadhi C, Khouja ML et al (2015) In vitro antimicrobial activity of *Pistacia lentiscus L.* edible oil and phenolic extract. Nat Prod Res 29:565–570. https://doi.org/10.1080/14786419.2014.952232

Mighri H, Hajlaoui H, Akrout A et al (2010) Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. C R Chim 13:380–386. https://doi.org/10.1016/j.crci.2009.09.008

Miyamoto T, Okimoto T, Kuwano M (2014) Chemical composition of the essential oil of mastic gum and their antibacterial activity against drug-resistant *Helicobacter pylori*. Nat Prod Bioprospect 4:227–231. https://doi.org/10.1007/s13659-014-0033-3

Mohamed MSM, Saleh AM, Abdel-Farid IB et al (2017) Growth, hydrolases and ultrastructure of *Fusarium oxysporum* as affected by phenolic rich extracts from several xerophytic plants. Pestic Biochem Physiol 141:57–64. https://doi.org/10.1016/j.pestbp.2016.11.007

Mohana L, Sandhya R, Kiran UA (2012) Review on diabetes milletus and the herbal plants used for its treatment. Asian J Pharm Clin Res 5:15–21. https://doi.org/10.1016/S2222-1808(14)60585-5

Morkhade DM (2017) Evaluation of gum mastic (*Pistacia lentiscus*) as a microencapsulating and matrix forming material for sustained drug release. Asian J Pharm Sci 12:424–432. https://doi.org/10.1016/j.ajps.2017.05.002

Moufid A, Eddouks M (2012) *Artemisia herba alba*: a popular plant with potential medicinal properties. Pak J Bio Sci 15:1152–1159. https://doi.org/10.3923/pjbs.2012.1152.1159

Moulos P, Papadodima O, Chatziioannou A et al (2009) A transcriptomic computational analysis of mastic oil-treated Lewis lung carcinomas reveals molecular mechanisms targeting tumor cell growth and survival. BMC Med Genet 2(68). https://doi.org/10.1186/1755-8794-2-68

Munné-Bosch S, Peñuelas J (2003) Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus L.* grown under Mediterranean field conditions. Ann Bot 92:385–391. https://doi.org/10.1093/aob/mcg152

Naouar MS, Mekki LZ, Charfi L et al (2016) Preventive and curative effect of *Pistacia lentiscus* oil in experimental colitis. Biomed Pharmacother 83:577–583. https://doi.org/10.1016/j.biopha.2016.07.021

Nedjimi B, Beladel B (2015) Assessment of some chemical elements in wild Shih (*Artemisia herba-alba Asso*) using INAA technique. J Appl Res Med Aromat Plants 2:203–205. https://doi.org/10.1016/j.jarmap.2015.06.003

Negro C, De Bellis L, Miceli A (2015) Chemical composition and antioxidant activity of *Pistacia lentiscus* essential oil from southern Italy (Apulia). J Essent Oil Res 27:23–29. https://doi.org/10.1080/10412905.2014.973614

Nessa F, Khan SA (2014) Evaluation of antioxidant and xanthine oxidase inhibitory activity of different solvent extracts of leaves of *Citrullus colocynthis*. Pharm Res 6:218–226. https://doi.org/10.4103/0974-8490.132599

Nile SH, Khobragade CN, Park SW (2012) Optimized and comparative antioxidant assays and its applications in herbal and synthetic drug analysis as an antioxidants. Mini Rev Med Chem 12:1007–1014. https://doi.org/10.2174/138955712802762310

Nischwitz V, Mogwasi R, Zor S et al (2015) First comprehensive study on total contents and hot water extractable fraction of selected elements in 19 medicinal plants from various locations in Nyamira County, Kenya. J Trace Elem Med Biol 39:54–61. https://doi.org/10.1016/j.jtemb.2016.08.001

Nmila R, Gross R, Rchid H et al (2000) Insulinotropic effect of *Citrullus colocynthis* fruit extracts. Planta Med 66:418–423. https://doi.org/10.1055/s-2000-8586

Oryan A, Hashemnia M, Hamidi AR et al (2014) Effects of hydro-ethanol extract of *Citrullus colocynthis* on blood glucose levels and pathology of organs in alloxan-induced diabetic rats. Asian Pac J Trop Dis 4:125–130. https://doi.org/10.1016/S2222-1808(14)60328-5

Ostovan F, Gol A, Javadi A (2017) Investigating the effects of *Citrullus colocynthis* pulp on oxidative stress in testes and epididymis in streptozotocin-induced diabetic male rats. Int J Reprod Biomed (Yazd) 15:41–48. https://doi.org/10.29252/ijrm.15.1.41

Ouelbani R, Bensari S, Mouas TN et al (2016) Ethnobotanical investigations on plants used in folk medicine in the regions of Constantine and Mila (North-East of Algeria). J Ethnopharmacol 194:196–218. https://doi.org/10.1016/j.jep.2016.08.016

Paraschos S, Magiatis P, Mitakou S et al (2007) In vitro and In vivo activities of chios mastic gum extracts and constituents against *Helicobacter pylori*. Antimicrob Agents Chemother 51:551–559. https://doi.org/10.1128/AAC.00642-06

Paraschos S, Mitakou S, Skaltsounis AL (2012) Chios gum mastic: a review of its biological activities. Curr Med Chem 19:2292–2302. https://doi.org/10.2174/092986712800229014

Pérez-Vizcaíno F, Ibarra M, Cogolludo AL et al (2002) Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. J Pharmacol Exp Ther 302:66–72. https://doi.org/10.1124/jpet.302.1.66

Qabaha K, Ras SA, Abbadi J et al (2016) Anti-inflammatory activity of *Eucalyptus spp.* and *Pistascia lentiscus* leaf extracts. Afr J Tradit Complement Altern Med 13:1–6. https://doi.org/10.21010/ajtcam.v13i5.1

Qiao J, Xu LH, He J et al (2013) Cucurbitacin E exhibits anti-inflammatory effect in RAW 264.7 cells via suppression of NF-κB nuclear translocation. Inflamm Res 62:461–469. https://doi.org/10.1007/s00011-013-0598-z

Qnais E, Raad D, Bseiso Y (2014) Analgesic and anti-inflammatory effects of an extract and flavonoids from *Artemisia Herba-Alba* and their mechanisms of action. Neurophysiology 46:238–246. https://doi.org/10.1007/s11062-014-9435-4

Quartu M, Serra MP, Boi M et al (2012) Effect of acute administration of *Pistacia lentiscus L.* essential oil on rat cerebral cortex following transient bilateral common carotid artery occlusion. Lipids Health Dis 11:8. https://doi.org/10.1186/1476-511X-11-8

Rafiq R, Hayek SA, Anyanwu U et al (2016) Antibacterial and antioxidant activities of essential oils from *Artemisia herba-alba Asso.*, *Pelargonium capitatum* × radens and *Laurus nobilis L.* Foods 5(28). https://doi.org/10.3390/foods5020028

Rahbar AR, Nabipour I (2010) The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia. Pak J Biol Sci 13:1202–1207. https://doi.org/10.3923/pjbs.2010.1202.1207

Ramzi S, Sahragard A, Zibaee A (2014) Effects of *Citrullus colocynthis* agglutinin on intermediary metabolism of *Ectomyelois ceratoniae* Zeller (Lepidoptera: *Pyralidae*). J Asia Pac Entomol 17:273–279. https://doi.org/10.1016/j.aspen.2014.01.005

Rehman MSU, Kamran SH, Ahmad M et al (2015) Anti-diabetic activity of crude *Pistacia lentiscus* in alloxan-induced diabetes in rats. Bangladesh J Pharmacol 10:543–547. https://doi.org/10.3329/bjp.v10i3.23225

Remila S, Atmani-Kilani D, Delemasure S et al (2015) Antioxidant, cytoprotective, anti-inflammatory and anticancer activities of *Pistacia lentiscus* (*Anacardiaceae*) leaf and fruit extracts. Eur J Integr Med 7:274–286. https://doi.org/10.1016/j.eujim.2015.03.009

Sahua B, Rathinam A, Javid MA et al (2017) Preparation of fatliquor having antifungal activity using the oil of *Citrullus colocynthis* for application in leather processing. Ind Crop Prod 108:553–557. https://doi.org/10.1016/j.indcrop.2017.06.029

Said MA, Vanloot P, Bombarda I et al (2016) Analysis of the major chiral compounds of *Artemisia herba-alba* essential oils (EOs) using reconstructed vibrational circular dichroism (VCD) spectra: en route to a VCD chiral signature of EOs. Anal Chim Acta 903:121–130. https://doi.org/10.1016/j.aca.2015.11.010

Saidi SA, Ncir M, Chaaben R et al (2017) Liver injury following small intestinal ischemia reperfusion in rats is attenuated by *Pistacia lentiscus* oil: antioxidant and anti-inflammatory effects. Arch Physiol Biochem 123:199–205. https://doi.org/10.1080/13813455.2017.1302961

Saitta M, Giuffrida D, Bella GD et al (2011) Chapter 107 – compounds with antioxidant properties in pistachio (*Pistacia vera L.*) seeds. Nuts and seeds in health and disease. Prevention:909–918. https://doi.org/10.1016/B978-0-12-375688-6.10107-0

Sakagami H, Kishino K, Kobayashi M et al (2009) Selective antibacterial and apoptosis-modulating activities of mastic. In Vivo 23:215–223

Salido S, Valenzuela L, Altarejos J et al (2004) Composition and infraspecific variability of *Artemisia herba-alba* from southern Spain. Biochem Syst Ecol 32:265–277. https://doi.org/10.1016/j.bse.2003.09.002

Sanz MJ, Terencio MC, Paya M (1992) Isolation and hypotensive activity of a polymeric procyanidin fraction from *Pistacia lentiscus L.* Pharmazie 47:466–467

Satyavani K, Gurudeeban S, Ramanathan T et al (2011) Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis (L.) Schrad*. J Nanobiotechnology 9(43). https://doi.org/10.1186/1477-3155-9-43

Schafferman D, Beharav A, Shabelsky E et al (1998) Evaluation of *Citrullus colocynthis*, a desert plant native in Israel, as a potential source of edible oil. J Arid Environ 40:431–439. https://doi.org/10.1006/jare.1998.0454

Sebbagh N, Cruciani-Guglielmacci C, Ouali F et al (2009) Comparative effects of *Citrullus colocynthis*, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats. Diabetes Metab 35:178–184. https://doi.org/10.1016/j.diabet.2008.10.005

Selmi S, Rtibi K, Grami D et al (2016) Antioxidant properties of *Artemisia herba-alba* and *Eucalyptus camaldulensis* essentials oils on malathion-induced reproductive damage in rat. RSC Adv 6:110661–110673. https://doi.org/10.1039/C6RA18268B

Shaikh J, Shaikh D, Rahman AB et al (2016) Antimicrobial and toxicological studies on fruit pulp of *Citrullus colocynthis* L. Pak J Pharm Sci 29:9–15

Shamsi-Baghbanan H, Sharifian A, Esmaeili S et al (2014) Hepatoprotective herbs, avicenna viewpoint. Iran Red Crescent Med J 16:e12313. https://doi.org/10.5812/ircmj.12313

Sharif Sharifi M, Hazell SL (2009) Fractionation of mastic gum in relation to antimicrobial activity. Pharmaceuticals (Basel) 2:2–10. https://doi.org/10.3390/ph2010002

Shi C, Karim S, Wang C et al (2014) A review on antidiabetic activity of *Citrullus colocynthis Schrad*. Acta Pol Pharm 71:363–367

Si Y, Zhang C, Meng S et al (2009) Gene expression changes in response to drought stress in *Citrullus colocynthis*. Plant Cell Rep 28:997–1009. https://doi.org/10.1007/s00299-009-0703-5

Sivaci A, Duman S (2014) Evaluation of seasonal antioxidant activity and total phenolic compounds in stems and leaves of some almond (*Prunus amygdalus L.*) varieties. Biol Res 47:1–5. https://doi.org/10.1186/0717-6287-47-9

Skiker M, Mekhfi H, Ami M et al (2010) *Artemisia herba-alba Asso* relaxes the rat aorta through activation of NO/cGMP pathway and KATP channels. J Smooth Muscle Res 46:165–174. https://doi.org/10.1540/jsmr.46.165

Somorin Y, Akinyemi A, Bertuzzi T et al (2016) Co-occurrence of aflatoxins, ochratoxin A and citrinin in "egusi" melon (*Colocynthis citrullus L.*) seeds consumed in Ireland and the United Kingdom. Food Addit Contam Part B Surveill 9:230–235. https://doi.org/10.1080/19393210.2016.1183051

Sterer N (2006) Antimicrobial effect of mastic gum methanolic extract against *Porphyromonas gingivalis*. J Med Food 9:290–292. https://doi.org/10.1089/jmf.2006.9.290

Sun C, Zhang M, Shan X et al (2010) Inhibitory effect of cucurbitacin E on pancreatic cancer cells growth via STAT3 signaling. J Cancer Res Clin Oncol 136:603–610. https://doi.org/10.1007/s00432-009-0698-x

Tan RX, Zheng WF, Tang HQ (1998) Biologically active substances from the genus *Artemisia*. Planta Med 64:295–302. https://doi.org/10.1055/s-2006-957438

Tannin-Spitz T, Bergman M, Grossman S (2007) Cucurbitacin glucosides: antioxidant and free-radical scavenging activities. Biochem Biophys Res Commun 364:181–186. https://doi.org/10.1016/j.bbrc.2007.09.075

Taştekin D, Atasever M, Adigüzel G et al (2006) Hypoglycaemic effect of *Artemisia herba-alba* in experimental hyperglycaemic rats. Bull Vet Inst Pulawy 50:235–238

Telli A, Esnault MA, Ould El Hadj Khelil A (2016) An ethnopharmacological survey of plants used in traditional diabetes treatment in South-Eastern Algeria (Ouargla province). J Arid Environ 127:82–92. https://doi.org/10.1016/j.jaridenv.2015.11.005

Tilaoui M, Ait Mouse H, Jaafari A et al (2015) Comparative phytochemical analysis of essential oils from different biological parts of *Artemisia herba alba* and their cytotoxic effect on cancer cells. PLoS One 10:e0131799. https://doi.org/10.1371/journal.pone.0131799

Triantafyllou A, Chaviaras N, Sergentanis TN et al (2007) Chios mastic gum modulates serum biochemical parameters in a human population. J Ethnopharmacol 111:43–49. https://doi.org/10.1016/j.jep.2006.10.031

Twaij HA, Al-Badr AA (1988) Hypoglycemic activity of *Artemisia herba alba*. J Ethnopharmacol 24:123–126. https://doi.org/10.1016/0378-8741(88)90143-2

Ullah N, Khan MA, Asif AH et al (2013) *Citrullus colocynthis* failed to combat against renal derangements, in spite of its strong antioxidant properties. Acta Pol Pharm 70:533–538

Vakiloddin S, Fuloria N, Fuloria S et al (2015) Evidences of hepatoprotective and antioxidant effect of *Citrullus colocynthis* fruits in paracetamol induced hepatotoxicity. Pak J Pharm Sci 28:951–957

Vernin G, Merad O, Vernin GMF et al (1995) GC–MS analysis of *Artemisia herba-alba Asso* essential oils from Algeria. Dev Food Sci 37:147–205. https://doi.org/10.1016/S0167-4501(06)80157-3

Villar A, Sanz MJ, Paya M (2008) Hypotensive effect of *Pistacia lentiscus L.* Int J Crude Drug Res 25:1–3. https://doi.org/10.3109/13880208709060902

Vuorinen A, Seibert J, Papageorgiou VP et al (2015) *Pistacia lentiscus* oleoresin: virtual screening and identification of masticadienonic and isomasticadienonic acids as inhibitors of 11β-Hydroxysteroid dehydrogenase 1. Planta Med 81:525–532. https://doi.org/10.1055/s-0035-1545720

Wakelin SH (2001) Allergic contact dermatitis from mastic in compound mastic paint. Contact Dermatitis 45:118. https://doi.org/10.1034/j.1600-0536.2001.045002118.x

Yar Saglam AS, Alp E, Elmazoglu Z et al (2016) Treatment with cucurbitacin B alone and in combination with gefitinib induces cell cycle inhibition and apoptosis via EGFR and JAK/STAT pathway in human colorectal cancer cell lines. Hum Exp Toxicol 35:526–543. https://doi.org/10.1177/0960327115595686

Yasir M, Sultana B, Nigam PS et al (2016) Antioxidant and genoprotective activity of selected cucurbitaceae seed extracts and LC–ESIMS/MS identification of phenolic components. Food Chem 199:307–313. https://doi.org/10.1016/j.foodchem.2015.11.138

Yemmen M, Landolsi A, Ben Hamida J et al (2017) Antioxidant activities, anticancer activity and polyphenolics profile, of leaf, fruit and stem extracts of *Pistacia lentiscus* from Tunisia. Cell Mol Biol (Noisy-le-Grand) 63:87–95. https://doi.org/10.14715/cmb/2017.63.9.16

Yoshikawa M, Morikawa T, Kobayashi H et al (2007) Bioactive Saponins and glycosides. XXVII. Structures of new Cucurbitane-type triterpene glycosides and Antiallergic constituents from *Citrullus Colocynthis*. Chem Pharm Bull 55:428–434. https://doi.org/10.1248/cpb.55.428

Younsi F, Trimech R, Boulila A et al (2016) Essential oil and phenolic compounds of *Artemisia herba-alba* (Asso.): composition, antioxidant, Antiacetylcholinesterase, and antibacterial activities. Int J Food Prop 19:1425–1438. https://doi.org/10.1080/10942912.2015.1079789

Zaini NAM, Anwar F, Hamid AA et al (2011) Kundur [*Benincasa hispida* (Thunb.) Cogn.]: a potential source for valuable nutrients and functional foods. Food Res Int 44:2368–2376. https://doi.org/10.1016/j.foodres.2010.10.024

Zeggwagh NA, Farid O, Michel JB et al (2008) Cardiovascular effect of *Artemisia herba alba* aqueous extract in spontaneously hypertensive rats. Methods Find Exp Clin Pharmacol 30:375–381. https://doi.org/10.1358/mf.2008.30.5.1186081

Zeggwagh NA, Michel JB, Eddouks M (2014) Acute hypotensive and diuretic activities of *Artemisia herba alba* aqueous extract in normal rats. Asian Pac J Trop Biomed 4:S644–S648. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0136

Zhang M, Sun C, Shan X et al (2010) Inhibition of pancreatic cancer cell growth by cucurbitacin B through modulation of signal transducer and activator of transcription 3 signaling. Pancreas 39:923–929. https://doi.org/10.1097/MPA.0b013e3181ce719e

Zhang M, Bian ZG, Zhang Y et al (2014) Cucurbitacin B inhibits proliferation and induces apoptosis via STAT3 pathway inhibition in A549 lung cancer cells. Mol Med Rep 10:2905–2911. https://doi.org/10.3892/mmr.2014.2581

Zhang ZR, Gao MX, Yang K (2017) Cucurbitacin B inhibits cell proliferation and induces apoptosis in human osteosarcoma cells via modulation of the JAK2/STAT3 and MAPK pathways. Exp Ther Med 14:805–812. https://doi.org/10.3892/etm.2017.4547

Zheng Q, Liu Y, Liu W et al (2014) Cucurbitacin B inhibits growth and induces apoptosis through the JAK2/STAT3 and MAPK pathways in SH-SY5Y human neuroblastoma cells. Mol Med Rep 10:89–94. https://doi.org/10.3892/mmr.2014.2175

Ziani BEC, Calhelha RC, Barreira JCM et al (2015) Bioactive properties of medicinal plants from the Algerian flora: selecting the species with the highest potential in view of application purposes. Ind Crop Prod 77:582–589. https://doi.org/10.1016/j.indcrop.2015.09.034

Ziyyat A, Legssyer A, Mekhfi H et al (1997) Phytotherapy of hypertension and diabetes in oriental Morocco. J Ethnopharmacol 58:45–54. https://doi.org/10.1016/S0378-8741(97)00077-9

V. Actinobactéries des niches microbiennes étonnantes et non exploitables du désert algérien : Importance biotechnologique et environnementale.

V. Actinobactéries des niches microbiennes étonnantes et non exploitables du désert algérien : Importance biotechnologique et environnementale.

« Actinobacteria from amazing and non-exploitable microbial niches in the Algerian desert: Biotechnological and environmental importance »

(Article en cours de révision dans la revue Biologia - Springer Nature).

En plus de la nanotechnologie, les techniques de CRISPR/Cas et l'ARNi utilisées pour éradiquer les microorganismes résistants, la recherche de nouvelles espèces d'actinobactéries productrices de nouvelles substances bioactives peut aussi constituer une alternative prometteuse. Cette approche a connu un progrès remarquable suite au développement des instruments analytiques et de la biologie moléculaire. Actuellement, les espèces bactériennes des sols des zones arides, des sols glaciers, des sédiments et des caves des anciens volcans sont recherchées. Ces zones sont plus visées pour des études approfondies en comparaison avec d'autres régions du globe terrestre en raison de leurs conditions abiotiques et biotiques extrêmes, à savoir les hautes températures, la sécheresse, la faible humidité relative, l'UV élevé, le manque de nutriment et le manque de couverture végétale. Par ailleurs, ces milieux extrêmes participent fortement dans la sélection de communautés microbiennes et favorisent le développement des communautés les plus résistantes.

Les nouvelles techniques de biologie moléculaire, en particulier la métagénomique et le séquençage à haut débit ont permis de mettre en évidence la richesse des zones extrêmement arides en taxons microbiens dont le phylum *Actinobacteria.* Certains taxons de ce phylum sont rares et nouvellement classés.

Les nouvelles espèces d'actinomycètes identifiées sont considérées comme une nouvelle source de composés éventuellement dotés d'activités biologiques. Durant ces dernières années, plusieurs substances bioactives additionnées au dictionnaire des produits naturels sont synthétisées par des actinobactéries, dont certaines sont produites par des espèces rares isolées des zones extrêmes.

Le Sahara algérien est l'un des plus chauds déserts au monde. Il est caractérisé par des systèmes oasiens s'étalant sur de vastes surfaces agricoles en formant des niches écologiques avec

des microclimats particuliers. Il héberge également d'importantes familles de plantes médicinales spontanées dont certaines, endémiques et connu pour leurs vertus thérapeutiques, elles sont utilisées en médecine traditionnelle locale.

Par ailleurs, la géologie du Sahara algérien le transforme en mosaïque exceptionnelle hébergeant des niches écologiques microbiennes jamais exploitées. Le massif d'Atakor situé au sud algérien est l'une des régions désertiques superficiellement étudiée. Ce massif formé d'immenses roches volcaniques a dévoilé par le peu d'études effectuées ces dernières années, sa richesse en de nouveaux taxons appartenant au phylum des actinobactéries. Les roches et les sols sédimentaires du Tassili n'ont fait l'objet d'aucune étude pouvant dévoiler leurs communautés microbiennes. D'autre part, de nouveaux taxons d'actinobactéries producteurs de nouveaux composés bioactifs ont été détectés dans les sols de Béchar et d'Adrar. Il a été démontré que les cratères d'impacts renferment aussi des taxons d'actinobactéries radio-tolérantes. Les cartes géographiques satellitaires ont révélé que le Sahara algérien renferme d'importants cratères d'impact dont les plus connus sont ceux de Tindouf et de Tamanrasset.

Mise à part l'importance biotechnologique et pharmaceutique des substances bioactives des actinobactéries, les streptomycètes isolés des sols pollués par les hydrocarbures ont montré un excellent pouvoir dépolluant. Ceux isolés des Oasis ont de grands intérêts en agriculture. Les actinobactéries endophytes des plantes médicinales spontanées possédant un grand intérêt pour la plante ont aussi des intérêts biotechnologiques.

Partie expérimentale.

I. Plan expérimental.

I. Plan expérimental

Les méthodes expérimentales utilisées dans ce travail sont résumées dans les Figure 1-10. Trois espèces végétales à savoir *P. lentiscus*, *A. heba-alba* et *C. colocynthis* poussant dans trois différentes régions du pays à savoir la région de Tifrit (34° 56' 04.2" N, 0° 22' 48.9" E), la région de Maamoura (34° 42' 06.7" N, 0° 28' 58.7" E) situé à Saida et la région de Oued N'sa (32° 48' 13.7" N, 3° 48' 13.6" E) situé à Ouargla font le premier objectif de cette étude. La sélection des plantes est basée sur leurs vertus médicinales. L'identification botanique des espèces végétales est effectuée au niveau du laboratoire de physiologie végétale de l'Université de Saida par Pr. Hasnaoui en se basant sur une documentation relative à la taxonomie de ces espèces au sein du règne végétal.

Les échantillons de sol utilisés pour l'isolement des actinobactéries sont prélevés de deux sites ; le premier est localisé dans la forêt d'El-Ogbane (34° 49′ 04.9″ N 0° 09′ 26.5″ E) située à l'ouest de l'Algérie dans les zones steppiques de la wilaya de Saida, le deuxième est situé à la wilaya de Ouargla ; précisément dans la région d'oued N'sa (32° 48' 13.7" N, 3° 48' 13.6" E). Tous les échantillons sont prélevés durant le mois de Janvier 2016. La situation géographique des différents sites de prélèvement est représentée dans la figure 01.

Afin d'extraire les métabolites secondaires des plantes, les parties utilisées sont lavées puis séchées à l'air libre. Un protocole bien précis est adopté pour l'extraction des composés phytochimiques brutes (Figure 02). Le choix du méthanol et l'eau distillé comme solvant d'extraction est basé sur leur forte polarité permettant un bon rendement d'extraction.

L'isolement des actinobactéries est précédé d'un traitement des échantillons de sols. L'isolement est effectué par la méthode de dilution sur deux milieux de cultures différents (Figure 05). L'ensemble des boites sont incubées à 30°C pendant 7 jours.

Les propagules fongiques des flores de contamination interne et externe des graines de blé tendre sont isolées selon la méthode indirecte. Trois milieux de culture spécifiques des moisissures sont requis pour cette étape. L'ensemble des boites sont incubées à 27°C pendant 7 jours (Figure 03).

Les extraits de plantes sont évaporés à sec puis soumis à des tests phytochimiques afin d'évaluer leur richesse en substances bioactives (Figure 02). Les isolats d'actinobactéries obtenus des sols sahariens et steppiques ont tout d'abord été purifiés puis conservés. Les isolats sélectionnés sont soumis à une série de tests. Ces isolats d'actinobactéries sélectionnées sont identifiés génétiquement par un séquençage complet de leur séquence nucléotidique (ARNr 16s). L'analyse phylogénétique est réalisée avec le logiciel MEGA 7 (Figure 06). Les isolats bactériens sont ensuite identifiés sur la base de leurs caractères biochimiques, morphologiques, microscopiques et physiologiques (Figure 05).

Afin de pouvoir tester la capacité antagoniste des isolats, les métabolites secondaires sont extraits après avoir cultivé les souches dans deux milieux de cultures liquides durant une période de 10 jours (Figure 07). Il est à noter qu'une seule souche est sélectionnée pour cette étude.

Les isolats fongiques obtenus du blé tendre local sont identifiés phénotypiquement (Figure 03). Les espèces du genre *Aspergillus* ayant révélé des caractères toxinogènes sur la chromatographie sur couche mince sont sélectionnées pour cette étude (Figure 04).

Tous les extraits obtenus des plantes et d'actinobactéries sont examinés pour déterminer leurs activités biologiques. L'activité antioxydante de ces extraits est évaluée selon trois tests différents à savoir l'inhibition du radical cation $ABTS^{\cdot+}$, le piégeage du radical $DPPH^{\cdot}$ et le pouvoir réducteur du fer (FRAP : Ferric Reducing Ability of Plasma) (Figure 09-10). L'activité antifongique des extraits est testée sur des souches de référence et celles isolées du blé tendre par la méthode de micro-dilution sur milieu solide (Figure 08). La capacité antimycotoxinogène est déterminée pour tous les extraits (Figure 08). Les activités antibactérienne et antifongique sont évaluées par micro-dilution sur les microplaques de 96 puits (Figure 09). Les extraits ayant révélé une excellente activité antimicrobienne sont fractionnés par HPLC et les fractions obtenues sont testées. L'extrait de l'isolats V_{002} est soumis à une LC-MS afin de déterminer sa composition en substances bioactives.

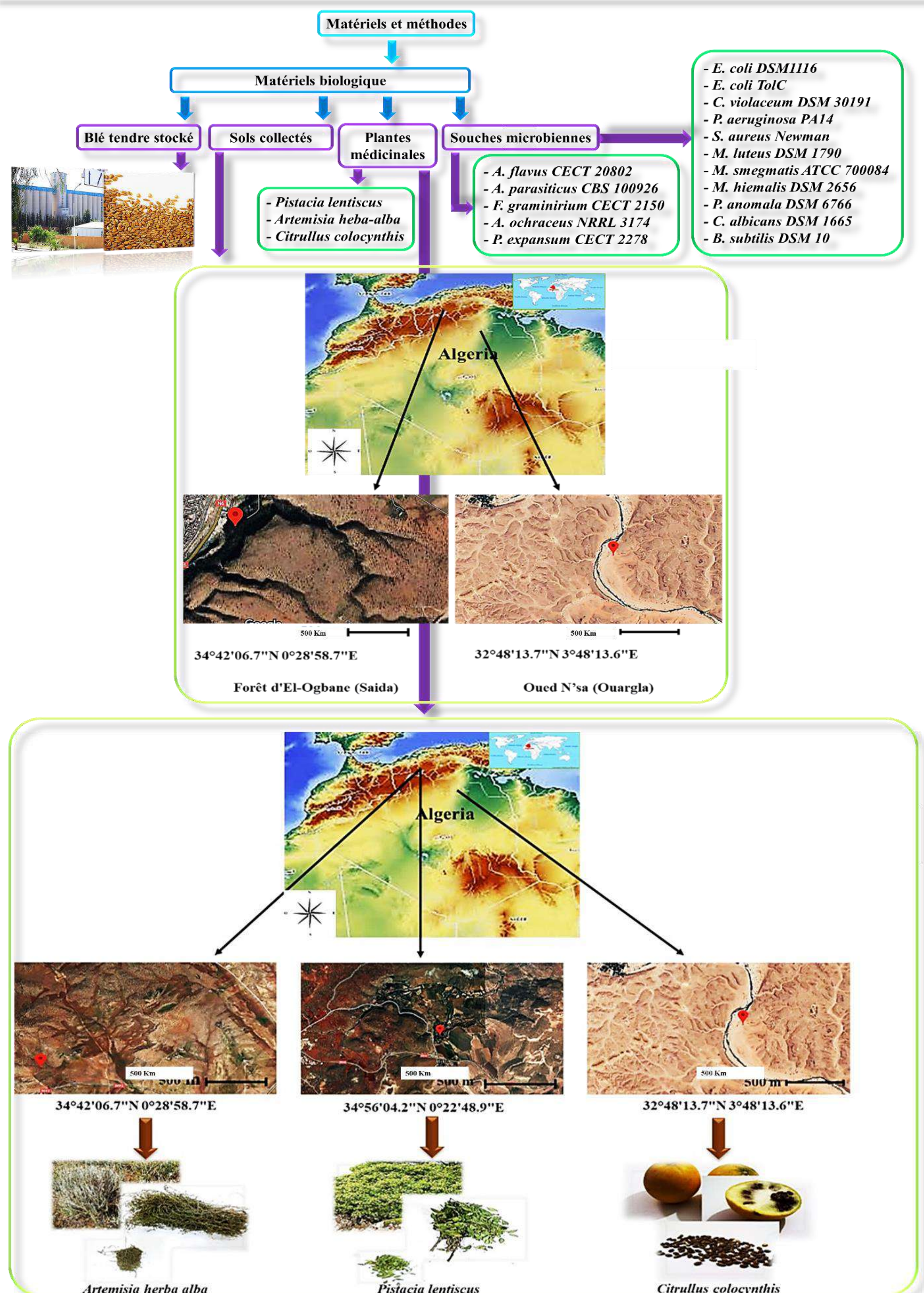


Figure 01. Localisation géographique des stations de récolte de plantes médicinales et des échantillons de sol.

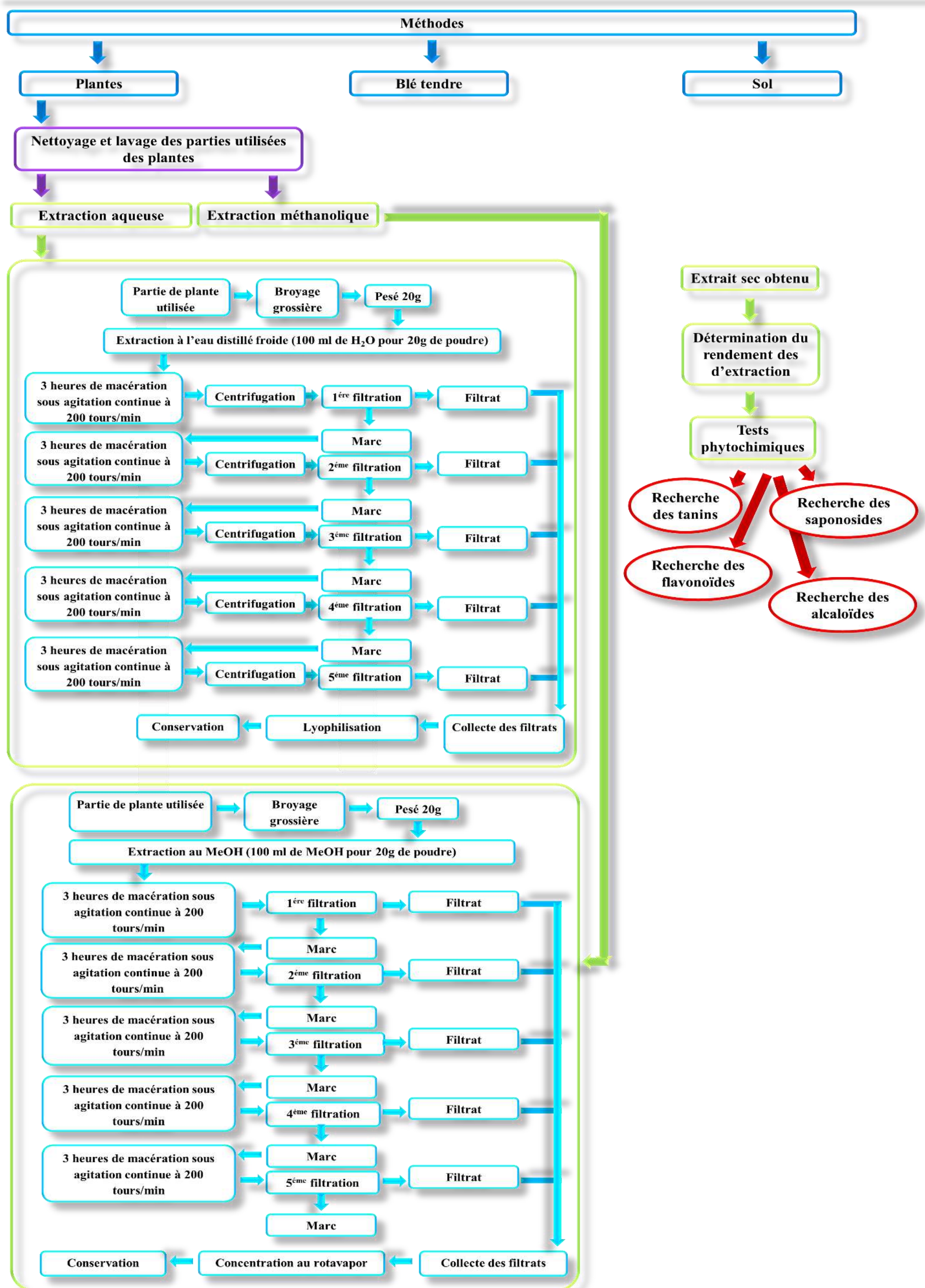


Figure 02. Préparation des extraits organiques et étude phytochimique.

