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THEME

Bioguided phytochemical study of extracts from some Saharan plants and their effect on *Fusarium oxysporum* f. sp. *albedinis* cellulase enzymes

Etude phytochimique bioguidée des extraits de quelques plantes sahariennes et leur effet sur les cellulases du *Fusarium oxysporum* f. sp. *albedinis*

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I discovered how science is truly a universal language, one that forges new connections among individuals and opens the mind to ideas that go far beyond the classroom.

Ahmed Hassan Zewail

(1946– 2016)

Noble Prize for Chemistry (1999)

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Abstract

Fusarium oxysporum f. sp. *albedinis* (Foa) is a soil-borne fungus causing the most serious disease of date palm (*Phoenix dactylifera* L.) called "Bayoudh'. In this study, the objective was to search for substances with an antifungal effect on Foa, as well as the enzymatic activity on its cellulases (the cell wall degradation enzymes of the host plant). For this, nine plants from the Algerian Sahara (South-West of Algeria) were chosen to evaluate their extracts, namely: *Acacia raddiana*, *Anabasis aretioides, Asteriscus graveolens, Calotropis procera, Citrullus colocynthis*, *Launeae arborescens, Limoniastrum feei*, *Nerium oleander,* and *Pergularia tomentosa*. The aerial parts of each plant were used to evaluate their extracts and fractions.

Antifungal activity evaluation was realized by direct bioautography. The cellulases inhibition test was realized by contact bioautography. Also, a phytochemical screening has been done for these extracts on TLC plates. Phytochemical screening was intended to know which metabolite is responsible for the antifungal activity through correlation with antifungal and anti-cellulases effects.

The phytochemical study revealed the presence of saponins, flavonoids, tannins, coumarins, and alkaloids in these extracts. The different extracts showed high variability in their phenolic and flavonoids contents as well as antifungal capacity, as in the case of ethyl acetate extracts. Detection of inhibition zones was revealed with ethanolic Gram's iodine. Inhibition zones were determined as dark brown spots. The results of enzymatic activity -by contact bioautography technique- showed that some extracts of *L. feei, L. arborescens,* and *A. graveolens* present a significant effect on cellulases of Foa with a zone inhibition diameter higher than 2 mm. Two bioactive compounds were identified, namely: methyl gallate and syringic acid from the ethyl acetate fractions of the aerial parts of *L. arborescens* and *A. graveolens* using NMR (^{1}H and ^{13}C) spectrophotometry and LC-MS.

Keywords: Medicinal plants, phytochemical study, *Phoenix dactylifera* L., *Fusarium oxysporum* f. sp. *albedinis*, cellulases, Bioautography.

دراسة كيميائية نباتية موجهة بيولوجيًا لمستخلصات بعض النباتات الصحراوية و تأثيرها على إنزيمات السيليولاز *Fusarium oxysporum* f. sp. *albedinis*

الملخص

Fusarium oxysporum f. sp. *albedinis* (Foa) هو فطر ينتقل عن طريق التربة ويسبب أخطر أمراض نخيل التمر (*Phoenix dactylifera* L.) ويسمى "البيوض". في هذه الدراسة ، كان الهدف هو البحث عن المواد ذات التأثير المضاد للفطريات على Foa ، بالإضافة إلى النشاط الإنزيمي على سليولازها (إنزيمات تحلل جدار الخلية للنبات المضيف). لهذا الغرض، تم اختيار تسعة نباتات من الصحراء الجزائرية (جنوب غرب الجزائر) لتقييم مستخلصاتها، وهي: *Acacia raddiana*, *Anabasis aretioides, Asteriscus graveolens, Calotropis procera, Citrullus colocynthis*, *Launeae arborescens, Limoniastrum feei*, *Nerium oleander,* و *Pergularia tomentosa* . تم استخدام الأجزاء الهوائية من كل نبتة لتقييم مستخلصاتها .

تم تقييم النشاط المضاد للفطريات عن طريق ، الكروماتوغرافيا الحيوية الذاتية (بيواوتوغرافيا) المباشرة. تم إجراء اختبار تثبيط أنزيمات السيليولاز عن طريق الكروماتوغرافيا الحيوية الذاتية (بيواوتوغرافيا) التلامسية. أيضًا، تم إجراء فحص كيميائي نباتي لهذه المستخلصات على صفائح TLC. كان الهدف من الفحص الكيميائي النباتي هو معرفة المركبات الأيضية المسؤولة عن النشاط المضاد للفطريات من خلال الارتباط مع التأثير المضاد للفطريات ومضادات أنزيمات السيليولاز.

كشفت الدراسة الكيميائية النباتية عن وجود مركبات الصابونين ,الفلافونويد ,العفص ,الكومارين والقلويدات في هذه المستخلصات. أظهرت المستخلصات المختلفة تباينًا كبيرًا في محتوياتها من الفينول والفلافونويدات بالإضافة إلى قدرتها المضادة للفطريات ، كما هو الحال في مستخلصات أسيتات الإيثيل. تم الكشف عن مناطق التثبيط باستخدام محلول يودي. تم تحديد مناطق التثبيط على أنها بقع بنية داكنة. أظهرت نتائج النشاط الإنزيمي - عن طريق تقنية البيواوتوغرافيا التلامسية - أن بعض مستخلصات *L. feei* و *L. arborescens* و *A. graveolens* لها تأثير قوي على أنزيمات السيليولاز.Foa وذلك عند منطقة التثبيط بقطر أعلى من 2 مم. تم تحديد مركبين نشطين بيولوجيين هما: ميثيل غالات و حمض السيرنجيك من أجزاء أسيتات الإيثيل للأجزاء الهوائية من *L. arborescens* و A. *graveolens* باستخدام مطياف الرنين المغناطيسي النووي (H^1 و C^{13}) و LC-MS.

الكلمات المفتاحية: نباتات طبية ، دراسة كيميائية نباتية ، *Fusarium oxysporum* f. sp. *albedinis* ، *Phoenix dactylifera* L. ، أنزيمات السيليولاز ، الكروماتوغرافيا الحيوية الذاتية (بيواوتوغرافيا).

Etude phytochimique bioguidée des extraits de quelques plantes sahariennes et leur effet sur les cellulases du *Fusarium oxysporum* f. sp. *albedinis*

Résumé

Fusarium oxysporum f. sp. *albedinis* (Foa) est un champignon du sol responsable de la maladie la plus grave du palmier dattier (*Phoenix dactylifera* L.) appelée «Bayoudh». Dans cette étude, l'objectif était de chercher des substances ayant un effet antifongique sur Foa, ainsi que l'activité enzymatique sur ses cellulases (les enzymes de dégradation de la paroi cellulaire de la plante hôte). Pour cela, neuf plantes du Sahara Algérien (Sud-Ouest de l'Algérie) ont été choisies pour évaluer leurs extraits, à savoir : *Acacia raddiana*, *Anabasis aretioides, Asteriscus graveolens, Calotropis procera, Citrullus colocynthis*, *Launeae arborescens, Limoniastrum feei*, *Nerium oleander,* et *Pergularia tomentosa*. Les parties aériennes de chaque plante ont été utilisées pour évaluer leurs extraits et fractions.

L'évaluation de l'activité antifongique a été réalisée par bioautographie directe. Le test d'inhibition des cellulases a été réalisé par bioautographie de contacte. En outre, un criblage phytochimique a été effectué pour les extraits sur des plaques CCM. Le criblage phytochimique visait à savoir quel métabolite est responsable de l'activité antifongique par corrélation avec l'effet antifongique et anti-cellulases.

L'étude phytochimique a révélé la présence des saponosides, flavonoïdes, tanins, coumarines et des alcaloïdes dans les extraits. Les différents extraits ont montré une grande variabilité dans leurs teneurs phénoliques et flavonoïdes ainsi qu'une capacité antifongique, comme dans le cas des extraits d'acétate d'éthyle. La détection des zones d'inhibition a été révélée avec la coloration iodine de Gram. Les zones d'inhibition ont été déterminées comme des taches brun foncé. Les résultats de l'activité enzymatique -par technique de bioautographie de contacte- ont montré que certains extraits de *L. feei*, *L. arborescens* et *A. graveolens* présentent un effet significatif sur les cellulases de Foa avec un diamètre de zone d'inhibition supérieur à 2 mm. Deux composés bioactifs ont été identifiés, à savoir : le gallate de méthyle et l'acide syringique à partir fractions d'acétate d'éthyle des parties aériennes de *L. arborescens* et *A. graveolens* par spectrophotométrie RMN (^{1}H et ^{13}C) et LC-MS.

Mots clés: Plantes médicinales, étude phytochimique, *Phoenix dactylifera* L., *Fusarium oxysporum* f. sp. *albedinis*, cellulases, bioautographie.

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ABBREVIATIONS

CC	Open-Column Chromatography
$CDCl_3$	Deuterated chloroform
CLA	Carnation Leaf Agar
CON	Control
COSY	Correlated Spectroscopy
CPC	Centrifugal Planar Chromatography
CWDE	Cell-Wall-Degrading Enzymes
DCM	Dichloromethane
EtOAc	Ethyl Acetate
EtOH	Ethanol
f. sp.	*Formae Speciales*
Foa	*Fusarium oxysporum* f. sp. *albedinis*
Hep	Heptane
Hex	Hexane
IR	Infrared
LC	Liquid Chromatography
LD	Low Dose
LD_{50}	Median Lethal Dose
MeOH	Methanol
MS	Mass Spectrometry

NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
PDA	Potatoes Dextrose Agar
PE	Petroleum Ether
PLC	Preparative Layer Chromatography
POSL	Phytochemistry and Organic Synthesis Laboratory
SEM	Standard Error Of Means
SNA	Synthetic Nutrient-Poor Agar
SPE	Solid-Phase Extraction
Syn.	Synonym
TLC	Thin-Layer Chromatography

General Introduction

General introduction

Historically, humans have always drawn from nature for food, clothing, and health care. On earth, four out of five people use plants to ward off their ailments; four out of five women, especially in emerging countries, do not have any other kind of medicine for their children. In Africa, Asia, and Latin America, many countries are using traditional medicine to meet some of their needs at the primary health care level. In Africa, up to 80% of the population uses traditional medicine for treatment (Chaabi, 2008). The use of medicinal plants from the traditional tropical pharmacopeia nowadays offers enriching research avenues (Sofowora, 2010).

In Southern Algeria, the development of date palm cultivation and the preservation of the already existing phoenicicultural heritage is a major concern of the farmers of these oases (Benabdelkader *et al.*, 2011). In the desert areas, dates have represented a providential fruit, one of the pillars of the economy of the oases, providing a large part of the food. Date palms (*Phœnix dactylifera* L.) are of ecological and socio-economic importance for the Saharan populations. Besides, they provide a suitable microclimate for other crops (fruit, cereals, etc.) and they also protect them against the wind. For this, palm trees represent food and ecological security measure (El Hadrami *et al.*, 1998). However, its culture is threatened by several pests and diseases such as *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *albedinis* (Ait Kettout *et al.*, 2010).

Many species of *Fusarium* are serious plant pathogens, causing symptoms such as necrotic lesions, putrefaction….etc. *Fusarium oxysporum* f. sp. *albedinis* (Foa) is the causative agent of Bayoudh, the disease that affects the date palm. According to previous studies since its first sighting over a century ago (before 1870), Bayoudh has killed millions of date palms in Algeria and Morocco (Ait Kettout *et al.*, 2010). It is known that the onset of the disease is the result of several factors called "pathogenicity factors". These factors are mainly enzymes and toxins. The most important enzymes from the point of view of Foa pathogenicity are the CWDEs (Cell Wall Degrading Enzymes). Among these enzymes, cellulases which hydrolyze cellulose (El Modafar *et al.*, 2000).

General introduction

This present work consists of studying the enzymatic inhibition of cellulose degradation. This study targeted the cellulases of *Fusarium oxysporum* f. sp. *albedinis*, enzymes that break down the cell walls of the host plant (*Phœnix dactylifera* L.). For this purpose, nine plants from the Bechar region (Southwest of Algeria) from various families were chosen: five medicinal plants: *Acacia raddiana, Asteriscus graveolens (Forsk.), Limoniastrum feei, Anabasis aretioides Moq . and Coss., Launeae arborescens* (Batt.) Murb .; and four poisonous plants: *Citrullus colocynthis (L.) Schrad, Calotropis procera Ait., Nerium oleander, Pergularia tomentosa*. The aerial parts of each plant were used to evaluate their extracts. The cellulase activity test was performed by contact bioautography, as well as the bioguided phytochemical study of bioactive extracts. Finally, the isolation and structural identification of the pure secondary metabolites of the bioactive extracts was performed.

This thesis is divided into four parts;

- The first chapter presents a bibliographical summary of the studied medicinal plants, their general description, their biological activities as well as their phytochemical data which are based on the methods of extraction, separation, and identification of natural products.

- The second chapter is reserved for a review of the natural products used to fight against *Fusarium oxysporum* f. sp. *albedinis*. This part presents the analysis of the main results of the antifungal activity against *F. oxysporum* of agents such as biofungicides (mainly from plants and microorganisms) or natural products derived from these organisms which have been carried out by studies during the last years.

- The third chapter presents materials and methods used in this study, hence describes the methods of preparation of extracts from the different plants studied, then the bioguided fractionation, as well as the study of phytochemical extracts. In another part, it explains the methods and techniques for evaluating antifungal activity against *Fusarium oxysporum* f. sp. *albedinis.* Besides, the experimental evaluation of parameters related to the cellulase activity of Foa was done. Finally, the isolation and structural identification of pure secondary metabolites from bioactive extracts, using spectroscopic techniques was carried out.

- The fourth chapter is devoted to the results obtained with discussions in two parts, the chemical part brings together the results of extraction yield and chemical composition of the extracts and the second part presents the results of the evaluation of the cellulase activity of *Fusarium oxysporum* f. sp. *albedinis* extracts.

And finally, a general conclusion to restore the main results obtained the limits of our work, and some perspectives required to complete and improve this work.

CHAPTER I

Phytochemistry and biological activities of studied plants

CHAPTER I: Phytochemistry and biological activities of studied plants

1. Introduction

Natural products such as plant extracts are a source of endless opportunities for the discovery of new drugs due to the wide chemical diversity present in plants (Cos *et al.*, 2006). Medicinal plants have been a valuable source of therapeutic agents, and still many of today's drugs are plant-derived natural products or their derivatives (Atanasov *et al.*, 2015). Phenolic compounds are an important class of secondary metabolites of plants possessing various pharmacological activities (Tsimogiannis *et al.*, 2019). These compounds have a significant role in human health with their pharmacological activities, such as anti-inflammatory, anti-allergic, antimicrobial, antiviral, anti-cancer, cardioprotective, and vasodilatory activities (Senhaji *et al.*, 2020).

In previous work of POSL laboratory, the existence of certain parameters and factors that can influence the growth of Foa has been shown (Boulenouar *et al.*, 2009; Boulenouar *et al.*, 2011; Boulenouar *et al.*, 2014; Boulenouar *et al.*, 2012; Ghazi *et al.*, 2020). In this context, cellulases can be targets of some factors and/or substances. So, in order to deepen our research in this niche, we propose in this research to perform an evaluation of parameters related to the cellulase activity of Foa and to study effects of secondary metabolites from some Saharan plants (*Acacia raddiana*, *Anabasis aretioides, Asteriscus graveolens*, *Calotropis procera*, *Citrullus colocynthis*, *Launeae arborescens*, *Limoniastrum feei*, *Nerium oleander,* and *Pergularia tomentosa*) on cellulases.

2. *Acacia raddiana*

2.1. General description and biological activities

Acacia tortilis (Forssk.) Hayne ssp. *raddiana* (Fabaceae) is present in the North and South of the Algerian Sahara. The height of this tree varies between 2 and 10 m and it favors sandy-gravelly substrates (Benhouhou, 2005).

In Algerian Sahara, *Acacia raddiana* tree (**Figure I.1**) provides food and shelter for many desert animals and is a major source of livestock feed and firewood for the native people (Talhi *et al.*, 2010). Also, *Acacia* is one of the plants that have been frequently used as

medicine to treat fever, diarrhea, leukorrhoea, hemoptysis, and throat infections (Embaby *et al.*, 2016).

Figure I. 1: *Acacia raddiana* in its natural habitat (Belhi Z., 2017).

2.2. Phytochemistry of *Acacia raddiana*

New hydrolysable tannin was isolated from the leaves of *A. raddiana*, together with three known galloylglucoses and six known flavonol glycosides. The known compounds are 1-*O*-galloyl-β-glucopyranose, 1,6-di-*O*-galloyl-β-glucopyranose, 1,3,6-tri- *O*-galloyl-β-glucopyranose, quercetin 3-*O*-gentiobioside, quercetin 3-*O*-glucosylgalactoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside **1**, quercetin 3-*O*-rutinoside **2**, and quercetin 3-*O*-galactoside **3** (**Figure I.2**) (El-Mousallamy *et al.*, 1991).

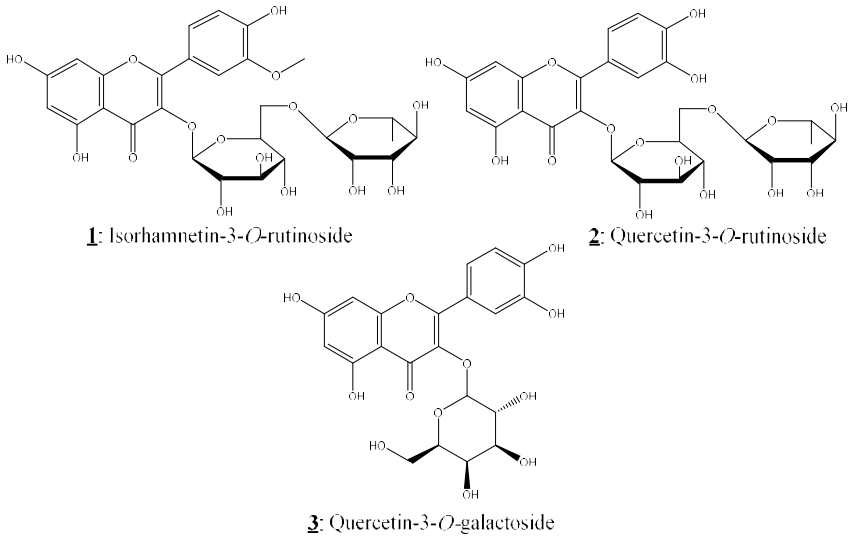


Figure I. 2: Chemical structures of compounds isolated from *Acacia raddiana* (El-Mousallamy *et al.*, 1991)

In a previous study, They reported the phytochemical screening of the bark's *Acacia* and the presence of important quantities of polyphenolic compounds (Flavonoids, Tannin) and other natural substances such as tritepenoids saponin, cellulose, hemicellulose, and lignin (Belhadjadji *et al.*, 2010).

Several hydrolyzable tannins have been reported from *A. raddiana* Savi, including 1,3-di-*O*-galloyl-4,6-(-)-hexahydroxydiphenoyl-β-glucopyranose,1-*O*-galloyl-β-glucosylpyranose **4**, 1,6-di-*O*-galloyl-β-D-glucosylpyranose **5**, and 1,3,6-tri-*O*-galloyl-β-glucosylpyranose **6** (**Figure I.3)** (Subhan *et al.*, 2018).

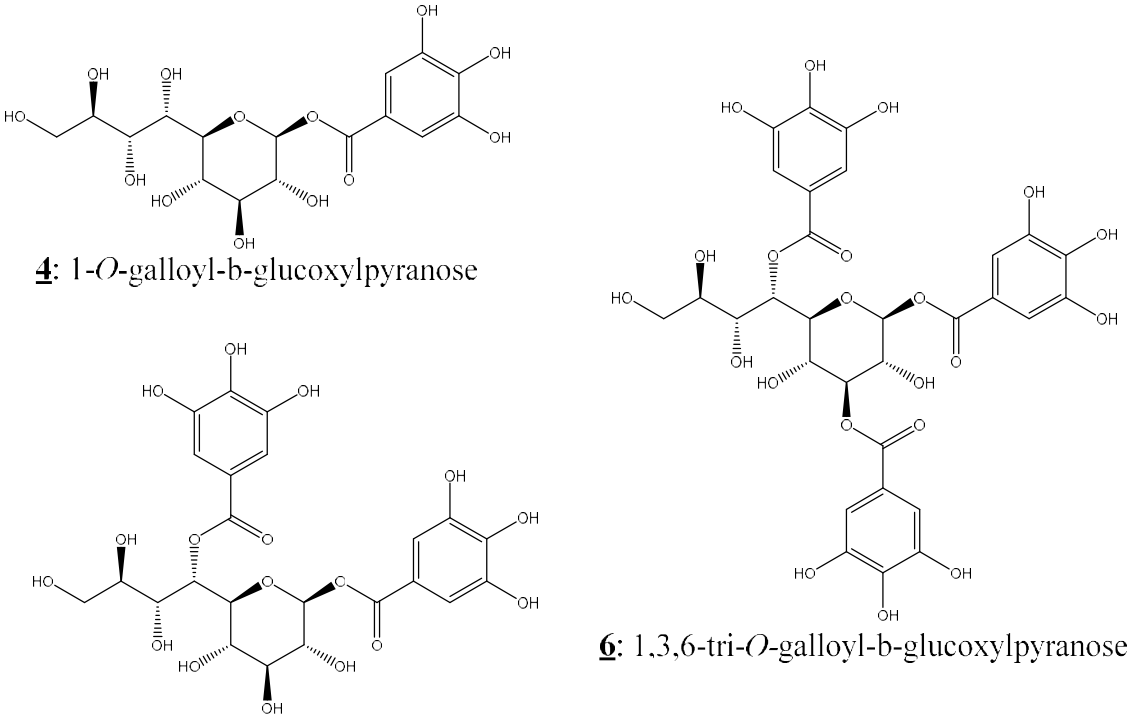


Figure I. 3: Compounds reported in *Acacia raddiana* (Subhan *et al.*, 2018).

3. *Anabasis aretioides*

3.1. General description and biological activities

Anabasis aretioides (Coss & Moq.) or *Fredolia aretioides*, locally called ‘‘Sellaa” or ‘‘degaa”, belonging to the Chenopodiaceae family, is an endemic plant of Morocco and Algeria. This cylindrical shrub is found on the rocky and stony plateau (reg and Hamada) (Benhouhou, 2005) (**Figure I.4**). It's common in the North-Western Sahara from the

Tafilalet, Tinghir in Morocco to Beni-Abbes in Algeria across Beni-Ounif, Ain Sefra, and Bechar (El-Haci *et al.*, 2013). Since ancient times; this plant has been used to manage various diseases (Farid *et al.*, 2018). The leaves of the plant are used as antirheumatismal and diuretic agents and as an antidote against poison (Fakchich *et al.*, 2014). The antimicrobial activity of *A. aretioides* has been studied (Amari *et al*., 2015; Bouchal *et al*., 2019; Boulenouar *et al*., 2012; Senhaji *et al*., 2020). *A. aretioides* aqueous extract exhibited an antihyperglycemic activity (Farid *et al.*, 2018). Also, the antioxidant activity of this herb has been reported (Bentabet *et al.*, 2014; El-Haci *et al.*, 2013; Rached *et al.*, 2010; Senhaji *et al.*, 2020).

Figure I. 4: *Anabasis aretioides* in its natural habitat (Belhi Z., 2017).

3.2. Phytochemistry of *Anabasis aretioides*

Despite the importance of this plant on the pharmacological side, there are not many studies on its chemical composition. Very little research on the contents of polyphenols and flavonoids of *A. aretioides* has been reported in the literature. However, there are a few studies to report.

According to UV, IR, and NMR spectral analysis, POSL team (Belboukhari *et al.*, 2013) separated and identified four natural compounds were from this plant. Three flavonoids 1, 2, 3, and one steroid 4. Namely, (1): 5,3’,4’,- trihydroxy,8-butyl,3-[3’’’,4’’’-dimethyl 2’’’-ene pentanoique-1yl] [2’’-methyl [5’’,6’’ :6,7]] tetra hydro pyrano flavone. (2): 5,7,4’-trihydroxy,3",3",4"–trimethyl- O-3’tetra hydro pyrano[2’,3’:3",4"]-C-2"-cyclo hexane [2’,2":2,3] flavones. (3): 5, 3’. 4’trihydroxy 8-[1-tetrahydrogeranyl]3-*O*-[β-D-glucoside-6 -*O*-acetyl] -*O*-7-[2’’,3’’-tetra methyl[5’’,6’’:6,7] tetrahydropyrano flavone. (4): Spirotano 4,6-diene-3-one,14 -Decyl ester.

El-Haci *et al.* (El-Haci *et al.*, 2013) found that the ethanolic extract of the aerial part of *A. aretioides* presented a high level of phenolic and flavonoid contents (231.85 ± 20.59 mg GAE/g and 132.8 ± 24.58 mg CEQ/g).

The phytochemical study realized by Bentabet *et al.* (Bentabet *et al.*, 2014; Nesrine *et al.*, 2014) allowed them to highlight the existence of alkaloids, flavonoids, tannins, reducing compounds, and saponins in the aqueous extract of roots of *A. aretioides*.

Another study, by Berrani *et al.*, revealed the presence of polyphenols, tannins, alkaloids, reducing compounds, and trace amounts of the proteins, carotenoids, coumarins, and cardiac glycosides but in the methanolic extracts (Berrani, Benassaoui, *et al.*, 2018). Thereafter chemical analysis, effectuated by the same authors, revealed 25 phenolic compounds (Berrani *et al.*, 2018).

Recently, Farid *et al.* (Farid *et al.*, 2018) revealed the presence of **12** polyphenolic compounds in the aqueous extract of the aerial part of *A. aretioides.* In this study, the structures of these molecules were illustrated, namely: Esculetin-6-*O*-glucoside **7**, Esculetin **8**, Esculetin-7-*O*-glucoside **9**, Neochlorogenicacid **10**, 3,4-Dihydroxybenzoicacid **11**, Chlorogenic acid **12**, Luteolin-7-*O*-glucuronide **13**, Cryptochlorogenic acid **14**, Apigenin-7-*O*-glucuronide **15**, 3,5-Dicaffeoylquinic acid **16**, Luteolin **17**, Hispidulin **18** (Figure I.5).

Lately, quantitative analysis showed that ethyl acetate extracts of the aerial part of *A. aretioides* presented a high level of total phenol contents 46.79 ± 0.75 µg GAE/mg E (Senhaji *et al.*, 2020).

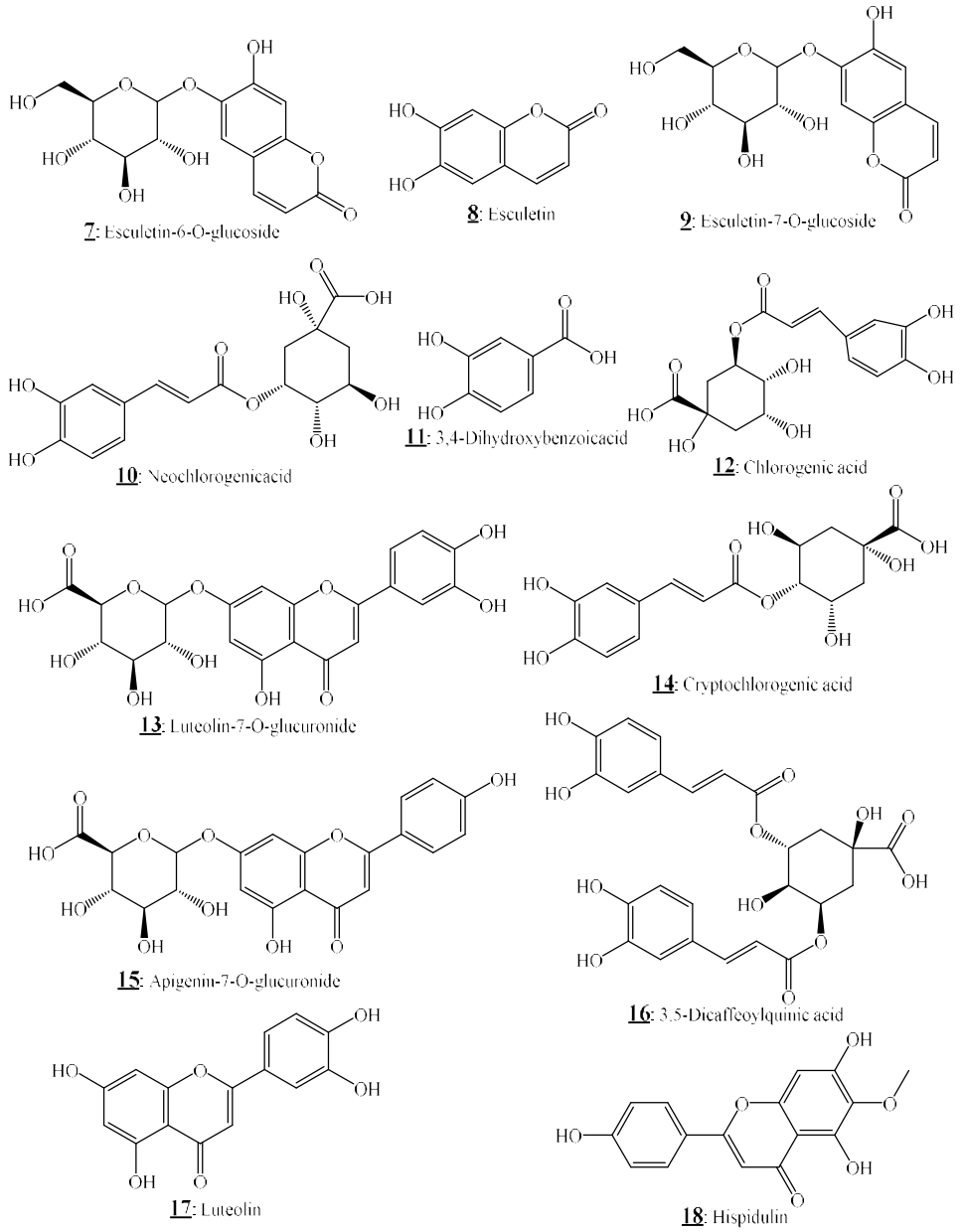


Figure I. 5: Structures of the polyphenolic compounds identified by HPLC–DAD–ESI–MS from aqueous extracts of *Anabasis aretioides* (Farid *et al.*, 2018).

4. *Asteriscus graveolens*

4.1. General description and biological activities

Asteriscus graveolens is an endemic medicinal plant, is a species belonging to the Asteraceae family, mainly distributed in the Southwest of Algeria and the Southeast of Morocco (Quezel *et al.*, 1963). *Asteriscus graveolens* (Forsk) Less. [Syn. *Bubonium*

graveolens, *Buphthalmum graveolens (*Forsk.), *Nauplius graveolens*, and *Bubonium odorum* (Schoub.)] (Cheriti *et al.*, 2007) is an herbaceous aromatic plant, growing in Saharan regions (**Figure I.6**), it is locally known as 'tafss' used in Saharan folk medicine for treating fever, cephalic pains, gastrointestinal tract complaints, stomachic, bronchitis, and an anti-inflammatory (Cheriti *et al.*, 2007; Znini *et al.*, 2011).

Figure I. 6: *Asteriscus graveolens* in its natural habitat (Belhi Z.., 2017).

Asteriscus graveolens extracts exhibited antifungal activity (Boulenouar *et al*., 2014; Boulenouar *et al*., 2012), antibacterial activity (ALrawashdeh *et al.*, 2019; Ramdane *et al.*, 2017), antioxidant, and Antileishmanial activities (Ramdane *et al.*, 2017), and cytotoxic activity (Triana *et al.*, 2016). This plant is also reported to have antimicrobial and hypoglycemic activities (Akssira *et al.*, 2006).

The essential oil of this species exhibits various biological activities such as antioxidant, antimicrobial (Chaib *et al.*, 2017; Melekmi *et al.*, 2006), anti-cancer (Aouissi *et al.*, 2018), and antifungal activities (Znini *et al.*, 2011), which could be a potential natural source of preservative and antifungal compound used in food, cosmetics and pharmaceuticals (Alilou *et al.*, 2014).

4.2. Phytochemistry of *Asteriscus graveolens*

Many studies have focused mainly on the essential oil of this plant, (include) (Aouissi *et al.*, 2018; Chaib *et al.*, 2017; Cheriti *et al.*, 2007; Cristofari *et al.*, 2012; Melekmi *et al.*, 2006; Said *et al.*, 2017; Znini *et al.*, 2011)

The phytochemical screening effectuated by the POSL team (Djelaila, 2007; Saad, 2006), of the leaves and flowers of *A. graveolens,* revealed the presence of alkaloids,

flavonoids, catechetical tannins, terpenes, coumarins, and cyanogenetic compounds. The characterization of molecules by UV spectrophotometry revealed the presence of caffeic acid, nevadensine, luteolin, and artemetin in the leaves of *Asteriscus graveolens* subsp. odorus (Alilou *et al.*, 2014). The presence of polyphenolics, flavonoids, and tannins in all fractions was attested (Ramdane *et al.*, 2017).

In 1991, Ahmed *et al.* isolated 11 flavonoids from the aerial parts of *A. graveolens*: namely, the previously unreported tamarixtin 3-*O*- β-D-robinobioside; the 3- *O* -β-D-glucosides, 3- *O* -β- D-galactosides, and 7- *O* -β- D-glucosides of both kaempferol and quercetin; the 7- *O* -β-D-glucoside of luteolin, and the three free aglycones, quercetin, quercetin 3,4'-dimethyl ether, and quercetagetin 3,6,3'-trimethyl ether.

A new sesquiterpene lactone named Naupliolide **19** was isolated by Akssira *et al.* from *A. graveolens*. Besides five known compounds, 6,7,9,10-tetradehydroasteriscanolide **20** and asteriscunolides A–D (**21a–d**) (**Figure I.7**).

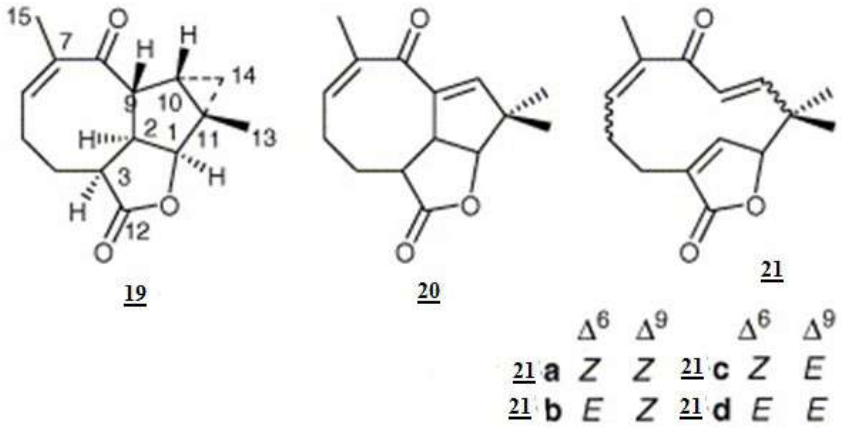


Figure I. 7: Structure of compounds isolated from *Asteriscus graveolens* subsp. Odorus (Akssira *et al.*, 2006)

The phytochemical investigation of an ethanol extract of the aerial parts of *A. graveolens* subsp. *stenophyllus* afforded four new humulenoid sesquiterpenes, namely, 6β,7β-epoxyasteriscunolide A **22**, 2α,3α-epoxyasteriscunolide C **23**, 6β-hydroxyasteriscunolide A **24**, and 6β-ethoxyasteriscunolide A **25**, and also a new asteriscanoid sesquiterpene, asteriscanolidenol **26**, along with the known sesquiterpenoids asteriscunolide A **27**, asteriscunolide C **28**, 8-oxo-6,7,9,10-tetrahydrohumulen-1,12-olide

29, asteriscanolide **30**, and 8-oxo-α-humula-6*E*,9*Z*-dien-12-oic acid **31**, as well as vanillin, stigmasterol, and tricin. Also, 1α,4β,6β-trihydroxyeudesmane **32**, and teuclatriol **33** (**Figure I.8**). (Triana *et al.*, 2016)

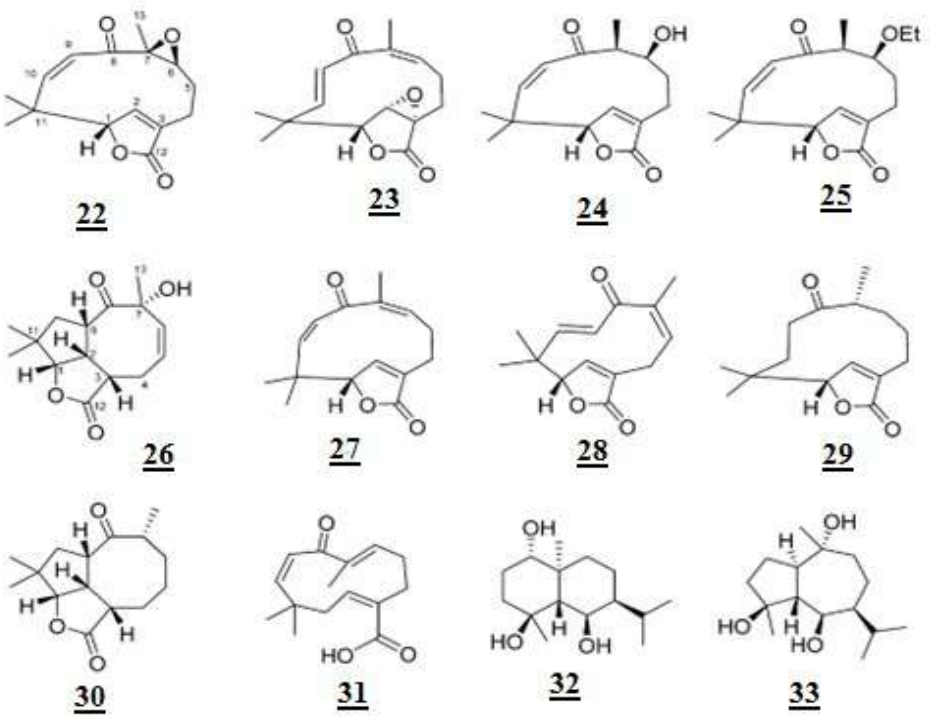


Figure I. 8: Structures of sesquiterpenoids identified from the aerial parts of *Asteriscus graveolens* subsp. *Stenophyllus* (Triana *et al.*, 2016).