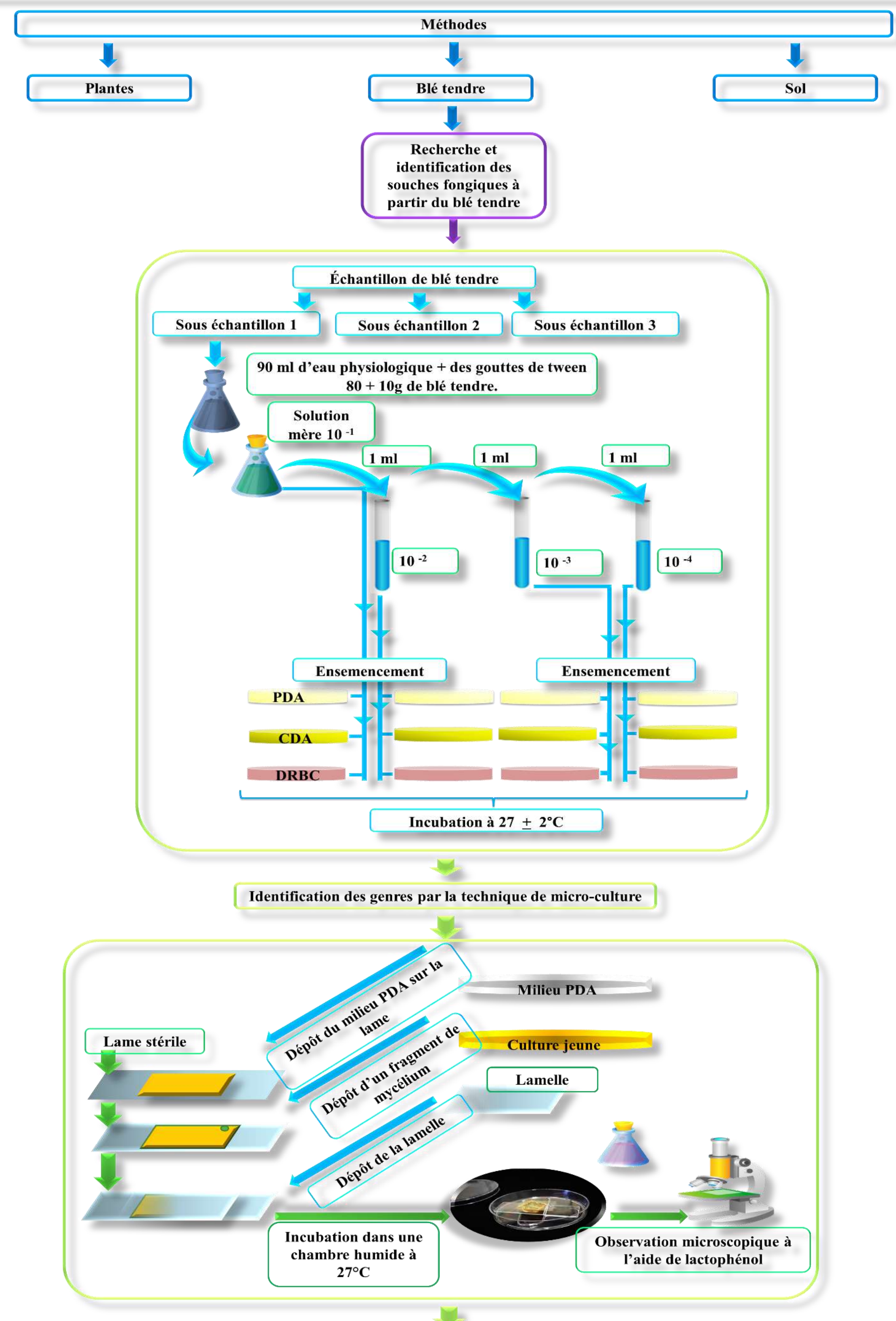


Figure 03. Techniques d'isolement et d'identification des genres fongiques.

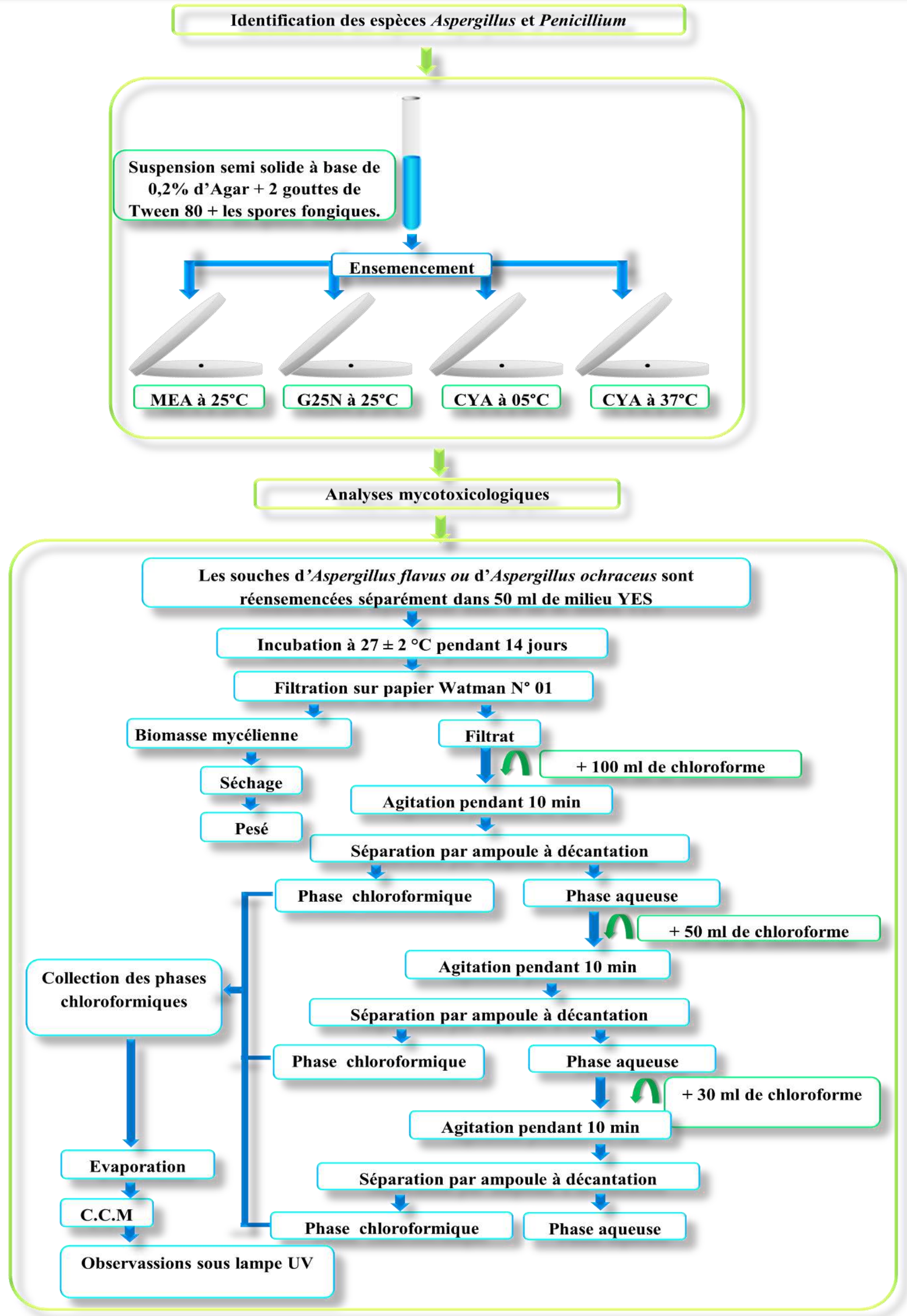


Figure 04. Détermination des espèces fongiques et identification des espèces productrices de mycotoxine.

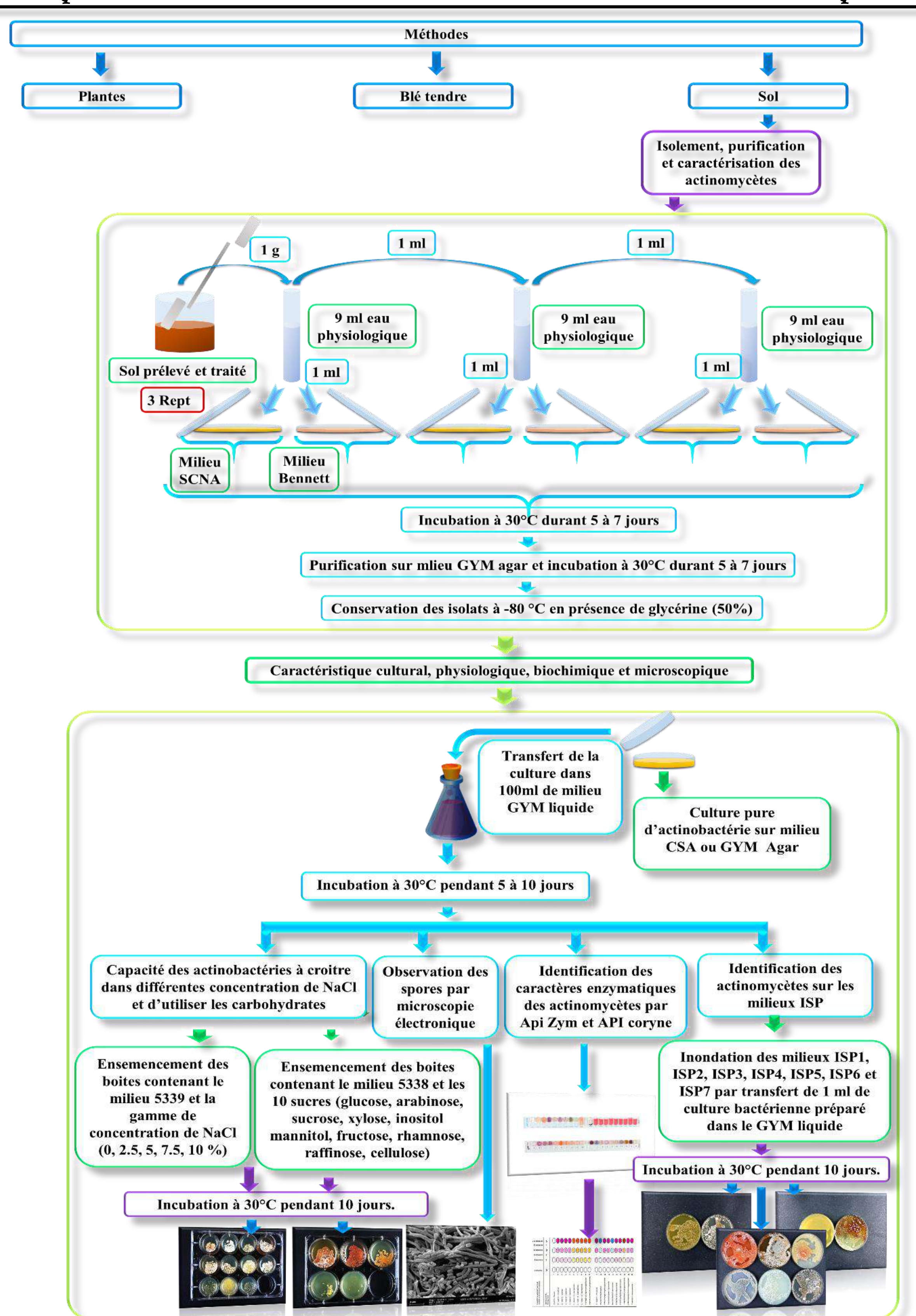


Figure 05. Technique d'isolement et d'identification phénotypique des actinobactéries.

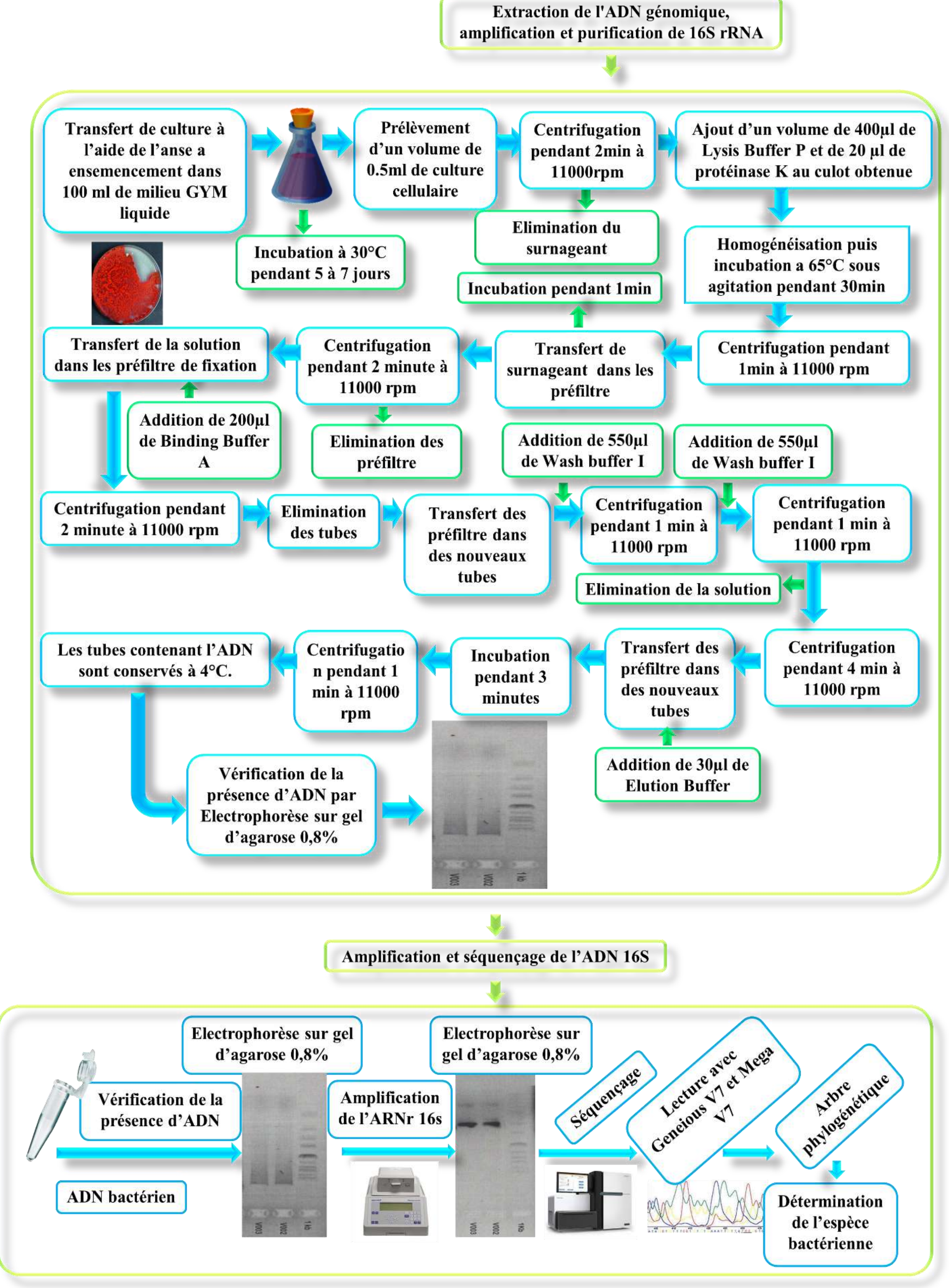


Figure 06. Technique d'extraction, d'amplification d'ADN 16S et d'identification phylogénétique des espèces d'actinobactéries.

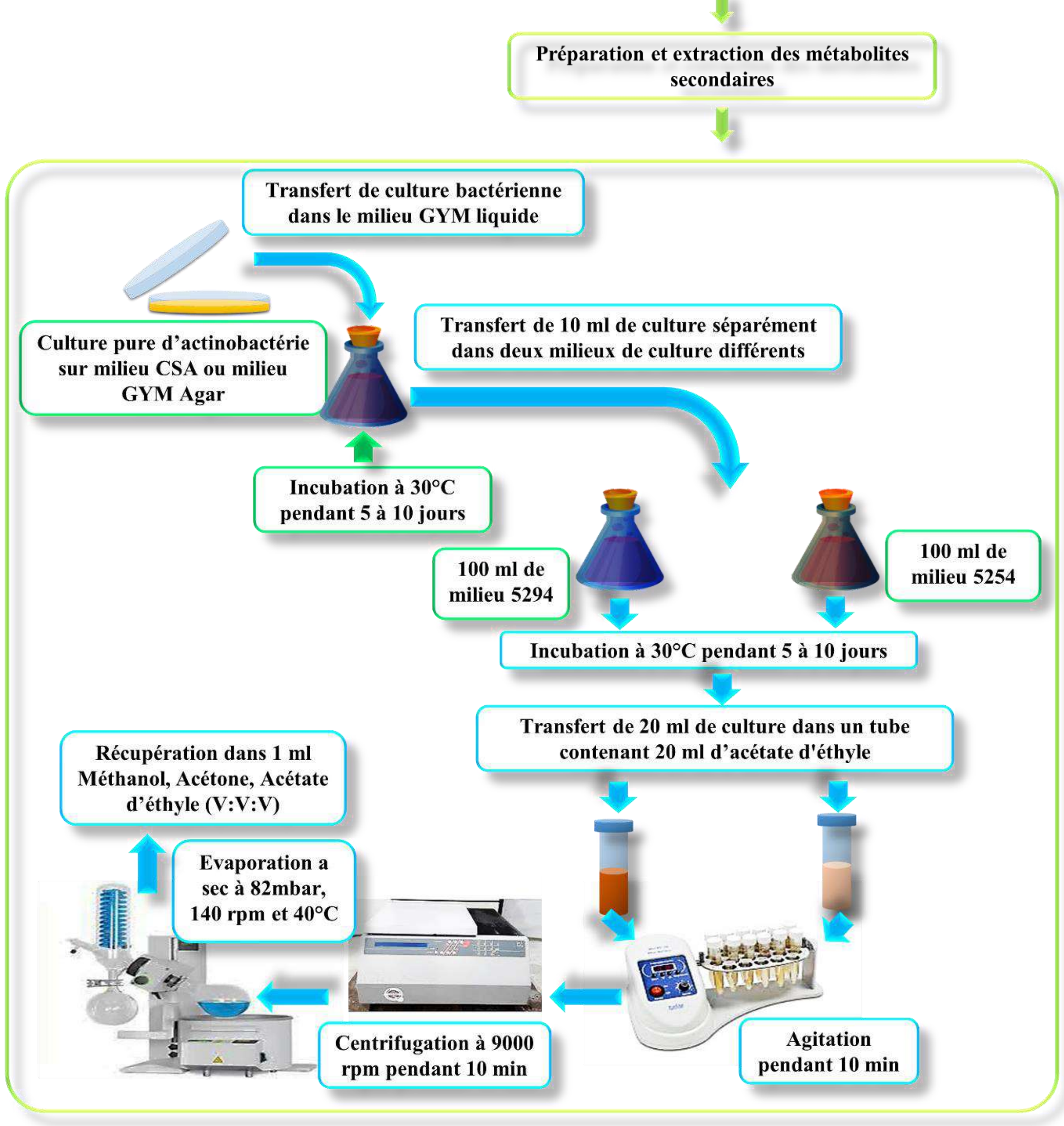


Figure 07. Technique d'extraction des métabolites secondaires à partir des espèces d'actinobactéries cultivées dans les milieux de cultures liquides.

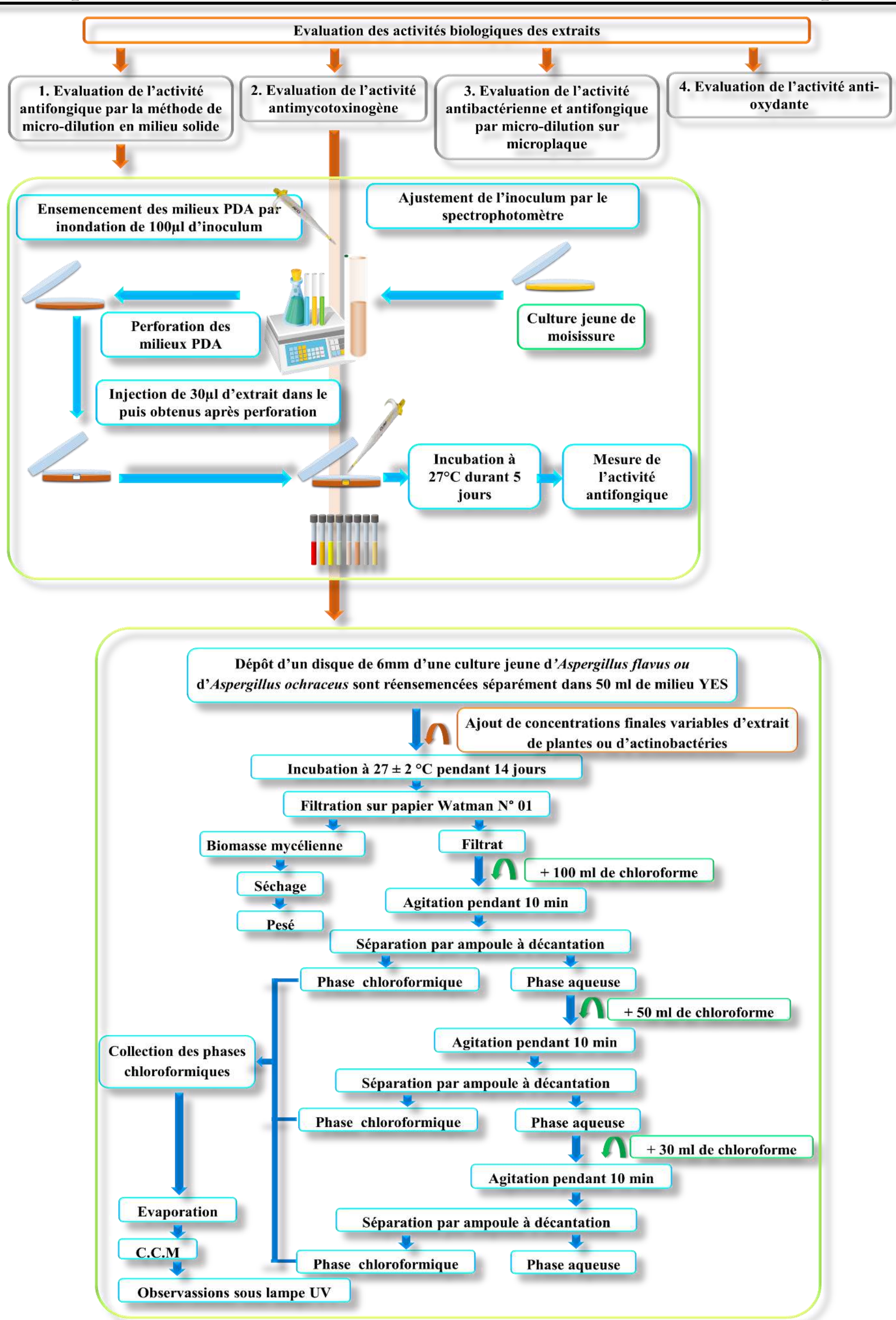


Figure 08. Détermination de l'activité antifongique et antimycotoxinogène des extraits organiques.

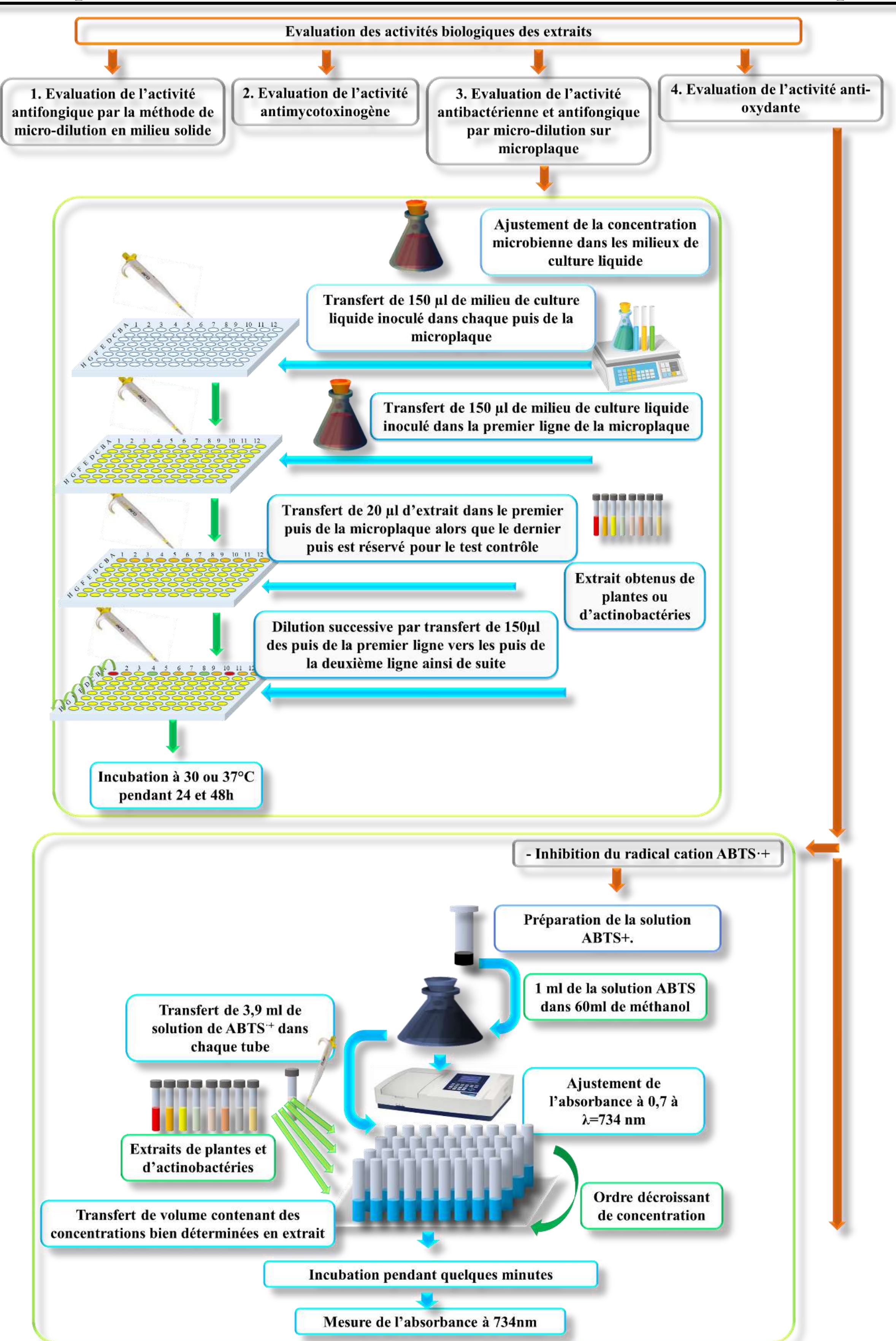


Figure 09. Détermination de l'activité antibactérienne et antioxydante des extraits organiques.

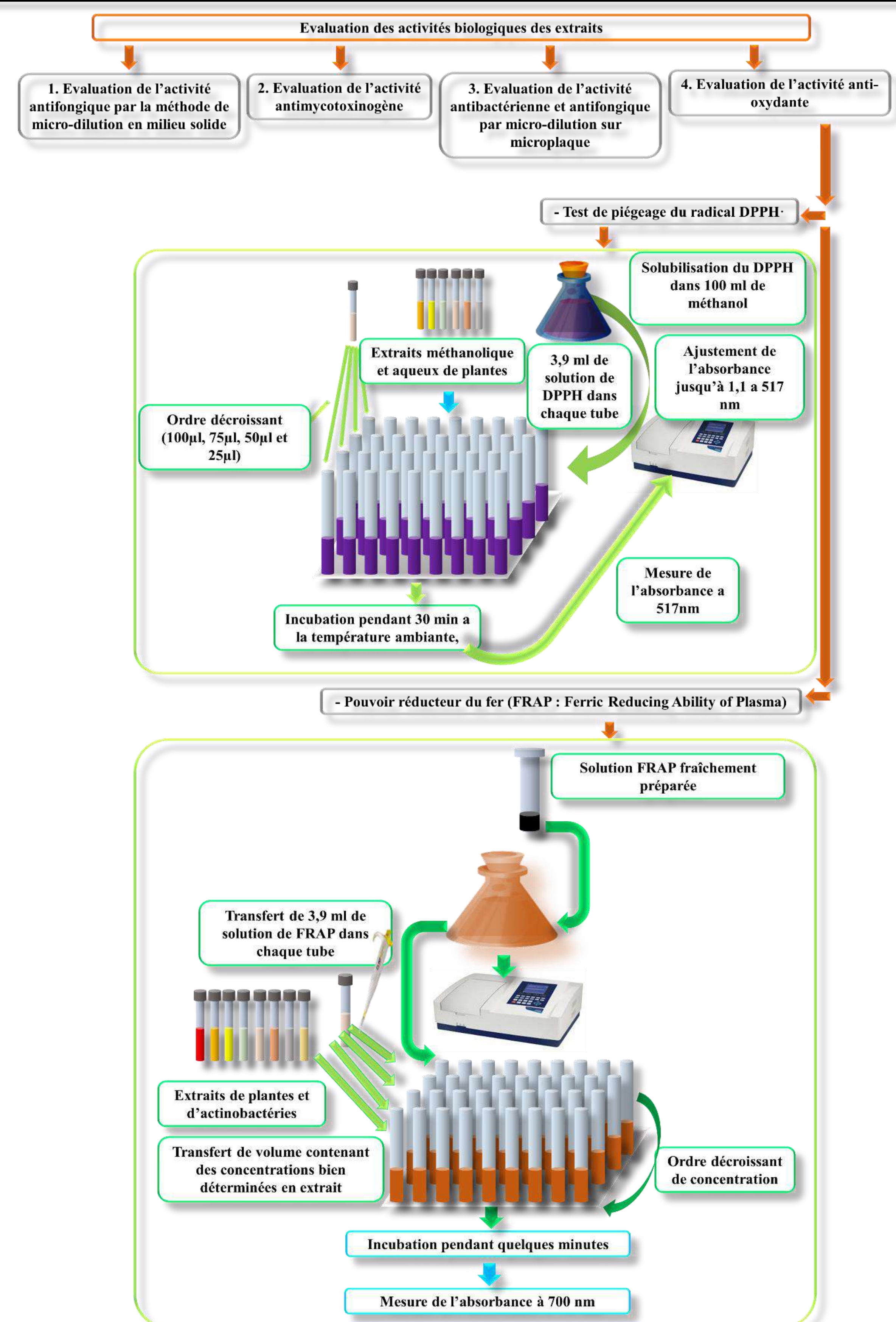


Figure 10. Détermination de l'activité antioxydante des extraits organiques.

II. Résultats et discussion.

II.1. Criblage phytochimique, activités antifongiques et antioxydantes de trois plantes médicinales de la steppe et du Sahara algériens (études préliminaires de criblage).

II.1. Criblage phytochimique, activités antifongiques et antioxydantes de trois plantes médicinales de la steppe et du Sahara algériens (études préliminaires de criblage).

« Phytochemical screening, antifungal and antioxidant activities of three medicinal plants from Algerian steppe and Sahara (preliminary screening studies) »

(Article publié en Novembre 2019 dans la revue SN Applied Sciences - Springer Nature).

Le présent chapitre est consacré à la détermination des activités antifongique et antioxydante de trois plantes médicinales récoltées de deux régions steppique et saharienne du l'Algérie.

Dans la présente partie du travail, les rendements en extraits secs obtenus après extraction méthanolique et aqueuse effectuée sur la partie aérienne de *P. lentiscus*, d'*A. heba-alba* et les graines de *C. colocynthis* sont évalués (**Tableau 1, Article1**).

Une différence remarquable en composés phytochimiques est constatée entre les trois plantes (**Tableau 1. Article1**). Cette différence dépend fortement de la méthode d'extraction, du solvant utilisé, des conditions expérimentales d'extraction, de la saison de la récolte, du climat de la région et de la composition du sol.

Les tests de l'activité antifongique ont montré que les extraits méthanoliques sont les plus actifs contre les souches fongiques de références sélectionnées. L'extrait méthanolique d'*A. heba-alba* est le plus actif. Les souches fongiques *P. expansum*, *A. ochraceus* et *F. graminearum* présentent une sensibilité remarquable aux extraits d'*A. herba-alba* (**Figure 4, 5, et 6, Tableau 2. Article 1**). Ces souches sont aussi considérées comme hautement productrices de patuline, citrinine et d'ochratoxine. *A. parasiticus* est l'espèce la plus résistante. La souche fongique *A. flavus* a montré une sensibilité moyenne aux extraits méthanolique et aqueux de *P. lentiscus* (**Figure 4 et 5, Tableau 2. Article1**).