Achoub *et al.* (2019) isolated three new sesquiterpenes from *A. graveolens* aerial parts (**Figure I.9**), among other known compounds.

Compound **34** was identified as 7, 12-dihydroxy-6, 7-dihydro-5, (6) E-dehydronerolidol. Compound **35** was identified as 9 β-hydroxy-11β, 13-dihydroparthenolide-9-*O*-β-D-glucopyranoside. Compound **36** was identified as 9α-hydroxy-11β, 13-dihydroparthenolide-9-*O*-β-D-glucopyranoside. The structures of known compounds were elucidated as 9α-hydroxyparthenolide, 9β-hydroxyparthenolide, quercetin 7-*O*-methylester (rhamnetin), borneol 2-*O*-β-D-glucopyranoside, spinacetin 3-*O*-β-D-glucopyranoside, roseoside, spinacetin 3-*O*-rutinoside, 5-*O*-feruloylquinic acid (Achoub *et al.*, 2019).

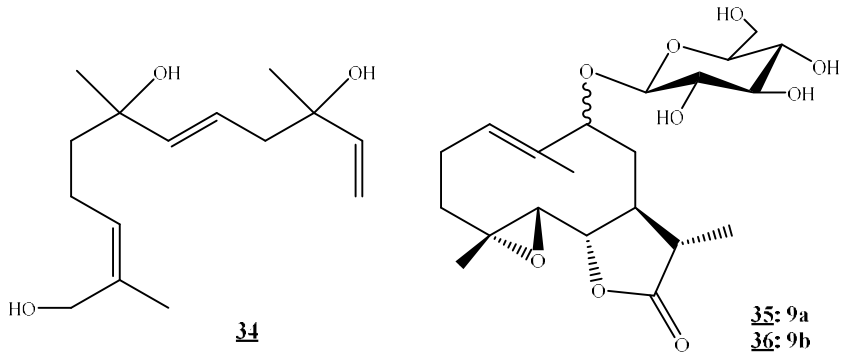


Figure I. 9: Chemical structures of sesquiterpenes isolated from *Asteriscus graveolens* aerial parts (Achoub *et al.*, 2019).

The major compound named myricetin 3'-*O*-rhamnoside **37** was isolated for the first time from the leaves of *A. graveolens* (**Figure I.10)**. Which has an antioxidant potential (Messaoudi *et al.*, 2018).

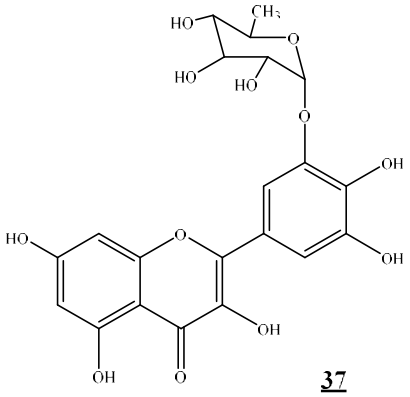


Figure I. 10: Structure of myricetin 3'-*O*-rhamnoside isolated from *Asteriscus graveolens* (Messaoudi *et al.*, 2018).

5. *Calotropis procera*

5.1. General description and biological activities

Calotropis procera (Ait.), a wild-growing plant of the family Asclepiadaceae (Khairnar *et al.*, 2012). The shrub *C. procera* is found mainly in tropical regions and to a lesser extent in sub-tropical areas. The plant is well-known for its great capacity of producing latex which exudates from damaged leaves (Cleverson *et al.*, 2007) (**Figure I.11**).

Figure I. 11: *Calotropis procera* in its natural habitat (Belhi Z., 2017).

Different parts of the plant have been reported to have many biological activities such as antimicrobial (Shobowale *et al.*, 2013), antioxidant (Tsala *et al.*, 2015), cytotoxic (de Lima *et al.*, 2011), anticancer (Shaker *et al.*, 2010), anti-fertility, an antidote for snakebite, anti-scabietic. Its flowers possess digestive and tonic properties (Meena *et al.*, 2010). *Calotropis* extracts functions were also used to treat skin infections, and inflammation (Rasik *et al.*, 1999).

This plant is anthelmintic, the ashes act as an expectorant. In African and Asian countries, the latex of *C. procera* is used as an arrow poison molluscicide, a fungicide, an anti-syphilitic, an anti-inflammatory, a purgative, and as anti-lepersy (Kazeem *et al.*, 2016).

5.2. Phytochemistry of *Calotropis procera*

Phytochemical studies on *Calotropis procera* have afforded several types of compounds such as cardenolide, triterpinoids, alkaloids, resins, anthocyanins, and proteolytic enzymes in latex, flavonoids, tannins, sterol, saponins, and cardiac glycosides (Hassan *et al.*, 2015).

The leaves contain mainly the α-amyrin **38**, ß-sitosterol **39**, α-amyrin acetate **40**, calotropin **41**, calotropagenin **42**, and urosolic acid **43** (**Figure I.12**) (Meena *et al.*, 2010). The phytochemical screening shows that flavonoids, reducing sugars and steroids are present in both the ethanolic and aqueous extracts of *Calotropis procera* leaves while tannins were detected in acetone and aqueous extracts. However, saponin was present in the aqueous extract only (Kazeem *et al.*, 2016).

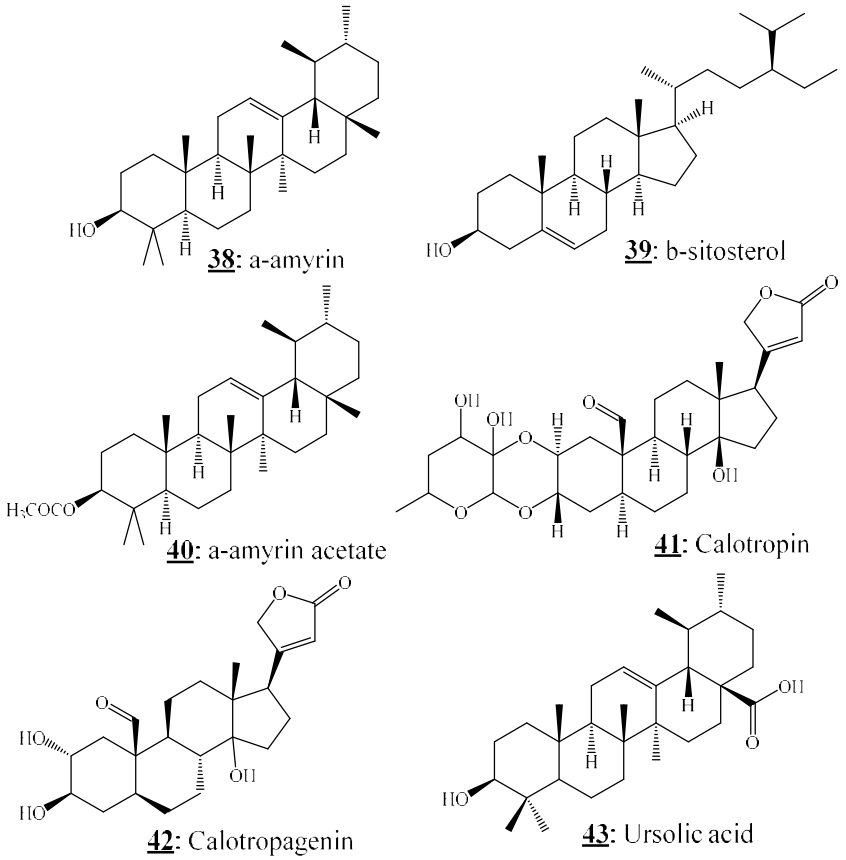


Figure I. 12: Chemical structures of compounds isolated from *Calotropis procera* leaves (Meena *et al.*, 2010).

The flower contains the flavonoids, queretin- 3- ratinoside, sterol, calactin, calotoxin, calotropagenin **42**, calotropin **41**, polysaccharides with D-arabinose, glucose, glucosamine, and L-rhamnose (Meena *et al.*, 2010). Flowers also contain enzymes 3-proteinase terpenes, multiflorenol, and cyclisadol (Hassan *et al.*, 2015). Pentacyclic triterpenes, phytosterols, and triterpenoids saponins have been isolated from the roots of *C. procera*. Chemical investigations of the seeds indicate the occurrence of coroglaucigenin, frugoside, and corotoxigenin (L. M. Hassan *et al.*, 2015).

In addition, the latex contains caoutchouc, calotropin **41**, calotoxin **44** 0.15%, calactin **45** 0.15%, uscharin **46** 0.45%, voruscharin **47** (**Figure I.13)**, trypsin, uzarigenin, syriogenin, and proceroside (Meena *et al.*, 2010). *C. procera* has been studied because its latex is rich in peptidases (Cleverson *et al.*, 2020).

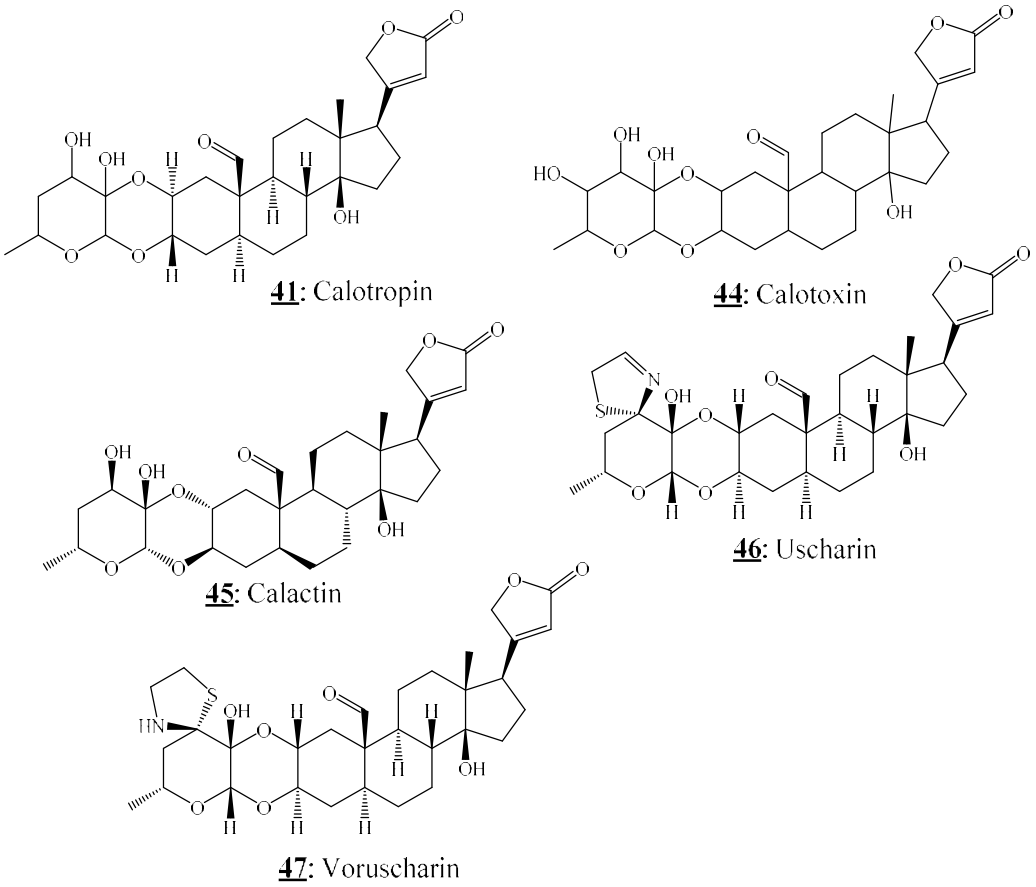


Figure I. 13: Chemical structures of compounds isolated from *Calotropis procera* latex.

6. *Citrullus colocynthis*

6.1. General description and biological activities

Citrullus colocynthis (L.) Schrad belongs to the Cucurbitaceae family. The plant is widely available in the Sahara and Arabian deserts, Sudan, and the Southern parts of Asia (Al-Ghaithi *et al.*, 2004) (**Figure I.14)**. The plant has been reported to have a wide range of traditional medicinal uses including in diabetes, leprosy, common cold, cough, asthma, bronchitis, jaundice, joint pain, cancer, toothache, wounds, mastitis, and gastrointestinal disorders such as indigestion, constipation, dysentery, gastroenteritis, colic pain and different microbial infections (Hussain *et al.*, 2014). *C. colocynthis* fruits are usually recognized for their wide range of medicinal uses as well as pharmaceutical and nutraceutical potential (Marzouk *et al.*, 2010).

Figure I. 14: *Citrullus colocynthis* in its natural habitat (Belhi Z., 2017).

6.2. Phytochemistry of *Citrullus colocynthis*

The POSL researchers since 2009 (Belhi, 2012; Boulenouar *et al.*, 2009; Boulenouar *et al.*, 2014; Ghazi *et al.*, 2020) contributed to the study of the composition of *Citrullus colocynthis.*

Many bioactive compounds of *C. colocynthis* fruit have been recorded in the literature. They are assembled as glycosides, flavonoids, alkaloids, carbohydrates, fatty acids, and essential oils. However, there are only a few reports on the isolation and identification of individual chemical constituents. Cucurbitacins (Cucurbitacin E **48**, Cucurbitacin I **49**, Cucurbitacin L **50**, and Cucurbitacin J **51**) have been reported as the main components of *C. colocynthis* fruits (**Figure I.15)** (Hussain *et al.*, 2014).

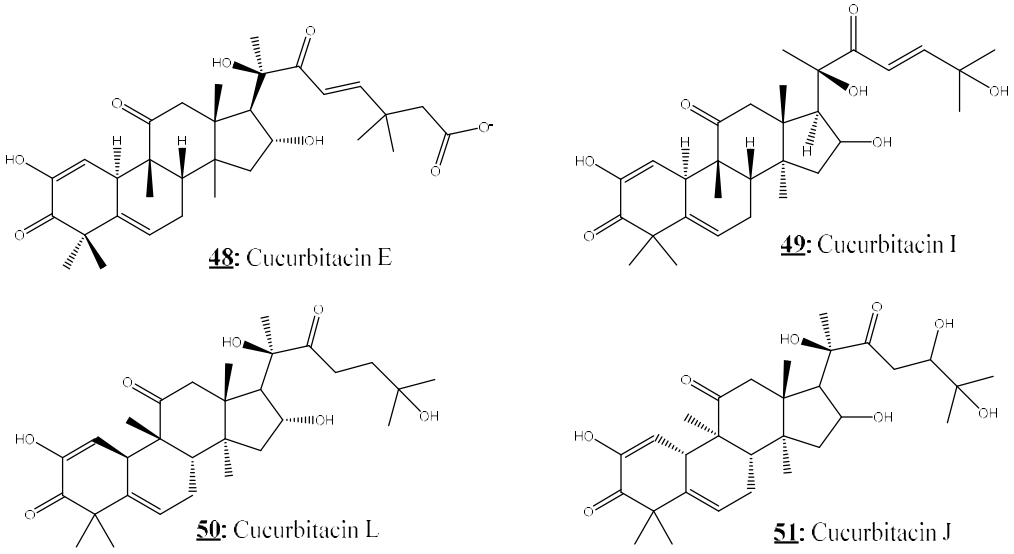


Figure I. 15: Structure of some compounds isolated from *Citrullus colocynthis* (Hussain *et al.*, 2014).

Ghazi *et al.* (2020) isolated and identified six compounds (**Figure I.16**). as 2-*O*-β-D-glucopyranosylcucurbitacin E **52**; Isosaponarin **53**; Isovitexin **54**; Isoorientin 3'-*O*-methyl ether **55,** 2-(Nonan-8-one)-(1H)-4-quinolone **56**; 2-(Nonan-8-one)-4-methoxy-quinoline **57**.

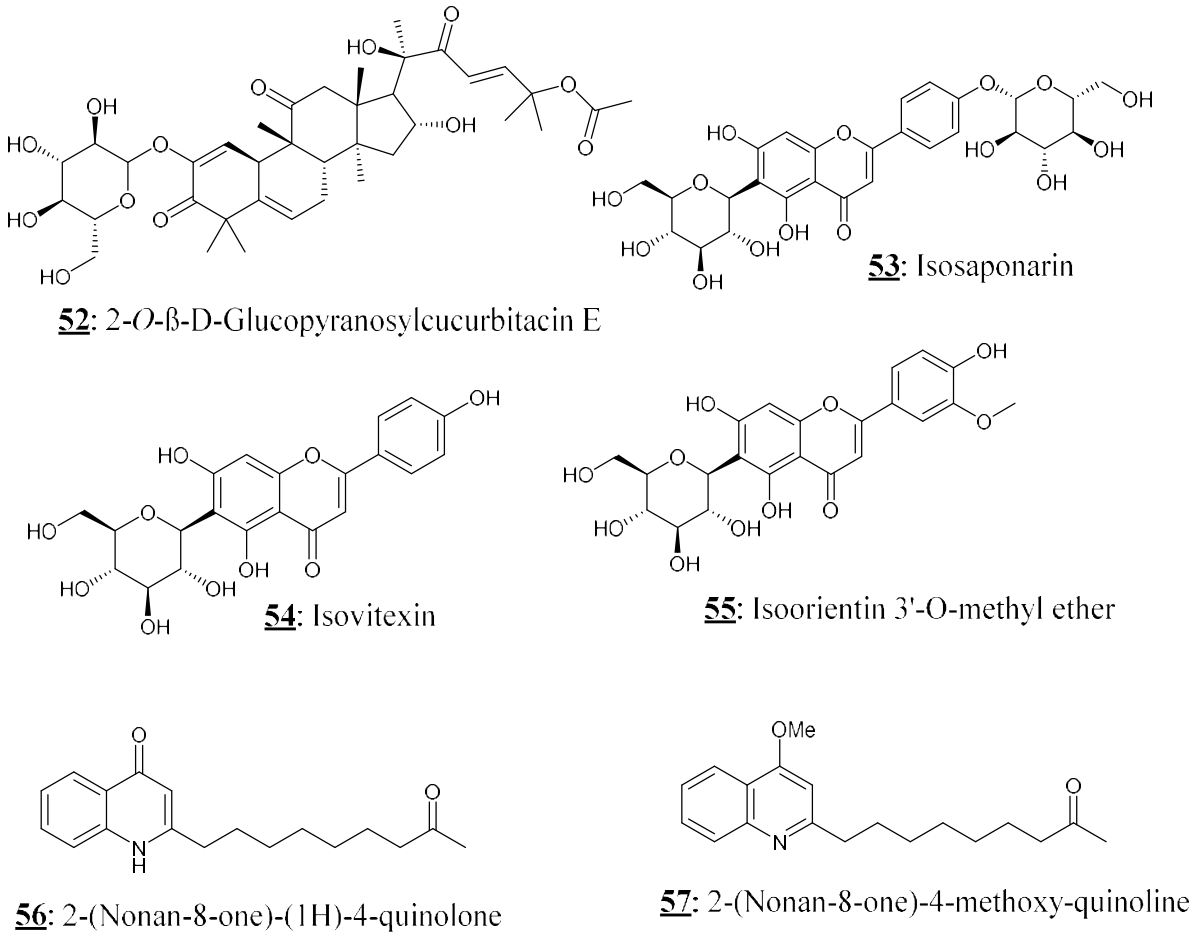


Figure I. 16: Compounds isolated from *Citrullus colocynthis* fruits peels (Ghazi *et al.*, 2020).

7. *Launaea arborescens*

7.1. General description and biological activities

Launeae arborescens (local name "Oum Lbina") is a herbaceous plant belonging to the Asteraceae family, which is widely distributed in the West Algerian Sahara (Belboukhari *et al.*, 2006) (**Figure I.17)**. This plant is commonly used in popular medicine as an antidiarrheal and antispasmodic, to relieve fever, and as a vermifuge in children. The latex

is applied locally to cure sore throats and in the treatment of furuncles. The powdered root mixed with *Artemisia herba-alba* is taken for diabetes (Cheriti *et al.*, 2012). Despite the biological interest in this plant commonly used in the Saharan popular medicine against diarrhea and abdominal spasms, very few chemical studies on *L. arborescens* have been so far reported.

Figure I. 17: *Launeae arborescens* in its natural habitat (Belhi Z., 2017).

7.2. Phytochemistry of *Launaea arborescens*

Researchers from POSL were the first initiators on the phytochemical study of the Algerian sample of *Launaea arborescens* (Belboukhari *et al*., 2006). The methanolic extract of aerial parts of this species provided four compounds: two flavonoids, 3- acetyl-5-methoxy7,3',4'-trihydroxyflavan-3-ol-8-*O*-glycoside **58**, 5,7,4'-trihydroxy-3'-methoxyflavone (chrysoeriol) **59**, one lignan, 4,4'-dihydroxy-3,3'-dimethoxy7,9':7,9'-diepoxylignan **60**, and a diterpene, methyl-15,16-epoxy-12-oxo-8(17), 13(16), 14ent-labdatrien-19-oate **61** (**Figure I.18**).

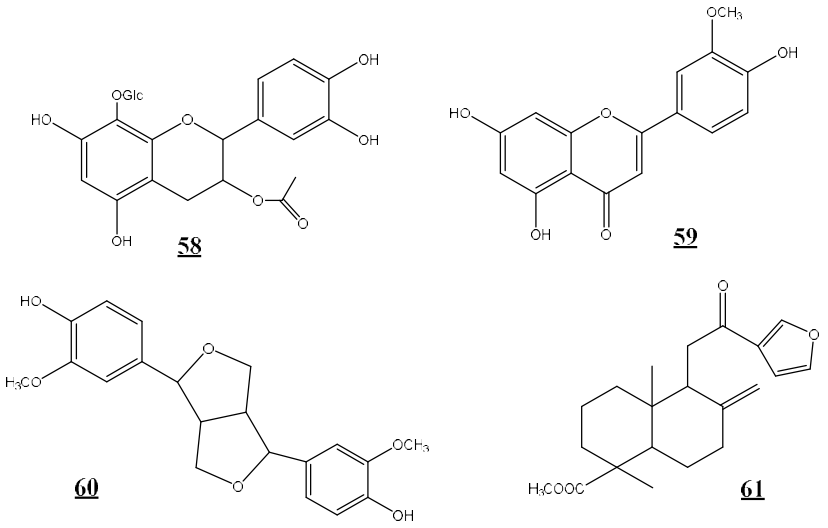


Figure I. 18: Compounds isolated from *Launaea arborescens* (POSL, 2006).

In their chemical investigation on the liposoluble extracts of both roots and aerial parts of Algerian specimens of *L. arborescens*, Bitam *et al.* obtained 27 pure metabolites (sesquiterpenes and triterpenes, five of which were novel compounds: 3β-hydroxy-11α-ethoxy-olean-12-ene **62**, 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C **63**, 9α-hydroxy-4α,15-dihydro-zaluzanin C **64**), 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside **65**, and 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-p-hydroxyphenylacetate **66** (**Figure I.19**) (Bitam *et al.*, 2008).

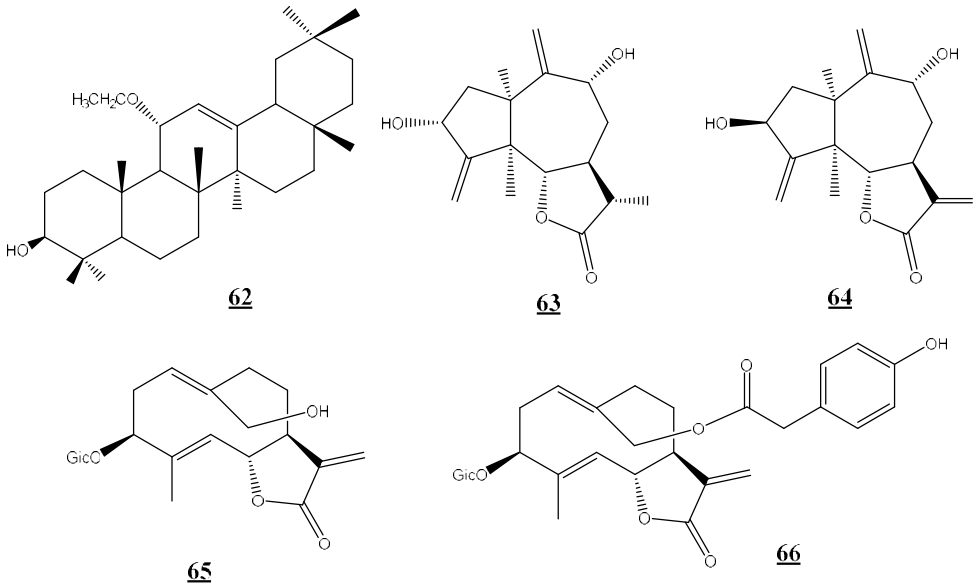
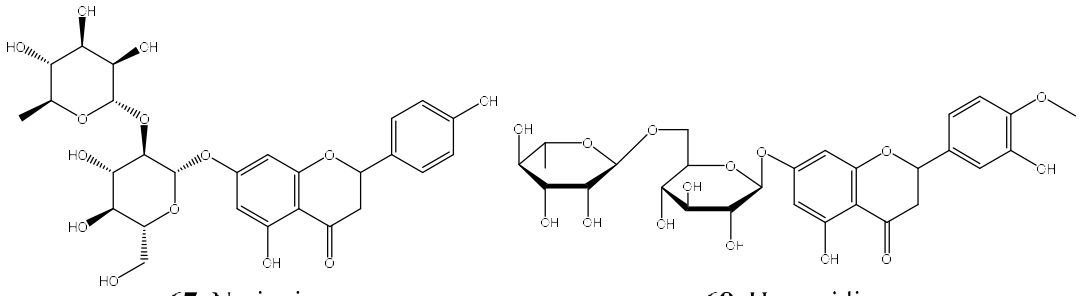


Figure I. 19: Terpenoids isolated from the Algerian plant *Launaea arborescens* (Bitam *et al.*, 2008)

Two flavanone glycosides were isolated from the aerial part of *Launeae arborescens* (Asteraceae), naringin **67**, and hesperetin 7-rutinoside (Hesperidin) **68** (**Figure I.20)** (Belboukhari *et al.*, 2010). In this study, they were interested in the chiral separation of hesperidin and naringin by HPLC methods.



67: Naringin **68**: Hesperidin

Figure I. 20: Structures of naringin and hesperidin from the aerial part of *Launeae arborescens* (Belboukhari *et al.*, 2010).

In continuation of the work on this plant, the POSL team isolated and identified 08 flavonoids from the butanolic fraction of the aerial part of *Launeae arborescens*. The isolated compounds were identified as 7-*O*-[α-rhamnopyranosyl 4', 5, 6-Trihydroxy flavone **69**, 4',5'-Di-Methoxy 7-(5''-Me Hexan)1-oyl flavanone **70**, 3"-isopropyl pyrano [1":7,4":6]3',4',5',5-Tetrahydroxy flavanone **71**, 5, 4', 5'-Tri-Hydroxy 7-(3''-Me butan) -yl flavanone **72,** 5, 7-Dihydroxy-2', 4', 5'–trimethoxy-isoflavanone **73**, 5, 6, 7,4'-tetrahydroxy flavonol **74**, 7-*O*-[α-rhamnopyranosyl-(1->6)-β-glucopyranosyl]- 4',5,7-tri-hydroxy-flavanone **75**, 7-*O*-[α-rhamnopyranosyl-(1->6)-β-glucopyranosyl] 3',5-Dihydroxy 4'-Methoxy flavanone **76 (Figure I.21)** (Sekkoum *et al.*, 2014).

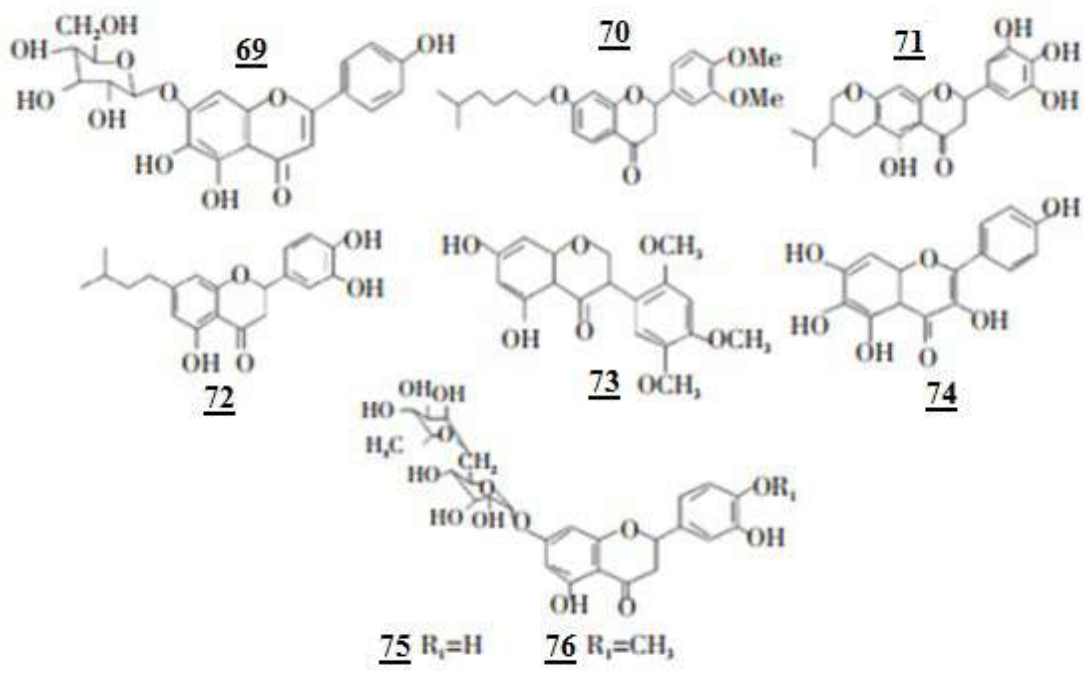


Figure I. 21: Structures of flavonoids isolated from the aerial part of *Launaea arborescens* (Sekkoum *et al.*, 2014)

Seven compounds have been chemically characterized and identified by Belboukhari *et al.* (2016) from the aerial parts of *Launaea arborescens* using spectroscopic methods. The identified compounds are: **77:** ursane-12-ene-3,6,16,21-tetraol-3-*O*-β-glucopyranoside , **78:** β—sitostérol, **79:**olean-12-ene-3b,16b-diol, **80:**7-*O*-(α-ramnopyranosyl-(1-6)-β-glycopyranosyl)- 6-Geranyl-5,2',4'-trihydroxyflavanone , **81:** 5,7,4' trihydroxy-3-(3 ,6-dimethyl-2,5-heptadienyl) flavones, **82:** 2'',3'',6,8 –Tetrahydroxy-3',4',5',5tetramethoxy-10,4- cyclolignan and **83:** 3,4-Di-*O*-galloylglucopyranoside (**Figure I.22)** (Belboukhari *et al.*, 2016).

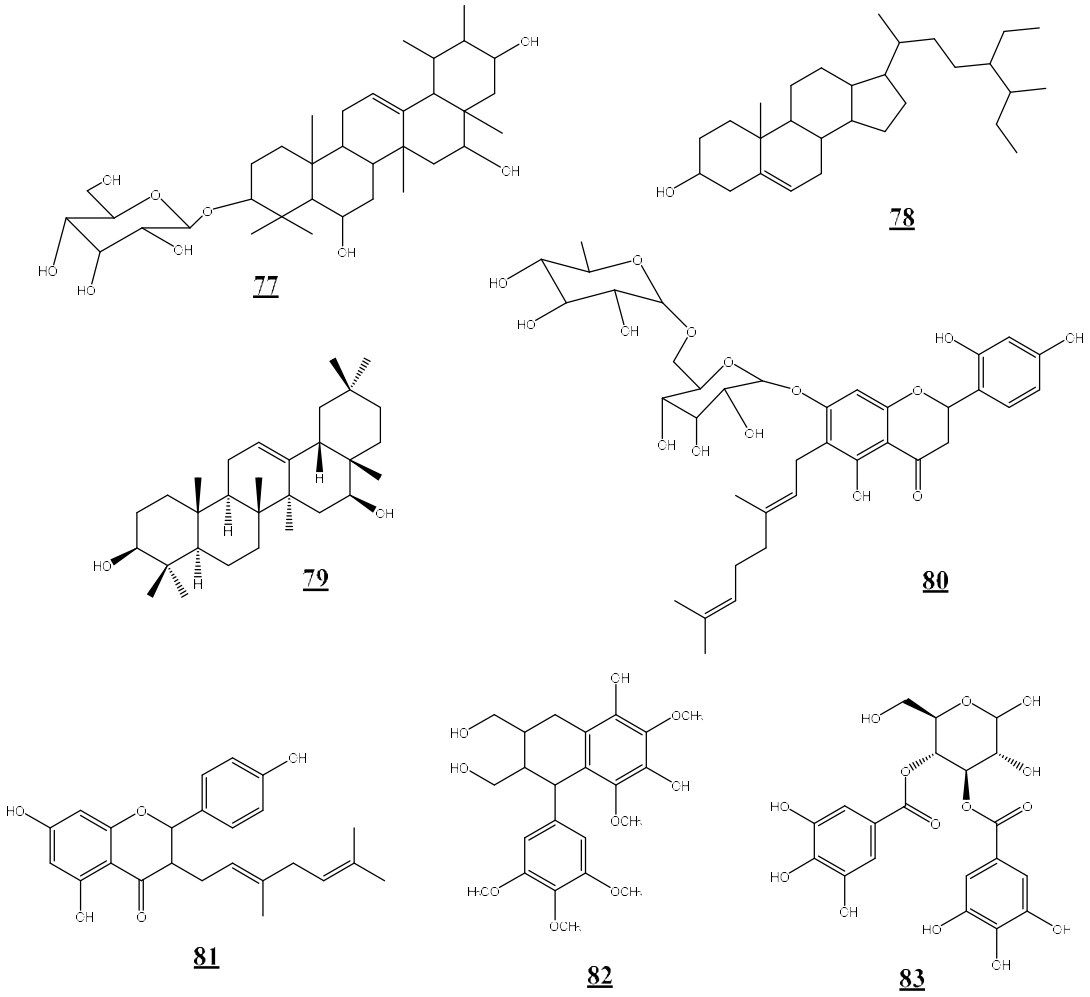


Figure I. 22: Structure of compound 1-7 isolated from *Launaea arborescens* (Belboukhari *et al.*, 2016).

8. *Limoniastrum feei*

8.1. General description and biological activities

Limoniastrum feei is a species belonging to the Plumbaginaceae family, a perennial shrub with densely ramified stems at the base, rather small, not exceeding 40 cm. This hardy shrub thrives particularly on the stony grounds of the djebels. Its occurrence on gravelly sandy wadi beds is less frequent. It can survive years of severe drought when the rainfall is less than 50 mm (Benhouhou, 2005) (**Figure I.23**). It is a Saharan medicinal plant, used in Saharan ethnopharmacopeae to treat gastric tract, hepatic disorder, and cough (Rahmani *et al.*, 2012). The other uses of *L. feei* are as an antibacterial, for the treatment of bronchitis and stomach infection (Belboukhari *et al.*, 2007). Due to the

notable medicinal value of *Limoniastrum feei*, it was considered of interest to carry out phytochemical and antimicrobial investigations of this species (Belboukhari *et al.*, 2005).

Figure I. 23: *Limoniastrum feei* in its natural habitat
(Belhi Z., 2017).

Some studies made at the Phytochemistry and Organic Synthesis Laboratory (POSL, Bechar University, Algeria) have demonstrated that this plant contains secondary metabolites with biological activities (Belboukhari *et al.*, 2005; Belboukhari *et al.*, 2007; Belboukhari *et al.*, 2009).