Tous les extraits testés ont révélé une bonne activité antioxydante. L'extrait méthanolique et aqueux d'*A. herba-alba* a une excellente activité antioxydante évaluée par le test FRAP (**Figure 7, Article1**). La plus faible IC_{50} de 0.91±0.92 $mg.ml^{-1}$ est déterminée par le test FRAP effectué sur

l'extrait méthanolique d'*A. herba-alba.* (**Tableau 3. Article1**). Cependant, les IC_{50} déterminées par les trois méthodes (ABTS, DPPH et FRAP) sont inférieures à celle de l'antioxydant standard (Trolox). Les différences observées entre les dosages semblent être dues à l'utilisation d'un mécanisme de réaction différent entre les tests.

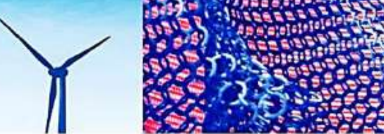
Les extraits d'*A. herba-alba* possèdent l'activité antifongique la plus élevée par rapport à *C. colocynthis* et de *P. lentiscus*. D'autres recherches effectuées sur les mêmes espèces végétales ont rapporté que ces espèces sont dotées de cette aptitude antifongique.

La sensibilité des cellules fongiques aux extraits de plantes dépend fortement des interactions entre les composés bioactifs des plantes et les composants cellulaires fongiques. Ces composés bioactifs peuvent inhiber la formation de la paroi cellulaire, provoquer la rupture de la membrane cellulaire, le dysfonctionnement des mitochondries, l'inhibition de la division cellulaire, l'inhibition de la réplication de l'ADN, l'inhibition de la synthèse protéique et l'inhibition des pompes d'efflux.

La résistance des souches fongiques est essentiellement due à la dégradation des composés bioactifs par les enzymes fongiques ou à l'élimination des substances bioactives par les systèmes d'efflux. Elle est aussi due à l'insensibilité du site ciblé par la molécule. Les résultats des dosages d'activité antioxydante présenté dans cette étude sont cohérents avec d'autre étude réalisée dans d'autre région.

La résistance des moisissures mycotoxinogènes aux agents antifongiques est à l'origine de la recherche de l'aptitude des plantes médicinales à inhiber la croissance de ces espèces microscopiques à travers de nouvelles plateformes de recherche installées récemment dans ce domaine.

La capacité des extraits des espèces étudiées à inhiber la croissance de trois espèces fongiques réputées toxinogènes est confirmée. Ces propriétés ouvrent des perspectives prometteuses pour leur application en tant qu'agents antimycotoxinogène contre certaines moisissures des denrées alimentaires.



Research Article

Phytochemical screening, antifungal and antioxidant activities of three medicinal plants from Algerian steppe and Sahara (preliminary screening studies)

Mohamed Amine Gacem[1,2] · Alia Telli[1] · Hiba Gacem[3] · Aminata Ould-El-Hadj-Khelil[1]

Received: 4 October 2019 / Accepted: 26 November 2019
© Springer Nature Switzerland AG 2019

Abstract
The contamination of foodstuffs by fungi and their mycotoxins, emergence of resistance to fungicides and unwanted side effects of pesticides on human and animal health currently present a challenge for researchers to create and innovate new and better solutions to fight against mycotoxigenic fungi with less harmful effects on the consumer and the environment. In the context of the valorization of natural products, this study aimed, first, to determine the phytochemical composition of methanolic and aqueous extracts of *Pistacia lentiscus, Artemisia herba-alba* and *Citrullus colocynthis* and, second, to evaluate the antifungal potential and antioxidant capacity of these extracts. The extraction of crude active compounds from selected plant species was carried out by methanolic and aqueous maceration. Antifungal activity against five fungal strains (*Aspergillus flavus, Aspergillus parasiticus, Fusarium graminirium, Aspergillus ochraceus* and *Penicillium expansum*) was evaluated by the dilution method in solid medium. Analysis of antioxidant activity was carried out by three tests, namely, the inhibition of $ABTS^{\cdot+}$radical cation, trapping of $DPPH^{\cdot}$ radical and reduction of Fe^{2+} (FRAP). The results show that the crude extracts were rich in bioactive compounds and that the crude methanolic and aqueous extracts of *A. herba-alba* were the most active against the tested fungal strains and had a percentage of inhibition exceeding 8%. Regarding antioxidant activity, all the extracts exhibited remarkable antioxidant potential in the tests used. The methanolic extract of *A. herba-alba* had the highest iron-reducing capacity. The results show that the studied medicinal plants could be a good alternative for protecting foodstuffs against toxigenic fungi.

Keywords *Pistacia lentiscus* · *Artemisia herba-alba* · *Citrullus colocynthis* · Antifungal activity · Antioxidant activity

1 Introduction

Mycotoxins are secondary metabolites produced by six fungal genera, namely, *Aspergillus, Alternaria, Claviceps, Fusarium, Penicillium* and *Stachybotrys*. These metabolites commonly occur in foodstuffs and cause diseases in consumers [1]. Depending on the administered dose, the toxicity can spread to all vital human and animal organs and cause oxidative stress [2], which leads to nephrotoxicity [3], genotoxicity [4], neurotoxicity [5], hepatotoxicity [6], immunotoxicity [7], gastrointestinal toxicity [8], and cardiotoxicity [9]. Long periods of exposure to mycotoxins contribute to cancer development [10].

To limit the presence of mycotoxinogenic fungi in food and the mycotoxicoses caused by the consumption of contaminated foods, a control policy has been applied in recent years that is based on prevention of contamination, on decontamination and on protection against the

✉ Mohamed Amine Gacem, biologieamine@yahoo.fr | [1]Laboratory of Ecosystems Protection in Arid and Semi-Arid Area, University of Kasdi Merbah, 30000 Ouargla, Algeria. [2]Department of Biology, Faculty of Science, University of Amar Tlidji, 03000 Laghouat, Algeria. [3]Epidemiology Service and Preventive Medicine, Hassani Abdelkader University Hospital Center, Faculty of Medicine, University of Djillali Liabes, 22000 Sidi-Bel-Abbès, Algeria.

SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1

Published online: 28 November 2019

SN Applied Sciences
A SPRINGER NATURE journal

Research Article SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1

toxicities of mycotoxins by the application of several physical, chemical and biological techniques [11]. However, it is very difficult to destroy mycotoxins by physical techniques without altering food quality [12]; moreover, the use of chemical processes also has undesirable effects because chemical compounds participate in the generation of free radicals and, therefore, the induction of oxidative stress [13, 14]. At the same time, the massive use of antibiotics in agriculture is a cause for alarm with respect to public health [15] due to antibiotic-induced oxidative stress and to the emergence of multiresistant strains [16–18]. To resolve this problem, several studies investigated the use of essential oils and medicinal plant extracts as a safe alternative in the field of phytoprotection, which is the fight against toxigenic moulds and mycotoxicoses [19, 20]. Reddy et al. [21] showed that aqueous extracts of some plant species (*Allium cepa*, *Allium sativum*, *Curcuma longa*, *Ocimum sanctum*, *Syzigium aromaticum*, etc.) are rich in bioactive chemical compounds that inhibit the biosynthesis of aflatoxin. A study conducted by Elsherbiny et al. [22] demonstrated that the essential oils of *Ocimum basilicum* completely inhibited the growth of several species of *Bipolaris* and the germination of *Bipolaris hawaiensis* spores. However, the use of medicinal plants from arid and semi-arid regions of Algeria as an alternative in the process of food product decontamination has not been extensively studied.

Many medicinal plant species from steppe and desert regions are known for their therapeutic virtues and are traditionally used by the local populations to treat various diseases. These steppe and Saharan species, which include *Pistacia lentiscus* (*Anacardiaceae*), *Artemisia herba-alba* (*Asteraceae*) and *Citrullus colocynthis* (*Cucurbitaceae*) have remarkable antidiabetic, antibacterial, anti-tumoural, anti-inflammatory and anticancer properties [23–29]. Therefore, the objective of this study was to determine the phytochemical composition of the aqueous and methanolic extracts of *P. lentiscus*, *A. herba-alba* and *C. colocynthis* and to evaluate their antifungal and antioxidant potency.

2 Materials and methods

2.1 Harvest and preparation of medicinal plants

The plants that are the focus of this study are known for their therapeutic virtues in traditional Algerian medicine [30]. The leaves of *P. lentiscus* were harvested in the region of Tifrit (34° 56′ 04.2″ N, 0° 22′ 48.9″ E) in December 2017. For the second species, the aerial part of *A. heba-alba* was harvested in the region of Maamoura (34° 42′ 06.7″ N, 0° 28′ 58.7″ E) in March 2017. Regarding *C. colocynthis* species, fruit were harvested in December 2016 from the desert region of Oued N'sa (32° 48′ 13.7″ N, 3° 48′ 13.6″ E). The geographical location of harvesting sites is illustrated in Fig. 1. The botanical identification of plant species was carried out by botanists from the plant biology laboratory of Saida University (Algeria). To achieve the targeted aim of the study, the plant parts used were the leaves of *P. lentiscus* and *A. heba-alba* and the seeds of *C. colocynthis*.

2.2 Extraction and phytochemical screening

P. lentiscus and *A. heba-alba* leaves and *C. colocynthis* seeds were first washed with distilled water and disinfected for 30 min by immersion in a 2% solution of sodium hypochlorite. The plant material was then rinsed with distilled water to remove residual hypochlorite. After open air drying away from light, the plant material was crushed roughly using a grinder (Moulinex Grinder AR110027) [31], and the powders obtained from each species were divided into two quantities for subsequent methanolic and aqueous extractions.

A quantity of 20 g of each plant powder was mixed with 100 ml of solvent (methanol [Sigma-Aldrich-Germany] or water) for 3 h under continuous stirring (200 rpm) in MS Orbital Shaker at room temperature. The mixture was then filtered using Whatman filter paper (No 4). This operation was repeated four times with renewal of the solvent to deplete the marc and increase the yield. At the end of the extraction, the fractions of each extraction were collected, and the methanolic extracts were evaporated to dryness using a rotavapor (Heidolph-Germany) [32], while the aqueous extracts were lyophilized. Extract powders were kept in dark bottles at 4 °C until use. Figure 2 shows the methanolic and aqueous extraction steps followed in this study. The qualitative detection of alkaloids, saponins, flavonoids, and tannins was carried out following the protocols described in the study done by Al-Daihan et al. [33].

2.2.1 Test for alkaloids

Each dry extract powder (100 mg) was dissolved in 5 ml of methanol and then filtered. 5 ml of hydrochloric acid (1%) was mixed with 2 ml of the filtrate, and then 1 ml of the mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent (potassium iodide-bismuth nitrate) were added in the tube and appearance of orange-red precipitate was taken as positive. Few drops of Mayer's reagent (composed of mercuric chloride and potassium iodide dissolved in distilled water) were added to the second tube and appearance of buff-colored precipitate designates the existence of alkaloids.

SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1 Research Article

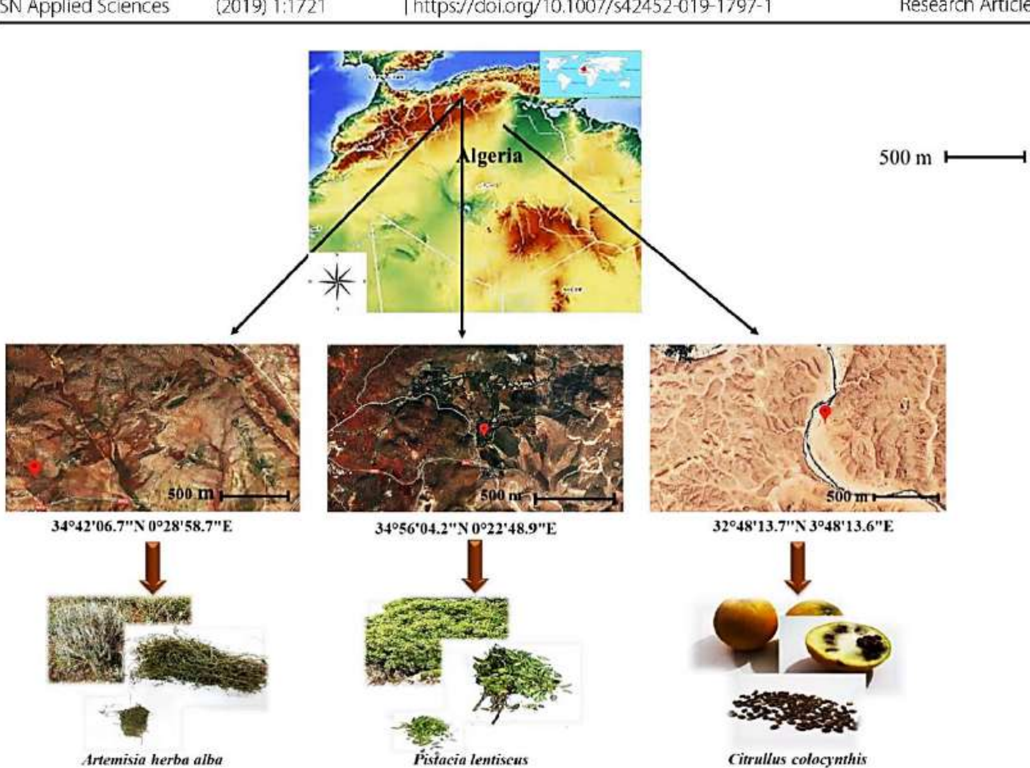


Fig. 1 Geographic location of medicinal plant harvesting stations

2.2.2 Test for saponins

10 ml of distilled water was mixed with 1 g of powdered dry extract and then boiled and filtered. The filtrate was mixed again with 3 ml of distilled water and shaken for 5 min. Appearance of foam after shaking designates the existence of saponins.

2.2.3 Shinoda's test for flavonoids

500 mg of powdered dry extract was added to 5 ml of ethanol, the mixture is slightly heated and then filtered. The filtrate was added to some pieces of magnesium chips, a few drops of concentrated HCl was then added to the mixture. Occurrence of a pink, orange, or red to purple coloration designates the existence of flavonoids.

2.2.4 Test for tannins

500 mg of powdered dry extract was added to 10 ml of distilled water, the mixture is then filtered and few drops of 1% ferric chloride solution are added to the filtrate. Occurrence of a blue-black, green or blue-green precipitate designates the existence of tannins.

2.3 Assessment of antifungal activity

Five fungal strains were selected to evaluate the antifungal activity of the different extracts: *A. flavus* CECT 20802 (producing aflatoxin B_1 (AFB_1), AFB_2, AFM_1 and AFM_2), *A. parasiticus* CBS 100926, *F. graminirium* CECT 2150, *A. ochraceus* NRRL 3174 (producing ochratoxin) and *P. expansum* CECT 2278 (producing patulin and citrinin). The purity of the strains was verified by the microculture chamber (slide culture) with some modifications [34], this technique takes place in a few steps; (1) using a sterile blade cut out the PDA medium (10 × 10 mm) to fit under a coverslip, (2) deposit the PDA block on a sterile blade, (3) inoculate the four sides of the PDA block with spores of the strain to be grown, (4) cover the PDA block with a flamed coverslip, (5) incubate the plate at 30 °C during 15 days, (6) transfer the coverslip on a new clean glass slide containing a small drop of lactophenol blue solution, (7) examine the slides under optical microscope.

Research Article SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1

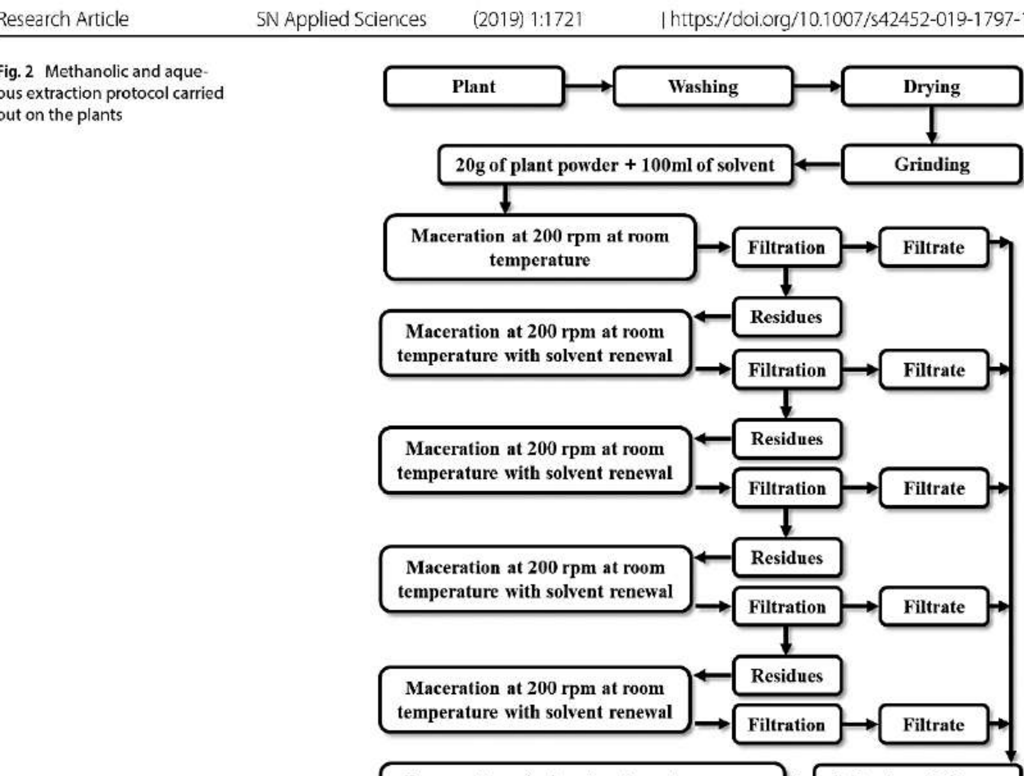


Fig. 2 Methanolic and aqueous extraction protocol carried out on the plants

Antifungal activity was measured by the protocol described by Magnusson and Schnürer [35] with some modifications. As described by Espinel-Ingroff and Cantón [36], spore solutions were prepared from seven-day-old cultures of each strain, and their concentrations were adjusted with a spectrophotometer (Jenway 6315- United Kingdom) set to 530 nm to obtain suspensions of 5×10^6 viable conidia or sporangiospores per milliliter. A volume of 100 µl of each suspension (diluted to approximately 5×10^4 CFU.ml^{-1}) was used to flood-inoculate PDA medium that had been solidified in Petri dishes. A dilution series (25, 50, 75 and 100 mg ml^{-1}) was prepared for each plant extract, and 40 µl of extract dilution was injected into the wells of inoculated PDA medium. The dishes were incubated for 5 days at 27 ± 2 °C. Antifungal activity was calculated by dividing the surface area of growth inhibition due to the extract by the total surface area of the petri dish and multiplying by 100% [35]. The results were categorized as follows:

- If the inhibition was between 0.1 and 3%, the antifungal activity was considered low.
- If the inhibition was between 3 and 8%, the antifungal activity was considered average.
- If the inhibition was greater than 8%, the antifungal activity was considered to be high.

2.4 Determination of antioxidant activity

2.4.1 ABTS radical scavenging assay

To measure the antioxidant capacity by monitoring inhibition of the $ABTS^{\cdot+}$ radical cation, the method described by Surveswaran et al. [37] was followed. The generation of $ABTS^{\cdot+}$ radical cation was carried out by a chemical reaction between a solution of ABTS (7 mM) and a solution of potassium persulfate (2.45 mM) incubated for 16 h in the dark at room temperature. The resulting $ABTS^{\cdot+}$ solution was diluted with methanol (at a ratio of 1/50) in order to obtain an absorbance of 0.700 ± 0.005 at a wavelength of 734 nm. A volume of 100 µl of plant extract solution prepared at different concentrations (10, 5, 2, 1, 0.66 or 0.4 mg ml^{-1}) was added to 3.9 ml of $ABTS^{\cdot+}$ solution (absorbance equal to 0.700 ± 0.005). After 10 min of

SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1 Research Article

reaction, the absorbance was measured by a spectrophotometer (Jenway 6315-United Kingdom) set to 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard antioxidant at a concentration range of 0–500 µM. The results were expressed in µM Trolox equivalent per gram of extract (µM TE g^{-1}).

2.4.2 DPPH radical scavenging assay

The antiradical activity of plant extracts was determined by the DPPH radical scavenging assay according to the method adopted by Orphanides et al. [38]. A volume of 3.9 ml of DPPH˙ solution (0.3 mM) was mixed with 100 µl of plant extract prepared at different concentrations (10, 5, 2, 1, 0.66 or 0.4 mg ml^{-1}) and incubated for 30 min at room temperature. The absorbances were then measured by a spectrophotometer (Jenway 6315-United Kingdom) set to 517 nm. Trolox was used as a standard antioxidant at a concentration range of 0–500 µM. The results were expressed in µM TE g^{-1}.

2.4.3 Ferric reducing ability (FRAP)

The ability of the various extracts to reduce ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) was determined according to the protocol described by Orphanides et al. [38]. Dilutions of plant extracts and Trolox were prepared at the same concentrations as used in the antioxidant assays above. A volume of 100 µl of extract was mixed with 3.9 ml of a freshly prepared FRAP solution [0.3 M acetate buffer, pH = 3.6, 10 mM TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine) and 20 mM $FeCl_3 \cdot 10H_2O$ at a ratio of 10:1:1 (v/v/v)]. The mixture was then incubated at 37 °C for 4 min, and the absorbance was measured at a 593 nm. Trolox was used as a standard antioxidant at a concentration range of 0–800 µM. The results were expressed in µM TE g^{-1}.

2.4.4 Statistical analyses

Each experiment was performed in triplicate, and the results were expressed as the mean ± standard deviation. Variance analysis was used to test the differences between extracts and selected plant species as well as among the various techniques used to evaluate the antioxidant activity. Linear regression was used to determine the IC_{50} and CE_{50}. All analyses were done by XLSTAT 2009.

3 Results

3.1 Extraction yield and phytochemical analysis

Water and methanol are the most polar solvents used in phytochemical extraction methods. Both solvents are also recommended in the literature to extract a maximum of bioactive compounds from plants [39, 40]. According to the results presented in Table 1, the highest yields were recorded for the methanolic and aqueous extracts of *P. lentiscus* leaves, which were 22.30% and 17.44%, respectively, followed in descending order by the methanolic and aqueous extracts of *A. herba-alba* and *C. colocynthis* seeds, which had the lowest yields with percentages of 8.14, 5.25, 3.20 and 2.26%, respectively. Concerning the phytochemical screening of the studied species, Table 1 indicates all sought bioactive substances (alkaloids, flavonoids, tannins and saponosides) were detected in *A. herba-alba*; however, saponosides were not detected in *C. colocynthis* seeds, while alkaloids were not detected in *P. lentiscus* leaves.

3.2 Antifungal activity

The results confirming the purity of the selected fungal strains are shown in Fig. 3. An examination of micro-culture slides stained with lactophenol blue solution under an optical microscope clearly demonstrated that fungal strains were pure with no other microbial or fungal contamination.

In the assays measuring the antifungal effects of the plant extracts against five fungal strains, the aqueous and methanolic extracts of *A. herba-alba* were the most active (Figs. 4, 5, 6), and at concentrations greater than or equal to 50 mg ml^{-1}, growth inhibition exceeded 8% (Figs. 4, 5). The most sensitive fungal strains were *P. expansum* and *A. ochraceus* followed by *F. graminearum* (Figs. 4, 5). According to the same figures, aqueous and methanolic extracts of the selected plant species had no effect on the growth of *A. parasiticus* and *A. flavus* except for the aqueous and methanolic extracts of *P. lentiscus*, which had an average

Table 1 Extraction yield and phytochemical screening

	Extraction yield %		Alkaloids	Flavonoids	Tannins	Saponins
	Met	Aq				
Plant						
P. lentiscus	22.30	17.44	−	+	+	+
A. herba-alba	8.14	5.25	+	+	+	+
C. colocynthis	3.20	2.26	+	+	+	−

Research Article SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1

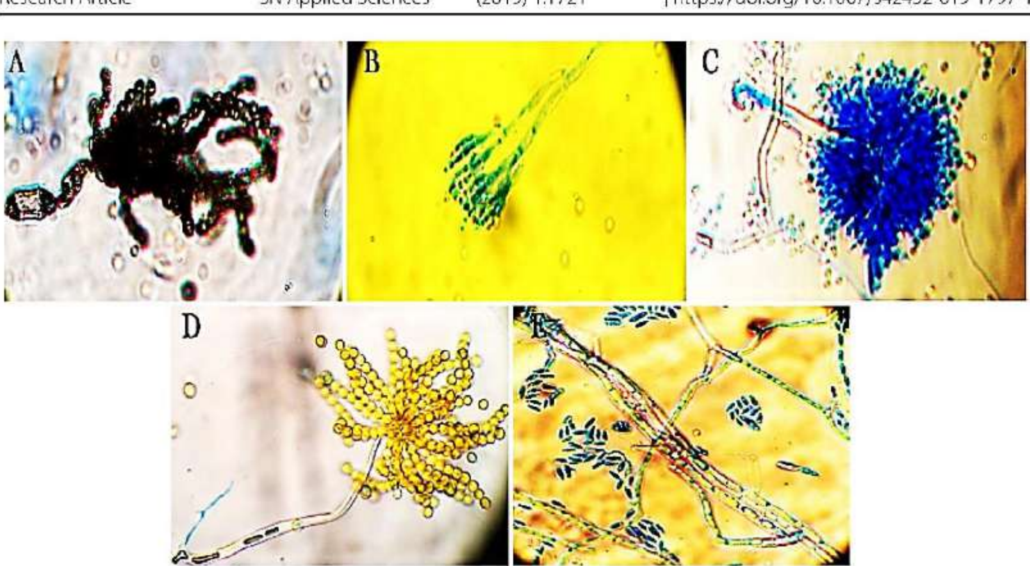


Fig. 3 Morphological appearance of fungal strains under optical microscope. **a** *A. flavus CECT 20802*, **b** *P. expansum CECT 2278*, **c** *A. parasiticus CBS 100926*, **d** *A. ochraceus NRRL 3174*, **e** *F. graminirium CECT 2150*

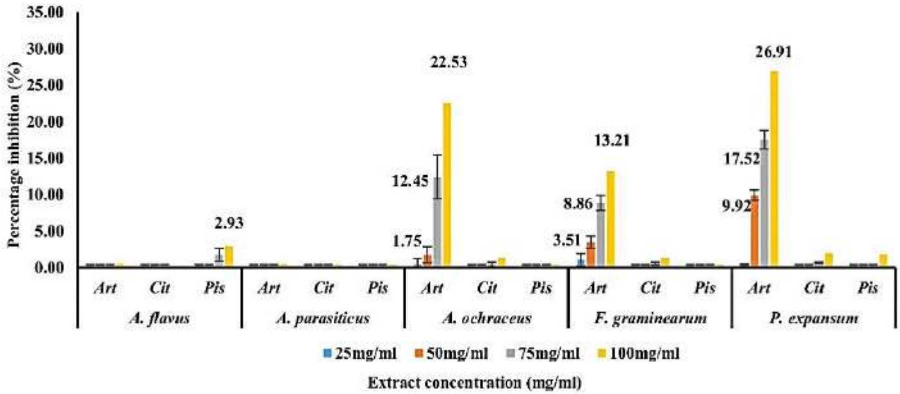


Fig. 4 Antifungal activity of aqueous extracts of the plant species. (*Art*: *A. heba-alba*, *Cit*: *C. colocynthis* and *Pis*: *P. lentiscus*)

inhibitory activity not exceeding 4% against *A. flavus*. A summary of the antifungal activity of plant extracts is shown in Table 2.

3.3 Antioxidant activity

The antioxidant activity of methanolic and aqueous extracts of the plants was determined in vitro by three techniques, namely, the ABTS, DPPH and FRAP assays. According to the results shown in Figs. 7, 8 and 9, the plant extracts exhibited remarkable antioxidant activity expressed in μM TE g^{-1}. The methanolic and aqueous extracts of *A. herba-alba* showed the highest antioxidant activity in the FRAP assay; their reducing powers were 6080.67 μM TE g^{-1} and 5315.11 μM TE g^{-1}, respectively. The antioxidant potentials of *P. lentiscus* leaves and *C. colocynthis* seeds measured by the same assay were lower in comparison with *A. herba-alba*, the values were 5300.48 μM TE g^{-1}, 4123.33 μM TE g^{-1} and

SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1 Research Article

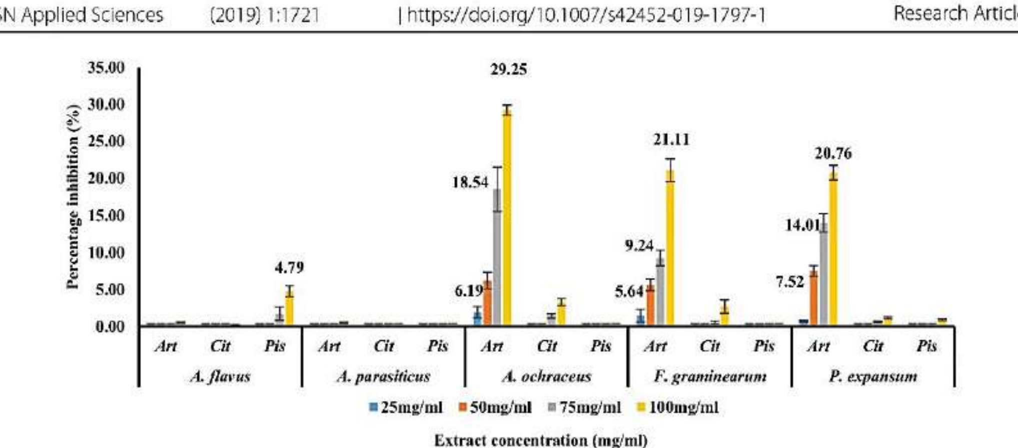
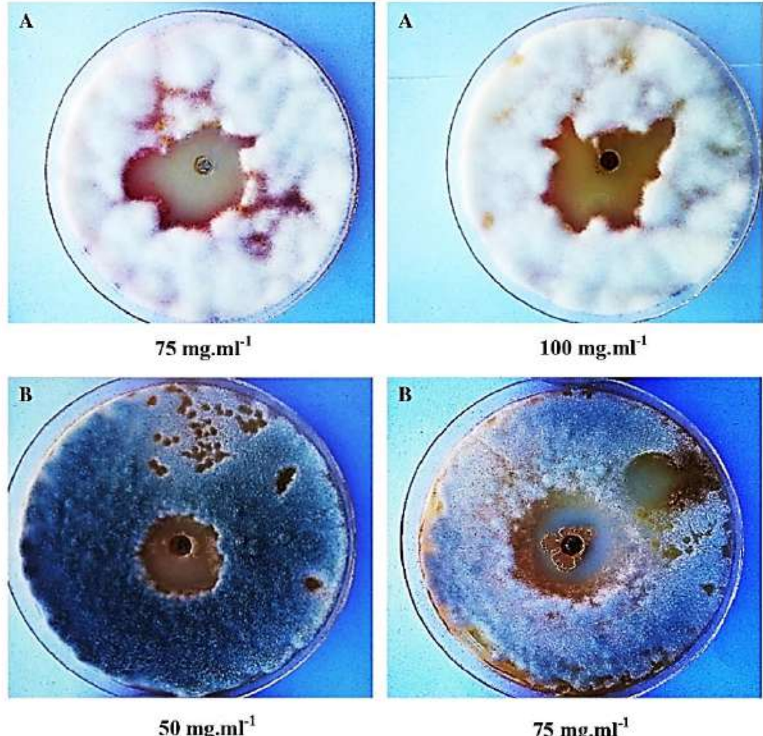


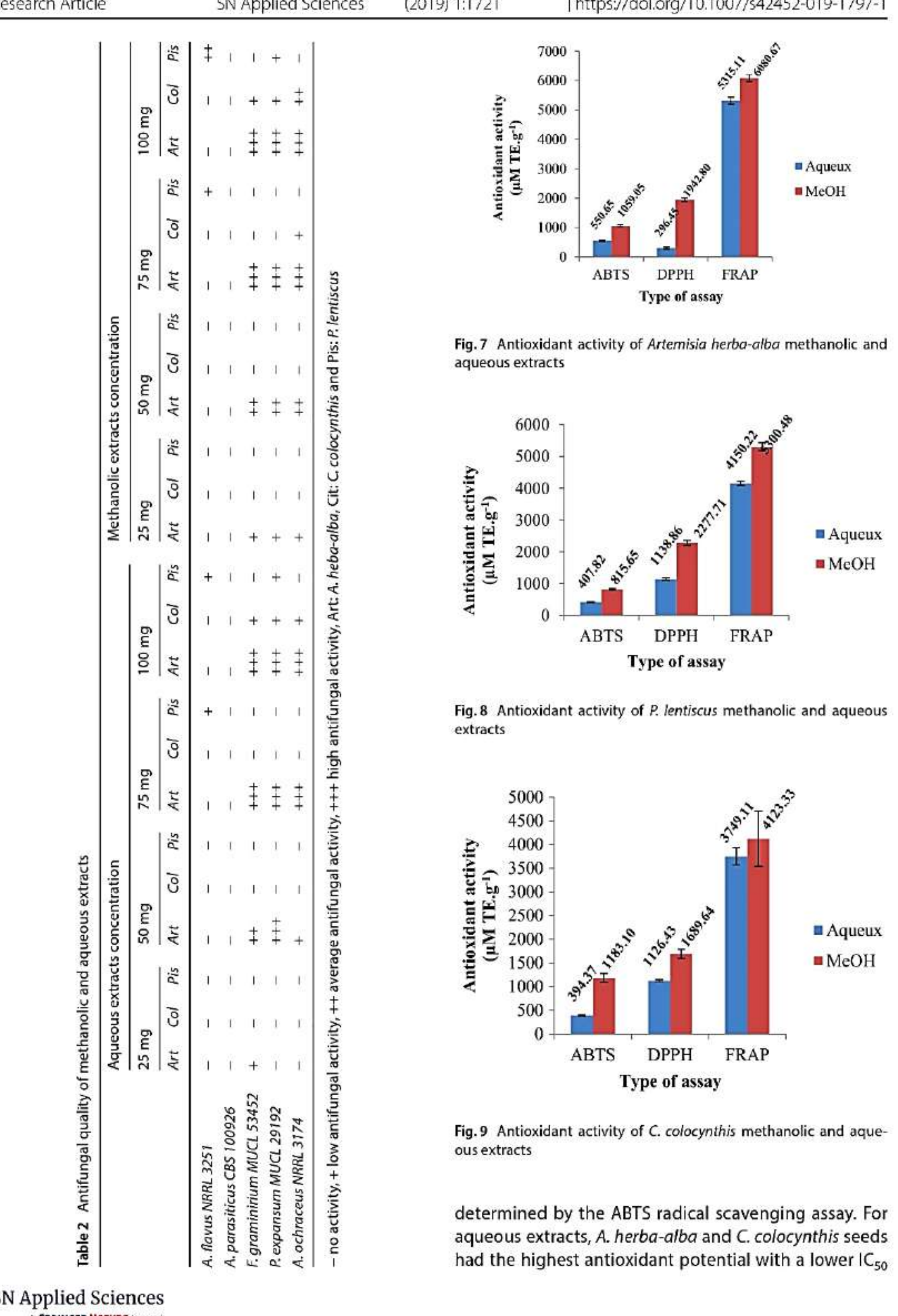
Fig. 5 Antifungal activity of methanolic extracts of the plant species. (*Art*: *A. heba-alba*, *Cit*: *C. colocynthis* and *Pis*: *P. lentiscus*)

Fig. 6 Antifungal activity of *A. herba-alba* methanolic extract. **a** *F. graminirium CECT 2150*, **b** *P. expansum CECT 2278*



4150.22 µM TE g^{-1}, 3749.11 µM TE g^{-1} for the methanolic and aqueous extracts, respectively in the FRAP assay.