A previous investigation revealed that methanol extract from *L. feei* leaves contained potential antifungal activity against *Candida albicans* and antibacterial effects against *E. coli* (Belboukhari *et al.*, 2009). *L. feei* extracts exhibited antifungal activity against *F. oxysporum* f. sp. *albedinis* (Boulenouar *et al*., 2012), antioxidant (Chaabi *et al.*, 2008), and anti-inflammatory (Rahmani *et al.*, 2016). Also, antioxidant and antiradical-scavenging activities were evaluated (Keffous *et al.*, 2016).

8.2. Phytochemistry of *Limoniastrum feei*

Cheriti's team since 2007 contributed to the study of the characterization of flavonoids extracted from the stems of *L. feei* (Cheriti, 2019). They identified four flavonoids: 6, 3', 4'-Tri-methoxy 3, 5, 5'-trihydroxy flavonol **84**, 3-(6"-malonyl 2"-ramnosyl glucosil) 6, 3',4'-tri-methoxy 5, 5'-dihydroxy flavonol **85**, Tetraacetate 7-dihydroxy-4'-Methoxy 8-*O*-β-glucopyranoside isoflavone **86,** Tetraacetate 7, 4'-diMethoxy 8-*O*-β-glucopyranoside isoflavone **87 (Figure I.24)**.

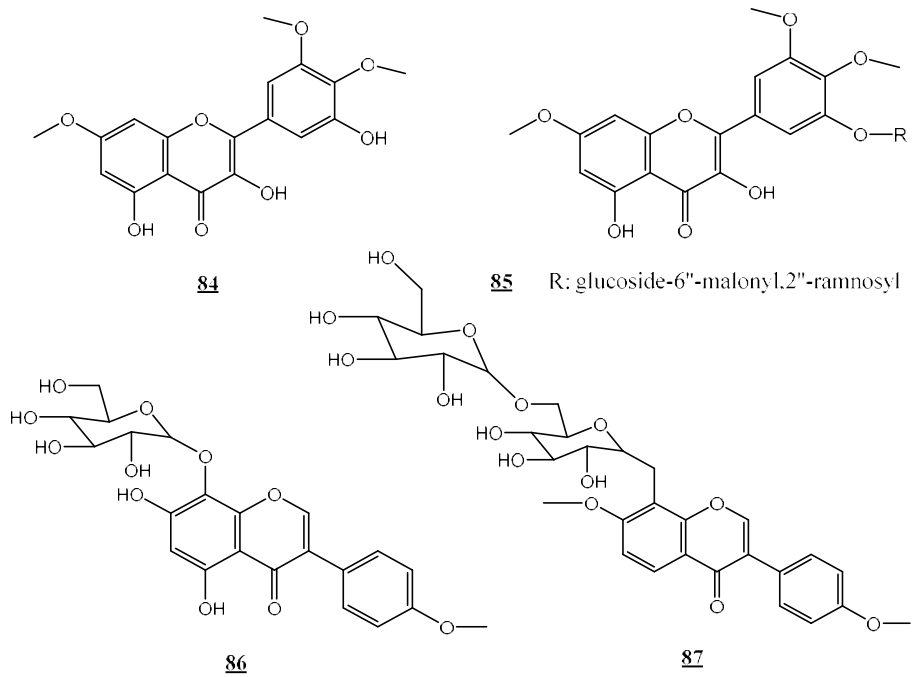


Figure I. 24: Flavonoids from the stems of *Limoniastrum feei* (84-87) (Belboukhari *et al.*, 2007).

In another study, two molecules of the saponins family were extracted and identified by Belboukhari and Cheriti (2009) **(Figure I.25)** from the stems of *L. feei*, namely:

2α ,3 β ,23-Trihydroxy-30-acetylolean-12-ene **88**($C_{32}H_{52}O_5$) and 1-*O*-[α -L-Rhamnopyranosyl-(1)-6-*O*-acetylβ -D-galactopyranosyl]-1 β ,3 β ,22 ξ -26-tetrahydroxyfurost-5(6)-ene-26-*O*β -D-glucopyranosid **89**($C_{50}H_{82}O_{19}$).

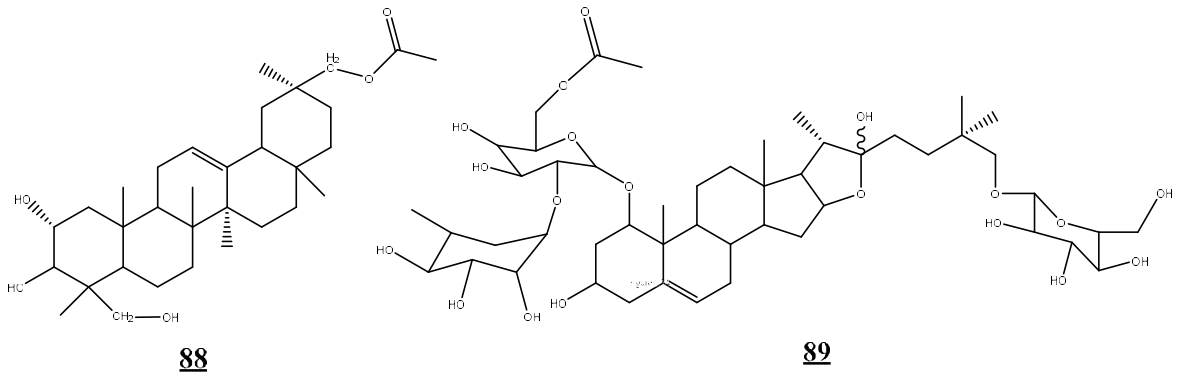
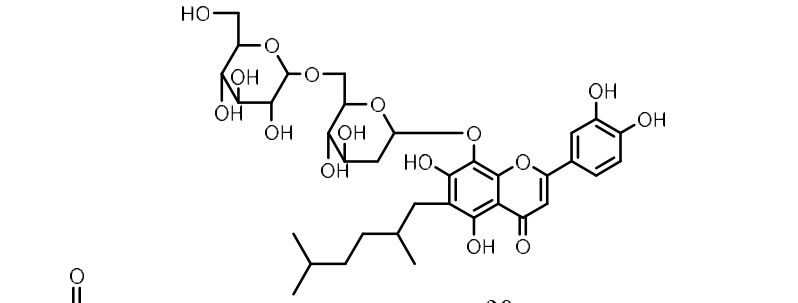


Figure I. 25: Saponins from the stems of *Limoniastrum feei* (88 and 89) (Belboukhari *et al.*, 2009).

A previous study carried out by Rahmani *et al.* (2013, 2014) (Rahmani *et al.*, 2014) reported the presence of eleven polyphenol compounds and glucoside flavonoids (**Figure I.26)** in the extract of the aerial part of *L. feei*. From the results obtained by El-Haci *et al.* (2017), polar organic extracts: methanol, ethanol, and acetone of *L. feei* presented considerable levels of phenols and flavonoids. These levels ranged from 127.07 to 262.11 mg GAE/g and 84 to 157.88 mg CEQ/g, respectively (El Haci *et al.*, 2017).



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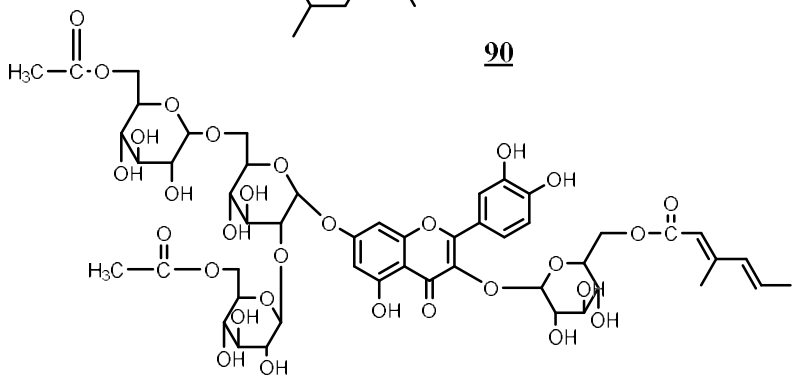

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Figure I. 26: Glucoside flavonoids from *Limoniastrum feei*

(Rahmani *et al.*, 2013)

9. *Nerium oleander*

9.1. General description and biological activities

Nerium oleander L. is a perennial shrub that can form clumps 2-4 m. tall with erect stems and branches, a poisonous tree, or shrub that belongs to the Apocynaceae family (Benhouhou, 2005) (**Figure I.27**). This plant is well known for its toxicity (Bandara *et al.*, 2010; Rubini *et al.*, 2019). This species is widely cultivated as an ornamental shrub throughout the tropical and subtropical parts of the world (Dhinesh *et al.*, 2018).

Figure I. 27: *Nerium oleander* L. in its natural habitat
(Belhi Z., 2017).

Despite its toxicity, the flowers and leaves of *N. oleander* are commonly used in folk medicine as an abortifacient, as well as therapeutic agents for heart failure, leprosy, malaria, ringworm infections, indigestion, and venereal diseases (Gnanathasan, 2015). *N. oleander* L. extracts have potent anti-cancer, antimicrobial, nematicide, pesticide, analgesic, antioxidant activity due to the presence of several phytoconstituents (Mouhcine *et al.*, 2019). *N. oleander* extracts exhibited antifungal activity (Boulenouar *et al.*, 2009), anti-inflammatory (Kumar *et al.*, 2010), and antitumoral activities (Namian *et al.*, 2013), anti-HIV activity (Singh *et al.*, 2013).

9.2. Phytochemistry of *Nerium oleander*

Several studies have shown that *N. oleander* is rich in secondary metabolites. The phytochemical analysis, effectuated by Bhuvaneshwari *et al.* (Bhuvaneshwari *et al.*, 2007), showed the presence of alkaloids, terpenoids, cardiac glycosides, saponins, tannins, and carbohydrates in all the solvents.

Santhi *et al.* (Santhi, 2011) screened the ethanolic extract of *N. oleander* leaves and found it to contain carbohydrates, proteins, amino acids, alkaloids, and cardiac glycosides. Flavonoids and terpenoids were absent. Fifty-eight bioactive phytochemical compounds were identified in the solvent-free extract of *N. oleander* L. root using GC-MS (Hase *et al.*, 2017).

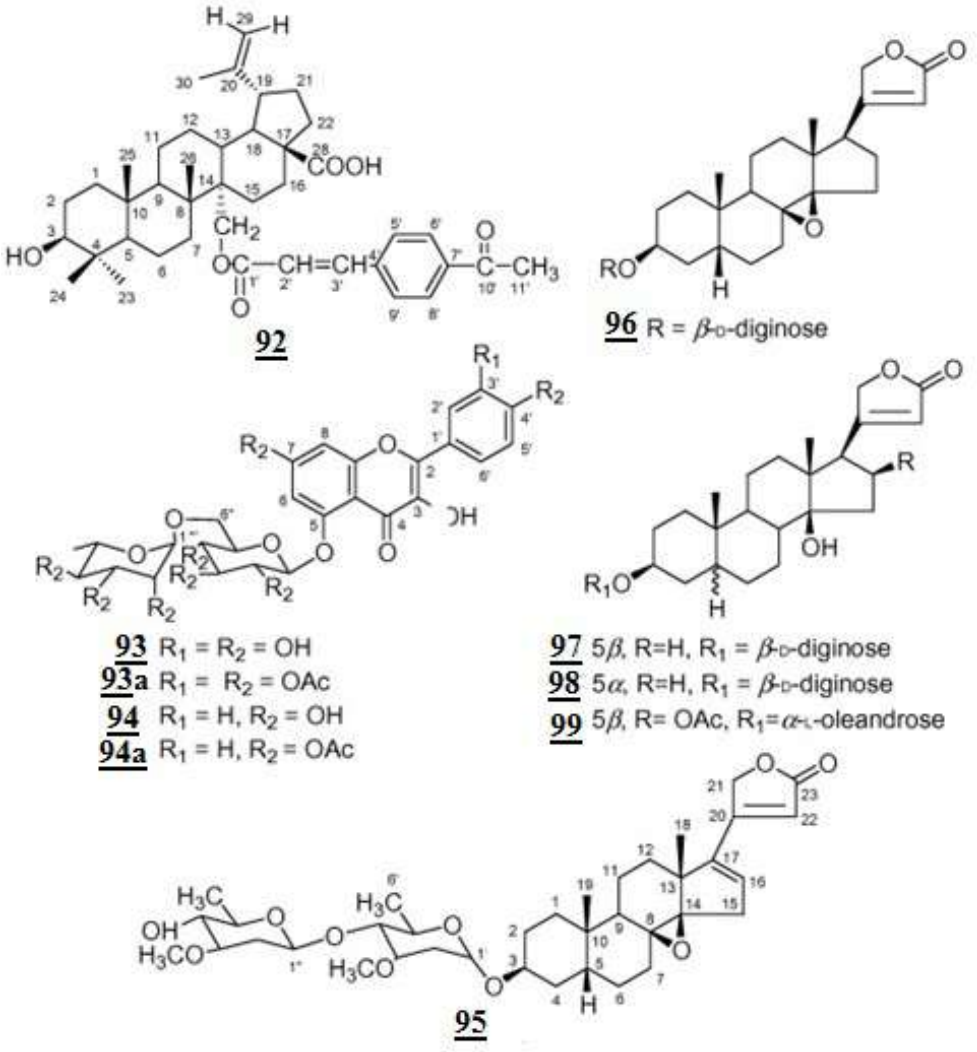


Figure I. 28: Structures of some compounds identified from the methanolic extract of *Nerium oleander* L. leaves (Siddiqui *et al.*, 2012).

Siddiqui *et al.* (Siddiqui *et al.*, 2012) reinvestigated the methanol extract of the leaves and isolated a new pentacyclic triterpene, oleanderocioic acid **92**, two new flavonoidal glycosides, quercetin-5-*O*-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] **93** and kaempferol-5-*O*-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside] **94**, and a new cardenolide, oleandigoside **95**, together with 11 known compounds, adynerigenin, adynerin **96**, odoroside A **97**, odoroside B **98**, oleandrin **99** **(Figure I.28)**, 27-*p*-(*E*)-coumaroyloxyursolic acid, β-neriursate, ursolic acid, stigmasterol, β-sitosterol, and neristigmol.

All parts of *N. oleander* contain very toxic cardiac glycosides, including oleandrin **100**, digitoxigenin **101**, neriine**102**, folinerin **103**, and rosagenin (Figure I.29**)** (Anadón *et al.*, 2018).

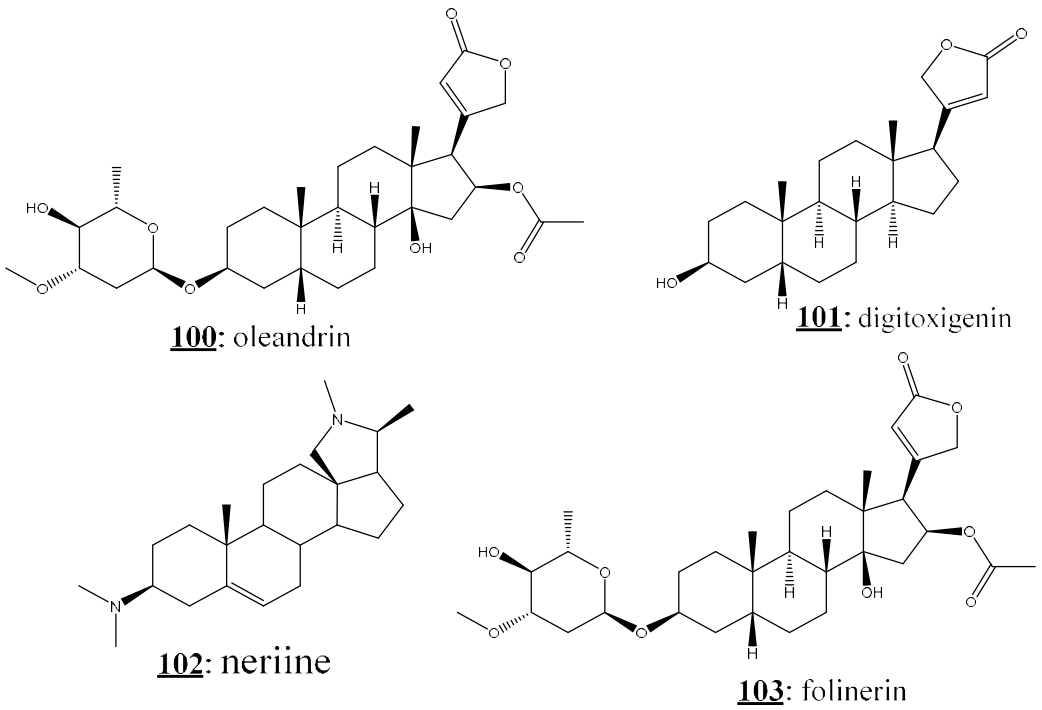


Figure I. 29: Structures of some cardiac glycosides present in *Nerium oleander* L. (Anadón *et al.*, 2018)

In a recent study, six new cardenolides, including three 14-hydroxylated cardenolides and three 14-carbonylated cardenolides were isolated from the dried aerial parts of *N. oleander* in addition to twenty-seven known compounds. Their structures were elucidated based on extensive spectroscopic evidence and single-crystal X-ray diffraction analysis. The new identified cardenolides are: 8-hydroxy-oleandrigenin-3-O-*β*-D-diginoside **104**, 5*α*-oleaside A **105**, 14-carbonyl-neriaside **106**, 3*β*-*O*-(*β*-D-diginosyl)-14*β*-hydroxy-5*β*,14*β*-card-8,16,20(22)-trienolide **107**, 21-hydroxy-neriaside **108**, 16-hydroxy-oleaside A **109 (Figure I.30)** (Cao *et al.*, 2018).

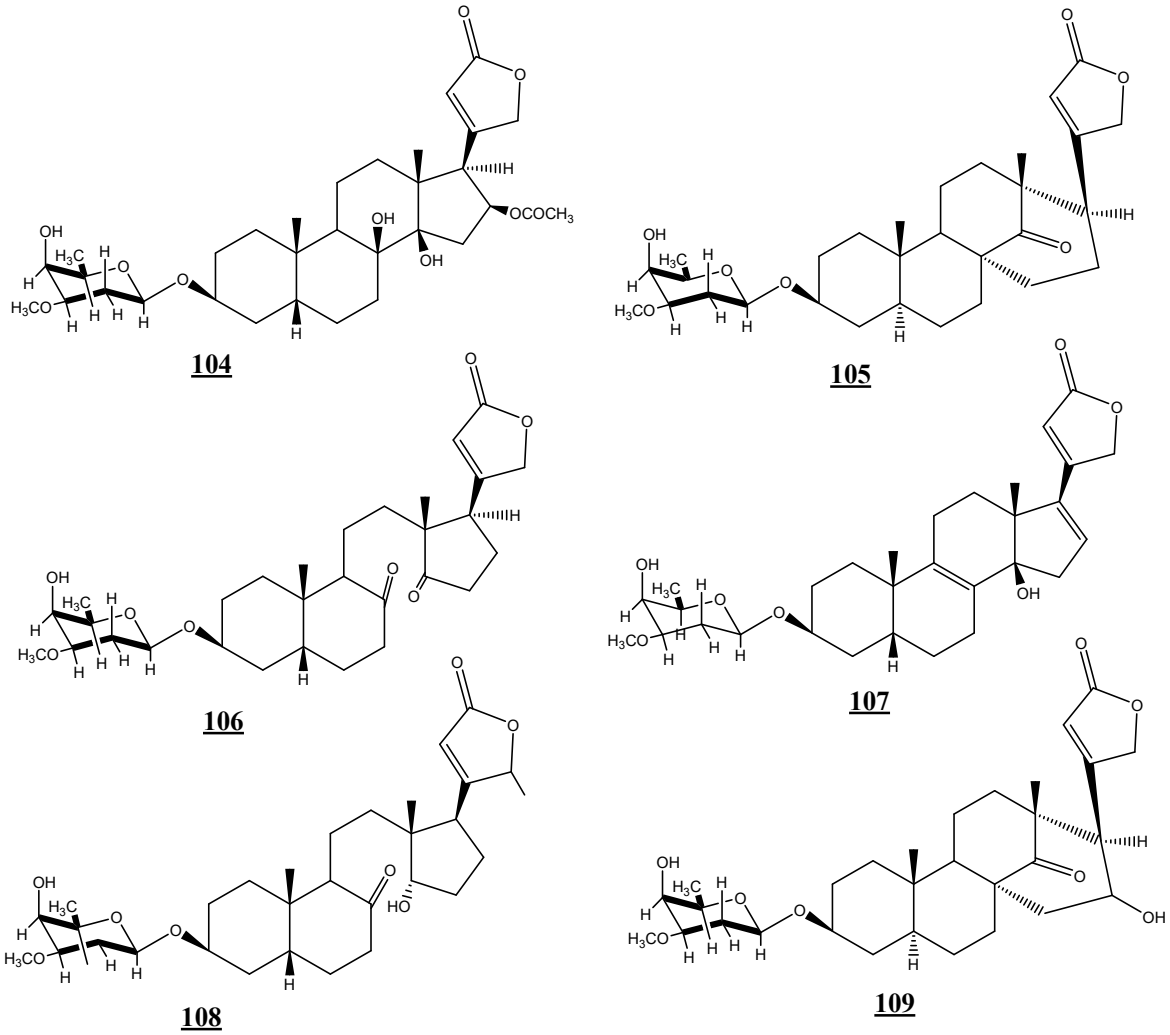


Figure I. 30: Structures of cardenolides identified from ethanolic extracts of the dried aerial parts of *Nerium oleander* (Cao *et al.*, 2018).

10. *Pergularia tomentosa*

10.1. General description and biological activities

Pergularia tomentosa L. (Apocynaceae) is climbing to semi-erect perennial herb (Benhouhou, 2005) (**Figure I.31**). *P. tomentosa* has been exploited in traditional medicine as a laxative, tumors, warts, depilatory, abortifacient, skin diseases, and anthelmintic agents. Moreover, antioxidant (Lahmar *et al.*, 2017), antidiabetic (Hamza *et al.*, 2019), antifungal (Boulenouar *et al.*, 2009), antibacterial, insecticidal and cytotoxic activities of this species are documented (Alghanem *et al.*, 2017).

Figure I. 31: *Pergularia tomentosa* in its natural habitat (Belhi Z., 2017).

10.2. Phytochemistry of *Pergularia tomentosa*

A wide range of chemical compounds including cardenolides, cardenolide glycoside, and taraxasterol-type triterpenes, etc. have been isolated from this plant.

Hassan *et al.* studied the nutritive value, phytochemical constituents, and antifungal activity of leaf, root, and stem of *P. tomentosa* L. extracts. Phytochemicals detected in the leaf and stem extracts were alkaloids, cardiac glycosides, cyanogenic glycosides, saponin, flavonoids, tannins, and anthraquinones. The roots contain trace amounts of these compounds. Mineral element composition of the plant showed a higher amount of phosphorous and potassium in the root and stem and sodium, magnesium, and calcium in the leaf extracts (Hassan *et al.*, 2007).

Twenty-Five compounds were isolated and identified from various parts of the *P. tomentosa.* The structure of these compounds has been presented in **Figure I.32 and I.33**. The main class secondary metabolites were isolated from this species include cardenolides, cardenolide glycoside, and taraxasterol-type triterpènes (Al-Mekhlafi *et al.*, 2017).

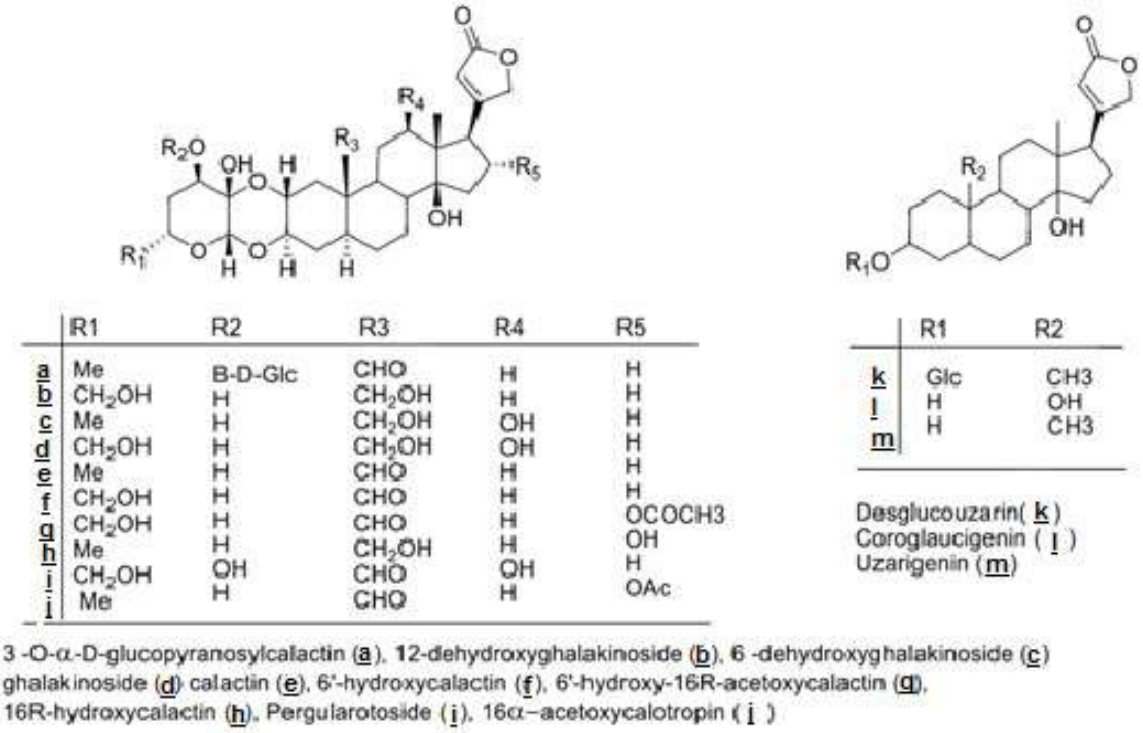


Figure I. 32: Cardiac glycosides from *Pergularia tomentosa* plant (Al-Mekhlafi *et al.*, 2017).

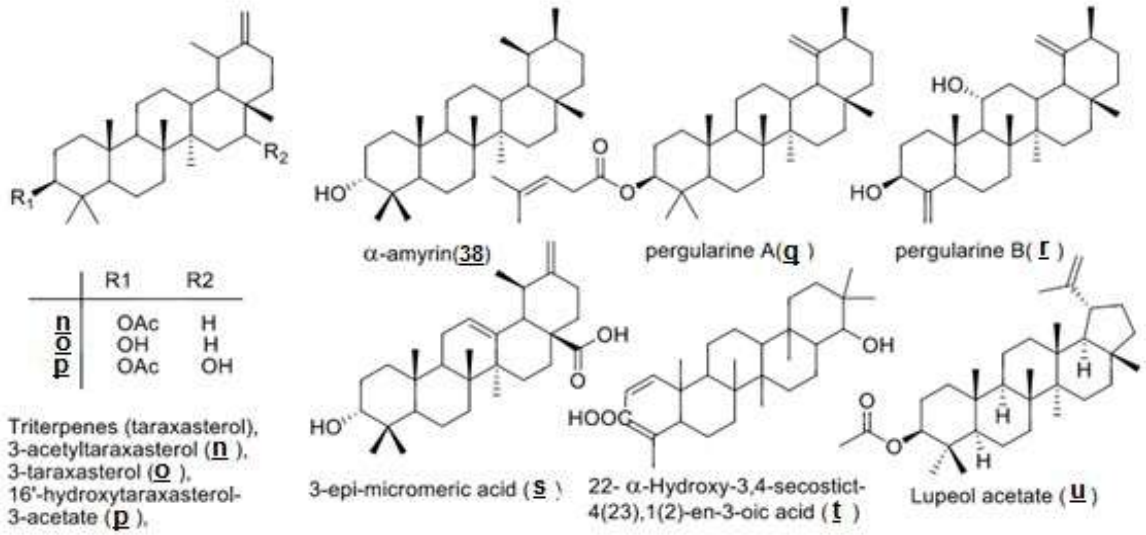


Figure I. 33: Triterpenes from *Pergularia tomentosa* plant (Al-Mekhlafi *et al.*, 2017).

11. Conclusion

In this chapter, we have described the existing traditional uses of the studied plants (*Acacia raddiana*, *Anabasis aretioides, Asteriscus graveolens*, *Calotropis procera*, *Citrullus colocynthis*, *Launeae arborescens*, *Limoniastrum feei*, *Nerium oleander,* and *Pergularia tomentosa*) and summarized recent research into phytochemistry and biological studies.

The literature shows that these plants have a wide range of medicinal uses and have been well studied for their antidiabetic, anticancer, antioxidant, antimicrobial, and anti-inflammatory activities, etc.

Several traditional uses of these plants have been validated by phytochemical and modern biological studies; however, some of these studies were only tested *in vitro*. Thus the effectiveness of plant extracts and isolated compounds needs to be further investigated for their efficacy and safety using *in vivo* assays.

CHAPTER II

The Use of Natural Products against *Fusarium oxysporum*

CHAPTER II: The Use of Natural Products against *Fusarium oxysporum*

1. Introduction

Plant pathogens such as fungi, bacteria, nematodes, and viruses cause various diseases in plants that may damage them. Plant diseases cause at least 10% of the losses of agricultural products (Davari *et al.*, 2017). More importantly, fungi are the main pathogen that harms the plants. Fungal diseases cause a considerable loss of crop yields in agricultural industries worldwide (Mahlo *et al.*, 2016). Fungi are a health concern for humans and animals. They are also associated with food spoilage, causing odor, rancidity, flavor changes, and loss of nutrients (Al-Asmari *et al.*, 2017).

Fusarium spp. and *Aspergillus* spp. are considered important phytopathogens world-wide. However, their adverse effects through mycotoxin production highlight their importance in food safety (Davari *et al.*, 2017). Fungi such as *Fusarium* spp., growing on plants, can produce mycotoxins that can seriously harm consumers (Mahlo *et al.*, 2016).

Fusarium species can survive in plant debris or soils through resistant structures such as chlamydospores for a long time or in the form of spores or mycelium in infected or dead tissues. The plant rhizosphere is an important ecological environment in the soil for plant-microbe interactions. These interactions with plants could be beneficial, neutral, or detrimental, resulting in plant diseases (Zhao *et al.*, 2018).

Currently, it is common the integral use of all kinds of control methods, a practice that is known as integrated pest management. All methods have advantages and disadvantages, but the choice of a specific one for a particular crop depends on the state of the disease (Prieto *et al.*, 2013). In the development of the agro-food chain, the utilization of synthetic fungicides to protect post-harvested fruits, grains and vegetables has caused an increase in environmental and human health concerns. However, in consideration of this and of antifungal resistance, novel alternatives, such as physical treatments, natural extracts, and biocontrol microbes are used instead to control post-harvest diseases (Confortin *et al.*, 2019).

There is a growing interest in the research of the possible use of natural products, such as plant extracts, which may be less damaging for fungal pathogens control.

The antifungal activity of plant extracts is well documented and it is postulated to be the effect of their major compounds or a synergistic effect of various compounds forming a mixture (Bartmańska *et al.*, 2018). Over 50% of all modern clinical drugs have their origins in natural products, which play an important role in drug-development programs in the pharmaceutical industry (Singh *et al.*, 2017).

Studies focusing on finding new antifungal agents are urgently required, as there is a limited therapeutic arsenal available for *Fusarium* spp. infections. Alternatives include natural products, especially as they are inexpensive and have low toxicity (Galletti *et al.*, 2017). An alternative solution to synthetic fungicides is the use of natural products for the management of fungal diseases in plants.

Through this chapter, we present and analyze the main results of antifungal activity against *F. oxysporum formae speciales* of candidates as biofungicides (principally from plants and microorganisms) or natural products derived from these organisms that have been carried out by studies during the last years (2017-2020).

2. General information on the genus *Fusarium*

Fusarium is a large genus of filamentous fungi that are widely distributed in soil, plants, and other organic substrates (Yoshida *et al.*, 1989). The food borne *Fusarium* species are characterized by fast growing colonies with a velvety floccose aerial mycelium. In general, *Fusarium* species prefer humid conditions, i.e. water activity higher than 0.86, and grow well at temperatures of around 0–37°C, however, no *Fusarium* species is thermophilic (Ulf Thrane, 1999). The genus *Fusarium* is one of the most important mycotoxigenic fungal genera in food and feed (U. Thrane, 2014).

Colony pigmentation varies from pale, rose, and burgundy to bluish violet depending on species and growth conditions. Conidia often are produced in sporodochia, which will appear as slimy dots in the culture (Ulf Thrane, 1999).

3. Microscopic morphology of *Fusarium oxysporum*

Fusarium oxysporum is well-known as a plant pathogen causing severe damage in many crops, both in the field and during postharvest storage. Strains of *F. oxysporum* can grow under very low oxygen tensions and often have been detected as recontaminants in

ultrahigh-temperature processed fruit juices. Some strains are known to produce the fumonisin mycotoxins (U. Thrane, 2014).

The members of this species produce both macro-and microconidia. The macroconidia are typically 25-35 µ long 3-5 µ wide, dorsi-ventrally curved, sickle- shaped 3-5 septated, with thin walls and tapering toward the ends which are quite pointed (in fact the term *oxysporum* roughly translates to pointed spores). The widest part of the spore tends to be about a third of the distance from the apex to the base. The basal cell possesses a marked foot or sometimes an appendage (Smith, 2007).

Figure II.1 contains drawings of some typical *F. oxysporum* spores made over the years from isolates grown on typical laboratory media. They, by no means, describe the whole range of spores that may be seen of various isolates on different media and conditions but are merely examples to accompany the foregoing discussion (Smith, 2007).

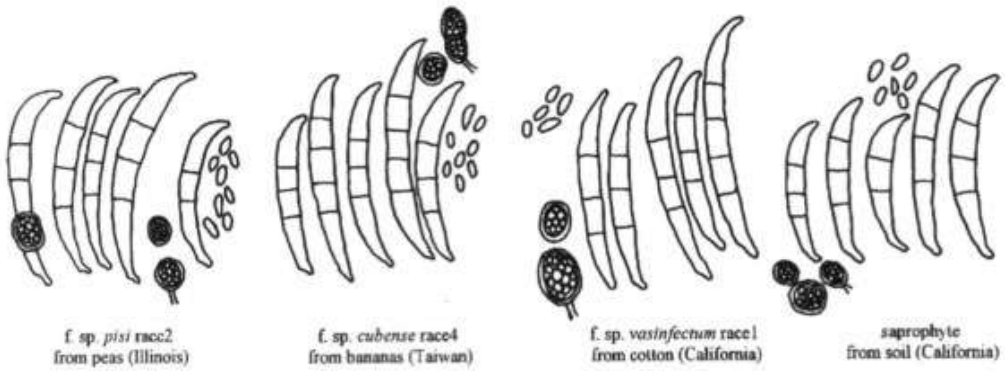


Figure II. 1: *Fusarium oxysporum* forms from PDA cultures. (Approx. 250X). (Smith, 2007)

F. oxysporum also produces microconidia abundantly, which tend to be 5-12 * 3-5 µ in size, oval to ellipsoidal, occurring singly or in aggregates as false heads held together by moisture, which dries out to become a powdery substance dispersed in the mycelium. *F. oxysporum* is light in color or takes on tints of the stroma coloring, commonly of purple-red, blueish-grey, or yellowish-tan. The stroma is plectochymatic and may erupt with blue-black or brown sclerotia. Besides masses of sporodochial macroconidia, pionnotes can be seen macroscopically as small yellow to orange upraised mats borne on the tops of sclerotia in a culture (Smith, 2007).

4. Methods of identification

For identification of *Fusarium* species, the purified SNA or CLA cultures should be inoculated on a fresh SNA dish for morphological observations, on potato dextrose agar (PDA), to determine colony diameter after 4 days at 25 ºC and cultural appearance. The use of metabolite profiles may provide a useful supplemental character, and a cultural extract is easily prepared by extraction of 6 mm plugs from 7- to 14-day-old cultures grown on PDA and yeast extract sucrose agar at 25 ºC **(Figure II.2)**. The extracts can be analyzed by state-of-the-art chromatography, mostly liquid chromatography, for which the detection of individual compounds can be combined with confirmatory identification of the compounds. This can be done to some extent by (UV-diode array detection) or preferably by mass spectroscopy (U. Thrane, 2014).

Figure II. 2: Culture of *Fusarium oxysporum* f. sp. *albedinis* on PDA medium.
(Belhi Z., 2017)

5. Formae speciales of *Fusarium oxysporum*

Fusarium wilt pathogens are hosted specific and, denoting this, the term forma specialis followed by another name designating the host, is added to the binomial *Fusarium oxysporum*. Thus, the pathogen wilting garden pea is called *Fusarium oxysporum* forma specialis pisi (*F. oxysporum* f. sp. *pisi*), that of tomato is *F. oxysporum* f. sp. *lycopersici,* etc. This same type of nomenclature is used in host-specific forms among many plant pathogenic fungal species (Smith, 2007).