For all extracts, the lowest IC_{50} were observed in the methanolic extracts of *A. herba-alba* in the FRAP assay, and IC_{50} values was 0.91 ± 0.92 mg ml^{-1} followed by the methanolic extracts of *P. lentiscus* leaves with IC_{50} 2.12 ± 0.49 measured by FRAP assay. The methanolic extracts of *C. colocynthis* seeds showed an IC_{50} values of 3.03 ± 0.51



Research Article SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1

Table 2 Antifungal quality of methanolic and aqueous extracts

	Aqueous extracts concentration												Methanolic extracts concentration											
	25 mg			50 mg			75 mg			100 mg			25 mg			50 mg			75 mg			100 mg		
	Art	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*
A. flavus NRRL 3251	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	++
A. parasiticus CBS 100926	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F. graminirium MUCL 53452	+	–	–	++	–	–	+++	–	–	+++	+	–	+	–	–	++	–	–	+++	–	–	+++	+	–
P. expansum MUCL 29192	–	–	–	+++	–	–	+++	–	–	+++	+	+	+	–	–	++	–	–	+++	–	–	+++	+	+
A. ochraceus NRRL 3174	–	–	–	+	–	–	+++	–	–	+++	+	–	+	–	–	++	–	–	+++	+	–	+++	++	–

– no activity, + low antifungal activity, ++ average antifungal activity, +++ high antifungal activity, Art: *A. heba-alba*, Cit: C. *colocynthis* and Pis: *P. lentiscus*

Fig. 7 Antioxidant activity of *Artemisia herba-alba* methanolic and aqueous extracts

Fig. 8 Antioxidant activity of *P. lentiscus* methanolic and aqueous extracts

Fig. 9 Antioxidant activity of *C. colocynthis* methanolic and aqueous extracts

determined by the ABTS radical scavenging assay. For aqueous extracts, *A. herba-alba* and *C. colocynthis* seeds had the highest antioxidant potential with a lower IC_{50}

SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1 Research Article

(2.42 ± 0.11 and 3.89 ± 0.44, respectively) followed by *P. lentiscus* leaves with IC_{50} values of 4.48 ± 0.52. However, the values of antioxidant activities evaluated by ABTS, DPPH and FRAP assays of all extracts were lower than the standard antioxidant used in this study (Trolox) (Table 3).

Variance analyses indicated that there are highly significant differences ($P < 0.01$) among the results of three antioxidant activity assays for the same species. Differences were also recorded among the three studied species ($P < 0.05$). The observed differences in the antioxidant activity among the different plant species is probably due to differences in their chemical composition. The differences among the assays seem to be due to the use of a different reaction mechanism by each assay.

4 Discussion

The fight against toxigenic fungi and mycotoxins is one of the difficulties of concern to scientists, and the results of extensive research in the field of mycotoxin toxicity are alarming and disturbing due to the health damage caused by mycotoxicosis and the economic losses caused by decreased food quality [41, 42]. As a result, researchers are abandoning conventional approaches and are exploring new biological control strategies against fungi and mycotoxicosis that include the use of medicinal plants [19, 20]. The three plants selected for this study were collected from semi-arid and arid regions of Algeria, which occupy more than 87% of the country's surface area. The desert regions are characterized by arid climates, in which rainfall is almost absent, with high temperatures, and in some areas of these desert regions, the climate is characterized by extreme aridity. In terms of flora, these arid and semi-arid areas are habitats for more than 2800 species [43], and this biodiversity is why these sites were chosen for plant sampling.

The study of the biological activities of aromatic and medicinal plants is based on extraction, and its principle, which is the crucial stage of any study, is that well-described precautions must be taken into consideration in order to avoid the loss, deformation or destruction of extracted molecules; in this case, the choice of solvent and extraction conditions depends on the targeted bioactive compound and it nature [44]. Regarding yield, the study of Barbouchi and his collaborators [45] demonstrated that the infusion extraction, or Soxhlet, method gave a higher yield without neglecting the nature of the solvent or the area where the leaves were harvested. Gupta and his team obtained a lower extraction yield compared to our yield for *C. colocynthis* seeds [46].

The organic extractions of this study were carried out by maceration, and the two solvents used were of polar

Table 3 IC_{50} of *P. lentiscus*, *A. herba-alba* and *C. colocynthis* methanolic and aqueous extracts obtained by FRAP, ABTS and DPPH assay

	ABTS				DPPH				FRAP			
	Art	Pis	Cit	Trolox*	Art	Pis	Cit	Trolox*	Art	Pis	Cit	Trolox
IC_{50} (mg ml^{-1}) of aqueous extract	6.74 ± 0.84	12.33 ± 1.29	5.33 ± 0.59	0.98 ± 0.1	7.58 ± 0.22	8.98 ± 1.34	3.89 ± 0.44	025 ± 0.03	2.42 ± 0.11	4.48 ± 0.52	5.12 ± 0.64	0.35 ± 0.04
IC_{50} (mg ml^{-1}) of methanolic extract	5.13 ± 0.63	9.78 ± 1.16	3.03 ± 0.51		3.27 ± 0.64	6.89 ± 0.88	3.23 ± 0.38		0.91 ± 0.92	2.12 ± 0.49	3.98 ± 0.5	

Research Article SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1

nature; consequently, the metabolites indentified were polar nature (alkaloids, flavonoids, tannins and saponins). In this context, Barbouchi's study demonstrated that organic extracts of *P. lentiscus* leaves are rich in catechic tannins, gallic tannins, flavonoids and saponins but not alkaloids [45]. Khlifi's study demonstrated that the hydromethanolic extract of *A. herba-alba* is rich in compound phenolics, flavonoids, tannins and anthocyanins [27]. Regarding the seeds of *C. colocynth*, Benariba et al. [47] revealed that catechic tannins and flavonoids are abundant in hydromethanol extracts, and according to Najafi et al. [48], the ethanolic extract contains tannins, alkaloids, flavonoids, and saponins. These differences in results may be attributed to differences in harvesting region, soil composition, climate, harvest season, solvents and experimental conditions of extraction.

In our assays, *A. herba-alba* exhibited the highest antifungal activity, followed in descending order by *C. colocynthis* seed and *P. lentiscus* leaf extracts. *F. graminearum*, *P. expansum* and *A. ochraceus* were the most sensitive moulds. *F. graminearum* is an opportunistic pathogen of cereals responsible for yield losses and contamination by its mycotoxins [49]. *P. expansum*, also known as blue mould, is responsible for the most important diseases of pome fruits and causes significant economic losses [50]. *A. ochraceus* is responsible for chronic granulomatous diseases [51]. *A. flavus and A. parasiticus* are known in mycology for their negative impact on health and for economic damage [41, 52]. In this study, these two fungi were the most resistant to the various prepared extracts.

Our results are in agreement with previous studies. *P. lentiscus* is considered to be an antimicrobial plant [53, 54], and ethanolic extracts of its fruits and leaves are used as ingredients in pork sausage to reduce microbial spoilage [55]. Some studies found that organic extracts of *C. colocynthis* are more active in comparison to aqueous extracts [47] and that hydromethanolic extracts are less toxic against *F. oxysporum* and its enzymes (CMCase, pectinase, and protease) [56] and exhibit a moderate antifungal activity against *A. fumigatus* DSM790 and *A. niger* DSM1988 [57].

The antifungal activities of the extracts are largely attributed to the solvent extraction capacity and the concentration of the active compounds in the crude extracts [58]. The sensitivity of microorganisms is due to the intolerance of cells to phytochemicals and their interactions with fungal cell components, which can cause, for example, the inhibition of cell wall formation, rupture of the cell membrane, mitochondria dysfunction, inhibition of cell division, inhibition of DNA replication, inhibition of protein synthesis or inhibition of efflux pumps [59]. However, the resistance of fungal strains may be due to degradation of bioactive compounds by fungal enzymes, removal of bioactive substances by efflux systems, insensitivity of the site targeted by the molecule [60] or by mutations in the genes encoding fungicide targets [61]. Tannins due to their structure interact with ergosterol and damage cell membranes [62]. Flavonoids and alkaloids are also known for their antimicrobial and spasmolytic activities [63, 64].

The complexity and multifunctionality of phytochemicals make the use of a single test insufficient to evaluate the antioxidant activity [65, 66]. The antioxidant activity assays carried out in this study made it possible to measure the capacities of the various bioactive molecules present in the crude extracts to donate an electron to a radical or to an ion, which leads to the inhibition or reduction of oxidants. The plant extracts prepared in this study showed good antioxidant potential in the three assays, and the ability of these extracts to reduce free radicals can be attributed to their phenolic and flavonoid compounds [67, 68], whose chemical structures and concentrations determine the antioxidant activity [69].

The results of the antioxidant activity assays in this study are consistent with those of a recent study done on *A. herba-alba* from another region [70], and another study supports our results and demonstrates that this plant reduces the oxidative damage induced by alloxan [71]. Khlifi et al. [27] reported that the hydromethanolic extract had IC_{50} values of 20.64 ± 0.84 mg ml^{-1} and 36.60 ± 1.03 mg ml^{-1} in the DPPH and ABTS assays, respectively.

Several other studies support our results and state that *P. lentiscus* has good antioxidant activity revealed by the DPPH and ABTS assays [72–75]. Barbouchi et al. [45] reported that the antioxidant activity of *P. lentiscus* leaves varies according to the location of harvest and observed IC_{50} values ranging from 0.50 ± 0.00 to 0.09 ± 0.00 mg ml^{-1} and from 0.57 ± 0.00 to 1.13 ± 0.00 mg ml^{-1} for the aqueous and methanolic extracts, respectively. Botsaris et al. [55] demonstrated that the methanolic extracts prevent lipid oxidation. Some authors showed that this activity is strongly due to the presence of gallic acid, 1,2,3,4,6-pentagalloyl glucose, 5-*O*-galloyl, 3,5-di-*O*-galloyl and 3,4,5-tri-*O*-galloyl and observed an IC_{50} equal to 3.9 µM [76, 77].

For *C. colocynthis*, several in vivo and in vitro studies reported good antioxidant activity in the fruits [47, 78]. Marzouk et al. [79] stated that the IC_{50} of aqueous seed extracts is between 0.020 and 0.021 mg ml^{-1} and attributed this difference to the different stages of fruit ripening. In contrast, Jayaraman and Christina [80] found that the maximum inhibition of DPPH radicals by the methanolic extract was approximately 62% at 0.8 mg ml^{-1}, whereas we observed only 57.11% of DPPH radical scavenging at 10 mg ml^{-1} methanolic extract. Tannin-Spitz et al. [81] reported that cucurbitacin B glucoside and cucurbitacin

E glucoside widely detected in *C. colocynthis* are powerful antioxidants.

5 Conclusion

Ethnopharmacological surveys based on scientific approaches and research oriented towards the exploitation of medicinal plants have been growing steadily in recent years due to pharmaceutical and medical interest in the bioactive substances isolated from these plants and characterized by less lethal side effects. These preliminary studies are of great importance in the development of plant-based pharmaceuticals and in the progression of green nanotechnology leading to the synthesis of new complex nanoparticles doped with bioactive substances active against multiresistant fungal strains. The results of the current study confirm that *Artemisia herba-alba*, *Pistacia lentiscus* and *Citrullus colocynthis* are potential sources of bioactive substances that could be used in the control of food contamination and of oxidative stress and the prevention of mycotoxicoses. Additional studies are in progress to characterize the structure of bioactive compounds and to elucidate their antifungal mechanisms in vitro by molecular docking.

Acknowledgements We are thankful to the researchers from the Laboratory of Ecosystems Protection in Arid and Semi-Arid Area, University of Kasdi Merbah-Ouargla, Algeria for their support during this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Milićević DR, Skrinjar M, Baltić T (2010) Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. Toxins 2(4):572–592
2. Hou YJ, Zhao YY, Xiong B, Cui XS, Kim NH, Xu YX, Sun SC (2013) Mycotoxin-containing diet causes oxidative stress in the mouse. PLoS ONE 8(3):e60374
3. Limonciel A, Jennings P (2014) A review of the evidence that ochratoxin A is an Nrf2 inhibitor: implications for nephrotoxicity and renal carcinogenicity. Toxins 6(1):371–379
4. Theumer MG, Henneb Y, Khoury L, Snini SP, Tadrist S, Canlet C, Puel O, Oswald IP, Audebert M (2018) Genotoxicity of aflatoxins and their precursors in human cells. Toxicol Lett 287:100–107
5. Mehrzad J, Malvandi AM, Alipour M, Hosseinkhani S (2017) Environmentally relevant level of aflatoxin B1 elicits toxic pro-inflammatory response in murine CNS-derived cells. Toxicol Lett 279:96–106
6. Gayathri L, Karthikeyan BS, Rajalakshmi M, Dhanasekaran D, Li AP, Akbarsha MA (2018) Metabolism-dependent cytotoxicity of citrinin and ochratoxin A alone and in combination as assessed adopting integrated discrete multiple organ co-culture (IdMOC). Toxicol In Vitro 46:166–177
7. Al-Anati L, Petzinger E (2006) Immunotoxic activity of ochratoxin A. J Vet Pharmacol Ther 29(2):79–90
8. Loiseau N, Debrauwer L, Sambou T, Bouhet S, Miller JD, Martin PG, Viadère JL, Pinton P, Puel O, Pineau T, Tulliez J, Galtier P, Oswald IP (2007) Fumonisin B1 exposure and its selective effect on porcine jejunal segment: sphingolipids, glycolipids and trans-epithelial passage disturbance. Biochem Pharmacol 74(1):144–152
9. Constable PD, Smith GW, Rottinghaus GE, Haschek WM (2000) Ingestion of fumonisin B1-containing culture material decreases cardiac contractility and mechanical efficiency in swine. Toxicol Appl Pharmacol 162(3):151–160
10. Magnussen A, Parsi MA (2013) Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol 19(10):1508–1512
11. Varga J, Kocsubé S, Péteri Z, Vágvölgyi C, Tóth B (2010) Chemical, physical and biological approaches to prevent ochratoxin induced toxicoses in humans and animals. Toxins 2(7):1718–1750
12. Calado T, Fernández-Cruz ML, Cabo Verde S, Venâncio A, Abrunhosa L (2018) Gamma irradiation effects on ochratoxin A: degradation, cytotoxicity and application in food. Food Chem 240:463–471
13. Štikarová J, Kotlín R, Riedel T, Suttnar J, Pimková K, Chrastinová L, Dyr JE (2013) The effect of reagents mimicking oxidative stress on fibrinogen function. Sci World J 2013:359621
14. Fatokun AA, Stone TW, Smith RA (2006) Hydrogen peroxide-induced oxidative stress in MC3T3-E1 cells: the effects of glutamate and protection by purines. Bone 39(3):542–551
15. Chang Q, Wang W, Regev-Yochay G, Lipsitch M, Hanage WP (2015) Antibiotics in agriculture and the risk to human health: how worried should we be? Evol Appl 8(3):240–247
16. Kohanski MA, Dwyer DJ, Wierzbowski J, Cottarel G, Collins JJ (2008) Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. Cell 135(4):679–690
17. Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CT, Lobritz MA, Braff D, Schwarz EG, Ye JD, Pati M, Vercruysse M, Ralifo PS, Allison KR, Khalil AS, Ting AY, Walker GC, Collins JJ (2014) Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci USA 111(20):E2100–E2109
18. Fink-Gremmels J (2008) The impact of mycotoxins in animal feeds. In: Leslie JF, Bandyopadhyay R, Visconti A (eds) Mycotoxins. Detection methods, management, public health and agricultural trade. CABI, Wallingford, pp 163–165
19. Owumi SE, Oloidi AC, Oloye CO, Oladeji OO, Obadare MO, Odunola OA (2015) Toxicological and phytoprotective effect of *Keayodendron bridelioides* and *Monodora myristica* extracts in Wister rats. Pharmacognosy Res 7(Suppl 1):S26–S33
20. Njoki LM, Okoth SA (2017) Wachira PM (2017) Effects of medicinal plant extracts and photosensitization on aflatoxin producing *Aspergillus flavus* (Raper and Fennell). Int J Microbiol 5:1–9
21. Reddy KRN, Muralidharan K, Reddy CS (2009) Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control 20(2):173–178
22. Elsherbiny EA, El-Khateeb AY, Azzaz NA (2016) Chemical composition and fungicidal effects of *Ocimum basilicum* essential oil on *Bipolaris* and *Cochliobolus* species. J Agric Sci Technol 18(4):1143–1152
23. Barghamdi B, Ghorat F, Asadollahi K, Sayehmiri K, Peyghambari R, Abangah G (2016) Therapeutic effects of *Citrullus colocynthis*

fruit in patients with type II diabetes: a clinical trial study. J Pharm Bioallied Sci 8(2):130–134
24. Gowri SS, Priyavardhini S, Vasantha K, Umadevi M (2009) Antibacterial activity on *Citrullus colocynthis* Leaf extract. Anc Sci Life 29(1):12–13
25. Chowdhury K, Sharma A, Kumar S, Gunjan GK, Nag A, Mandal CC (2017) Colocynth extracts prevent epithelial to mesenchymal transition and stemness of breast cancer cells. Front Pharmacol 8:593
26. Moufid A, Eddouks M (2012) *Artemisia herba alba*: a popular plant with potential medicinal properties. Pak J Biol Sci 15(24):1152–1159
27. Khlifi D, Sghaier RM, Amouri S, Laouini D, Hamdi M, Bouajila J (2013) Composition and anti-oxidant, anti-cancer and anti-inflammatory activities of *Artemisia herba-alba*, *Ruta chalpensis L.* and *Peganum harmala L.* Food Chem Toxicol 55:202–208
28. Zeggwagh NA, Michel JB, Eddouks M (2014) Acute hypotensive and diuretic activities of *Artemisia herba- alba* aqueous extract in normal rats. Asian Pac J Trop Biomed 4(2):S644–S648
29. Bozorgi M, Memariani Z, Mobli M, Salehi Surmaghi MH, Shams-Ardekani MR, Rahimi R (2013) Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): a review of their traditional uses, phytochemistry, and pharmacology. Sci World J 2013:219815
30. Telli A, Esnault MA, Khelil OEH (2016) An ethnopharmacological survey of plants used in traditional diabetes treatment in south-eastern Algeria (Ouargla province). J Arid Environ 127:82–92
31. Jasso de Rodriguez D, Hernández-Castillo D, Rodriguez-Garcia R, Angulo-Sánchez JL (2005) Antifungal activity in vitro of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. Ind Crops Prod 21(1):81–87
32. Senhaji O, Faid M, Elyachioui M, Dehhaoui M (2005) Antifungal activity of different cinnamon extracts. J Med Mycol 15(4):220–229
33. Al-Daihan S, Al-Faham M, Al-shawi N, Almayman R, Brnawi A, Zargar S, Bhat RS (2013) Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. J King Saud Univ 25(2):115–120
34. Aylmore RC, Todd NK (1984) A microculture chamber and improved method for combined light and electron microscopy of filamentous fungi. J Microbiol Methods 2(6):317–322
35. Magnusson J, Schnürer J (2001) *Lactobacillus coryniformis subsp.* coryniformis strain si3 produces a broad-spectrum proteinaceous antifungal compound. Appl Environ Microbiol 67(1):1–5
36. Espinel-Ingroff A, Cantón E (2007) Antifungal susceptibility testing of filamentous fungi. In: Schwalbe R, Steele-Moore L, Goodwin AC (eds) Antimicrobial susceptibility testing protocols, 1st edn. CRC Press, Boca Raton, pp 209–241
37. Surveswaran S, Cai YZ, Corke H, Sun M (2007) Systemic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem 102(3):938–953
38. Orphanides A, Goulas V, Gekas V (2013) Effect of drying method on the phenolic content and antioxidant capacity of spearmint. Czech J Food Sci 31(5):509–513
39. Liharaka Kidaha M, Alakonya AE, Nyende AB (2013) Bioactivity determination of methanol and water extracts for roots and leaves of Kenyan *Psidium guajava L* landraces against pathogenic bacteria. Springerplus 2:670
40. Seo J, Lee S, Elam ML, Johnson SA, Kang J, Arjmandi BH (2014) Study to find the best extraction solvent for use with guava leaves (*Psidium guajava L.*) for high antioxidant efficacy. Food Sci Nutr 2(2):174–180
41. Gacem MA, Ould El Hadj-Khelil A (2016) Toxicology, biosynthesis, bio-control of aflatoxin and new methods of detection. Asian Pac J Trop Biomed 6(9):808–814
42. Mitchell NJ, Bowers E, Hurburgh C, Wu F (2016) Potential economic losses to the US corn industry from aflatoxin contamination. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 33(3):540–550
43. Halla N, Boucherit K, Boucherit Otmani Z, Touati FZ, Rahmani N, Aid I (2018) *Ammodaucus leucotrichus* and *Citrullus colocynthis* from Algerian Sahara: Ethnopharmacological application, phytochemical screening, polyphenols content and antioxidant activity of hydromethanolic extracts. J King Saud Univ Sci. https://doi.org/10.1016/j.jksus.2018.03.018
44. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 8(1):1–10
45. Barbouchi M, Elamrani K, El Idrissi M, Choukrad M (2018) A comparative study on phytochemical screening, quantification of phenolic contents and antioxidant properties of different solvent extracts from various parts of *Pistacia lentiscus* L. J King Saud Univ Sci. https://doi.org/10.1016/j.jksus.2018.05.010
46. Gupta SC, Tripathi T, Paswan SK, Agarwal AG, Rao CV, Sidhu OP (2018) Phytochemical investigation, antioxidant and wound healing activities of *Citrullus colocynthis* (bitter apple). Asian Pac J Trop Biomed 8(8):418–424
47. Benariba N, Djaziri R, Bellakhdar W, Belkacem N, Kadiata M, Malaisse WJ, Sener A (2013) Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. Asian Pac J Trop Biomed 3(1):35–40
48. Najafi S, Sanadgol N, Sadeghi NB, Ashofteh BM, Sanadgol E (2010) Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus*. J Med Plants Res 4(22):2321–2325
49. Boedi S, Berger H, Sieber C, Münsterkötter M, Maloku I, Warth B, Sulyok M, Lemmens M, Schuhmacher R, Güldener U, Strauss J (2016) Comparison of *Fusarium graminearum* transcriptomes on living or dead wheat differentiates substrate-responsive and defense-responsive genes. Front Microbiol 7:1113
50. Sánchez-Torres P, Vilanova L, Ballester AR, López-Pérez M, Teixidó N, Viñas I, Usall J, González-Candelas L, Torres R (2018) Unravelling the contribution of the *Penicillium expansum* PeSte12 transcription factor to virulence during apple fruit infection. Food Microbiol 69:123–135
51. Moazam S, Denning DW (2017) *Aspergillus nodules* in chronic granulomatous disease attributable to *Aspergillus ochraceus*. Med Mycol Case Rep 17:31–33
52. Sánchez E, Heredia N, García S (2005) Inhibition of growth and mycotoxin production of *Aspergillus flavus* and *Aspergillus parasiticus* by extracts of Agave species. Int J Food Microbiol 98(3):271–279
53. Mezni F, Aouadhi C, Khouja ML, Khaldi A, Maaroufi A (2015) *In vitro* antimicrobial activity of *Pistacia lentiscus* L. edible oil and phenolic extract. Nat Prod Res 29(6):565–570
54. Bonifácio BV, dos Santos Ramos MA, da Silva PB, Bauab TM (2014) Antimicrobial activity of natural products against *Helicobacter pylori*: a review. Ann Clin Microbiol Antimicrob 13:54
55. Botsaris G, Orphanides A, Yiannakou E, Gekas V, Goulas V (2015) Antioxidant and antimicrobial effects of *Pistacia lentiscus* L. extracts in pork sausages. Food Technol Biotechnol 53(4):472–478
56. Mohamed MSM, Saleh AM, Abdel-Farid IB, El-Naggar SA (2017) Growth, hydrolases and ultrastructure of *Fusarium oxysporum* as affected by phenolic rich extracts from several xerophytic plants. Pestic Biochem Physiol 141:57–64
57. Eidi S, Azadi HG, Rahbar N, Mehmannavaz HR (2015) Evaluation of antifungal activity of hydroalcoholic extracts of *Citrullus colocynthis* fruit. J Herb Med 5(1):36–40

SN Applied Sciences (2019) 1:1721 | https://doi.org/10.1007/s42452-019-1797-1 Research Article

58. Iloki-Assanga SB, Lewis-Luján LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL, Haines DD (2018) Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. BMC Res Notes 8:396
59. Lagrouh F, Dakka N, Bakri Y (2017) The antifungal activity of Moroccan plants and the mechanism of action of secondary metabolites from plants. J Med Myc 27(3):303–311
60. Morrissey JP, Osbourn AE (1999) Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol Mol Biol Rev 63(3):708–724
61. Deising HB, Reimann S, Pascholati SF (2008) Mechanisms and significance of fungicide resistance. Braz J Microbiol 39(2):286–295
62. Lopes G, Pinto E, Andrade PB, Valentão P (2013) Antifungal activity of phlorotannins against dermatophytes and yeasts: approaches to the mechanism of action and influence on *Candida albicans* virulence factor. PLoS ONE 8(8):e72203
63. Panche AN, Diwan AD, Chandra SR (2017) Flavonoids: an overview. J Nutr Sci 5:e47
64. Khan H, Mubarak MS, Amin S (2017) Antifungal potential of alkaloids as an emerging therapeutic target. Curr Drug Targets 18(16):1825–1835
65. Škrovánková S, Mišurcová L, Machů L (2012) Antioxidant activity and protecting health effects of common medicinal plants. Adv Food Nutr Res 67:75–139
66. Ortega-Ramirez LA, Rodriguez-Garcia I, Leyva JM, Cruz-Valenzuela MR, Silva-Espinoza BA, Gonzalez-Aguilar GA, Siddiqui W, Ayala-Zavala JF (2014) Potential of medicinal plants as antimicrobial and antioxidant agents in food industry: a hypothesis. J Food Sci 79(2):R129–R137
67. Pourreza N (2013) Phenolic compounds as potential antioxidant. Jundishapur J Nat Pharm Prod 8(4):149–150
68. Huyut Z, Beydemir Ş, Gülçin İ (2017) Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. Biochem Res Int 2017:1–10
69. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 22(3):296–302
70. Dif MM, Benali Toumi F, Boukaaza H, Mokaddem F, Benyahia M, Bouazza S (2018) Phenolic content and antioxidant activity of *Artemisa herba-alba*, A medicinal plant from Algerian arid zone. Phytothérapie 16(2):91–95
71. Sekiou O, Boumendjel M, Taibi F, Boumendjel A, Messarah M (2018) Mitigating effects of antioxidant properties of *Artemisia herba alba* aqueous extract on hyperlipidemia and oxidative damage in alloxan-induced diabetic rats. Arch Physiol Biochem 125(2):163–173
72. Papada E, Forbes A, Amerikanou C, Torović L, Kalogeropoulos N, Tzavara C, Triantafillidis JK, Kaliora AC (2018) Antioxidative efficacy of a *Pistacia Lentiscus* supplement and its effect on the plasma amino acid profile in inflammatory bowel disease: a randomised, double-blind, placebo-controlled trial. Nutrients 10(11):pii: 1779
73. Yemmen M, Landolsi A, Ben Hamida J, Mégraud F, Trabelsi Ayadi M (2017) Antioxidant activities, anticancer activity and polyphenolics profile, of leaf, fruit and stem extracts of *Pistacia lentiscus* from Tunisia. Cell Mol Biol (Noisy-le-grand) 63(9):87–95
74. Belhachat D, Aid F, Mekimene L, Belhachat M (2017) Phytochemical screening and in vitro antioxidant activity of *Pistacia lentiscus* berries ethanolic extract growing in Algeria. Med J Nutr Metab 10(2):273–285
75. Beghlal D, El Bairi K, Marmouzi I, Haddar L, Mohamed B (2016) Phytochemical, organoleptic and ferric reducing properties of essential oil and ethanolic extract from *Pistacia lentiscus* (L.). Asian Pac J Trop Dis 6(4):305–310
76. Abdelwahed A, Bouhlel I, Skandrani I, Valenti K, Kadri M, Guiraud P, Steiman R, Mariotte AM, Ghedira K, Laporte F, Dijoux-Franca MG, Chekir-Ghedira L (2007) Study of antimutagenic and antioxidant activities of gallic acid and 1,2,3,4,6-pentagalloylglucose from *Pistacia lentiscus*. Confirmation by microarray expression profiling. Chem Biol Interact 165(1):1–13
77. Baratto MC, Tattini M, Galardi C, Pinelli P, Romani A, Visioli F, Basosi R, Pogni R (2003) Antioxidant activity of galloyl quinic derivatives isolated from *P. lentiscus* leaves. Free Radic Res 37(4):405–412
78. Vakiloddin S, Fuloria N, Fuloria S, Dhanaraj SA, Balaji K, Karupiah S (2015) Evidences of hepatoprotective and antioxidant effect of *Citrullus colocynthis* fruits in paracetamol induced hepatotoxicity. Pak J Pharm Sci 28(3):951–957
79. Marzouk Z, Marzouk B, Mahjoub MA, Haloui E, Mighri Z, Aouni M, Fenina N (2010) Screening of the antioxidant and the free radical scavenging potential of Tunisian *Citrullus colocynthis* Schrad. from Mednine. J Food Agric Environ 8(2):261–265
80. Jayaraman R, Christina AJM (2013) Evaluation of *Citrullus colocynthis* fruits on in vitro antioxidant activity and in vivo DEN/PB induced hepatotoxicity. Int J Appl Res Nat Prod 6(1):1–9
81. Tannin-Spitz T, Bergman M, Grossman S (2007) Cucurbitacin glucosides: antioxidant and free-radical scavenging activities. Biochem Biophys Res Commun 364(1):181–186

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

II.2. Effets antimicrobiens et antioxydants d'une actinobactérie forestière V_{002} en tant que nouvelle productrice de spectinabiline, undécylprodigiosine et métacycloprodigiosine.

II.2. Effets antimicrobiens et antioxydants d'une actinobactérie forestière V_{002} en tant que nouvelle productrice de spectinabiline, undécylprodigiosine et métacycloprodigiosine.

«Antimicrobial and Antioxidant Effects of a Forest Actinobacterium V_{002} as New Producer of Spectinabilin, Undecylprodigiosin and Metacycloprodigiosin»

(Article publié en Mai 2020 dans la revue Current Microbiology - Springer Nature).

Le second chapitre se concentre sur l'identification des *Streptomyces* cultivables isolées deux de site de prélèvement différents. Les sites d'échantillonnage choisis pour cette étude n'ont pas été étudiés précédemment. L'un des sites est situé dans la forêt d'El-Ogbane de la ville de Saida localisé dans la région steppique du pays, alors que le second site est situé en plein désert. En effet, l'adaptation des actinobactéries aux conditions environnementales biotiques et abiotiques sont les atouts à l'origine du choix des sites.

Dans cette partie du projet, au total, 110 isolats distincts ont été obtenues des sites de prélèvement. Les isolats sont sélectionnés sur la base des caractéristiques morphologiques et culturels des actinobactéries. Les isolats obtenus de la forêt d'El-Ogbane sont marqués de V_{000} à V_{053}, cependant, ceux isolés de la région désertique sont marqué ON_{000} à ON_{055}. Tous les isolats sont conservés à -80°C dans 50% de glycérol pour les tests ultérieurs.

Après le screening antimicrobien préliminaire, un seul isolat à savoir V_{002} est sélectionné pour la poursuite de ce travail. La caractérisation phénotypique de l'isolat V_{002} a démontré que ce dernier est Gram positive, aérobie avec un mycélium aérien et culturel. La microscopie électronique a démontré que le mycélium aérien de la souches désignée V_{002} était différencié par des spores complètement mûres en longues chaînes droites et flexibles. Les spores étaient en forme de bâtonnet avec une surface rigoureuse et non mobile (**Figure 1. Matériel supplémentaire. Article 2**). L'identification phénotypique sur les milieux ISP a démontré que V_{002} possède une bonne croissance sur les milieux GYM, ISP3, ISP4, ISP5, ISP7, SSM+T et SSM-T. Une croissance modérée est notée sur les milieux ISP2 et ISP6. L'aptitude de la croissance et la couleur des mycéliums aériens et de substrat de V_{002} sur les milieux ISP sont décrites dans le tableau 1 et la figure 1 (**Matériel supplémentaire. Article 2**).

L'isolat V_{002} a démontré une tolérance significative à NaCl et une capacité enzymatique lui permettant de dégrader l'ensemble de la gamme de glucides sélectionnés (**Tableau 2, Figure 1. Matériel supplémentaire. Article 2**). La détermination de l'activité enzymatique par l'utilisation des plaques d'Api Zym® et d'Api Coryne® a dévoilé un potentiel enzymatique élevée chez V_{002} (**Tableau 3, Figure 1. Matériel supplémentaire. Article 2**). La caractérisation génotypique par séquençage de l'ADN 16s de l'isolat V_{002} a démontré que la souche appartenait au genre *Streptomyces* (**Figure 1, Article 2**). La séquence du gène de l'ARNr 16S de la souche V_{002} est déposé dans la base NCBI sous le numéro d'accès MH298058.