F. oxysporum has received considerable attention from plant pathologists for more than a century owing to its broad host range and the economic losses it causes. The narrow host specificity of pathogenic strains has led to the concept of *formae speciales*, each *forma*

specialis grouping strains with the same host range (Edel-Hermann *et al.*, 2019). As shown in **Table II.1,** different *formae speciales* of *F. oxysporum* were studied in recent years (2017-2020)

Some *Fusarium* strains may also produce diseases in animals and humans, especially in immunocompromised patients. Besides, the fungi from this taxonomic group stand out because of their complex secondary metabolism. Many of the produced metabolites are mycotoxins, with harmful consequences for humans or animals when they contaminate plant-derived products (Rodríguez-Ortiz *et al.*, 2012). Diseases caused by *F. oxysporum* are widespread in the world. They are harmful to many vegetables (tomato, cucurbit ...) and ornamental (carnation) plants, as well as for field crops such as cotton, banana (Panama disease), and date palm (Bayoud disease) (El Hadrami *et al.*, 1998).

Fusarium species are typically found in plants prior to harvest attacking cereals and often forming mycotoxins in the kernels. *F. oxysporum* causes primarily vascular wilts on many crops, whereas numerous species, especially *F. solani*, cause root and stem rots and rots of seeds that are accompanied by the production of mycotoxins (Agrios, 2005).

F. to their host range (Armstrong *et al.*, 1981). More than 150 *formae speciales* (f. sp.) have been reported. These *formae speciales* are part of the *F. oxysporum* complex**,** show narrow host adaptation, and exploit a broad range of niches. Many *F. oxysporum* isolates are non-pathogenic soil saprophytes and some have even been *oxysporum* isolates are highly host-specific and have been grouped into *formae speciales* according exploited as biocontrol agents (Armitage *et al.*, 2018).

Forty-one percent (41.00 %) of analyzed studies (**Table II.1)** did not indicate the special form of *F. oxysporum*, 19.18 % had focused on *F. oxysporum* f. sp. *lycopersici.* This may be due to its propagation and because it is one of the most damaging soil-borne diseases of tomatoes. Nirmaladevi *et al.* (2016) (Nirmaladevi *et al.*, 2016) reported that *F. oxysporum* f. sp. *lycopersici* is the most serious soil-borne disease and becoming more common in greenhouse and field tomato production.

Table II. 1: *Formae speciales* of *F. oxysporum* analyzed in this chapter.

Forma specialis	**Host species**	**Percentage**	**References**
F. oxysporum f. sp. *lentis*	*Lens culinaris*	1.37 %	(Davari *et al.*, 2017)
F. oxysporum	NS	41 %	(Adnan *et al.*, 2018; Agarwal *et al.*, 2017; Ahmad *et al.*, 2017; A. K. Al-Asmari *et al.*, 2017; F. Al-Asmari *et al.*, 2017; Bartmańska *et al.*, 2018; Carvalho *et al.*, 2018; X. Chen *et al.*, 2019; Confortin *et al.*, 2019; Galletti *et al.*, 2017; Kälvö *et al.*, 2018; Kamel *et al.*, 2020; Mahlo *et al.*, 2016; Ramos *et al.*, 2018; Sarwar, Brader, *et al.*, 2018; Sarwar, Hassan, *et al.*, 2018; Schalchli *et al.*, 2017; Silva *et al.*, 2017; M. K. Singh *et al.*, 2017; Srivastava *et al.*, 2019; Tao *et al.*, 2020; Tarhriz *et al.*, 2020; Wu *et al.*, 2019; X. Wu *et al.*, 2018; Z. Wu *et al.*, 2018; Xiong *et al.*, 2017; Yoo *et al.*, 2020; Yuan *et al.*, 2017; Zandvakili *et al.*, 2017; Y.-K. Zheng *et al.*, 2017)
F. oxysporum f. sp. *canariensis*	*Phoenix canariensis*	1.37 %	(Bentrad *et al.*, 2017)
F. oxysporum f. sp. *albedinis*	*Phoenix dactylifera*	4.11%	(Belhi *et al*., 2020; Bentrad *et al*., 2017; Ghazi *et al*., 2020)
F. oxysporum f. sp. *phaseoli*	*Phaseolus vulgaris*	1.37 %	(Bentrad *et al.*, 2017)
F. oxysporum f. sp. *lycopersici*	*Solanum lycopersicum* (or *Lycopersicon esculentum* Mill.)	19.18 %	(Bentrad *et al.*, 2017; Bouayad Alam *et al.*, 2017; Hsu *et al.*, 2017; Kalleli *et al.*, 2020; Lahmar *et al.*, 2017; Li *et al.*, 2017; López-Seijas *et al.*, 2020; Medina-Romero *et al.*, 2017; Mohamed *et al.*, 2017; Montenegro *et al.*, 2018; Yoo *et al.*, 2020; Yu *et al.*, 2017; Zehra *et al.*, 2017; Zhu *et al.*, 2018)
F. oxysporum f. sp. *melonis*	*Cucumis melo*	2.74 %	(Bentrad *et al.*, 2017; Hsu *et al.*, 2017)
F. oxysporum f. sp. *cubense*	*Musa acuminata*	1.37 %	(Hsu *et al.*, 2017)

NS: not specified.

Table II.1 (continued): *Formae speciales* of *F. oxysporum* analyzed in this chapter.

Forma specialis	**Host species**	**Percentage**	**References**
F. oxysporum f. sp. *cucumerinum*	*Cucumis sativus*	8,22 %	(Gao *et al.*, 2017; Han *et al.*, 2019; Huang *et al.*, 2017; Limbadri *et al.*, 2018; Wang *et al.*, 2017; J. Zhang *et al.*, 2018)
F. oxysporum f. sp. *fragariae*	*Fragaria ananassa*	2.74 %	(Kim *et al.*, 2019; Park *et al.*, 2017)
F. oxysporum f. sp. *momordicae*	*Momordica charantia*	1.37 %	(Limbadri *et al.*, 2018)
F. oxysporum f. sp. *iridacearum*	*Iris germanica*	1.37 %	(Mihalache *et al.*, 2018)
F. oxysporum f. sp. *glycines*	*Zea mays*	2.74 %	(Patiño *et al.*, 2018; Xego *et al.*, 2017)
F. oxysporum f. sp. *tulipae*	*Tulipa gesneriana* L.	1.37 %	(Roşca-Casian *et al.*, 2017)
F. oxysporum f. sp. *pisi*	*Pisum sativum* L.	1.37 %	(Sen *et al.*, 2017)
F. oxysporum f. sp. *niveum*	*Citrullus lanatus*	11 %	(Chen *et al.*, 2018; Sun *et al.*, 2017; Tan, Li, *et al.*, 2017; Tan, Zhang, *et al.*, 2017; Tan *et al.*, 2018; J. Zhang *et al.*, 2018; M. Zhang *et al.*, 2018; G. Zheng *et al.*, 2018)
F. oxysporum	*Panax notoginseng.*	4.11%	(X. Chen *et al.*, 2019; Xu *et al.*, 2019; Zhao *et al.*, 2018)
F. oxysporum f. sp. *fabae*	*Vicia faba L.*	1.37 %	(Q. Zhao *et al.*, 2018)
F. oxysporum f. sp. *capsici*	*Capsicum annuum*	1.37 %	(Bashir *et al.*, 2018)
F. oxysporum	*Olea europaea*	1.37 %	(Bouzoumita *et al.*, 2020)
F. oxysporum	*Solanum tuberosum* L.	1.37 %	(Lastochkina *et al.*, 2020)
F. oxysporum f. sp. *cepae*	*Allium cepa*	1.37 %	(Akhtar *et al.*, 2020)

Sun *et al.* (2017) described that *F. oxysporum* f. sp. *niveum* is difficult to eliminate from the soil. This is due to the fact that *F. oxysporum* f. sp. *niveum* can survive for several years in soil as chlamydospores and many hosts are symptomless.

The low percentage for some *formae speciales* does not reflect their less importance but this is due to some reasons. Among them, the geographic limit of diseases such as *F. oxysporum* f. sp. *albedinis*.

6. Production of cellulolytic enzymes by *Fusarium oxysporum*

The pathogenic strains are known to secrete cellulolytic enzymes which make it possible for the fungal cells to penetrate into the host plant cells by decomposing the cell walls and causing pathogenesis in the host plant (Yoshida *et al.*, 1989). Several fungal genera are associated with mycotoxin contamination of raw and processed foods; these include *Aspergillus, Penicillium, Byssochlamys*, *Alternaria,* and *Fusarium.*

Mycotoxin production depends both on fungal characteristics and environmental factors. *Fusarium oxysporum* was described as a good producer of cellulase (Panagiotou *et al.*, 2003).

More than 50% of *Fusarium* species are toxigenic and produce harmful secondary metabolites (SM), such as the pigments fusarubins and bikaverin, as well as the mycotoxins, fumonisins, fusarins, and fusaric acid. In the progression of the infection, *Fusarium* species damage host plants through the intrusion of hyphae into the host vascular system, secretion of hydrolytic enzymes, and mycotoxins (Sun *et al.*, 2017).

Fusarium species could infect plants through the root, and then colonize the root system and lower stem, and finally result in the disease. Toxins and enzymes generated by fungi in the process of invading plants could interfere with the metabolism of the host plant (Zhao *et al.*, 2018).

F. oxysporum acquires the exceptional ability of bioconverting cellulose directly to ethanol through the consecutive steps of hydrolysis of cellulose and fermentation of the resulting oligosaccharides (Christakopoulos *et al.*, 1995).

The mechanism of cellulose decomposition by microbial cells has not been clarified precisely because of the participation of several distinct enzymes. It is postulated that

cellulose is decomposed through the synergistic actions of enzymes such as endo-p-l,4-glucanase, exo-p-I,4-glucanase, and p-glucosidase (Yoshida *et al.*, 1989).

7. Antifungal products against *F. oxysporum* f. sp. from natural sources

There are different sources : plants, animals, and microorganisms (including bacteria, fungi, and viruses) or natural products derived from these organisms, which are used to suppress pest populations and pathogens (Sun *et al.*, 2017).

The following table presents the sources of natural products evaluated against different *F. oxysporum formae speciales* (**Table II.2**).

Medicinal plants are widely used for therapy; the screening of medicinal plants is also another alternative that may produce chemical fungicides that are relatively non-toxic and cost-effective (Mahlo *et al.*, 2016). Many analyzed studies (41.00 %) had used plants as a source of antifungal agents against *F. oxysporum* f. sp.

Fungi are also an important source of secondary metabolites and novel bioactive compounds for different biological applications (Adnan *et al.*, 2018). In recent years, fungi inhabiting insect organs have been recognized as abundant sources of biologically active natural products with novel structures (Wu *et al.*, 2018). Among analyzed studies, 23.30 % had used fungi to fight against *F. oxysporum*.

Many studies (23.30 %) have shown that bacteria and their metabolites can play a vital role in the biocontrol of plant fusarioses. Active compounds produced by microorganisms (bacteria and fungi) are used to control plant pathogens may be due to their diversity, flexibility in operation, and more ecofriendly than chemical agents.

Al-Asmari *et al.* (2017) have used venoms of animal origin (Scorpion venom). This venom showed significant activity in a dose-dependent manner from 5–20 mg/mL, against *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans,* and *Candida glabrata*, unfortunately, no significant activity was observed against *F. oxysporum*. Recently, Ortiz *et al.* (2015) reported the anticancer potential of toxins and venoms of animal origin. Other studies have reported that the venom of scorpion exhibits strong antimicrobial activities (Alajmi *et al.*, 2019).

Table II. 2: Sources of antifungal agents against *F. oxysporum.*

Antifungal source	Percentage (%)	References
Plant	41 %	(Akhtar *et al*., 2020; F. Al-Asmari *et al*., 2017; Bartmańska *et al*., 2018; Belhi *et al*., 2020; Bentrad *et al*., 2017; Bouayad Alam *et al*., 2017; Confortin *et al*., 2019; Davari *et al*., 2017; Ghazi *et al*., 2020; Kalleli *et al*., 2020; Lahmar *et al*., 2017; Mahlo *et al.*, 2016; Mohamed *et al.*, 2017; Montenegro *et al.*, 2018; Park *et al.*, 2017; Ramos *et al.*, 2018; Roşca-Casian *et al.*, 2017; Silva *et al.*, 2017; M. K. Singh *et al.*, 2017; Sun *et al.*, 2017; Tao *et al.*, 2020; Wang *et al.*, 2017; Wu *et al.*, 2019; Xego *et al.*, 2017; Xiong *et al.*, 2017; Zandvakili *et al.*, 2017; M. Zhang *et al.*, 2018; Q. Zhao *et al.*, 2018; Y.-M. Zhao *et al.*, 2018; G. Zheng *et al.*, 2018)
Animal	1.37 %	(A. K. Al-Asmari *et al.*, 2017)
Bacteria	23.30 %	(Agarwal *et al.*, 2017; Ahmad *et al.*, 2017; Bouzoumita *et al.*, 2020; X. Chen *et al.*, 2019; Gao *et al.*, 2017; Han *et al.*, 2019; Hsu *et al.*, 2017; Kim *et al.*, 2019; Lastochkina *et al.*, 2020; López-Seijas *et al.*, 2020; Mihalache *et al.*, 2018; Sarwar, Brader, *et al.*, 2018; Sarwar, Hassan, *et al.*, 2018; Tarhriz *et al.*, 2020; Xu *et al.*, 2019; Yoo *et al.*, 2020; Yu *et al.*, 2017)
Fungi	23.30 %	(Adnan *et al.*, 2018; Carvalho *et al.*, 2018; Kälvö *et al.*, 2018; Kamel *et al.*, 2020; Li *et al.*, 2017; Limbadri *et al.*, 2018; Medina-Romero *et al.*, 2017; Patiño *et al.*, 2018; Schalchli *et al.*, 2017; Sen *et al.*, 2017; X. Wu *et al.*, 2018; Z. Wu *et al.*, 2018; Xiong *et al.*, 2017; Yuan *et al.*, 2017; Zehra *et al.*, 2017; Zheng *et al.*, 2017; Zhu *et al.*, 2018)
Other	13.7 %	(Bashir *et al.*, 2018; Y. Chen *et al.*, 2018; Galletti *et al.*, 2017; Huang *et al.*, 2017; Montenegro *et al.*, 2018; Srivastava *et al.*, 2019; Tan, Li, *et al.*, 2017; Tan, Zhang, *et al.*, 2017; Tan *et al.*, 2018; J. Zhang *et al.*, 2018)

On the other hand, 13.7 % of analyzed studies had tested the antifungal activity against *F. oxysporum* by using different sources, as synthesis products (Chen *et al.*, 2018;

Montenegro *et al.*, 2018; Srivastava *et al.*, 2019; Tan, Zhang, *et al.*, 2017; Tan *et al.*, 2018; Zhang *et al.*, 2018) or Bio-organic fertilizer (Huang *et al.*, 2017).

On contact with the soil, some bacteria and fungi produce antifungal metabolites, which can suppress soil-borne plant pathogens, including *Fusarium* (Bending, 2017).

In open fields, it is usually not economically feasible to apply the biological control agents (BCAs: natural products, and beneficial microorganisms) evenly at the dose needed for efficacy. Whatever the modes of action of the BCA, the success of biological control depends on the activity of the BCA at the root level, in the rhizosphere. Moreover, this capacity is plant-specific, and therefore a given BCA might be effective in controlling a given pathogen in one crop but not in another (Alabouvette *et al.*, 2009).

The success of natural products application needs an adequate understanding of the action modes of the antifungal agent and its interactions with the plant, the pathogen, and the rest of the microbiota.

8. Natural products as antifungal agents for inhibiting *F. oxysporum*

This part presents an analysis of the main results of antifungal activity from crude extracts, essential oils, pure compounds, and fractions (**Table II.3**).

The antifungal activity was observed in crude extracts, pure compounds, and fractions of variable polarity, and essential oils but with different percentages.

Table II. 3: Natural products with antifungal activity against *F. oxysporum.*

Crude extract		**Essential oil**		**Pure compound**		**Fraction**	
Number	**Percentage (%)**	**Number**	**Percentage (%)**	**Number**	**Percentage (%)**	**Number**	**Percentage (%)**
14	19.18 %	04	5.48 %	36	49.30 %	01	1.37 %

Among analyzed studies, only 19.18 % had used the crude extract for antifungal tests. Pure compounds were the most used among all-natural products (49.30 %). Some studies ((Chen *et al.*, 2019; Davari *et al.*, 2017; Gao *et al.*, 2017; Ghazi *et al.*, 2020; Han *et al.*, 2019; Kälvö *et al.*, 2018; Kim *et al.*, 2019; Li *et al.*, 2017; Limbadri *et al.*, 2018; Montenegro *et al.*, 2018; Sarwar, Hassan, *et al.*, 2018; Sun *et al.*, 2017; Wang *et al.*, 2017;

G. Zheng *et al.*, 2018; Zhu *et al.*, 2018)) have identified the individual compounds responsible for the activity (**Table II.4**, **Figure II.3 and Figure II.4**).

Table II. 4: Some chemical constituents isolated from natural sources with antifungal activity against *F. oxysporum.*

Source species (plant, microorganism, animal,…	**Isolated Compounds**	**References**
Phlebiopsis gigantean	*O*-orsellinaldehyde	(Kälvö *et al.*, 2018)
Streptomyces strain	Strepphenazine A1	(X. Chen *et al.*, 2019)
	Strepphenazine B2	
	Strepphenazine C3	
Citrullus colocynthis	(C1): 2-*O*-β-D-Glucopyranosylcucurbitacin E;	(Ghazi *et al.*, 2020)
	(C2): 2-(Nonan-8-one)-(1H)-4-quinolone;	
	(C3): 2(Nonan-8-one) 4-methoxy-quinoline,	
	(C4): Isosaponarin;	
	(C5): Isovitexin;	
	(C6): Isoorientin 3'-*O*-methyl ether.	
Aspergillus clavatus	4,4'-dimethoxy-5,5'-dimethyl-7,7'-oxydicoumarin (1),	(Li *et al.*, 2017)
	7(γ, γ-dimethylallyloxy)-5-methoxy-4-methylcoumarin (2),	
	Kotanin (3)	
	orlandin (4)	
	(S)-5-hydroxy-2,6-dimethyl-4H-furo[3,4-g]benzopyran-4,8(6H)-dione (5),	
	24-hydroxylergosta-4,6,8(14),22-tetraen-3-one (6)	
Drimys winteri	Polygodial (1)	(Montenegro *et al.*, 2018)
	Drimenol (2)	
Fusarium sp.	Fusarihexin A	(Zhu *et al.*, 2018)
	Fusarihexin B	

Table II.4 (continued): Some chemical constituents isolated from natural sources with antifungal activity against *F. oxysporum.*

Source species (plant, microorganism, animal,…	Isolated Compounds	References
Bacillus subtilis	Mycosubtilin, Plipastatin and Surfactin	(Gao *et al.*, 2017; Mihalache *et al.*, 2018; Sarwar, Hassan, *et al.*, 2018)
Bacillus amyloliquefaciens	Peptide P852	(Han *et al.*, 2019)
Streptomyces griseus S4-7	Lantipeptides	(Kim *et al.*, 2019)
Aspergillus fumigatus	Fumigatoside E	(Limbadri *et al.*, 2018)
Cuminum cyminum L.	Cuminic acid	(Sun *et al.*, 2017; Wang *et al.*, 2017)
Ageratina adenophora	2,5,7-tri-substituted benzofuran **(1)**	(G. Zheng *et al.*, 2018)

Despite the benefits of essential oils for the control of *F. oxysporum* f. sp., their use as antifungal products was limited (5.48 %) (Bouayad *et al.*, 2017; Davari *et al.*, 2017; Kalleli *et al.*, 2020; Park *et al.*, 2017). This may be due to its high cost-benefit ratio (low extraction yields) and difficulties related to applications against phytopathogens.