L'évaluation de l'activité antimicrobienne des extraits brutes obtenus sur les microplaques a démontré que l'extrait obtenu à partir du milieu 5294 ($Ext_{5294.V002}$) était le plus active. Les souches pathogènes à Gram positive à savoir *Staphylococcus aureus* Newman et *Micrococcus luteus* étaient les plus sensibles (**Tableau 1. Article 2**). Le fractionnement par HPLC et l'analyse LC-MS a dévoilé que l'$Extrait_{5294.V002}$ est formé de trois composés à savoir spectinabiline, undécylprodigiosine et métacycloprodigiosine, cependant, seul undécylprodigiosine et métacycloprodigiosine étaient les plus active (**Figure 2, Figure 3. Matériel supplémentaire. Article 2**).

L'analyse de l'activité antioxydant de l'$Ext_{5294.V002}$ évalué par le test ABTS, DPPH et FRAP a démontré que l'extrait renferme un bon pouvoir antioxydant (**Tableau 2. Matériel supplémentaire. Article 2**). Les résultats du test de FRAP n'ont pas été obtenus.

Il est a noté que les extraits $Ext_{5294.V002}$ et l'$Ext_{5254.V002}$ non démontré aucune activité antifongique sur les deux souches fongiques (*Aspergillus flavus* et *Aspergillus ochraceus*) isolées du blé tendre. Par ailleurs, les mêmes extraits non démontré aucune activité antifongique sur les souches fongiques de références à savoir *Aspergillus flavus* CECT 20802, *Aspergillus parasiticus* CBS 100926, *Fusarium. graminirium* CECT 2150, *Aspergillus ochraceus* NRRL 3174 et *Penicillium expansum* CECT 2278.

Afin de combattre la résistance bactérienne aux antibiotiques, la recherche de l'aptitude des actinobactéries à synthétiser des composées bioactives est une excellente plateforme pour la recherche. Les résultats de notre travail ont confirmé la possibilité d'isoler des actinobactéries possédant des caractéristiques et des capacités à produire des substances bioactives rechercher. L'isolat V_{002} est considéré comme nouveau producteur de la spectinabiline, undécylprodigiosine et métacycloprodigiosine ce qui confirme l'intérêt biotechnologique de la souche.

Current Microbiology
https://doi.org/10.1007/s00284-020-02007-1

Antimicrobial and Antioxidant Effects of a Forest Actinobacterium V_{002} as New Producer of Spectinabilin, Undecylprodigiosin and Metacycloprodigiosin

Mohamed Amine Gacem[1,2,3] · Aminata Ould-El-Hadj-Khelil[2] · Badreddine Boudjemaa[3] · Joachim Wink[1]

Received: 4 December 2019 / Accepted: 24 April 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

The aim of the study is the research and identification of a *Streptomyces* strain as a new producer of spectinabilin, undecylprodigiosin and metacycloprodigiosin. Among 54 actinomycete isolates isolated from El-Ogbane forest soils in Algeria, only one isolate, designated V_{002}, was selected for its ability to produce prodigiosins. The selected strain was analysed for its ability to produce three different secondary metabolites as well as their biological activities. V_{002} belongs to the *Streptomyces* genus and has significant antimicrobial and antioxidant activities. The taxonomic position of V_{002} by 16S rRNA sequence analysis showed a similarity of 99.93% with *Streptomyces lasiicapitis* DSM 103124^T and 98.96% with *Streptomyces spectabilis* DSM 40512^T. Fractionation of crude secondary metabolites produced by the strain using HPLC–MS revealed the presence of spectinabilin, undecylprodigiosin and metacycloprodigiosin, which demonstrated significant activity. Strain V_{002} is considered a new producer of spectinabilin, undecylprodigiosin and metacycloprodigiosin with significant antimicrobial and antioxidant activity.

Introduction

The identification of antibiotics was a wondrous innovation and discovery that humanity recognized upon its appearance, but the flame of this miracle was quickly extinguished just after the appearance of antimicrobial resistance in pathogens [1]; moreover, the phenomenon of bio-resistance has not ceased to develop and spread in microorganisms [2]. In confronting this serious problem, cancer constitutes another challenge that humanity has tried to conquer, although its pathology is perhaps more serious following the failure of its eradication because of cytotoxicity of chemicals used during treatment against normal cells [3]. These disturbing situations have pushed researchers to leave classical research platforms to pursue other new strategies based on the detection of new microbial strains, in particular *Actinobacteria* strains producing new bioactive compounds [4]. New actinobacterial strains can be isolated from marine [5], desertic [6] and glacier soil ecosystems [7], or volcanic fumaroles [8]. Following this strategy, several new bioactive compounds have been discovered in recent years, citing for example α-pyrone [9], the polyhydroxyl macrolide lactones PM100117 and PM100118 [10], novonestmycins A and B [11] and venturicidin C [12].

Other previously discovered substances, in particular spectinabilin, undecylprodigiosin and metacycloprodigiosin, are well known for their biological activities. Spectinabilin is a rare polyketide metabolite, and its chemical structure is substituted by a nitrophenyl; spectinabilin was isolated for the first time from a crude streptovaricin complex produced by *Streptomyces spectabilis* [13]. Undecylprodigiosin and metacycloprodigiosin are red pigments, belonging to the prodigiosins; these compounds are produced by certain species of the *Streptomyces* genus, such as *S. coelicolor* A3 and *S. longisporus ruber* M-3 [14, 15]. Given the importance and the attractive activities of these three compounds, some

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00284-020-02007-1) contains supplementary material, which is available to authorized users.

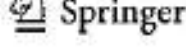
✉ Mohamed Amine Gacem
biologicamine@yahoo.fr

[1] Department of Microbial Strain Collection, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

[2] Laboratory of Ecosystems Protection in Arid and Semi-Arid Area, University of Kasdi Merbah, 30000 Ouargla, Algeria

[3] Department of Biology, Faculty of Science, University of Amar Tlidji, 03000 Laghouat, Algeria

Published online: 05 May 2020



researchers tried to develop techniques using plug-and-play scaffolding in order to reactivate the silencing gene cluster for spectinabilin in *Streptomyces orinoci* [16].

Algeria is the largest country in Africa, and the Mediterranean Sea is the limit of the country in the north, while the Sahara Desert is the limit in the south. Between these two borders, the climate is complex and ranges from wet to arid. This study aims to isolate and identify strains belonging to the *Streptomyces* genus as new producers of spectinabilin, undecylprodigiosin and metacycloprodigiosin from forest soils located in a semi-arid zone in western Algeria.

Materials and Methods

Sampling Location

Soil samples were taken from El-Ogbane forest (34° 49′ 04.9″ N 0° 09′ 26.5″ E) located in the west of the country in Saida city. The samples were taken from sedimentary lands on the banks of the forest river. After removing stones, tree and root debris, the samples were dried in the open air, then crushed with a mortar and kept in plastic containers.

Isolation and Purification of Actinomycetes

Isolation of actinobacteria was performed by the standard dilution method [17]. First, one gram of soil sample was aseptically diluted in 9 ml of sterile distilled water (10^{-1}), followed by agitation of the liquid for a few minutes, and a series of decimal dilutions (10^{-2}, 10^{-3} and 10^{-4}) was then generated. An aliquot of 100 µl was spread on the surface of starch casein nitrate agar medium (SCNA supplemented with nalidixic acid (20 mg l^{-1}) and nystatin (50 mg l^{-1})) at the rate of three plates for each dilution [18, 19]. The plates were finally incubated at 30 °C for 5 to 10 days. According to the morphological and cultural characteristics, typical colonies were selected and then sub-cultured on GYM agar medium and incubated at 30 °C for 5 to 10 days [20]. Once the aerial mycelium was formed, the isolates were labelled and stored at − 80 °C in 50% glycerin.

Determination of Culture, Physiological, Biochemical and Microscopic Characteristics of Typical Strains

To identify and compare the isolates from different strains, a series of tests was carried out. Culture characteristics such as colony colour, aerial mycelium production and soluble pigment synthesis were revealed on ISP medium (International Streptomyces Project) by flooding GYM medium, yeast extract-malt extract agar (ISP_2), oatmeal agar (ISP_3), inorganic salt–starch agar (ISP_4), glycerol-asparagine agar (ISP_5), peptone-yeast extract iron agar (ISP_6), tyrosine agar (ISP_7), SSM + T and SSM − T medium with two drops of a fresh culture of the selected isolates [20–22]. After growth, the ISCC-NBS colour chart was used to determine the colour of the colonies, aerial mycelium and soluble pigment. Growth at different temperatures (5 to 40 °C, with intervals of 5 °C) was resolved in ISP_3 agar over 15 days of incubation. Growth at different pH values was revealed in GYM broth (glucose 1%, yeast extract 1%, $K_2HPO_4 \cdot 3H_2O$ 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, weight/volume) over a pH range from 4 to 12 (with intervals of 1.0 pH unit) followed by incubation at 20 °C for 15 days in a rotary shaker [23].

The use of carbohydrates (glucose, arabinose, sucrose, xylose, inositol, mannitol, fructose, raffinose, cellulose and rhamnose) as a single carbon source was determined on 5338 medium ($(NH_4)2SO_4$, KH_2PO_4, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, agar and trace element solution 5342), which is a basic medium. Incubation was carried out at 30° C for 10 to 15 days [20]. Growth on different concentrations of NaCl (0 to 10%, with intervals of 2.5%) was performed on 5339 medium (casein peptone, yeast extract and agar) as a basic medium. Incubation was carried out at 30 °C for 10 to 15 days. Enzymatic characteristics were revealed on Api Zym® and Api Coryne® plates [24–26] according to the protocol accompanying the kits. The preparation of the strains selected for scanning electron microscopy was carried out on agar containing a bacterial lawn fixed in glutaraldehyde using cultures after growth for 4 weeks at 30 °C on ISP_3 medium [27].

Genomic DNA Extraction, Amplification, Purification and Sequencing of the 16S rRNA Gene

Genomic DNA extraction was performed by using 0.5 ml of liquid culture using a DNA extraction kit (Stratec Molecular, Invisorb Spin plant, mini Kit, Berlin, Germany) according to the supplied protocol. Verification of the DNA extraction efficiency was performed by agarose gel electrophoresis (0.8% of agarose gel, 70 V, 400 mA, 40 min). Once the genomic DNA was extracted, amplification of 16S rRNA was carried out in a thermocycler (Eppendorf, Mastercycler gradient) using two universal primers (27F: 5′-AGT TTGATCCTGGCTCAG-3′ and 1492R: 5′-ACGGCTACC TTGTTACGACTT-3′) [28]. Cleanup was then carried out using a cleanup kit (Macherey–Nagel, Nucleo Spin, gel and PCR clean up, Düran, Germany). The cleanup of PCR products was confirmed by electrophoresis according to the same previous conditions. Sequencing of PCR products was performed using five primers, 27F, 518R, 1100F, 1100R and 1492R, at the DSMZ (Braunschweig. Germany). The obtained DNA sequences were viewed and edited by Geneouis software V7. The 16S rRNA sequence similarities between the strains were calculated by pairwise

alignment using the EzTaxon-e server [29]. Phylogenetic trees were produced with the maximum likelihood [30] and neighbour-joining algorithms [31] using Mega software V7 [32]. The stability of the topology of the phylogenetic trees was evaluated by the 1000 repetition bootstrap method [33]. A distance matrix was generated using Kimura's model of two parameters [34]. All positions with gaps and missing data were eliminated.

Extraction of Secondary Metabolites and Screening of Biological Activities

Screening of Antimicrobial Activity of Crude Extracts

Screening of the antimicrobial activity of crude extracts was accomplished for several pathogenic bacteria and fungi, specifically three microscopic fungi, Gram-negative bacteria and Gram-positive bacteria. All selected pathogenic strains were obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and ATCC (American Type Culture Collection, Manassas, VA 20110, USA).

To prepare the crude extracts, 20 ml of ethyl acetate (Sigma-Aldrich, USA) was mixed with 20 ml of bacterial culture from the previously prepared selected isolate (culture incubated under agitation for 10 days in two different liquid media, namely: 5294 and 5254). The mixtures were agitated for 12 min, and then the media were separated by centrifugation at 9000 rpm for 10 min. The solvent containing the metabolites was subsequently transferred to a rotavapor (Heidolph, Germany) in order to evaporate it completely. Once evaporation was completely achieved, the weight of the extract is calculated and then recovered in 1 ml of methanol.

The measurement of antimicrobial activity was carried out by microdilution in 96-well microplates. Three liquid culture media were used: Mueller–Hinton (MH), Mycosel (Myc) and Middlebrook (Mid). The choice of medium was determined for each of the pathogenic strains, for which the concentration adjustment was set to 0.01 McFarland units. The dose of extract in the well (A) of the first line corresponded to 1 µl of extract from 14 µl of inoculated culture medium. By successive dilution, the concentration decreased twice in the wells (B–H). The results were obtained by visual observation after 24 and 48 h of incubation at different temperatures [35].

Screening of the Antioxidant Activity of Crude Extracts

ABTS Radical Scavenging Assay To measure the antioxidant capacity of the crude active extract by $ABTS^{\cdot+}$ radical cation inhibition, the method described by Surveswaran et al. [36] was applied. $ABTS^{\cdot+}$ generation was carried out by a chemical reaction between ABTS solution (7 mM) and potassium persulfate solution (2.45 mM) for 16 h in obscurity and at room temperature. The resulting solution ($ABTS^{\cdot+}$) was diluted in methanol (in a ratio of 1/50) in order to obtain an absorbance of 0.700 ± 0.005 at a wavelength of 734 nm. A concentration of 3.75 µg μl^{-1} of crude extract was prepared at different volumes (25, 50, 75, 100 µl) and was reacted with 3.9 ml of $ABTS^{\cdot+}$ solution, and methanol was added to achieve the same volume for all prepared solutions (4 ml). After 10 min of reaction, the absorbance was measured by a spectrophotometer set at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard antioxidant at a concentration range of 0–500 µM. Neutralization of the $ABTS^{\cdot+}$ was calculated according to the following formula:

$$\text{Percentage of } ABTS^{\cdot+} \text{ radical scavenging} = |\text{absorbance of control}| - |\text{absorbance of sample}| / |\text{absorbance of control}| \times 100.$$

DPPH Radical Scavenging Assay The antiradical activity of the crude extracts was determined by a $DPPH^{\cdot}$ radical scavenging assay according to the method adopted by Orphanides et al. [37]. A volume of 3.9 ml of $DPPH^{\cdot}$ solution (0.3 mM) was mixed with 3.75 µg μl^{-1} of crude extract dissolved in methanol at different volumes (25, 50, 75, 100 µl) and incubated for 30 min in obscurity and at room temperature. Methanol volumes were added to achieve the same volume for all prepared solutions (4 ml). The absorbances were then measured by a spectrophotometer set at 517 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard antioxidant at a concentration range of 0–500 µM. The neutralization of the $DPPH^{\cdot}$ radical was calculated according to the following formula:

$$\text{Percentage of } DPPH^{\cdot} \text{ radical scavenging} = |\text{absorbance of control}| - |\text{absorbance of sample}| / |\text{absorbance of control}| \times 100.$$

Fractionation of Crude Extracts by HPLC and LC–MS

Only the extracts showing good antimicrobial potency were selected for HPLC fractionation. In this study, the HPLC used was an Agilent 1100, with an X-Bridge C18 3.5 µm, 2.1 × 100 mm Column (Waters, Milford, USA) ,and a DAD detector (200–400 nm). Initial pressure was adjusted at 33 bar, and monitoring of the wavelength was

located between 210 and 360 nm. The liquid phase was composed of two elution buffers: A1 (950 ml of H_2O, 50 ml of C_2H_3N + 0.05 mM (385 mg l^{-1}) $C_2H_7NO_2$ + 40 µl of $C_2H_4O_2$) and B2 (50 ml of H_2O, 950 ml of C_2H_3N, 0.05 mM (385 mg l^{-1}) $C_2H_7NO_2$ + 40 µl of $C_2H_4O_2$). After 40 min, the fractions were recovered in a 96-well microplate at a rate of 150 µl per well. Once fractionation was completely achieved, a chromatogram showing the position of the fractions present in the extract was obtained from the HPLC. All obtained fractions were evaporated in a MiniVap (Porvair Sciences, UK) under heated nitrogen (40 °C). After drying the microplates, each well was filled with 150 µl of inoculum containing pathogen adjusted to 0.01 McFarland. After incubation for 24–48 h, the results were determined by visual observation.

To identify the active fractions, HPLC results were compared with those obtained from LC–MS; the instrument used was an LC–MS Agilent 1200 with a DAD detector (200–600 nm) combined with a maXis mass spectrometer (UHR-TOF, Bruker Daltonics, USA). The column was a Waters Acquity UPLC BEH C18 (2.1×50 mm, 1.7 µm). The mobile phase was composed of two solvents: A (H_2O with 0.1% CH_2O_2) and B (CH_3CN with 0.1% CH_2O_2) with a flow rate of 0.6 ml min^{-1}. The equilibration time between samples was 5 min [35].

Results

Selection of Isolated Strains

A total of approximately 54 distinct isolates, depending on the colony morphology and pigmentation characteristics of *Actinobacteria*, were isolated by the dilution method on SCNA medium supplemented with nalidixic acid and nystatin. All isolates were labelled from V_{000} to V_{053} and then stored at − 80 °C in 50% glycerol. In the present study, only one isolate (V_{002}) was selected due to its particular cultural characteristics, indicating suspected production of prodigiosins.

Phenotypic Characterization of Isolates

The isolate V_{002} was observed to be Gram positive, aerobic with abundant mycelium and well developed in the substrate. The aerial mycelium was typical of that of the *Streptomyces* genus. Scanning electron microscopy allowed visualization of the complex architecture of the isolate, and several morphological characteristics related to the colonies were observed: the aerial mycelia was differentiated by fully matured spores into long chains that were straight and flexible, and the spores were in stick form with a rigorous and non-motile surface. Complete septation of the air chains led to a collapse of mature spores (Fig. 1 in Supplementary Material).

The isolate V_{002} showed good growth on GYM, ISP_3, ISP_4, ISP_5, ISP_7, SSM+T ,and SSM-T medium. Moderate growth was observed on ISP_2 and ISP_6. The colour of the aerial mass varied between yellow orange and red violet. No diffusible pigment or melanin was observed in the medium. The growth and colour of the aerial and substrate mycelia of V_{002} on ISP medium are described in Table 1 and Fig. 1 (Supplementary Material). The optimum temperature and pH for mycelial growth were 30 °C and 7.0, respectively. V_{002} showed significant growth in the absence and presence of 2.5 and 5% NaCl, respectively, and possessed important genetic material that allowed the strain to degrade the whole range of carbohydrates as a single source of carbon (Table 2 and Fig. 1 Supplementary Material).

The enzymatic activity determined by the use of Api Zym® and Api Coryne® showed significant enzymatic potential; V_{002} exhibited high enzymatic activities in the presence of leucin arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, phosphatase acid, naphtyl-AS-BI-phosphohydrolase, phosphatase alcaline, beta galactosidase, alpha glucosidase, N-acetyl-beta-glucoseamidase and alpha mannosidase. The enzymatic activity of nitrate reductase, pyraziamidase, beta glucuronidase, esculin and urease was almost negative. V_{002} was unable to ferment sugars; however, it was able to hydrolyse gelatine (Table 3 and Fig. 1 Supplementary Material).

Genotypic Characterization of V_{002}

EzTaxon-e analysis of 16S rRNA gene sequences demonstrated that the bacterial isolate labelled V_{002} should be classified in the *Streptomyces* genus. The analysis also showed that the sequence of V_{002} (1509 bp) had high similarity to *Streptomyces lasiicapitis* DSM 103124^T (99.93%, 1/1494) and *Streptomyces spectabilis* DSM 40512^T (98.96%, 15/1444). A phylogenetic analysis of maximum likelihood confirmed the results (Fig. 1). The 16S rRNA gene sequence of V_{002} is deposited under accession number MH298058.

Evaluation of Antibacterial Activity and Determination of Bioactive Compounds

According to the results presented in Table 1, $Ext_{5294.V002}$ presented low antimicrobial activity (A–B) against *C. violaceum*, *M. smegmatis* and *P. anomala*, minimum inhibitory concentrations (MIC) were 0.125, 0.125 and 0.25 µg $µl^{-1}$, respectively. An average antimicrobial activity (C–F) was registered against *B. subtilis*, whereas high activity was registered against *S. aureus* Newman and *M. luteus*. Minimum inhibitory concentrations were 0.781 × 10^{-2}, 0.195 × 10^{-2} and 0.195 × 10^{-2} µg $µl^{-1}$, respectively. $Ext_{5254.V002}$ exhibited moderate activity against *B. subtilis*. The active extract ($Ext_{5294.V002}$) was subjected to HPLC fractionation and LC–MS analysis to determine which compounds were active.

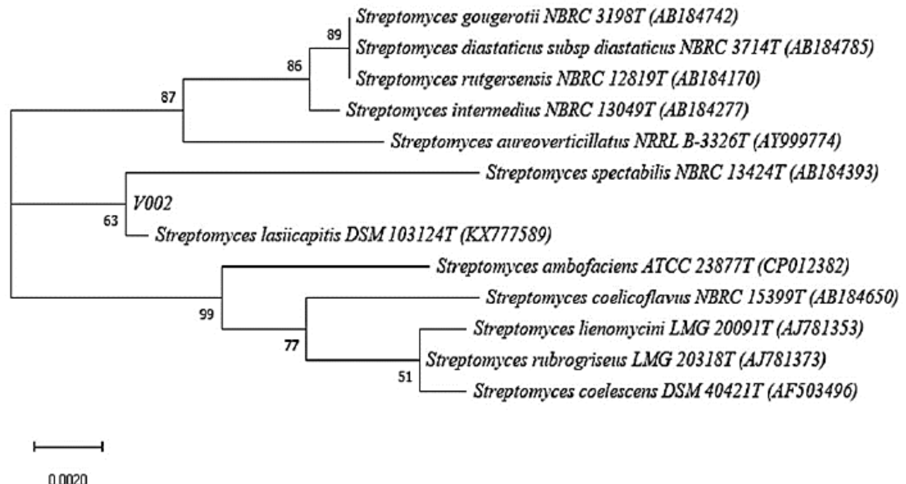


Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain V_{002} and the related taxa. Numbers at nodes are bootstrap values (percentages of 1000 replications); only values > 50% are shown. Asterisks indicate branches that were also recovered in the maximum likelihood tree. Bar, 0.0020 nucleotide substitutions per site

Table 1 Screening of the antimicrobial activity of crude extracts ($Ext_{5254.V002}$ and $Ext_{5294.V002}$)

	Dilution stages (A–H)[a] and MIC			
	Strain V_{002}			
	Ext_{5254}	Ext_{5294}	MIC_{5254} (μg μl^{-1})	MIC_{5294} (μg μl^{-1})
E. coli DSM1116	–	–	–	–
E. coli TolC	–	–	–	–
Chromobacterium violaceum DSM 30191	–	B	–	0.125
Pseudomonas aeruginosa PA14	–	–	–	–
Staphylococcus aureus Newman	–	H	–	0.195×10^{-2}
Micrococcus luteus DSM 1790	–	H	–	0.195×10^{-2}
Mycobacterium smegmatis ATCC 700084	–	B	–	0.125
Mucor hiemalis DSM 2656	–	–	–	–
Pichia anomala DSM 6766	–	A	–	0.25
Candida albicans DSM 1665	–	–	–	–
Bacillus subtilis DSM 10	D	F	0.312×10^{-1}	0.781×10^{-2}

MIC, minimum inhibitory concentration; –, no activity

[a]The dilution steps represent the successive dilution of crude extracts from well B until well H

The range of the inhibited wells after fractionation of $Ext_{5294.V002}$ revealed that the fractions exerted strong antimicrobial activity against *S. aureus*, *M. luteus*, *B. subtilis* and *C. violaceum* For *B. subtilis*, the correlation test between the peak activity of $Ext_{5294.V002}$ after HPLC fractionation (Fig. 2 Supplementary Material) and the LC–MS chromatogram (Fig. 3 Supplementary Material) revealed that the active fractions were obtained between 21.5 and 34 min, and the LC–MS data suggested that the peaks correlated with spectinabilin, undecylprodigiosin and metacycloprodigiosin. For *M. luteus* and *S. aureus*, peak data from HPLC and LC–MS revealed that active fractions were located between retention time 26.5 and 33.5 min, which suggested that metacycloprodigiosin was the active fraction against these pathogens. For *C. violaceum*, peak data from HPLC and LC–MS revealed that the active fractions were obtained between 24 and 25 min, which suggested that spectinabilin was the active fraction.

Table 2 Antioxidant activity of crude extracts obtained from $Ext_{S294.V002}$

Extract volume (μg μl^{-1})	Antioxidant activity of $Ext_{S294.V002}$					
	DPPH˙ radical scavenging			ABTS˙+ radical scavenging		
	Antioxidant activity (%)	IC_{50} (μg μl^{-1})	IC_{50}-trolox (μg μl^{-1})	Antioxidant activity (%)	IC_{50} (μg μl^{-1})	IC_{50}-trolox (μg μl^{-1})
0.023	7.39 ± 0.41	0.834 ± 0.04	0.25 10^{-3} ± 0.03	71.48 ± 1.30	5.8 10^{-3} ± 0.001	0.98 10^{-3} ± 0.1
0.046	13.24 ± 1.43			96.25 ± 0.82		
0.070	18.06 ± 0.36			96.14 ± 0.25		
0.093	25.45 ± 0.29			97.43 ± 0.87		

IC_{50}, the half maximal inhibitory concentration

Antioxidant Activity of Crude Extracts

The antioxidant potential of $Ext_{S294.V002}$ as revealed by DPPH˙ and ABTS˙+ radical assays is presented in Table 2. The figure clearly shows that the volume range between 25 and 100 μl had significant antioxidant activity, which was visible by a change in the solution colour. The percentage of ABTS˙+ radical scavenging ranged from 71.48 ± 1.30% to 97.43 ± 0.87%. The ABTS˙+ radical assay showed consistent activity between 50 to 100 μl. The percentage of DPPH˙ radical scavenging ranged from 7.39 ± 0.41% to 25.45 ± 0.29%.

Discussion

Microorganisms of the genus *Streptomyces* are considered potential producers of secondary metabolites with interesting biological activities and beneficial effects on human health. The research and exploitation of *Streptomyces* from unexplored environments is one of the most effective approaches for discovering new bioactive metabolites [28]. *Streptomyces* sp. V_{002} was isolated from El-Ogbane forest, located in the semi-arid zone in Algeria; this forest contains plants and trees with muddy soils in winter and high temperatures in summer. The river that divides the forest creates a unique environment that contains a diversity of flora, freshwater sediments and microorganisms.

The search for new strains producing spectinabilin and prodigiosins requires reliable screening and identification. Despite the specificity of the 16S rRNA region, a dendrogram showed that *Streptomyces* sp. V_{002} had a high concordance with the grouping and topology of *S. lasiicapitis* and *S. spectabilis*; however, the comparison of cultural and biochemical characteristics revealed the dissimilarity of both types of strains. LC–MS results demonstrated that *Streptomyces* sp. V_{002} isolated from the sedimentary lands on the banks of the forest river was considered a new producer of spectinabilin, metacycloprodigiosin and undecylprodigiosin; however, *S. lasiicapitis* identified by Ye produced kanchanamycin [38], and *S. spectabilis* produced spectinabilin and metacycloprodigiosin [13, 39].

Only a few compounds containing nitro groups are known and among them is spectinabilin which is a rare polyketide metabolite substituted with a nitrophenyl group; also, few studies have unveiled the antimicrobial activity of spectinabilin, metacycloprodigiosin and undecylprodigiosin [40]. Evaluation of the antimicrobial activity of secondary metabolites of crude and fractionated extracts revealed good inhibitory activity. Spectinabilin possesses significant biological activities against *P. falciparum* K1 [39], and this compound also has significant nematocidal activity against *Bursaphelenchus xylophilus*, with an LC_{50} equal to 0.84 μg ml^{-1} [41]. Spectinabilin isolated from *Streptomyces* sp. ZQ4BG demonstrated suppression of *C. albicans* with an MIC of 12.5 μg ml^{-1} [42]. The antimicrobial activity of undecylprodigiosin was confirmed in the study of Stankovic and his team, in which the compound was able to inhibit the growth of *M. luteus* and *B. subtilis* at a concentration of 50 μg ml^{-1}, while for *C. albicans* ATCC 10231 and *C. albicans* ATCC 10259, the inhibition concentrations were 100 and 200 μg ml^{-1}, respectively [43]. In the study of Zainal Abidin et al., undecylprodigiosin demonstrated strong antibacterial activities against *S. aureus*, *B. subtilis* and *C. albicans*, with algicidal activity against *A. minutum* and *P. bahamense* [44]. Metacycloprodigiosin is known for its anti-malaria activity [39], and it also induces cell death in β-catenin-mutated tumour cells [45]. Metacycloprodigiosin and undecylprodigiosin possess anticancer activity against several human cancer cell lines (P388, HL60, A-549, BEL-7402 et SPCA4) [46].

The complexity and multifunctionality of bioactive compounds in an extract make it difficult to choose a single assay to detect antioxidant activity, although the analyses performed by DPPH˙ and ABTS˙+ radical scavenging assays are robust and simple to perform [28]. In this study, both assays were used for a preliminary screening to determine the antioxidant capacity of the crude extract. The antioxidant reactions were measured by a hydrogen atom transfer or an election transfer on probe molecules [47]. The most active crude extracts against selected

microorganisms revealed good antioxidant potential, and these results may be pursued by further research to determine which compound is more active than the others and to develop important products. The radical scavenging activity of the extract was proportionally related to the compositions of secondary metabolites and the concentration of bioactive compounds, which was corroborated by Tan and his team, who said that antioxidant activity correlates with content for bioactive compounds [28].

Several studies reported that extracts from *Streptomyces* provided antioxidant potential; Raghava Rao and Raghava Rao [48] demonstrated that *Streptomyces* isolated from mangrove soil of the Visakhapatnam region was endowed with antioxidant activity, while another study confirmed our findings and stated that ethyl acetate extract obtained from *Streptomyces* sp. AM-S1 possessed antioxidant potential against DPPH· and $ABTS^{·+}$ with IC_{50} values of 90.2 and 13.2 µl ml^{-1}, respectively [49]. *Streptomyces* V_{002} is recognized as a good source of antioxidants. With the results obtained, these antioxidants may prevent the progression of various disorders related to oxidative stress, and they have good potential to avoid cell damage resulting from a redox imbalance following the rise of $O_2^{·-}$ products after exceeding the cell defence capacity [28]. Undecylprodigiosin has demonstrated its antioxidant activity against the oxidation of linoleic acid [43], and it can also exert gastroprotective effects and attenuate induced gastric lesions via antioxidant and anti-inflammatory mechanisms by decreasing the levels of inflammatory mediators and apoptotic markers [50].

Adaptation of microorganisms of the El-Ogbane forest to climatic and environmental conditions allows them to develop metabolic capacities and to synthesize prodigious compounds that allow them to survive in this forest ecosystem. In this study, the ability of *Streptomyces* sp. V_{002} isolated from sedimentary lands on the banks of the forest river to produce spectinabilin, undecylprodigiosin and metacycloprodigiosin was confirmed. Previously published results strongly suggest that the three substances could be selected as important lead molecules for the development of chemotherapy treatment. The present study has demonstrated the antimicrobial and antioxidant activities of the three molecules, which clarifies their importance with the producing strain. In-depth investigation of the underlying mechanism of the antimicrobial effect of spectinabilin, undecylprodigiosin and metacycloprodigiosin would be valuable in the future.

Acknowledgements We are thankful to the researchers and technician from microbial strain collection department, Helmholtz Centre for Infection Research for their support during this work.

Author Contributions MAG carried out experimental work. MAG and BB co-wrote the manuscript. AOHK and JW conceived and designed the study. All authors contributed to interpretation of results, read and approved the final draft.

Compliance with Ethical Standards

Conflict of interest We declare that we have no conflict of interest.

References

1. Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol 74:417–433
2. Friedman ND, Temkin E, Carmeli Y (2016) The negative impact of antibiotic resistance. Clin Microbiol Infect 22:416–422. https://doi.org/10.1016/j.cmi.2015.12.002
3. McLachlan A, Kekre N, McNulty J, Pandey S (2005) Pancratistatin: a natural anti-cancer compound that targets mitochondria specifically in cancer cells to induce apoptosis. Apoptosis 10:619–630. https://doi.org/10.1007/s10495-005-1896-x
4. Mahajan GB, Balachandran L (2012) Antibacterial agents from actinomycetes—a review. Front Biosci 4:240–253. https://doi.org/10.2741/373
5. Hassan SS, Anjum K, Abbas SQ, Akhter N, Shagufta BI, Shah SA, Tasneem U (2017) Emerging biopharmaceuticals from marine actinobacteria. Environ Toxicol Pharmacol 49:34–47. https://doi.org/10.1016/j.etap.2016.11.015
6. Yang ZW, Salam N, Mohany M, Chinnathambi A, Alharbi SA, Xiao M, Hozzein WN, Li WJ (2018) *Microbacterium album* sp. nov. and *Microbacterium deserti* sp. nov., two halotolerant actinobacteria isolated from desert soil. Int J Syst Evol Microbiol 68:217–222. https://doi.org/10.1099/ijsem.0.002485
7. Hauptmann AL, Stibal M, Bælum J, Sicheritz-Pontén T, Brunak S, Bowman JS, Hansen LH, Jacobsen CS, Blom N (2014) Bacterial diversity in snow on North Pole ice floes. Extremophiles 18:945–951. https://doi.org/10.1007/s00792-014-0660-y
8. Medrano-Santillana M, Souza-Brito EM, Duran R, Gutierrez-Corona F, Reyna-López GE (2014) Bacterial diversity in fumarole environments of the Paricutín volcano, Michoacán (Mexico). Extremophiles 21(3):499–511. https://doi.org/10.1007/s00792-017-0920-8
9. Yang N, Song F (2018) Bioprospecting of novel and bioactive compounds from marine actinomycetes isolated from south china sea sediments. Curr Microbiol 75:142–149. https://doi.org/10.1007/s00284-017-1358-z
10. Pérez M, Schleissner C, Fernández R, Rodríguez P, Reyes F, Zuñiga P, de la Calle F, Cuevas C (2016) PM100117 and PM100118, new antitumor macrolides produced by a marine *Streptomyces caniferus* GUA-06-05-006A. J Antibiot 69:388–394. https://doi.org/10.1038/ja.2015.121
11. Wan Z, Fang W, Shi L, Wang K, Zhang Y, Zhang Z, Wu Z, Yang Z, Gu Y (2015) Novonestmycins A and B, two new 32-membered bioactive macrolides from *Streptomyces phytohabitans* HBERC-20821. J Antibiot 68:185–190. https://doi.org/10.1038/ja.2014.123
12. Shaaban KA, Singh S, Elshahawi SI, Wang X, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS (2014) Venturicidin C, a new 20-membered macrolide produced by *Streptomyces* sp. TS-2-2. J Antibiot 67:223–230
13. Kakinuma K, Hanson CA, Rinehart Jr KL (1976) Spectinabilin, a new nitro-containing metabolite isolated from *Streptomyces spectabilis*. Tetrahedron 32:217–222. https://doi.org/10.1016/0040-4020(76)87004-4
14. Sevcikova B, Kormanec J (2004) Differential production of two antibiotics of *Streptomyces coelicolor* A3(2), actinorhodin and

undecylprodigiosin, upon salt stress conditions. Arch Microbiol 181:384–389. https://doi.org/10.1007/s00203-004-0669-1
15. Wasserman HH, Rodgers GC, Keith DD (1969) Metacycloprodigiosin, a tripyrrole pigment from *Streptomyces longisporus* ruber. J Am Chem Soc 91:1263–1264. https://doi.org/10.1021/ja01033a065
16. Shao Z, Rao G, Li C, Abil Z, Luo Y, Zhao H (2013) Refactoring the silent spectinabilin gene cluster using a plug-and-play scaffold. ACS Synth Biol 2:662–669
17. Valanarasu M, Duraipandiyan V, Agastian P, Ignacimuthu S (2009) *In vitro* antimicrobial activity of *Streptomyces* from Western Ghats rock soil (India). J Mycol Med 19:22–28. https://doi.org/10.1016/j.mycmed.2008.12.002
18. Tanasupawat S, Jongrungruangchok S, Kudo T (2010) *Micromonospora marina* sp. nov., isolated from sea sand. Int J Syst Evol Microbiol 60:648–652. https://doi.org/10.1099/ijs.0.014068-0
19. Songsumanus A, Tanasupawat S, Thawai C, Suwanborirux K, Kudo T (2011) *Micromonospora humi* sp. nov., isolated from peat swamp forest soil. Int J Syst Evol Microbiol 61:1176–1181. https://doi.org/10.1099/ijs.0.024281-0
20. Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340. https://doi.org/10.1099/00207713-16-3-313
21. Kämpfer P, Glaeser SP, Parkes L, van Keulen G, Dyson P (2014) The family Streptomycetaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The prokaryotes, 4th edn. Springer, Berlin, pp 889–1010
22. Suter MA (1978) Isolierung von Melanin-negativen Mutanten aus *Streptomyces glaucescens*, thesis no. 6276. Eidgenossische Technische Hochschule, Switzerland
23. Jia F, Liu C, Wang X, Zhao J, Liu Q, Zhang J, Gao R, Xiang W (2013) *Wangella harbinensis* gen. nov., sp. nov., a new member of the family *Micromonosporaceae*. Antonie Van Leeuwenhoek 103:399–408. https://doi.org/10.1007/s10482-012-9820-1
24. MacFaddin JF (2000) Biochemical tests for the identification of medical bacteria, 3rd edn. Lippincott Williams & Wilkins, Philadelphia
25. Humble MW, King A, Phillips I (1977) API ZYM: a simple rapid system for the detection of bacterial enzymes. J Clin Pathol 30:275–277. https://doi.org/10.1136/jcp.30.3.275
26. Kilian M (1978) Rapid identification of *Actinomycetaceae* and related bacteria. J Clin Microbiol 8:127–133
27. Wink J (2002) Polyphasic taxonomy and antibiotic formation in some closely related genera of the family *Pseudonocardiaceae*. Recent Res Dev Antibiot 2:97–140
28. Tan LT, Chan KG, Khan TM, Bukhari SI, Saokaew S, Duangjai A, Pusparajah P, Lee LH, Goh BH (2017) *Streptomyces* sp. MUM212 as a source of antioxidants with radical scavenging and metal chelating properties. Front Pharmacol 8:276
29. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. https://doi.org/10.1099/ijs.0.038075-0
30. Kim SB, Brown R, Oldfield C, Gilbert SC, Iliarionov S, Goodfellow M (2000) *Gordonia amicalis* sp. nov., a novel dibenzothiophene-desulphurizing actinomycete. Int J Syst Evol Microbiol 50:2031–2036. https://doi.org/10.1099/00207713-50-6-2031
31. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
32. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197
33. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x
34. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120. https://doi.org/10.1007/BF01731581
35. Charousová I, Medo J, Hleba L, Javoreková S (2018) *Streptomyces globosus* DK15 and *Streptomyces ederensis* ST13 as new producers of factumycin and tetrangomycin antibiotics. Braz J Microbiol 49:816–822
36. Surveswaran S, Cai YZ, Corke H, Sun M (2007) Systemic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem 102:938–953. https://doi.org/10.1016/j.foodchem.2006.06.033
37. Orphanides A, Goulas V, Gekas V (2013) Effect of drying method on the phenolic content and antioxidant capacity of spearmint. Czech J Food Sci 31:509–513
38. Ye L, Zhao S, Li Y, Jiang S, Zhao Y, Li J, Yan K, Wang X, Xiang W, Liu C (2017) *Streptomyces lasiicapitis* sp. nov., an actinomycete that produces kanchanamycin, isolated from the head of an ant (*Lasius fuliginosus* L.). Int J Syst Evol Microbiol 67:1529–1534. https://doi.org/10.1099/ijsem.0.001756
39. Isaka M, Jaturapat A, Kramyu J, Tanticharoen M, Thebtaranonth Y (2002) Potent *in vitro* antimalarial activity of metacycloprodigiosin isolated from *Streptomyces spectabilis* BCC 4785. Antimicrob Agents Chemother 46:1112–1113
40. Choi YS, Johannes TW, Simurdiak M, Shao Z, Lu H, Zhao H (2010) Cloning and heterologous expression of the spectinabilin biosynthetic gene cluster from *Streptomyces spectabilis*. Mol Biosyst 6:336–338. https://doi.org/10.1039/b923177c
41. Liu MJ, Hwang BS, Jin CZ, Li WJ, Park DJ, Seo ST, Kim CJ (2019) Screening, isolation and evaluation of a nematicidal compound from actinomycetes against the pine wood nematode, *Bursaphelenchus xylophilus*. Pest Manag Sci 75:1585–1593. https://doi.org/10.1002/ps.5272
42. Wang W, Song T, Chai W, Chen L, Chen L, Lian XY, Zhang Z (2017) Rare polyene-polyol macrolides from mangrove-derived *Streptomyces* sp. ZQ4BG. Sci Rep 7:1703
43. Stankovic N, Radulovic V, Petkovic M, Vuckovic I, Jadranin M, Vasiljevic B, Nikodinovic-Runic J (2012) *Streptomyces* sp. JS520 produces exceptionally high quantities of undecylprodigiosin with antibacterial, antioxidative, and UV-protective properties. Appl Microbiol Biotechnol 96:1217–1231. https://doi.org/10.1007/s00253-012-4237-3
44. Zainal Abidin ZA, Ahmad A, Latip J, Usup G (2016) Marine *Streptomyces* sp. UKMCC_PT15 producing undecylprodigiosin with algicidal activity. J Teknologi 78:55–60
45. Ikeda H, Shikata Y, Watanapokasin R, Tashiro E, Imoto M (2017) Metacycloprodigiosin induced cell death selectively in β-catenin-mutated tumor cells. J Antibiot 70:109–112. https://doi.org/10.1038/ja.2016.75
46. Liu R, Cui CB, Duan L, Gu QQ, Zhu WM (2005) Potent *in vitro* anticancer activity of metacycloprodigiosin and undecylprodigiosin from a sponge-derived actinomycete *Saccharopolyspora* sp. nov. Arch Pharm Res 28:1341–1344. https://doi.org/10.1007/BF02977899
47. Apak R, Özyürek M, Güçlü K, Çapanoğlu E (2016) Antioxidant activity/capacity measurement. 2. Hydrogen atom transfer (HAT)-based, mixed-mode (electron transfer (ET)/HAT), and lipid peroxidation assays. J Agric Food Chem 64:1028–1045. https://doi.org/10.1021/acs.jafc.5b04743
48. Raghava Rao KV, Raghava Rao TJ (2013) Molecular characterization and its antioxidant activity of a newly isolated *Streptomyces coelicoflavus* BC 01 from mangrove soil. J Young Pharm 5(4):121–126

49. Sowndhararajan K, Kang SC (2013) Evaluation of in vitro free radical scavenging potential of *Streptomyces* sp. AM-S1 culture filtrate. Saudi J Biol Sci 20:227–233. https://doi.org/10.1016/j.sjbs.2012.12.003
50. Abdelfattah MS, Elmallah MIY, Ebrahim HY, Almeer RS, Eltanany RMA, Abdel Moneim AE (2019) Prodigiosins from a marine sponge-associated actinomycete attenuate HCl/ethanol-induced gastric lesion via antioxidant and anti-inflammatory mechanisms. PLoS ONE 14:e0216737

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary material

Figure

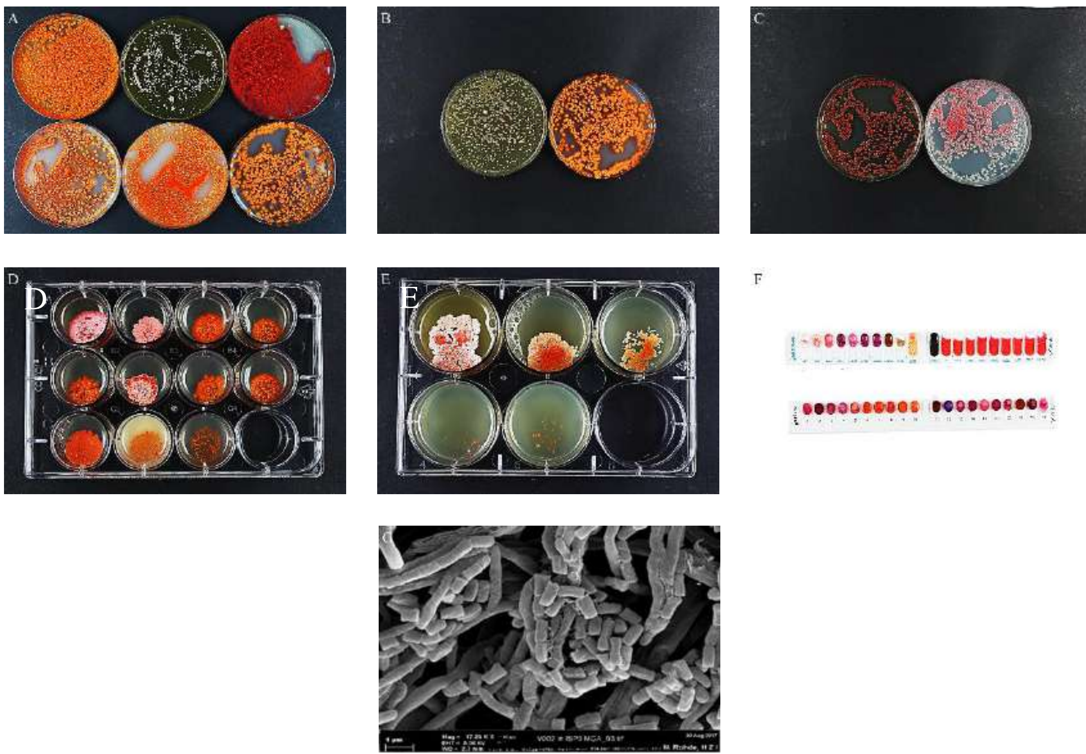


Fig 1. Culture, physiological and microscopic characteristics of V_{002}.

A: From top left to bottom right: GYM, ISP_2, ISP_3, ISP_4, ISP_5, ISP_7. **B**: From left to right: ISP_6, ISP_7. **C**: From left to right: SSM+T, SSM-T. **D**: Carbon utilization (from top left to bottom right: glucose, arabinose, sucrose, xylose, inositol, mannose, fructose, rhamnose, raffinose, cellulose). **E**: Sodium chloride tolerance test (from top left to bottom right: 0%, 2,5%, 5%, 7,5%, 10%). **F:** Enzymatic characteristics on Api Zym® and Api Coryne® plates. **G**: Microscopic appearance under scanning electron microscopy.

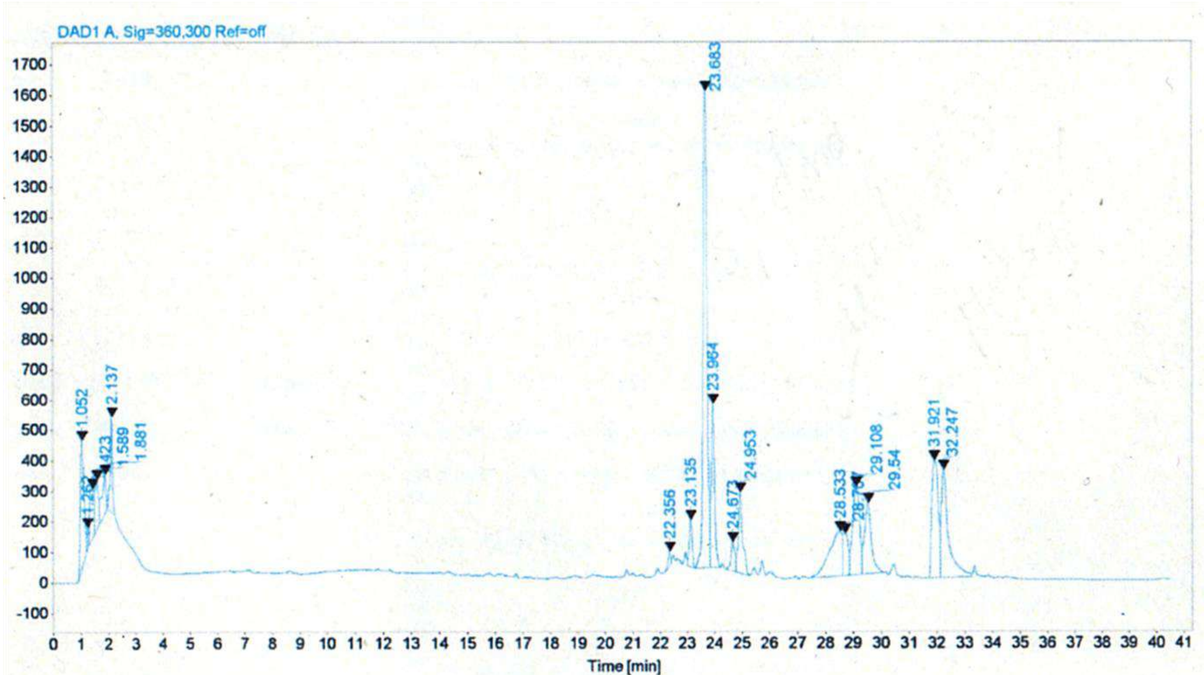


Fig 2. Chromatogram of HPLC fractionation of $Ext_{5294.V002}$.

The chromatogram obtained from the HPLC reveals that the extract contains different fractions characterized by distinct retention times.

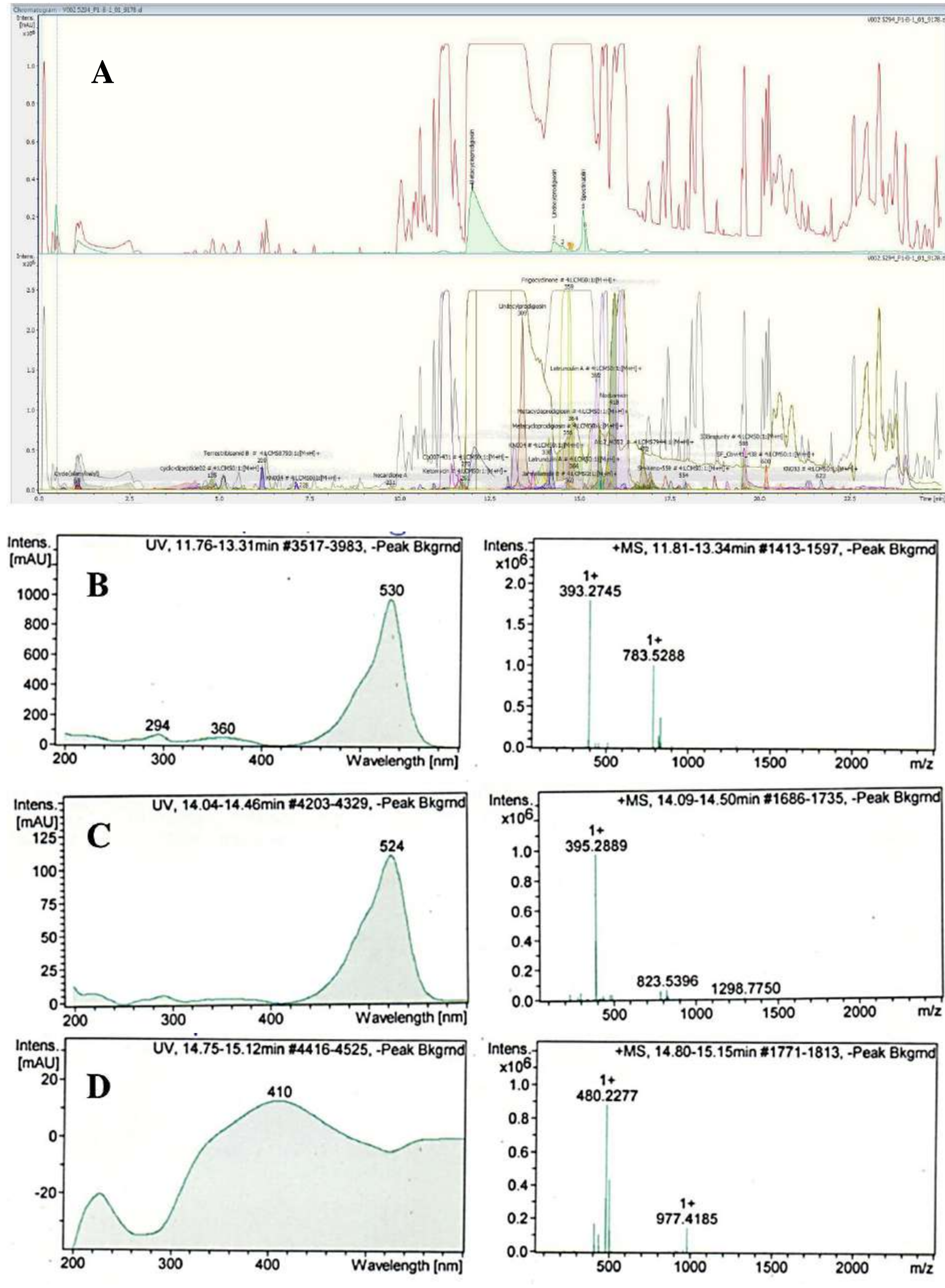


Fig 3. Determination of fractions using the dictionary of natural product.

A: Compound spectrum list rapport, **B**: compound-1, RT=12.02 min: Metacycloprodigiosin. **C**: compound-2, RT=14.30 min: Undecylprodigiosin. **D**: compound-3, RT=15.11 min: Spectinabilin.

Table

Table 1. Culture characteristics of V_{002} in ISP medium.

Medium		Strains		
		V_{002}	*S. lasiicapitis* **DSM 103124T**	*S. spectabilis* **DSM 40512T**
Agar	ISP 2 – G	Not good	Good	Good
Agar	ISP 2 – C	Beige red	**Nd**	Red
Agar	ISP 2 – A	Beige red, light pink	Light orange	White/red
Agar	ISP 2 – S	None	None	None
Agar	ISP 3 - G	Good	Good	Good
Agar	ISP 3 - R	Rose, strawberry red	**Nd**	Orange
Agar	ISP 3 - A	Strawberry red, traffic red	Light pink	Sparse/white
Agar	ISP 3 - S	None	None	None
Agar	ISP 4 - G	Good	Good	Good
Agar	ISP 4 - R	Salamon orange	**Nd**	Orange
Agar	ISP 4 - A	Deep orange, signal orange, traffic orange	Pinkish-white	White
Agar	ISP 4 - S	None	None	None
Agar	ISP 5 - G	Good	Moderate growth	Good
Agar	ISP 5 - R	Salamon orange	**Nd**	Red
Agar	ISP 5 - A	Pure orange, pastel orange, vermillion	None	White/red
Agar	ISP 5 - S	None	None	None
Agar	ISP 6 - G	Not good	Moderate growth	Good
Agar	ISP 6 - R	Saffron yellow	**Nd**	Yellow
Agar	ISP 6 - A	Sun yellow	**Nd**	None
Agar	ISP 6 - S	None	None	None
Agar	ISP 7 - G	Good	Good	Good
Agar	ISP 7 - R	Bright red orange, ruby red, strawberry red	**Nd**	Red
Agar	ISP 7 - A	Yellow orange	Light pink	White/red
Agar	ISP 7 - S	None	None	None
Agar	suter with tyrosine - G	Good	**Nd**	**Nd**
Agar	suter with tyrosine - R	Red violet	**Nd**	**Nd**
Agar	suter with tyrosine - A	Red violet, raspberry red	**Nd**	**Nd**
Agar	suter with tyrosine - S	None	**Nd**	**Nd**
Agar	suter without tyrosine - G	Good	**Nd**	**Nd**
Agar	suter without tyrosine - R	Heather violet	**Nd**	**Nd**
Agar	suter without tyrosine - A	Rose, heather violet	**Nd**	**Nd**
Agar	suter without tyrosine - S	None	**Nd**	**Nd**

Determination of the cultural characteristics of V_{002} on ISP medium and comparison of the results with the cultural characteristics of the type strains, namely: *S. lasiicapitis* DSM 103124^{T} and *S. spectabilis* DSM 40512^{T}

G: growth, **C:** colony, **A:** aerial mycelium, **S:** soluble pigment, **Nd:** not determined.

Table 2. Biochemical characteristics of V_{002}.

Biochemical test		V_{002}	*S. lasiicapitis* DSM 103124^T	*S. spectabilis* DSM 40512^T
Sodium chloride tolerance		**0-5%**	**0-9%**	**0-5%**
use of carbohydrates	glucose	+	+	+
use of carbohydrates	arabinose	+	+	+
use of carbohydrates	sucrose	+	+	±
use of carbohydrates	xylose	+	+	**-**
use of carbohydrates	inositol	+	+	+
use of carbohydrates	mannose	+	+	**-**
use of carbohydrates	fructose	+	+	**-**
use of carbohydrates	rhamnose	+	+	±
use of carbohydrates	raffinose	+	+	+
use of carbohydrates	cellulose	+	**-**	**Nd**

Tolerance to different concentrations of NaCl and use of carbohydrates by V_{002}, and comparison of the results to the types strains, namely *S. lasiicapitis* DSM 103124^T and *S. spectabilis* DSM 40512^T

+**:** good growth, ±**:** average growth**, -**: no growth, **Nd:** not determined.

Table 3. Enzymatic characteristics of V_{002}.

Biochemical test		V_{002}
Api zym	Phosphatase alcaline	**5**
Api zym	Esterase (C4)	**4**
Api zym	Esterase Lipase (C8)	**3**
Api zym	Lipase (C14)	**1**
Api zym	Leucin arylamidase	**5**
Api zym	Valine arylamidase	**5**
Api zym	Cystine arylamidase	**5**
Api zym	Trypsin	**5**
Api zym	Chymotrypsin	**5**
Api zym	Phosphatase acid	**5**
Api zym	Naphtol-AS-BI-phosphohydrolase	**5**
Api zym	alpha galactosidase	**2**
Api zym	beta galactosidase	**5**
Api zym	beta glucuronidase	**1**
Api zym	alpha glucosidase	**5**
Api zym	beta glucosidase	**1**
Api zym	N-acetyl-beta-glucoseamidase	**5**
Api zym	alpha mannosidase	**5**
Api zym	alpha fucosidase	**2**
Api coryne	Nitrate reduction	**-**
Api coryne	Pyraziamidase	**-**
Api coryne	Pyrrolidonyl arylamidase	+
Api coryne	Alkaline phosphatase	+
Api coryne	beta glucuronidase	**-**
Api coryne	beta galactosidase	+
Api coryne	alpha glucosidase	+
Api coryne	N-acetyl -beta glucoseamidase	+
Api coryne	Esculin	**-**
Api coryne	Urease	**-**
Api coryne	Gelatine (hydrolysis)	+
Api coryne	Glucose fermentation	**-**
Api coryne	Ribose fermentation	**-**
Api coryne	Xylose fermentation	**-**
Api coryne	Mannitol fermentation	**-**
Api coryne	Maltose fermentation	**-**
Api coryne	Lactose fermentation	**-**
Api coryne	Sucrose fermentation	**-**
Api coryne	Glycogen fermentation	**-**

Determination of the enzymatic and fermentable capacities of V_{002} by Api Zym® and Api Coryne®

0 to 5: level of enzymatic expression by the strain; from absence to excellence expression. +: presence of enzyme activity, **-**: no enzyme activity.

II.3. Activités antimycotoxinogène et antifongique des graines de Citrullus colocynthis contre Aspergillus flavus et Aspergillus ochraceus contaminant le blé tendre stocké.

II.3. Activités antimycotoxinogène et antifongique des graines de *Citrullus colocynthis* contre *Aspergillus flavus* et *Aspergillus ochraceus* contaminant le blé tendre stocké.

«Antimycotoxigenic and antifungal activities of *Citrullus colocynthis* seeds against *Aspergillus flavus* and *Aspergillus ochraceus* contaminating stored wheat »

(Article publié en Novembre 2013 dans la revue African Journal of Biotechnology-Academic Journals).

Ce troisième chapitre est consacré à la détermination de la capacité des extraits des plantes et des extraits de la souche d'actinobactérie V_{002} à inhiber la synthèse de l'aflatoxine et de l'ochratoxine produites par les moisissures isolées du blé tendre.

Les deux moisissures recherchées sont isolées du blé tendre stocké et identifiées, il s'agit d'*Aspergillus flavus* et *Aspergillus ochraceus*. Les deux souches sont identifiées selon leurs caractères microscopiques et macroscopiques en se référant aux clés d'identification (**Figure 1 et 2, Tableau 2. Article 3**). Les résultats de la chromatographie sur couche mince ont montré que les deux moisissures sont dotées d'un grand pouvoir toxinogène. *A. favus* synthétise l'aflatoxine B_1, alors que *A. ochraceus* produit de l'ochratoxine A.

La technique d'empoisonnement dans le milieu YES a révélé l'activité antifongique des extraits (aqueux et méthanolique) des graines *de Citrullus colocynthis* traduite par leur excellente activité biologique contre la souche *A. ochraceus*. Au-delà de 20 mg mL^{-1}, les extrait ont complétement inhibé la formation de biomasse mycélienne (**Figure 3. Article 3**). La souche *A. flavus* s'est avérée très résistante aux deux extraits des graines de *C. colocynthis* (**Figure 4. Article 3**).

A partir de 10 et 15 mg mL^{-1} d'extrait de *C. colocynthis*, la CCM a révélé une réduction de la synthèse de l'ochratoxine A produite par *A. ochraceus*. Au-delà de 15 mg mL^{-1}, la synthèse de l'ochratoxine A est complètement inhibée par les extraits dans le milieu YES (**Tableau 3. Figure 5. Article 3**).

Les extraits d'*A. herba alba* présentent une forte inhibition de la biosynthèse de l'ochratoxine. A partir de 5 mg mL^{-1} d'extrait organique ou aqueux, la CCM n'a dévoilé aucune

trace d'ochratoxine A produite par *A. ochraceus*. Les extraits organiques de *P. lentiscus* et les métabolites secondaires de l'isolat V_{002} n'ont pas montré d'activité antimycotoxinogène.

academicJournals

Vol. 12(43), pp. 6222-6231, 23 October, 2013
DOI: 10.5897/AJB2013.13163
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Antimycotoxigenic and antifungal activities of *Citrullus colocynthis* seeds against *Aspergillus flavus* and *Aspergillus ochraceus* contaminating wheat stored

GACEM Mohamed Amine[1]*, OULD EL HADJ KHELIL Aminata[1], GACEMI Bouabdallah[2], HALLA Noureddine[3], DJERBAOUI Amina Nesrine[1], BOUDERHEM Amel[1], HADEF Sawsen[1], BENREGUIEG Mokhtar[2,4] and ADLI Djalal Eddine Houari[4]

[1]Laboratory of Protection of Ecosystems in Arid and Semi-arid Area, University of Kasdi Merbah, Ouargla (30000), Algeria.
[2]Laboratory of Technology and Animal Production, University of Abdelhamid Ibn Badis, Mostaganem (27000), Algeria.
[3]Laboratory of Antibiotics Antifungal, Physical Chemistry, Synthesis and Biological Activity, University of Abou Bekr Belkaïd, Tlemcen (13000), Algeria.
[4]Departement of Biology, University of Tahar Moulay, Saida (20000), Algeria.

Accepted 27 September, 2013

Plant extracts and their constituents have a long history as antifungal agents, but their use in biotechnology as preservatives, due to the increasing resistance of fungi to fungicides, has been rarely reported. The aim of this study was to assess *in vitro* antifungal and antimycotoxigenic power of methanolic and aqueous extracts of *Citrullus colocynthis* seeds, an aromatic and medicinal plant, of Algerian flora, against two toxigenic species of the genera *Aspergillus* responsible of contamination of wheat stored. The antifungal and antimycotoxigenic activity of methanolic and aqueous extracts were screened against *Aspergillus ochraceus* and *Aspergillus flavus*. Dilution method was used to investigate the antimicrobial and antimycotoxigenic activity. These bioassays are preceded by a phytochemical screening. The phytochemical analysis of seeds extracts revealed the presence of some chemical groups (polyphenols, steroids and alkaloids) which can express the desired activities. The results suggest that the extracts showed a very good antifungal activity against *A. ochraceus*, but for *A. flavus* any antifungal activity was recorded. The extracts have good antiochratoxigenic power in liquid medium. This evaluation confirms that the extracts of *C. colocynthis* seeds used at low concentration may have significant potential for biological control of fungi and theirs toxins.

Key words: *Citrullus colocynthis*, methanolic extract, aqueous extract, phytochemical screening, antifungal activity, antimycotoxigenic activity, antiochratoxigenic activity.

INTRODUCTION

Fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect,

*Corresponding author. E-mail: biologieamine@yahoo.fr.

Abbreviations: AFs, Aflatoxins; **OTA,** ochratoxin A; **TLC,** thin-layer chromatography; **DRBC,** Dichloran Rose-Bengal chloramphenicol agar; **CDA,** Czapek dextrose agar; **PDA,** potatoes dextrose agar; **YES,** yeast extract sucrose; **MIC,** minimum inhibitory concentration; **MFC,** minimum fungicidal concentration.

nutritional value, organoleptic characteristics and limited shelf life (Yanes et al., 2012). In addition, fungi are responsible for allergic or toxic disorders among consumers because of the production of spores or mycotoxins (Dellavalle et al., 2011).

Mycotoxins are secondary metabolites produced by five fungal genera namely *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps*, they are synthesized under favorable conditions of temperature and humidity. They may be developed in several stages, in the field before harvest, during storage and even in the production chain (Petzinger and Weindenbach, 2002). These toxic substances are carcinogenic, nephrotoxic, hepatotoxic and immunosuppressive (Dellavalle et al., 2011; Korhonen et al., 2012). They are found in many food products such as coffee, cereals, wine and fermented products (Cynthia et al., 2012). Aflatoxins (AFs) are the most dangerous mycotoxins.

Five types of aflatoxins are known; AFB1, AFB2, AFG1, AFG2 and AFM, these toxins are produced by *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus pseudotamarii, Aspergillus bombycis, Aspergillus toxicarius, Aspergillus minisclerotigenes, Aspergillus parvisclerotigenus* and *Aspergillus arachidicola* (Samson et al., 2006; Pildain el al., 2008). *Aspergillus flavus* and *Aspergillus parasiticus* are the major producers of AFB1 (Gourama and Bullerman, 1995). Ochratoxin A (OTA) is the second important mycotoxin with fumonisin, zearalenone and trichothecene. OTA is produced by *Penicillium verrucosum, Aspergillus ochraceus, Aspergillus alliaceus, Aspergillus carbonarius, Aspergillus niger* and *Aspergillus melleus* (Da Rocha Rosa et al., 2002; Accensi et al., 2004; Bau et al, 2005; Bayman and Baker, 2006).

Fungi are generally controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Hermiche et al., 2012). The increased risk of high-level toxic residues in the products and the emergence of pathogens resistant to the products employed, justifies the search for novel actives molecules and new control strategies. Thus, there are a growing interest on the research of possible use of the plant extracts for control of the pest and diseases in agriculture which is less harmful to the health and environment (Nwosu and Okafor, 2000; Logardia et al., 2012).

Several works have demonstrated in laboratory trials that plants tissues, such as roots, leaves, seeds and flowers posses inhibitory properties against bacteria, fungi and insects (Thembo et al., 2010; Benariba et al., 2013). In front these very serious health problems, use of medicinal plants in biomedical research received great interest. This is because herbs are an inexhaustible source of bioactive natural compounds and fewer side effects than drugs (Dramane et al., 2010; Satyavani et al., 2012). Medicinal plants are now an endless source of interesting molecules for scientists and industry, which occur as secondary metabolites (Lozoya and Lozoya, 1989; Karthikeyan et al., 2009). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Molecules from these plants have similar active ingredients which have specific properties giving them an intrinsic behavior (Evon, 2008). A wide spectrum of biological substances extracted from medicinal plants, including oils were tested to replace some of the ways to fight against fungi. In this section, several authors have confirmed the effectiveness of the oils on toxigenic fungi (Ziyada and El Hussein, 2008; Yingying et al., 2008).

Citrullus colocynthis (Schrad), belonging to the family of *Cucurbitaceae*, is an endemic in the south of Algeria. This medicinal plant popularly known as *Handhal, Hdaj* or *Dellaa El-Wad*, is widely used in Algerian folk medicine for treating many diseases such as rheumatism, hypertension hyperglycemia and various contagious diseases, including dermatological problems and gynaecological, urinary and pulmonary infections (Le Flock, 1983; Boukef, 1986; Marzouk et al., 2009; Gurudeeban et al., 2010).

The objective of this work is to demonstrate the antifungal, antiaflatoxigenic and antiochratoxigenic effect of methanol and aqueous extracts of *C. colocynthis* seeds, after determining their chemical composition, against two toxigenic fungal strains namely: *A. flavus* and *A. ochraceus* isolated from wheat stored.

MATERIALS AND METHODS

Plant

C. colocynthis Schrad. fruits were collected in December (2010) near Ouargla, Algeria in the area of Oued N'sa. The identification was performed according to the flora of Tunisia (Pottier-Alapetite, 1981) and the botanists of Faculty of Biology of Saida University (Algeria).

Extraction protocol

The extractions were performed on the seeds of *C. colocynthis*. Plant materials were washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite. Afterwards, the seeds are ready for extraction (Jasso de Rodriguez et al., 2005). In this study, water and methanol are the two solvent used for extraction. These two solvent are polar and they can extract the maximum of bioactive substances.

Methanol extract

Twenty grams of seeds were ground with a mixer and added to 100 ml of methanol. After 3 h of maceration with continuous stirring at 200 rev/min, the mixture was then filtered using filter paper (Whatman No 1). This operation is repeated four times after each filtration with renewal of the solvent in order to exhaust the marc and increase the yield. At the end of extraction, the fractions obtained were collected in a vial and then were evaporated by rotavapor at a specific temperature to the solvent (Senhaji et al., 2005).

Aqueous extract

The aqueous extract is prepared by soaking 20 g of the ground seeds in 100 ml of cold distilled water for 3 h with continuous agitation. The mixture was then centrifuged at 3600 g for 30 min. The supernatant was recovered and then filtered through Whatman filter No. 1. This operation was repeated four times after each filtration with renewal of the solvent. At the end of extraction, the fractions obtained were collected in a vial, then, lyophilized or dried in the drying oven, giving the dry aqueous extract (Senhaji et al., 2005).

Determination of extraction yield

The yield is determined by the ratio of the weight of the dry extract after evaporation on the weight of the plant material used for extraction, multiplied by 100%.

$Rd\ \% = (m_1 \times 100) / m_0$

Where, m_1 is the Mass in grams of the dry extract; m_0 is the mass in grams of dry plant material; Rd is the yield.

Qualitative phytochemical screening

Tannins

One milliliter of extract was mixed with 10 ml of distilled water and filtered. Three drops of ferric chloride ($FeCl_3$) reagent (1% prepared in methanol) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic tannins or catechol tannins, respectively (Karumi et al., 2004).

Saponins

Ten milliliters of extract were placed in a test tube shaken for 15 s and then deposited for 15 min. A persistent foam height greater than 1 cm indicates the presence of saponins (N'Guessan et al., 2009).

Steroids

After addition of 5 ml of acetic anhydride to 5 ml of hot extract, the mixture was added to 0.5 ml of concentrated sulfuric acid. After stirring, the appearance of a purple or violet ring turning blue to green indicates the presence of steroids (Edeoga et al., 2005).

Flavonoids

Flavonoids were detected by reaction with cyaniding. 2 ml of each extract were evaporated and the residue was taken in 5 ml of alcohol hydrochloric dilute 2 times. By adding 2 to 3 magnesium chips, there is a heat release and an orange-pink coloration or purplish. The addition of 3 drops of isoamyl alcohol has intensified this color which confirmed the presence of flavonoids (N'Guessan et al., 2009).

Alkaloids

Alkaloids have been characterized using reagents of Mayer. 10 milliliters of extract were evaporated until a volume of 0.2 ml was obtained on which, 1.5 ml of HCl (2%) was added. After stirring the acid solution, 1 to 2 drops of reagent were added, and the appearance of a yellowish white precipitate indicates the presence of alkaloids (Mojab et al., 2003).

Anthraquinones

The method cited by Trease and Evans (1996) was used for the detection of anthraquinones. The presence of violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxy anthraquinones (Trease and Evans, 1996).

Coumarins

Coumarins were found from 5 ml of extract placed in a tube brought to boiling until obtaining a volume of 1 ml, this volume is added to 1 ml of hot water. After stirring, the total volume is divided into two volumes, one as a control and the other is added to 0.5 ml of NH_4OH (10%) and examined under a UV lamp. The fluorescence emission indicates the presence of coumarins (Rizk, 1982).

Antifungal activity of plant extracts

Fungal isolation

Dilution plating was used as isolation technique (Pitt and Hocking, 2009). 10 g of the sample were added to 90 ml of 0.1% peptone water. This mixture was then shaken on a rotary shaker for approximately 15 min and diluted 10^{-2}, 10^{-3} and 10^{-4} fold. Aliquots composing of 0.1 ml of each dilution were spread (in triplicate) on the surface of the dichloran Rose-Bengal chloramphenicol agar (DRBC), Czapek dextrose agar (CDA) and potatoes dextrose agar (PDA). All plates were incubated for 5 to 7 days at 28°C in the dark and under normal atmosphere. The identification of fungal strain is realized on the basis of morphological characteristics, under the microscope (Barnett and Hunter, 1972; Pitt and Hocking, 2009), and single spore method by colony characteristics after their culture on different culture media (Pitt, 1973; Pitt and Hocking, 2009).

Identification of strains producing mycotoxins

The strains of *A. flavus* and *A. ochraceus* identified were reseeded separately on 50 ml of yeast extract sucrose (YES) medium. After 14 days of incubation at 27 ± 2°C, the biomass formed is removed by filtering the medium through Whatman filter paper No. 01. The 50 ml of the filtrate are added to 100 ml of chloroform, the mixture is thoroughly stirred for 10 min and then allowed to settle by using a separating funnel. This operation is repeated by adding successively 50 and 30 ml of solvent to the aqueous phase recovered at each separation. The chloroform phase thus obtained is filtered through Whatman paper No. 01 and then concentrated by evaporation under vacuum using a rotary evaporator type (Heidolph efficient Laborota 4000) until a volume of 2 to 3 ml.

Thin-layer chromatography (TLC) is performed on a silica gel plate (silica gel 60 F254). The plate is then placed in a chromatographic tank dipped in a mixture of elution solvent consisting of toluene, ethyl acetate and formic acid with volume (5: 4: 1), respectively. After migration and evaporation of the elution product dry, the plate is examined under a UV lamp at a wavelength of 365 nm (Frayssinet and Cahagnier, 1982).

Evaluation of antifungal and antimycotoxigenic activity of organic extracts

The study of the antifungal and antimycotoxigenic activity of methanol and aqueous extracts were tested against two species *A ochraceus* and *A. flavus* on YES medium in order to be able to extract the mycotoxins produced. On an individual basis, each of

Table 1. Extraction yields (%) and phytochemical screening of *C. colocynthis* seeds.

Extract	Extraction yields (%)	Phytochemical substance						
		Flavonoid	Steroid	Alkaloid	Anthraquinon	Coumarin	Saponosid	Tannin
Methanol	4.89	+	+	+	-	-	+	+
Aqueous	2.72	+	+	+	-	-	-	+

+, Presence; -, absence.

Table 2. Identification of *Aspergillus ochraceus* and *Aspergillus flavus* by single spore method.

Genera species	Medium	Reading (Color)
Aspergillus flavus	MEA 25°C	Pistachio green
	CYA 37°C	Dark brown
	G25N 25°C	Greenish yellow
	AFAP	Orange back
Aspergillus ochraceus	MEA 25°C	Yellow gold
	CYA 37°C	Yellow
	G25N 25°C	Yellow

the two extracts (*C. colocynthis* seeds) was added to 50 ml of YES medium but to varying final concentrations are in the order of 1 to 25 mg/ml. After rigorous agitation, different media are inoculated with discs of 0.6 cm of diameter containing youth cultures of 3 to 7 days of *A. ochraceus* and *A. flavus*. Control tests are made for strains and for each test series (Ezzat and Sarhan, 1991; Al-Rahmah et al., 2011). After an incubation period of 14 days at 27 ± 2°C, the same steps mentioned above for the extraction and the revelation of mycotoxins have been followed. The biomass of the filtered mycelium was determined after drying at 70°C for 4 days till their weights remains constant. The percentage inhibition is calculated by the following formula:

Percentage of mycelial inhibition = [C - T / C] × 100

Where, C and T are the mycelial dry weight (mg) in control and treatment, respectively.

RESULTS

Extraction yield and phytochemical screening

The calculation of the chemical extractions yields relative to the total weight of the dry powder used displayed in Table 1 shows that the *C. colocynthis* seeds gave dry extracts masses greater than 1 g/100 g seed powder. From the point of view profitability by weight, methanolic extract gave the highest proportions by comparing it with the aqueous extract. On same Table 1, the qualitative chemical analysis tests that are designed to demonstrate the different phytochemical families existing in both extracts revealed a slight difference in the composition of the extracts. This difference is noticed by the lack of saponins which are absent in the aqueous extract while they are present in the methanol extract. Both extract react negatively with tests revealing anthraquinons and coumarins, whereas for other photochemical tests, the two types of extracts reacted positively.

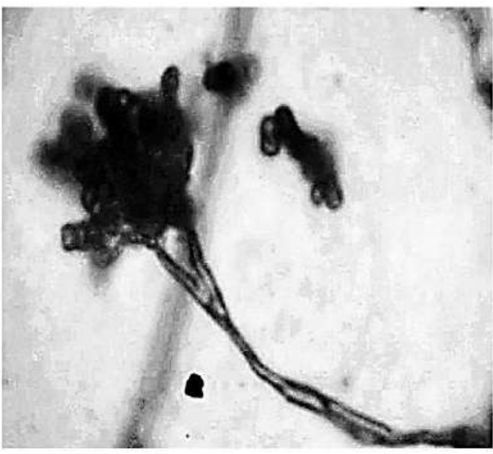
Identification of fungal strains

The different microscopic and macroscopic aspects of both fungal strains searched are demonstrated in Figures 1 and 2. The aspects of fungal colonies of the same strains by single spore method on different culture media are shown in Table 2. The results revealed strains producing mycotoxins on TLC which showed that the strain *A. flavus* is producing AFB1 and strain *A. ochraceus* is producing the OTA.

Antifungal and antimycotoxigenic activity of organic extracts

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were employed by poisoned food technique to assess fungistatic and fungicidal properties of the effective plant extract. As illustrated in Figure 3, the inhibitory plant extracts showed that the fungal strain *A. ochraceus* is very sensitive to both types of extracts. Beyond 15 mg/ml of methanol extract and 20 mg/ml of aqueous extract, the latter did not develop biomass in YES medium. Transplanting these mycelial discs that could not grow in the presence of extracts on other PDA medium (without extracts) did not provide any radial growth after 14 days of incubation at 25 ± 2°C, which explains that CMF is 15 mg/ml for

6226 Afr. J. Biotechnol.



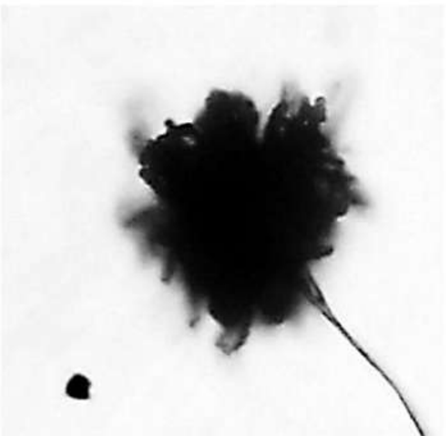


Figure 1. Identification of the genus *Aspergillus* by micro-culture method.

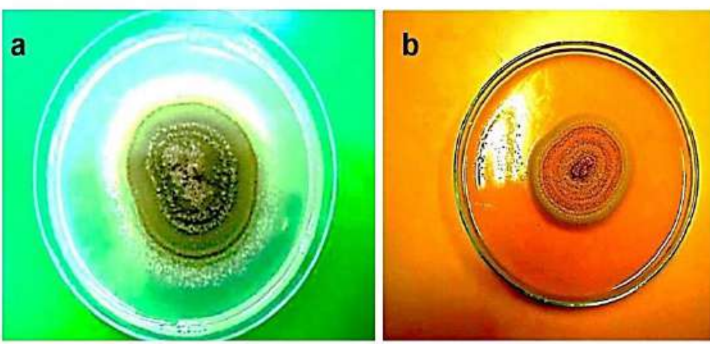


Figure 2. Identification of fungal species by Single Spore method. **a,** Colonies of *Aspergillus flavus* on PDA medium; **b,** colonies of *Aspergillus ochraceus* on PDA medium.

methanolic extract and 20 mg/ml for the aqueous extract. MICs are 10 mg/ml and 15 mg/ml for methanolic and aqueous extract, respectively. Below these two concentrations, the antifungal activity begins to decrease. Figure 4 showed that the strain *A. flavus* has proved highly resistant to two extracts of *C. colocynthis* seeds and no antifungal activity was recorded.

The results displayed in Table 3 achieve the last objective of this study by demonstrating that methanol and aqueous extracts tested against *A. flavus* to determine the power synthesis inhibitor of AFB1 showed no inhibitory activity against this toxin and TLC revealed the presence of a similar spot to the standard of pure AFB1 (Figure 5). For antiochratoxigenic activity (Table 3), methanol extract was able to reduce the synthesis of OTA produced by *A. ochraceus* from 10 mg/ml of extract in the YES medium explained by the reduction of the size of the spot toxin on the TLC plate. For the aqueous extract, the TLC detected a reduction of the synthesis of the toxin produced from 15 mg/ml of extract in the medium. At 15 mg/ml of methanol extract and 20 mg/ml aqueous extract, OTA was not detected on the TLC plate (Table 3).

DISCUSSION

Fungi are ubiquitous in the environment, and infection due to fungal pathogens which has become more common. The genus *Aspergillus* is widely distributed in nature and its species are among the most common destroyers of foodstuffs and grains during storage. It includes species that may damage crops in the field or cause post-harvest decay (Sun et al., 2012). In addition, the

Table 3. Antimycotoxigenic activities of methanol and aqueous extracts of *C. colocynthis* seeds.

Parameter	Concentrations (mg/ml)						
	0	1	5	10	15	20	25
Production of AFB_1 in the presence of Me. E	+	+	+	+	+	+	+
Production of AFB_1 in the presence of Aq. E	+	+	+	+	+	+	+
Production of OTA in the presence of Me. E	+	+	+	-/+	-	-	-
Production of OTA in the presence of Aq. E	+	+	+	+	-/+	-	-

AFB_1, Aflatoxine B_1, OTA, ochratoxine A; Me. E, methanol extract; Aq. E, aqueous extract; +, presence; -, absence.

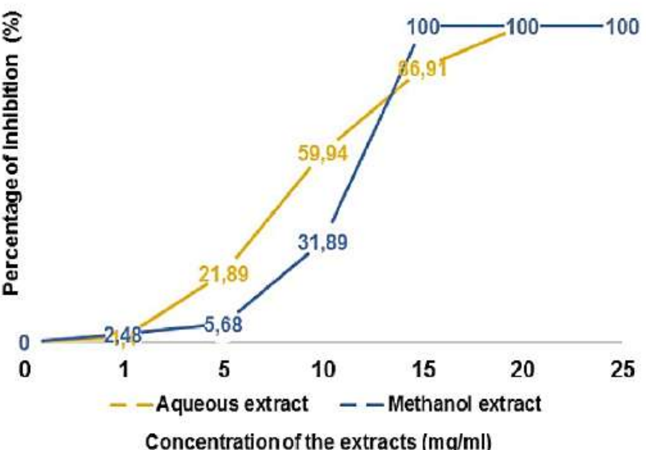


Figure 3. Antifungal activity of methanol and aqueous extracts against *Aspergillus ochraceus* on YES medium.

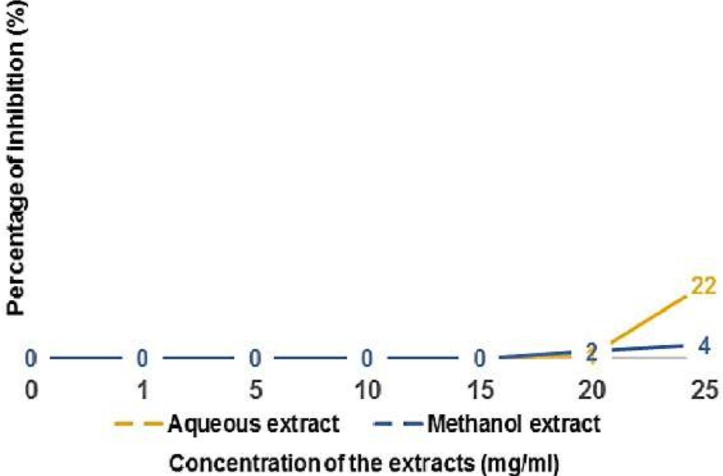


Figure 4. Antifungal activity of methanol and aqueous extracts against *Aspergillus flavus* on YES medium.

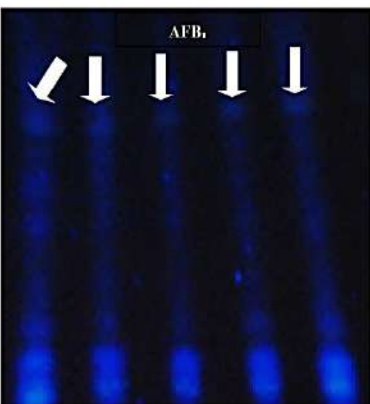


Figure 5. Antiaflatoxigenic activity of methanol extracts against *Aspergillus flavus*.

genus products mycotoxins and studies in the last decade have emphasized its toxicogenic properties. Indeed, the palette adverse effects of mycotoxins on the human and animal health is very extensive and sometimes unknown (Brochard and Le Bacle, 2009; Cynthia et al., 2012). Besides, acute toxic effects or chronic hemorrhagic, immunotoxic, hepatotoxic, nephrotoxic, neurotoxic and teratogenic, some mycotoxins have shown mutagenic and carcinogenic effects in laboratory animals and humans (Korhonen et al., 2012).

Over the last decades, concerns were expressed about the increasing prevalence of pathogenic fungi that are resistant and more precisely those producing mycotoxins. But the problem posed by the high cost and the increased toxic side effects of some synthetic substances coupled with their failure to be treated cannot be underestimated. For this reason, this last decade witnessed increased intensive studies of extracts and biologically active compounds isolated from natural plants (Mabrouk, 2012; Elamathi et al., 2012).

Despite numerous studies on the use of colocynth in the culinary field and that of traditional medicine, little work has been done on the antifungal effect of *C. colocynthis* seeds against fungal strains spoilage of the physicochemical and mycological quality of stored wheat (Gacem et al., 2013). Facing this situation, the aim of this work was to evaluate *in vitro* the antifungal and antimyctoxigenic potential of *C. colocynthis* seeds against toxigenic fungi producing mycotoxins namely *A. flavus* and *A. ochraceus* in order to check possible inhibition activity.

The extraction method performed on the powder of *C. colocynthis* seeds conducted at an ambient temperature can extract maximum compounds and prevent their denaturation or probable modification (Yagoub, 2008). The phytochemical screening of methanol and aqueous extracts reveal the richness of *C. colocynthis* seeds from a qualitative point by secondary metabolites such as steroids, flavonoids, alkaloids and tannins. These compounds have been reported in the *C. colocynthis* by several studies (Gurudeeban et al., 2010; Adebayo-Tayo et al., 2010).

Outcomes related to phytochemical screening of the last class of secondary metabolites show that the alkaloids are present in both extracts from seeds of *C. colocynthis*. This result is confirmed by Sultan et al. (2010) and Benariba et al. (2013) who detected the alkaloids in seeds of this species and Marzouk et al. (2010) who showed that the seeds of *C. colcoynthis* contain 1.64 mg of alkaloid per 100g of material dry. The absence of antraquinons is cited in the study of Suman (2010).

Ethanolic and aqueous extracts from *C. colocynthis* leaves and fruits contain alkaloids, flavonoids, glycosides and saponosides (Najafi et al., 2010). Likewise, the entire *C. colocynthis* plants contain 1.39 mg flavonoids, 0.52 mg saponosides, 1.64 mg alkaloids, 1.64 mg phenolic compounds and 30.12 mg ascorbic acid per 100 g (Sultan et al., 2010). Another study by Gill et al. (2011) documented the presence of alkaloids, steroids, terpenoids, flavonoids, as well as coumarins, glycosides in methanolic and hydromethanolic extracts of *C. colocynthis* seeds. The polyphenol compounds, represented in majority by tannins and flavonoids, are presently a major axis of research, because they are considered as potent antioxidants, anti-inflammatory, anti-bacterial, antiviral and anti-cancer agents (Oliver Chen and Blumberg, 2008).

For instance, Afifi et al. (1973) reported the presence in the whole plant of three alkaloids ($C_{10}H_{15}NO_3$, $C_{20}H_{32}NO$ and $C_{16}H_{24}NO_7$. Hatam et al. (1990) documented the presence of two sterols ($C_{29}H_{48}O$ and $C_{29}H_{50}O$) in a *C. colocynthis* fruits collected in Basra area in Iraq. Thus, *C. colocynthis* contain flavonoids such as quercetin, myricetin and kaemoferol (Oliver Chen and Blumberg, 2008).

Several study identified cucurbitacins in a methanol extract of *C. colocynthis* fruits (Sonja and Hermann, 2000; Seger et al., 2005; Nayab et al., 2006). Such cucurbitacins are relevant to the bitterness and toxicity of the plants, as well as their anti-inflammatory, purgative and anti-cancer activities, such as the inhibition of cell adhesion resulting from the cytoskeleton destabilizing in cancer cells exposed to cucurbitacin E (Jian et al., 2005).

Incidentally, minor differences between the results of distinct studies could be related to differences in local climate and soil composition. The distribution of phytoconstituents such as saponins, tannins, flavonoids and alkaloids, may also vary in distinct parts of *C. colocynthis*, in leaves, fruits, roots and seeds. The study of Gacem et al. (2013) revealed a good activity against strains of *Aspergillus*. The tests of antifungal activities of colocynth in YES medium against strains *A. flavus* and *A. ochraceus* isolated from wheat stored revealed effective-

ness of methanol extract of the seeds of this plant against *A. ochraceus*. The experiment revealed that the methanol extract has a more antagonistic effect than the aqueous extract. This effect is explained by the high yield of methanolic extraction, which is due to the presence of bioactive substances with high quantity. This strong antifungal activity of methanol extract was also reported by several authors (Hadizadeh et al., 2009; Gurudeeban et al., 2010; Gacem et al., 2013).

Chang et al. (2008) and Abdel Ghani et al. (2008) join the antifungal activity of extracts from *C. colocynthis* seeds with bioactive substances of the plant. The power of these phytochemicals compounds to exert higher activity is depending to their concentrations in the extracts (Yan et al., 2008). Among the phytochemicals compounds with antifungal activity, mainly cites alkaloids, polyphenols and steroids (Yan et al., 2008; Oliver Chen and Blumberg, 2008). The antifungal activity of the extracts of the plant depends on its composition, the plant organ to be tested, the nature of the extract and the fungal strains selected (Veldhuizen et al., 2006; Dan et al., 1998).

Several studies have been conducted to understand the mechanism action of plant extracts. Many researchers attribute this feature to phenolic compounds. These compounds can interfere with bio-membranes causing cell damage and causing leakage of cellular materials and finally the death of microorganisms (Veldhuizen et al., 2006; Abdel Ghani et al., 2008). This is a possible mechanism by which the mycelial growth can be reduced or completely inhibited by the effect of extracts acting on the function and structure of the cell membrane. Saponins are a special class of glycosides with a soapy characteristic and very good antifungal activity (Sikkema et al., 1995).

Flavonoids are also responsible for the inhibition of resistant microbes. They are responsible for the scavenging process or chelators and may disrupt microbial membranes. Furthermore, alkaloids contain a detoxifying effect and have a very good antifungal activity (Kessler et al., 2003). Terpens (steroids) affect not only the permeability but also other functions in cell membranes. These compounds can penetrate cell membranes, enter the interior of the cell, and interact with critical sites such as intracellular enzymes and proteins, leading to cell death (Omidbeygi et al., 2007).

The extracts obtained from the upper parts of plants have the ability to suppress the growth of toxigenic fungi and therefore toxin production (Thanaboripat et al., 1997). They can also completely block the biosynthesis of mycotoxins while fungal growth is not affected (Bhatnagar and Mccormick, 1988). These seed extracts of *C. colocynthis* are less important relative to the extracts of *Eucalyptus globolus*, *Olea europea* and *Thymus vulgaris* described in the study of Al-Rahmah et al. (2011), which proved a complete inhibition of AFB1 synthesis and the study of El-Nagerabi et al. (2012) who demonstrated the effect of *Hibiscus sabdariffa* extract and *Nigella sativa* oil for inhibiting the synthesis of AFB1. The phenolic compounds of *C. colocynthis* seeds cannot inhibit the biosynthesis steps of AFB1, explained by their absence in the lipids of the fungal cell wall membrane and mitochondria, disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occur (Cox et al., 2000; Burt, 2004).

Contrariwise, the methanolic extract of *C. colocynthis* seeds showed a very good inhibition of OTA and this extract is ranked higher than other extract as *Ferronia eluhantum, Lawsona innermis* and *Azadirachta indica* causing a reduction of the synthesis only (Warke et al., 2006). The use of this extract is best looked for other substances that have the same effect such as 4-hydroxy-3-(p-toluoyl)-6-(p-tolil)-2H-pyrane-2-one (DHT) and 5-bromo-4-hydroxy-3-(p-toluoyl)-6-(p-tolil)-2H-pyrane-2-one (BrDHT) (Durakovic et al., 1989). The advantage of herbal extracts is their bioactivity, a feature that makes them attractive for the protection of stored products such as cereals against fungal attack.

Conclusion

The results obtained are encouraging and confirm the value of the use of *C. colocynthis* seeds as an antifungal agent and in biotechnology as a preservative for the fight against toxigenic fungi and their mycotoxins. It is therefore interesting to continue this study in order to determine the mode of action of extracts on mold.

REFERENCES

Abdel Ghani SB, Weaver L, Zidan ZH, Hussein MA, Keevil CW, Brown RCD (2008). Microware-assisted synthesis and antimicrobial activities of flavonoid derivatives. Bioorg. Med. Chem. Lett. 18:518-522.

Accensi F, Abarca ML, Cabanes FJ (2004). Occurrence of *Aspergillus* species in mixed feeds and component raw materials and their ability to produce OTA. Food Microbiol. 21:623-627.

Adebayo-Tayo BC, Adegoke AA, Okoh AI, Ajibesin KK (2010). Rationalizing some medicinal plants used in treatment of skin diseases. Afr. J. Microbiol. Res. 4(10):958-963.

Afifi MD, Sayed MS, Balbaa SI (1973). Nitrogenous bases of different organ of *Citrullus colocynthis*. Planta Med. 24:260-265.

Al-Rahmah N, Mostafa A, Abdel-Megeed A (2011). Antifungal and antiaflatoxigenic activities of some plant extracts. Afr. J. Microbiol. Res. 5(11):1342-1348.

Barnett HL, Hunter BB (1972). Illustrated genera of Imperfect fungi. 3th Ed. Minnesota, USA: Burgess Publishing Company.

Bau M, Bragulat MR, Abarca ML, Minguez S, Cabanes FJ (2005). Ochratoxigenic species from Spanish wine grapes. Int. J. Food Microbiol. 98:125-30.

Bayman P, Baker JL (2006). Ochratoxins: a global perspective. Mycopathologia. 162:215-223.

Benariba N, Djaziri R, Bellakhdar W, Belkacem N, Kadiata M, Malaisse WJ, Sener A (2013). Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. Asian. Pac. J. Trop. Biomed. 3(1):35-40.

Bhatnagar D, Mccormick S (1988). The inhibitory effect of neem (*Azadirachta indica*) leaf extracts on aflatoxin synthesis in *Aspergillus parasiticus*. J. Am. Oil Chem. Soc. 65:1166-1168.

Boukef MK (1986), Traditional medicine and pharmacopoeia. Plants in the traditional Tunisian medicine, Agency for Cultural and Technical Cooperation. Paris. France.
Brochard G, Le Bacle C (2009). Mycotoxins in the workplace. Document for the doctor of labour. Studies Department and medical assistance. INRS P. 119:299-323.
Burt S (2004). Esential oils; their antimicrobial properties and potential applications in foods - a review. Int. J. Food Microbiol., 94:223-253.
Chang H, Cheng Y, Wu C, Chang S, Chang T, Su T (2008). Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. fomiosana Florin leaf against plant pathogenic fungi. Bioresour. Technol. 99:6266-6270.
Cox SD, Mann CM, Markham JL, Bell HC (2000). The mode of antimicrobial action of essential oil of Melaleuca alternifolia (tea tree oil). J. Appl. Microbiol., 88:170-175.
Cynthia AC, Suretha DK, Judith ZP, Mulunda M, Mary AE, Dutton MF (2012). Fungal and mycotoxin of south African commercial maize. J. Food. Afri. Environ. 10(2):296-303.
Da Rocha Rosa CA, Palacios V, Combina M, Fraga ME, De Oliveira Rekson A, Magnoli CE (2002). Potential OTA producers from wine grapes in Argentina and Brazil. Food. Addit. Contam. 19:408-414.
Dan S, Alex B, Ella S, Zohara Y (1998). Evaluation of *Citrullus colocynthis*, a desert plant native in Israel, as a potential source of edible oil. J. Arid. Environ. 40:43-439.
Dellavalle PD, Cabrera A, DiegOA, Larranaga P, Ferreira F, Rizza MD (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria spp.* Chilean. J. Agric. Res. 71(2):231-239.
Dramane S, Mamidou KW, Kamanzi K (2010). Evaluation of antimicrobial and free radical scavenging activities of some bioactive taxons from cote d'ivoire. Eur. J. Sci. Res. 40(2):307-317.
Durakovic S, Zagreb, Susnik I, Rajnovic P, Markov K, Pospisil O, Stilinovic L (1989). Antifungal and antiochratoxigenic properties of new synthetized analogues of dehydroacetic acid. Mikrobiologija. 26(1):1-13.
Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7):685-688.
Elamathi R, Kavitha R, Kamalakannan P, Deepa T, Sridhar S (2012). Preliminary phytochemical and antimicrobial studies on the leaf of *Ecbolium viride*. World. J. Pharm. Biol. Sci. 2(1):5-10.
El-Nagerabi SAF, Al-Bahry SN, Elshafie AE, AlHilali S (2012). Effect of *Hibiscus sabdariffa* extract and *Nigella sativa* oil on the growth and aflatoxin B1 production of *Aspergillus flavus* and *Aspergillus parasiticus* strains. Food. Control. 25:59-63.
Evon MP (2008). New biorefinery process of sunflower whole plant by splitting thermo-chemical-mecano twin-screw extruder:a study of the aqueous lipid extraction and shaping of a raffinate by theromoulage biomaterials. INRA Toulouse France. pp:12-25.
Ezzat SM, Sarhan MM (1991). Effect of different concentrations of sodium chloride on the growth and biochemical activities of *Aspergillus flavus* Link. Egy. J. Microbiol., 26(1):133-146.
Frayssinet C, Cahagnier B (1982). Detection and determination of toxins in grains and seeds and derived products:cereals, oilseeds, pulses, feed, Paris:technical and documentation (Lavoisier).
Gacem MA, Ould El Hadj Khelil Aminata, Gacemi B (2013). Evaluation of antifungal effect of organic extracts of Algerian *Citrullus colocynthis* seeds against four strains of *Aspergillus* isolate from wheat stored. J. Med. Plants. Res. 7(12):727-733.
Gill NS, Supreet K, Arora R, Bali M (2011). Screening of antioxydant and antiulcer potential of *Citrullus colocynthis* seed extract. Res. J. Phytochem. 5:98-110.
Gourama H, Bullerman LB (1995). *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxigenic fungi of concern in foods and feed - a review. J. Food. Protect. 58:1395-1404.
Gurudeeban S, Rajamanickam E, Ramanathan T, Satyavani K (2010). Antimicrobial activity of *Citrullus colocynthis* in gulf of mannar. Inter. J. Curr. Res. 2:078-081.
Hadizadeh F, Ebrahimzadeh MA, Hosseinzadeh H, Motamed-Shariaty V, Salami S, Bekhradnia AR (2009). Antidepressant and antioxidant activities of some 2-benzoxazolinone derivatives as bupropion analogues. Pharmacologyonline. 1:331-335.
Hatam NAR, Whiting DA, Yousif NJ (1990). Lipids and sterols of *Citrullus colocynthis*. Int. J. Crude. Drug. Res. 28:183-184.
Hermiche R, Daniela B, Dujardin B, Bergman P, Herman F (2012). EFSA's contribution to the implementation of the EU legislation on pesticides residues in food. EFSA J. 10(10):s(1011).
Jasso de Rodriguez D, Hernández-Castillo D, Rodriguez-Garcia R, Angulo-Sánchez JL (2005). Antifungal activity *in vitro* of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. Ind. Crop Prod. 21(1):81-87.
Jian CC, Chiu MH, Nie RL, Cordell GA, Qiu SX (2005). Cucurbitacins and cucurbitane glycosides: structures and biological activities. Nat. Prod. Rep. 22:386-399.
Karthikeyan A, Shanthi V, Nagasathaya A (2009). Preliminary phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica. L. Int. J. Green Pharm. 3:78-80.
Karumi Y, Onyeyili PA, Ogugbuaja VO (2004). Identification of active principals of *M. balsamina* (Balsam apple) leaf extract. J. Med. Sci. 4(3):179-182.
Kessler M, Ubeaud G, Jung L (2003). Anti-and prooxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol. 55(1):131-42.
Korhonen A, Seaghdha OD, Silins I, Sun L, Hogberg J, Ulla S (2012). Text mining for literature review and knowledge discovery in cancer risk assessment and research. PLoS One 7(4):e33427.
Le Flock E (1983), Contribution to ethnobotanical study of the Tunisian flora, Official Printing Office of the Republic of Tunisia.
Logardia T, Ramdani M, Figueredo G, Chalachat JC, Chalard P (2012). Essentiel oil composition and antimicrobial activity of *Genista microcephala* Coss. et Dur. Int. J. Med. Arom. Plants. 2(1):75- 79.
Lozoya M, Lozoya X (1989). Pharmacological properties in vitro of various extracts of *Mimosa pudica Linn.* Tepescohuite Arch Invest Mex. pp. 87-93.
Mabrouk MI (2012). Synergistic and antibacterial activity of six medicinal plants used in folklore medicine in Egypt against *E. coli* O157:H7. J. Appl. Sci. Res. 8(2):1321-1327.
Malec LS, Pamilio AB (2003). Herbivory effects on the chemical constituents of *Bromus pictus*. Mol. Med. Chem. 1:30-38.
Marzouk B, Marzouk Z, Décor R, Edziri H, Haloui E, Fenina N (2009). Antibacterial and anticandidal screening of Tunisian *Citrullus colocynthis Schrad.* from Medenine, J. Ethnopharmacol 125:344-349.
Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M (2010). Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. J. Ethnopharmacol. 128(1):15-19.
Mojab F, Kamalinejab M, Ghaderi N, Vahidipour HR (2003). Phytochemical screening of some species of Iranian plants. Iran. J. Pharm. Res. 7:77-82.
N'Guessan K, Kadja B, Zirihi G, Traoré D, Aké-Assi L (2009). Phytochemical screening of some ivorian medicinal plants used by the Krobou people (Agboville, Côte-d'Ivoire). Sci. Nat. 6(1):1-15.
Najafi S, Sanadgol N, Sadeghi NB, Ashofteh MB, Sanadgol E (2010). Phytochemical screening and antibacterial activity of *Citrullus colocynthis (Linn.) Schrad* against *Staphylococcus aureus*. J. Med. Plants. Res. 4(22):2321-2325.
Nayab D, Ali D, Arshad N, Malik AM, Choudhary I, Ahmed Z (2006). Cucurbitacin glucosides from *Citrullus colocynthis*. Nat. Prod. Res. 20:409-413.
Nwosu MO, Okafor JI (2000). Preliminary studies of the antifungal activitues of some medicinal plants against *basidiobolus* and some other pathogenic fungi. J. Ethnopharmacol. 72(1-2):111-117.
Oliver Chen CY, Blumberg JB (2008). Are there age-related changes in flavonoid bioavailability? Phytochemicals aging and health. New York: Taylor Francis Group.
Omidbeygi M, Barzegar M, Hamidi Z, Naihdibadi H (2007). Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste. Food. Control. 18:1518-1523.
Petzinger E, Weindenbach A (2002). Mycotoxins in the food chain:the role of ochratoxins. Livest. Prod. Sci. 76:245-250.
Pildain MB, Frisvad JC, Vaamonde G, Cabral D, Varga J, Samson RA (2008). Two novel aflatoxin-producing *Aspergillus* species from Argentinean peanuts. Int. J. Syst. Evol. Microbiol. 58:725-735.
Pitt JI (1973). An appraisal of identification methods for *Penicillium*

species. Novel taxonomic criteria based on temperature and water relations. Mycology. 65:1135-1157.

Pitt JI, Hocking AD (2009). Fungi and Food Spoilage. Blackie Academic and Professional, London.

Pottier-Alapetite G (1981) Flore De La Tunisie, Angiospermes-Dicotylédones: Gamopétales. Tunisia: Imprimerie officielle de la république tunisienne.

Rizk AM (1982). Constituents of plants growing in Qatar. Fitoterapia. 52(2):35-42.

Samson RA, Hong SB, Frisvad JC (2006). Old and new concepts of species differentiation in *Aspergillus*. Med. Mycol. 44:133-148.

Satyavani K, Gurudeeban S (2012). Toxicity Study of Silver Nanoparticles Synthesized from *Suaeda monoica* on Hep-2 Cell Line. Avicenna. J. Med. Biotechnol 4(1):35-39.

Seger C, Sturm S, Mair ME, Ellmerer EP, Stuppner H (2005). 1H and 13C NMR signal assignment of cucurbitacin derivatives from *Citrullus colocynthis (L.)* Schrader and *Ecballium elaterium* L. (Cucurbitaceae) Magn. Reson. Chem. 43:489-491.

Senhaji O, Faid M, Elyachioui M, Dehhaoui M (2005). Antifungal activity of different cinnamon extracts. J. Med. Mycol. 15:220-229.

Sikkema J, De Bont JAM, Poolman B (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Mol. Biol. Rev. 59:201-222.

Sonja S, Hermann S (2000). Analysis of Cucurbitacins in medicinal plant by high-pressure liquid chromatography-mass spectrometry. Phytochem. Anal. 11:121-127.

Sultan A, Farman UK, Iqbal H, Murad AK, Ihsan UK (2010). Evaluation of chemical analysisprofile of *Citrullus colocynthis* growing in southern Areas of Khyber Pukhtunkhwa, Pakistan. World Appl. Sci. J. 10(4):402-405.

Suman S (2010). Phytochemical investigation of *Sonchus oleraceus* leaves and *Citrullus colocynth* root. J. Herbal. Med. Toxicol. 4(2):159-162.

Sun J, Awakawa T, Noguchi H, Abe I (2012). Induced production of mycotoxins in an endophytic fungus from the medicinal plant *Datura stramonium L.* Bioorg. Med. Chem. Lett. 22(20):6397-6400.

Thanaboripat D, Nontabenjawan K, Leesin K, Teerapiannont D, Sukchareon O, Ruangrattanamatee V (1997). Inhibitory effects of garlic, clove and carrot on growth of *Aspergillus flavus* and aflatoxin production. J. Forest. Res. 8:39-42.

Thembo KM, Vismer HF, Nyazema NZ, Gelderblom WCA, Katerere DR (2010). Antifungal activity of four weedy plant extracts against selected mycotoxigenic fungi. J. Appl. Microbiol. 109(4):1479-1486.

Trease GE, Evans WC (1996). A textbook of pharmacognosy. 14th Ed. Bailliere Tindall Ltd. London.

Veldhuizen E, Tjeerdsma-Van Bokhoven C, Zweijtzer SA, Haagsman HP (2006). Structural requirements for the antimicrobial activity of carvacrol. J. Agric. Food. Chem. 54:1874-1879.

Warke S, Kalorey DR, Kurkure NV (2006). *In vitro* antiochratoxigenic and ochratoxin a neutralization potential of various aqueous herbal extracts. Ind. J. Comp. Microbiol. Immunol. Infect. Disea. 27(1):26-29.

Yagoub SO (2008). Anti-microbial activity of *Tamarindus indica* and *Adansonia digitata* extracts against *E. coli* Isolated from urine and water specimens. Res. J. Microbiol. 3(3):193-197.

Yan D, Jin C, Xiao XH, Dong XP (2008). Antimicrobial properties of berberines alkaloids in Franch *Coptis chinensis* by microcalorimetry, J. Biochem. Biophys. Methods. 70(6):845-849.

Yanes LR, Torres PI, Guevara-Gonzales RG, Hernandez-Zul MI, Quijano-Carranza JA, Rico-Garcia E (2012). The effect of climate change on plant diseases. Afr. J. Biotechnol. 11(10):2417-2428.

Yingying X, Clifford H, Charlene WH, Frank M (2008). Fungistatic activity of flaxseed in potato dextrose agar and a fresh noodlesystem. Int. J. Food. Microbiol. 121:262-267.

Ziyada AK, El Hussein SA (2008). Physical and chemical characteristics of *Citrullus lanatus var. colocynthis* seed oil. J. Phys. Sci. 19(2):69-75.

View publication stats

Conclusion générale et perspectives

Conclusion générale et perspectives

L'objectif de notre travail est la recherche des activités antifongique, antibactérienne, antioxydante et antimycotoxinogène des extraits méthanolique et aqueux de *Pistacia lentiscus*, *Artemisia herba alba*, des graines de *Citrullus colocynthis* et d'un isolat d'actinobactérie.

Les examens phytochimiques effectués ont permis de constater que les trois plantes médicinales sont une source privilégiée de molécules biologiquement actives. Parmi ces composés, les polyphénols sont représentés par les tanins hydrolysables, les flavonoïdes et les saponosides. Les stéroïdes et les alcaloïdes y sont aussi présents.

L'étude de l'activité antifongique des extraits des trois plantes étudiées a révélé une excellente activité antifongique des extraits méthanolique et aqueux de *C. colocynthis* et d'*A. herba-alba* liée à leur richesse en composés bioactifs largement répandus dans les plantes médicinales. L'extrait méthanolique possède une activité fongicide sur la majorité des souches fongiques sélectionnées à l'exception d'*A. flavus* et *A. parasiticus* qui se sont révélées résistantes. Une activité antiradicalaire remarquable des extraits organiques des trois plantes médicinales a été mise en évidence.

Selon les résultats de l'identification phénotypique et génotypique basé sur l'ARNr 16S, l'isolat d'actinobactérie désigné V_{002} appartient au genre *Streptomyces*.

Les extraits bruts de la souches V_{002} ont montré une importante activité antibactérienne contre les bactéries à Gram négatif. Ils ont aussi une excellente activité antioxydante.

Le fractionnement des extrait obtenus de l'isolat V_{002} par HPLC et la détection des composés actifs par LC-MS laissent constater que la souche V_{002} peut être considérée comme nouvelle productrice de spectinabiline, d'undecylprodigiosine et de metacycloprodigiosine.

Les extraits bruts d'*A. harba-alba* et des graines de *Citrullus colocynthis* testés montrent une activité antimycotoxinogène élevée puisqu'ils ont pu atteindre les sites de synthèse des toxines. Les extraits des deux plantes ont inhibé la synthèse d'ochratoxine A produite par *Aspergillus ochraceus* isolée du blé.

Les extraits des plantes médicinales et de l'isolat V_{002} peuvent donc constituer une nouvelle stratégie de décontamination pour la lutte contre les mycotoxines.

Les résultats présentés dans cette thèse, ont permis de mieux comprendre l'importance des trois plantes et de la souche d'actinobactérie isolée dans la lutte contre les bactéries et des moisissures pathogènes.

Par ailleurs, l'étude réalisée a révélé le rôle des extraits organique en tant qu'agent antioxydant permettant de minimiser le stress oxydatif dans la cellule.

A l'issue de cette étude et afin d'élucider certains points restés peu claires, il apparait nécessaire d'effectuer d'autres études approfondies qui se résument dans les points suivants :

- Identification moléculaire des souches fongiques isolées du blé tendre ;

- une étude *in vivo* pour obtenir une vue globale sur l'activité antifongique et antibactérienne des extraits testés ;

- extraction et caractérisation des composés actifs des trois plantes par des méthodes plus spécifiques ;

- réalisation d'une étude toxicologique avant toute application des extraits ;

- recherche de l'activité antioxydante de chaque fraction ;

- synthèse de nanoparticules vertes avec les molécules bioactives des extraits afin d'améliorer les activités biologiques des molécules bioactives.

Références bibliographiques.

Références bibliographiques

Adgigitov F, Kosichenko LP, Popandopulo PG et al (1984) Frequency of chromosome aberrations in bone marrow of monkeys and their F_1 after aflatoxin B_1 injection. Exp Pathol 26: 163–169.

Al-Anati L, Petzinger E (2006) Immunotoxic activity of ochratoxin A. J Vet Pharmacol Ther 29: 79–90.

Almeida-Ferreira GC, Barbosa-Tessmann IP, Sega R et al (2013) Occurrence of zearalenone in wheat- and corn-based products commercialized in the State of Paraná, Brazil Braz J Microbiol 44: 371–375.

Baoune H, Ould El Hadj-Khelil A, Pucci G, et al (2018) Petroleum degradation by endophytic *Streptomyces spp*. isolated from plants grown in contaminated soil of southern Algeria. Ecotoxicol Environ Saf 147: 602-609.

Barrett JR (2005) Liver cancer and aflatoxin: New information from the Kenyan outbreak. Environ Health Perspect 113: A837–A838.

Bárta I, Adámková M, Markarjan D et al (1984) The mutagenic activity of aflatoxin B_1 in the *Cricetulus griseus* hamster and *Macaca mulatta* monkey. J Hyg Epidemiol Microbiol Immunol 28: 149–159.

Bhatti AA, Haq S, Bhat RA (2017) Actinomycetes benefaction role in soil and plant health. Microb Pathog 111: 458-467.

Bouhet S, Hourcade E, Loiseau N et al (2004) The mycotoxin fumonisin B_1 alters the proliferation and the barrier function of porcine intestinal epithelial cells. Toxicol Sci 77: 165–171.

Calado T, Fernández-Cruz ML, Cabo Verde S et al (2018) Gamma irradiation effects on ochratoxin A: Degradation, cytotoxicity and application in food. Food Chem 240: 463–471.

Celik I, Oğuz H, Demet O et al (2000) Embryotoxicity assay of aflatoxin produced by *Aspergillus parasiticus* NRRL 2999. Br Poult Sci 41: 401–409.

Ciacci-Zanella JR, Merrill AH Jr, Wang E et al (1998) Characterization of cell-cycle arrest by fumonisin B_1 in CV-1 cells. Food Chem Toxicol 36: 791–804.

Constable PD, Smith GW, Rottinghaus GE et al (2000) Ingestion of fumonisin B_1-containing culture material decreases cardiac contractility and mechanical efficiency in swine. Toxicol Appl Pharmacol 162: 151–160.

De Jesus CL, Bartley A, Welch AZ et al (2018) High incidence and levels of ochratoxin A in wines sourced from the United States. Toxins 10: 1.

Fatokun AA, Stone TW, Smith RA (2006) Hydrogen peroxideinduced oxidative stress in MC3T3-E1 cells: The effects of glutamate and protection by purines. Bone 39: 542–551.

Friedman ND, Temkin E, Carmeli Y (2016) The negative impact of antibiotic resistance. Clin Microbiol Infect 22: 416-22.

Gagliano N, Donne ID, Torri C et al (2006) Early cytotoxic effects of ochratoxin A in rat liver: A morphological, biochemical and molecular study. Toxicology 15: 214–224.

Gayathri L, Dhivya R, Dhanasekaran D et al (2015) Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: In *vitro* study in HepG2 cell. Food Chem Toxicol 83: 151–163.

Goudjal Y, Toumatia O, Sabaou N, et al (2013) Endophytic actinomycetes from spontaneous plants of Algerian Sahara: Indole-3-acetic acid production and tomato plants growth promoting activity. World J Microbiol Biotechnol 29: 1821-9.

Greco MV, Pardo AG, Ludemann V et al (2012) Mycoflora and natural incidence of selected mycotoxins in rabbit and chinchilla feeds. Sci World J 2012: 956056.

Gupta GN, Srivastava S, Khare SK et al (2014) Extremophiles: an overview of microorganism from extreme environment. Int J Agricult Environ Biotechnol 7: 371-380.

Huffman J, Gerber R, Du L (2010) Recent advancements in the biosynthetic mechanisms for polyketide-derived mycotoxins. Biopolymers 93: 764–776.

Ikegwuonu FI (1983) The neurotoxicity of aflatoxin B_1 in the rat. Toxicology 28: 247–259.

Kurapova I, Zenova GM, Sudnitsyn II et al (2012) Thermotolerant and thermophilic actinomycetes from soils of Mongolia Desert steppe zone. Microbiology 81: 98-108.

Limonciel A, Jennings P (2014) A review of the evidence that ochratoxin A is an Nrf2 inhibitor: implications for nephrotoxicity and renal carcinogenicity. Toxins 6: 371–379.

Loiseau N, Debrauwer L, Sambou T et al (2007) Fumonisin B_1 exposure and its selective effect on porcine jejunal segment: Sphingolipids, glycolipids and trans-epithelial passage disturbance. Biochem Pharmacol 74: 144–152.

Magnussen A, Parsi MA (2013) Aflatoxins, hepatocellular carcinoma and public health. World J Gastroenterol 19: 1508–1512.

Mahajan GB, Balachandran L (2012) Antibacterial agents from actinomycetes - A review. Front Biosci 4: 240-53.

Mata AT, Ferreira JP, Oliveira BR et al (2015) Bottled water: analysis of mycotoxins by LC-MS/MS. Food Chem 176: 455–464.

Mehrzad J, Malvandi AM, Alipour M et al (2017) Environmentally relevant level of aflatoxin B_1 elicits toxic pro-inflammatory response in murine CNS-derived cells. Toxicol Lett 279: 96–106.

Mohammadipanah F, Wink J (2015) Actinobacteria from arid and Desert habitats: Diversity and biological activity. Front Microbiol 6: 1541.

Njoki LM, Okoth SA, Wachira PM (2017) Effects of medicinal plant extracts and photosensitization on aflatoxin producing *Aspergillus flavus* (Raper and Fennell). Int J Microbiol 5: 1–9.

Palabiyik SS, Erkekoglu P, Zeybek ND et al (2013) Protective effect of lycopene against ochratoxin A induced renal oxidative stress and apoptosis in rats. Exp Toxicol Pathol 65: 853–861.

Peraica M, Radić B, Lucić A et al (1999) Toxic effects of mycotoxins in humans. Bull World Health Organ 77: 754–766.

Perši N, Pleadin J, Kovačević D et al (2014) Ochratoxin A in raw materials and cooked meat products made from OTA-treated pigs. Meat Sci 96: 203–210.

Pizzolato Montanha F, Anater A, Burchard JF et al (2018) Mycotoxins in dry-cured meats: A review. Food Chem Toxicol 111: 494–502.

Sava V, Reunova O, Velasquez A et al (2006) Can low level exposure to ochratoxin-A cause parkinsonism? J Neurol Sci 249: 68–75.

Shirokikh IG, Shirokikh AA (2017) Soil actinomycetes in the national forest park in northeastern China. Eurasian Soil Sc 50: 78.

Smith GW, Constable PD, Tumbleson ME et al (1999) Sequence of cardiovascular changes leading to pulmonary edema in swine fed culture material containing fumonisin. Am J Vet Res 60: 1292–1300.

Štikarová J, Kotlín R, Riedel T et al (2013) The effect of reagents mimicking oxidative stress on fibrinogen function. Sci World J 2013: 359621.

Sur E, Celik I (2003) Effects of aflatoxin B_1 on the development of the bursa of Fabricius and blood lymphocyte acid phosphatase of the chicken. Br Poult Sci 44: 558–566.

Theumer MG, Henneb Y, Khoury L et al (2018) Genotoxicity of aflatoxins and their precursors in human cells. Toxicol Lett 287: 100–107.

Thuvander A, Breitholtz-Emanuelsson A, Olsen M (1995) Effects of ochratoxin A on the mouse immune system after subchronic exposure. Food Chem Toxicol 33: 1005–1011.

Varga J, Kocsubé S, Péteri Z, et al (2010) Chemical, physical and biological approaches to prevent ochratoxin induced toxicoses in humans and animals. Toxins 2: 1718-50.

Wu HC, Santella R (2012) The role of aflatoxins in hepatocellular carcinoma. Hepat Mon 12: e7238.

Annexe

Composition des milieux de culture

Milieu PDA (Potatoes Dextrose agar)

Pomme de terre (macération 500ml de filtrat)	200 g
Dextrose	10 g
Agar	15 g
Eau distillée	1000 ml

Milieu CDA (Czapek Dox Agar)

Sucrose	30 g
KH2PO4	1 g
KCL	0.5 g
MgSO4	0.5 g
FeSO4	0.01 g
NaNO3	3 g
Agar	15 g
Eau distillée	1000 ml

(Czapek Concentre)

NaNO3	30 g
KCL	5 g
MgSO4	5 g
FeSO4	0.1 g
Eau distillée	100 ml

Milieu MEA (Malt Extract Agar)

Matière Sèche	50 g
Agar	5 g
Eau distillée	1000 ml

Milieu YES (Yeast Extract Sucrose)

Sucrose	40 g
Extrait de Levure	20 g
Eau distillée	1000 ml

Milieu CYA (Czapek Yeast Agar)

Czapek Concentre	10 ml
KH2PO4	1 g
Extrait de levure	5 g
Sucrose	30 g
Agar	15 g
Eau distillée	1000 ml

Milieu G25N (25 % Glycérol Nitrate Agar)

KH2PO4	0.75 g
Czapek Concentre	7.5 ml
Extrait de levure	3.7 g
glycerol	250 g
Agar	12 g
Eau distillée	750 ml

Milieu AFPA (Milieu Selectif pour *A. flavus* et *A. parasiticus*)

Extrait de levure	20 g
peptone	10 g
Citrate ferrique ammoniacal	0.5 g
chloramphenicol	0.1 g
Dichloran solution ethaolique à 0.2%	1 ml
Agar	15 g
Eau distillée	1000 ml

Eau physiologique

NaCl	9 g
Eau distillée	1000 ml

Lactophenol

Phénol pur cristallisé	20 g
Acide lactique	20 ml
Glycérol pur	20 ml
Eau distillée	40 ml

Rose Bengal

Rose bengal	1 g
Eau distillée	100 ml

Bleu de coton

Lactophénol	0,5 g
bleu de méthylène	

Starch Casein Agar medium

Soluble starch	10.00 g
Casein	0.30 g
KNO_3	2.00 g
$MgSO_4.7H_2O$	0.05 g
K_2HPO_4	2.00 g
NaCl	2.00 g
CaCO3	0.02 g
$FeSO_4.7H_2O$	0.01 g
Agar	18.00 g
Eau distillée	1000 ml

GYM (Agar) medium

Glucose	4.0 g
Yeast extract	4.0 g
Malt extract	10.0 g
$CaCO_3$	2.0 g
Agar	12.0 g
Distilled water	1000.0 ml

GYM (Broth) medium

Glucose	4.0 g

Yeast extract	4.0 g
Malt extract	10.0 g
$CaCO_3$	2.0 g
Distilled water	1000.0 ml

ISP2 medium

Yeast extract	4.0 g
Malt extract	10.0 g
Dextrose	4.0 g
Agar	20.0 g
Distilled water	1000.0 ml

ISP3 medium

Oat Meal	20.00 g
Agar	18.000 g

Adjust pH to 7.2. Cook or steam 20.0 g in 1000.0 ml distilled water for 20 min. Filter through cheese cloth. Add 18.0 g agar and make up to 1000.0 ml. Add 1 ml of trace salts solution:

$FeSO_4$ x 7 H_2O	0.1 g/100ml
$MnCl_2$ x 4 H_2O	0.1 g/100ml
$ZnSO_4$ x 7 H_2O	0.1 g/100ml

ISP4 medium

Soluble starch	10.0 g
$(NH_4)_2SO_4$	1 g
$CaCO_3$	2.0 g
K_2HPO_4	1.0 g
$MgSO_4$ x 7 H_2O	1.0 g
NaCl	1.0 g
Agar	20 g
Distilled water	1000.0 ml

ISP5 medium

L-asparagine (anhydrous basis)	1.0 g
Agar 20.0 g	
Glycerol	10.0 g
K_2HPO_4 (anhydrous basis)	1.0 g
Distilled water	1000.0 ml
Trace salts solution:	1.0 ml
$FeSO_4$ x 7 H_2O	1 g/100ml
$MnCl_2$ x 4 H_2O	1 g/100ml
$ZnSO_4$ x 7 H_2O	1 g/100ml
pH 7.2	

ISP6 medium

Peptone	15.00 g
Proteose Peptone	5.00 g
Ferric Ammonium Citrate	0.50 g
Dipotassium Phosphate	1.00 g
Sodium Thiosulfate	0.08 g

Yeast Extract	1.00 g
Agar	20.00 g
Distilled water	1000.0 ml

ISP7 medium

L-Asparagine	1.00 g
L-Tyrosine	0.50 g
Glycerol	15 g
K_2HPO_4	0.5 g
NaCl	0.5 g
$FeSO_4$ x 7 H_2O	0.01 g
Trace salt solution:	1 ml
Distilled water	1000.0 ml
pH 7.3	

Synthetically Sulter Medium (SST)
Production of melanoid pigment

Glycerol	15 g
Tyrosine	1 g
L-arginine	5 g
L-glutamc acid	5 g
L-methionine	0.3 g
L-isoleucine	0.3 g
K_2HPO_4	0.5 g
$MgSO_4$ x 7 H_2O	0.2 g
Agar	20 g
Distilled water	1000.0 ml
Trace element solution:	1.0 ml
$CuSO_4$ x 5 H_2O	10 g
$CaCl_2$ x 2 H_2O	10 g
$FeSO_4$ x 7 H_2O	10 g
$ZnSO_4$ x 7 H_2O	10 g
$MnSO_4$ x 7 H_2O	40 g
Distilled water	1000.0 ml
pH 7.2	

Control medium is prepared without tyrosine

Medium 5294 metabolite production

Soluble starch	10 g
Yeast extract	2 g
Glucose	10 g
Glycerol	10 g
Corn steep liquor	2.5 g
Peptone	2 g
NaCl	1 g
$CaCO_3$	3 g
Distilled water	1000.0 ml
pH 7.2	

Medium 5254 metabolite production

Glucose	15 g

Soymeal	15 g
Corn steep	5 g
$CaCO_3$	2 g
NaCl	5 g
Distilled water	1000.0 ml
pH 7.2	

Basal medium for carbohydrate utilization

$(NH_4)_2SO_4$	2.64 g
K_2HPO_4	4.31 g
KH_2PO_4	2.38 g
$MgSO_4$ x 7 H_2O	1 g
Agar	15 g
Trace element solution:	1.0 ml
$CuSO_4$ x 5 H_2O	0.64 g
$FeSO_4$ x 7 H_2O	0.11 g
$ZnSO_4$ x 7 H_2O	0.15 g
$MnCl_2$ x 4 H_2O	0.79 g
Distilled water	1000.0 ml
pH 7.3	

- Carbohydrates solutions with 10% oh the 10carbohydrates (2.4) are prepared.
- After autoclaving, 100ml of one of the sterile filtrated carbohydrate solutions are added (2.4)

Sodium chlorite tolerance

Casein peptone	10 g
Yeast extract	5 g
Agar	20 g
Distilled water	
pH 7	

Screening phytochimique et activités antifongique et antibactérienne des substances bioactives de plantes médicinales et d'actinomycètes prélevées des sols sahariens.

Résumé

L'objectif de ce travail est de mettre en évidence l'intérêt biologique de trois plantes médicinales et d'un isolat d'actinobactérie dans le contrôle de quelques souches bactériennes et fongiques ainsi que l'inhibition de la synthèse de quelques mycotoxines.

Pistacia lentiscus, Citrullus colocynthis, Artemisia herba-alba et un isolat d'actinobactérie forestière ont été sélectionnés pour évaluer leur activités antibactérienne, antifongique, antimycotoxinogène et antioxydante. Les trois plantes sont prélevées des régions steppique et saharienne de l'Algérie. Les résultats du screening phytochimique ont révélé la richesse des trois espèces végétales en substances bioactives. Les résultats des tests biologiques suggèrent que les extraits méthanolique et aqueux d'*A. herba alba* sont dotés d'une excellente activité antifongique contre *F. graminirium* CECT 2150, *A. ochraceus* NRRL 3174 et *P. expansum* CECT 2278. La synthèse d'ochratoxine est significativement inhibée par les extraits méthanoliques d'*A. herba alba* et des graines de *C. colocynthis*. L'activité antioxydante des extraits organiques des trois plantes est démontrée.

L'actinobactérie est isolée d'un sol forestier de la région de Saida. L'identification moléculaire à base d'ARNr 16S a révélé que l'isolat d'actinobactérie désigné V_{002} appartient au genre *Streptomyces*. Cette souche est considérée comme nouvelle espèce productrice de spectinabiline, d'undecylprodigiosine et de metacycloprodigiosine. La souche *Streptomyces sp.* V_{002} est considérée comme un producteur prometteur de prodigiosine. Elle est également considérée comme agent antimicrobien efficace en raison de son rôle important dans l'inhibition des bactéries à Gram positif. Les métabolites secondaires de cette souche ont une puissante capacité antioxydante dans l'inhibition d'ABTS et le piégeage de DPPH.

Les métabolites secondaires de *P. lentiscus, C. colocynthis, A. herba alba* et de la souche V_{002} représentent une alternative prometteuse pour éliminer certains microbes pathogènes. Ils sont aussi considérés comme agents détoxifiant permettant d'éliminer certaines mycotoxines. L'excellente activité antioxydante des extraits leur confère une place importante dans le traitement des maladies causées par le stress oxydatif.

Mots-clés : *Pistacia lentiscus*; *Citrullus colocynthis*; *Artemisia herba-alba*; Actinobactérie; Activité antioxydante; Activité antibactérienne; Activité antifongique; Spectinabiline. Undecylprodigiosine. Metacycloprodigiosine.

Phytochemical screening and antifungal and antibacterial activity of bioactive substances from medicinal plants and actinomycetes collected from Saharan soils.

Abstract

The aim of this work is to demonstrate the biological interest of three medicinal plants and an isolate of actinobacteria in the control of certain bacterial and fungal strains as well as the inhibition of the synthesis of certain mycotoxins.

Pistacia lentiscus, Citrullus colocynthis, Artemisia herba-alba and a forest actinobacterium were selected to assess their antibacterial, antifungal, antimycotoxin and antioxidant activities. The three medicinal plants are collected from the steppes and the Saharan regions of Algeria. The results of the phytochemical screening revealed the richness of the three medicinal plant species in bioactive substances. The results of biological tests suggest that the methanolic and aqueous extracts of *A. herba-alba* have a significant antifungal activity against *F. graminirium* CECT 2150, *A. ochraceus* NRRL 3174 and *P. expansum* CECT 2278. Ochratoxin synthesis is significantly inhibited by methanolic extracts from *A. herba-alba* and *C. colocynthis* seeds. The antioxidant activity of organic extracts from the three plants has been demonstrated.

The actinobacterium is isolated from forest soil in the Saida region. Molecular identification using 16S rRNA revealed that the actinobacterium isolate designated V_{002} belongs to the genus *Streptomyces*. This strain is considered to be a new species producing spectinabilin, undecylprodigiosin and metacycloprodigiosin. The strain *Streptomyces sp*. V_{002} is considered as a promising producer of prodigiosin. It is also considered to be an effective antimicrobial agent due to its important role in inhibiting Gram-positive bacteria. The secondary metabolites of this strain have a potent antioxidant capacity to inhibit ABTS and scavenge DPPH.

The secondary metabolites of *P. lentiscus, C. colocynthis, A. herba alba* and strain V_{002} represent a promising alternative to eliminate certain pathogenic microbes. They are also considered to be detoxifying agents which eliminate certain mycotoxins. The excellent antioxidant activity of the extracts gives them an important place in the treatment of diseases caused by oxidative stress.

Keywords: *Pistacia lentiscus; Citrullus colocynthis; Artemisia herba-alba;* Actinobacterium; Antioxidant activity; Antibacterial activity; Antifungal activity; Spectinabilin; Undecylprodigiosin; Metacycloprodigiosin.

تشخيص المكونات الكيمائية النباتية والنشاطات الحيوية المضادة للفطريات والبكتيريا لبعض المواد الحيوية المستخلصة من النباتات الطبية ونوع من بكتيريا التربة (الأكتينوبكتيريا) المأخوذة من تربة الصحراء.

الملخص

الهدف من إنجاز هذا العمل هو إظهار الفوائد البيولوجية لثلاث أنواع من النباتات الطبية وكذا عزل الأكتينوبكتيريا وذلك من اجل مكافحة بعض السلالات البكتيرية والفطرية الضارة وكذلك تثبيط بعض السموم الفطرية.

تم اختيار شجرة الضرو، فاكهة الحنظل وعشبة الشيح إضافة الى عزل الأكتينوبكتيريا وذلك من اجل دراسة وتقييم أنشطتها المضادة للبكتيريا، للفطريات، لسموم بعض الفطريات وكذلك لتقيم نشاطها الحيوي المضاد للأكسدة. تم جمع النباتات الطبية الثلاث من مناطق السهوب والصحراء الجزائرية. أظهرت نتائج الفحص الكيميائي النباتي ثراء النباتية الطبية بالمواد البيوكيميائية النشطة، كما تشير نتائج الاختبارات البيولوجية إلى أن المستخلصات الميثانولية والمائية لنبات الشيح ان لها نشاط بيولوجي كبير مضاد للفطر
F. graminirium CECT 2150, A. ochraceus NRRL 3174 ,P. expansum CECT 2278

تشير نتائج الاختبارات البيولوجية أيضا ان المستخلصات الميثانولية لبذور الحنظل وعشبة الشيح لها القدرة على تثبيط السموم الفطرية (الأكراتوكسين)، كما تم إثبات النشاطات المضادة للأكسدة للمستخلصات العضوية للنباتات الطبية الثلاثة.

تم عزل الأكتينوبكتيريا من تربة غابة العقبان لولاية سعيدة حيث كشف التحديد الجيئي باستخدام ARNr 16S الى ان الأكتينوبكتيريا المعزولة V_{002} تنتمي الى سلالة *Streptomyces*

تعتبر هذه البكتيريا كمنتج جديد لسبيكتينابلين ، وندسيل بروديجيوسين وميتاسيكلوبروديجيوسين. كما تعتبر أيضا عاملًا فعالًا مضادًا للميكروبات نظرًا لدورها المهم في تثبيط البكتيريا+Gram .تحتوي المستقلبات الثانوية لهذه البكتيريا مضادات للأكسدة

تمثل المستقلبات الثانوية للنباتات الطبية الثلاث وكذا المستقلبات الثانوية للأكتينوبكتيريا المعزولة بديلاً واعدًا للقضاء على بعض الميكروبات المسببة للأمراض، كما تعتبر أيضًا عوامل محفزة للقضاء على الفطريات او تثبيط سمومها. يمنح النشاط الممتاز المضاد للأكسدة للمستخلصات مكانًا مهمًا في علاج الأمراض الناتجة عن الإجهاد التأكسدي.

الكلمات المفتاحية : شجرة الضرو، فاكهة الحنظل، عشبة الشيح، الأكتينوبكتيريا، مضاد للبكتيريا، مضادة للبكتيريا، مضاد للفطر، مضاد للأكسدة، سبيكتينابلين، ندسيل بروديجيوسين، ميتاسيكلوبروديجيوسين.