In some cases, the antifungal activity can be combined with insecticide, anti-inflammatory, antimicrobial, and antibacterial activities through synergistic or antagonistic interactions (Confortin *et al.*, 2019; Lastochkina *et al.*, 2020). Usually, pure compounds do not have fungi toxic activity by themselves but can have synergistic effects on the antifungal activity of other extracts (Mihalache *et al.*, 2018). These results demonstrate that no type of extract, fraction, or pure compound is responsible for the antifungal activity, but that they sometimes act synergistically and that it is, therefore, possible to use the whole extract at the same time, instead of pure compounds, which will cost less. The effects of all extracts and/ or compounds are complex, mainly including synergistic and antagonistic effects.

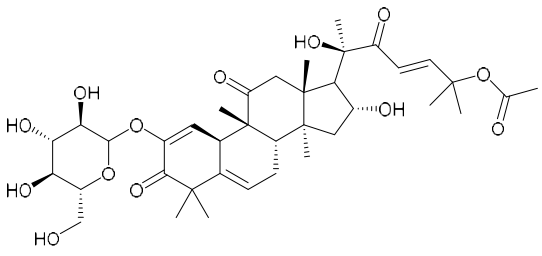
In recent years, the concept of synergy (or antagonism) in natural product mixtures has gained attention, and the importance of multi-target combination therapies has come to the forefront (Caesar *et al.*, 2019).

When working with natural product mixtures, constituents responsible for antifungal activity are often not known. The antifungal effect of crude extracts is considered as an initiatory step to pass through more advanced researches to detect and isolate the effective compound(s).

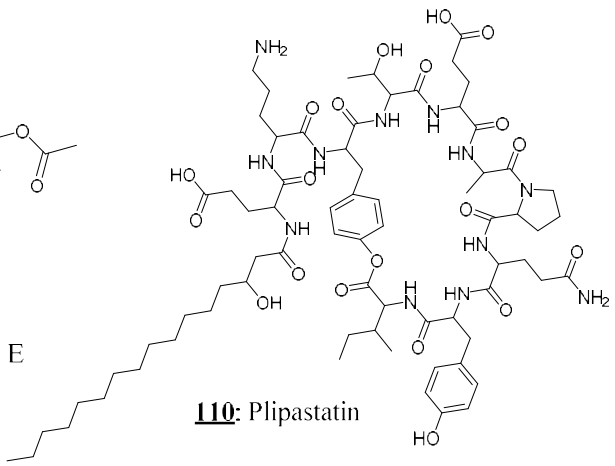
Natural products demonstrate advantageous traits compared to synthetic compounds such as their low danger for the environment and human health and their biodegradability. In addition, it can have specific activities following the existence of chiral centers in most natural products. These products are considered non-aggressive from the standpoint of health and are not dangerous for farmers.

Despite the advantages that have natural products for control of *F. oxysporum*, their action mechanisms are not clear. There are other disadvantages such as The high cost for isolation and identification of these bioactive natural products, their sensitivity, and instability in the case of certain products with fragile bonds such as lactone, spiro, peroxide, glycosides, azirine ring.

Finally, on an industrial scale, the demand for natural products can put natural resources under intensive exploitation, which has harmful effects on the environment.



52: 2-*O*-ß-D-Glucopyranosylcucurbitacin E



110: Plipastatin

Figure II. 3: Chemical structures of some important natural products with antifungal activity against *F. oxysporum*.

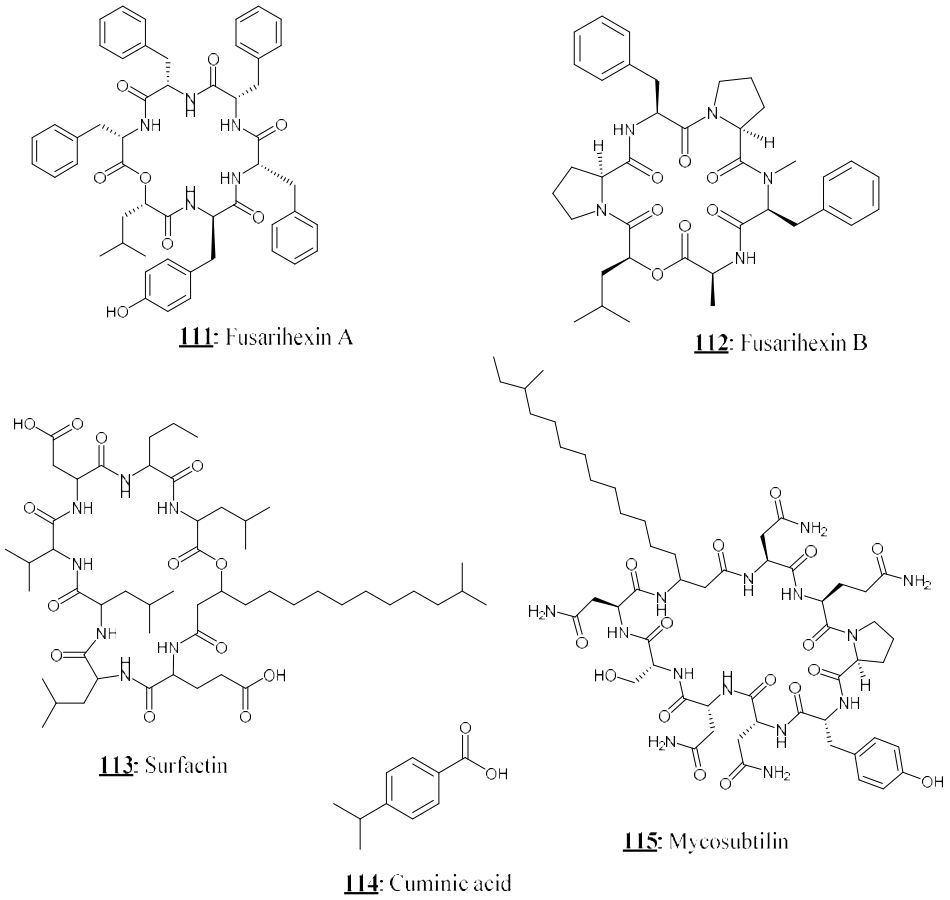


Figure II. 4: Chemical structures of some important natural products with antifungal activity against *F. oxysporum* (continuation).

2-*O*-β-D-Glucopyranosylcucurbitacin E **52** isolated from *Citrullus colocynthis* (Ghazi *et al.*, 2020)*;* Fusarihexin A **111** and Fusarihexin B **112** isolated from *Fusarium* sp (Zhu *et al.*, 2018)*;* Cuminic acid **114** isolated from *Cuminum cyminum* L. (Sun *et al.*, 2017; Wang *et al.*, 2017)*;* Surfactin **113** isolated from *Bacillus subtilis* (Gao *et al.*, 2017; Mihalache *et al.*, 2018; Sarwar, Hassan, *et al.*, 2018)*;* Plipastatin **110** isolated from *Bacillus subtilis* (Gao *et al.*, 2017; Mihalache *et al.*, 2018)*;* Mycosubtilin **115** isolated from *Bacillus subtilis* (Mihalache *et al.*, 2018).

9. Techniques for antifungal activity evaluation

The main antifungal assays employed to evaluate the fungicide potential of different substances are presented in **Table II.5**.

Table II. 5: Techniques used for antifungal tests.

Technique used for antifungal test	**Percentage (%)**	**References**
Dilution methods: microdilution, broth dilution, agar dilution method.	53.4 %	(Agarwal *et al.*, 2017; Akhtar *et al.*, 2020; Bartmańska *et al.*, 2018; Bentrad *et al.*, 2017; Carvalho *et al.*, 2018; X. Chen *et al.*, 2019; Davari *et al.*, 2017; Galletti *et al.*, 2017; Gao *et al.*, 2017; Han *et al.*, 2019; Hsu *et al.*, 2017; Kalleli *et al.*, 2020; Kälvö *et al.*, 2018; Lahmar *et al.*, 2017; Li *et al.*, 2017; Limbadri *et al.*, 2018; Mahlo *et al.*, 2016; Medina-Romero *et al.*, 2017; Mihalache *et al.*, 2018; Montenegro *et al.*, 2018; Patiño *et al.*, 2018; Roşca-Casian *et al.*, 2017; Sen *et al.*, 2017; Silva *et al.*, 2017; Sun *et al.*, 2017; Tan, Li, *et al.*, 2017; Tan *et al.*, 2018; Wang *et al.*, 2017; Wu *et al.*, 2019; X. Wu *et al.*, 2018; Z. Wu *et al.*, 2018; Xego *et al.*, 2017; Xiong *et al.*, 2017; Xu *et al.*, 2019; Yuan *et al.*, 2017; J. Zhang *et al.*, 2018; M. Zhang *et al.*, 2018; Y.-M. Zhao *et al.*, 2018; Zhu *et al.*, 2018)
Antagonism test (or Dual culture assay).	17.81 %	(Adnan *et al.*, 2018; Agarwal *et al.*, 2017; Ahmad *et al.*, 2017; Bouzoumita *et al.*, 2020; Kamel *et al.*, 2020; Kim *et al.*, 2019; López-Seijas *et al.*, 2020; Sarwar, Brader, *et al.*, 2018; Sarwar, Hassan, *et al.*, 2018; Yoo *et al.*, 2020; Yu *et al.*, 2017; Zehra *et al.*, 2017; Y.-K. Zheng *et al.*, 2017)
Diffusion methods: Agar disk-diffusion method, Agar diffusion method, Agar well bioassay, Poisoned food technique.	13.70 %	(Bashir *et al.*, 2018; Belhi *et al.*, 2020; Confortin *et al.*, 2019; Park *et al.*, 2017; Schalchli *et al.*, 2017; M. K. Singh *et al.*, 2017; Srivastava *et al.*, 2019; Tarhriz *et al.*, 2020; Zandvakili *et al.*, 2017; G. Zheng *et al.*, 2018)
Gas-phase method.	1.37 %	(Medina-Romero *et al.*, 2017)

Table II. 5 (continued): Techniques used for antifungal tests.

Technique used for antifungal test	**Percentage (%)**	**References**
Mycelium Growth Rate (MGR).	4.11 %	(Ghazi *et al.*, 2020; Mihalache *et al.*, 2018; Tao *et al.*, 2020)
Inhibition of Spores Germination (ISG).	5.48%	(Mihalache *et al.*, 2018; Yu *et al.*, 2017; Zandvakili *et al.*, 2017; Y.-M. Zhao *et al.*, 2018)
ELISA-like microtiter plates.	1.37 %	(Ramos *et al.*, 2018)
Radial growth assay.	9.6 %	(Bouayad Alam *et al.*, 2017; Y. Chen *et al.*, 2018; Mohamed *et al.*, 2017; Tan, Li, *et al.*, 2017; Tan, Zhang, *et al.*, 2017; Tan *et al.*, 2018; Wang *et al.*, 2017)
In vivo effect.	6.85 %	(Bouzoumita *et al.*, 2020; Lastochkina *et al.*, 2020; López-Seijas *et al.*, 2020; Medina-Romero *et al.*, 2017; Q. Zhao *et al.*, 2018)
Other	4.11 %	(A. K. Al-Asmari *et al.*, 2017; F. Al-Asmari *et al.*, 2017; Huang *et al.*, 2017)

Different techniques were used for the antifungal test. Dilution methods were the most used (53.4 %), these methods are the most appropriate ones for the determination of MIC values since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macrodilution or microdilution). Either the broth or agar dilution method may be used to quantitatively measure the *in vitro* antimicrobial activity against bacteria and fungi (Balouiri *et al.*, 2016).

Antagonism tests (17.81 %), as biological methods, become an option that reduces the risks to health and the ecosystem. These methods are defined as the use of living organisms to eliminate or control others. The biological control methods most commonly used involve the use of antagonistic organisms, particularly fungi and bacteria.

Biological control approaches based on the usage of normal microbial populations and their secondary metabolites are a promising and safe alternate to chemical fungicides used for pathogen control and might be successfully used in conjunction with chemicals (Sarwar, Hassan, *et al.*, 2018).

Further, convergent percentages were observed for diffusion methods and radial growth assay, 13.70 %, 9.60 % respectively. Diffusion methods (Agar disk-diffusion method, Agar diffusion method, Agar well bioassay, Poisoned food technique) are common and used in the microbiology research laboratories to screen extracts, fractions, or pure substances for their antimicrobial potency or to investigate the antagonism between microorganisms. Because of the slow fungal growth in culture media, radial growth assays become difficult. Such slow growth rates turn the storage very difficult, and the fungus culture is often impregnated with contaminants. Radial growth rate determination and diffusion methods may have their merits, but also their limitations concerning result comparability.

Most of the studies on the biological activity of antifungal compounds are based on *in vitro* models since the existing *in vivo* models are extremely time-consuming, labor-intensive, and expensive (Van Dijck *et al.*, 2018). *In vivo* tests are more relevant than these *in vitro* but in our analyzed studies only 05 studies (Bouzoumita *et al.*, 2020; Lastochkina *et al.*, 2020; López-Seijas *et al.*, 2020; Medina-Romero *et al.*, 2017; Q. Zhao *et al.*, 2018) used *in vivo* tests. Because these types of tests are expensive, low efficacy, difficult to

control, and difficult to interpret. Balouiri *et al.* (2016) proposed that the best way is to find a good correlation between the *in vitro* data and the *in vivo* evolution.

Other techniques were used but for a small rate: Gas-phase method (Medina-Romero *et al.*, 2017), Mycelium Growth Rate(Ghazi *et al.*, 2020; Mihalache *et al.*, 2018), ELISA-like microtiter plates (Ramos *et al.*, 2018).

10. Assessment of efficacy of natural compounds as antifungal agents against *F. oxysporum*

Natural products tested in these studies showed different antifungal efficacies against *F. oxysporum formae speciales*. Antifungal testing of fungi generally suffers from the incomparability of results. Therefore, it is not easy to classify and compare the analyzed results: because of the differences in the source, concentration, technique used, and efficacy. In order to bypass this problem, efficacy levels (low, medium, high, and very high) were used instead of comparing incomparable values. The results of these analyses are shown in **Table II.6**.

Table II. 6: Evaluation of the antifungal efficacy of different sources.

No efficacy	Low efficacy	Medium efficacy	High efficacy	Very high Efficacy
5.50 %	9.60 %	20.50 %	26. 00 %	38.36 %

Efficacy of essential oils has been reported in several studies against *F. oxysporum formae speciales*. Park *et al.* (2017) evaluated four essential oils namely: Cinnamn oil, Fennel oil, Origanum oil, and Thyme oil with concentrations of 0.5 µl/disc against *F. oxysporum* f. sp. *fragariae* through agar disc diffusion method. Origanum oil and Thyme oil expressed maximum inhibition (100 %) of fungus at 0.5 µl/disc and reduction in mycelia growth followed by Cinnamn oil and Fennel Oil with 89.40 and 84.40 %, respectively. This study showed significant results as compared to other essential oils fungicides, reported by Bouayad *et al.* (2017), Davari *et al.* (2017), and Kalleli *et al.* (2020). The efficacy of essential oils and major components varied with their concentration and the techniques used.

Cuminic acid (p-isopropyl benzoic acid), isolated from the seed of *Cuminum cyminum* L., was reported to possess good inhibition towards several plant pathogens. That was proved by Sun *et al.* (2017) and Wang *et al.* (2017) against *F. oxysporum*. In the study of Sun *et al.* (2017), results showed that the growth of *F. oxysporum* f. sp. *niveum* was strongly inhibited by cuminic acid in a concentration-dependent manner, with an EC_{50} value of 22.53µg/mL.

In the analyzed studies, some *F. oxysporum* was resistant (no efficacy) this is the case of crude plant extracts tested by Mahlo *et al.* (2016); some compounds isolated by Mihalache *et al.* (2018) from *Bacillus subtilis*; a Protein isolated by Patiño *et al.* (2018) from *F. graminearum*, and some alcohols isolated by Medina-Romero *et al.* (2017) from plants (*Nodulis porium* sp. and *Hypoxylon anthochroum*).

Most studies that used fungi as an antifungal source have shown effective results against *F. oxysporum*, using the growth inhibition technique (Carvalho *et al.*, 2018; Sen *et al.*, 2017; Wu *et al.*, 2019; X. Wu *et al.*, 2018; Yuan *et al.*, 2017). The study realized by Kalvo *et al.* (2018) (Kälvö *et al.*, 2018) also presents an effective effect (100 %) but by using inhibition of conidial germination.

In the study realized by Zhu *et al.* (2018), protein extracts (fusarihexin A and B) from a *Fusarium* strain had a Gas-phase antifungal activity (100%). In contrast, the study conducted by Patiño *et al.* (2018) using another *Fusarium* strain had given negative results.

Sarwar *et al.* (2018) observed that some compounds isolated from *Bacillus* species, as surfactin, were the most effective fungicides to inhibit the growth of *F. oxysporum*. Correspondingly, the results of contemporary studies are in line with Agarwal *et al.* (2017), Ahmad *et al.* (2017), Gao *et al.* (2017), and Han *et al.* (2019). Bacterial peptides are known to show antimicrobial activity against antibiotic-resistant bacteria due to their selectivity (Mani Chandrika *et al.*, 2020). Mihalache *et al.* (2018) evaluated lipopeptides from *Bacillus* species against *F. oxysporum* and found that Plipastatin, Peptide P852, and Mycosubtilin are the most effective fungicides against *Fusarium* wilt.

The chemical structures of some important natural products with antifungal activity against *F. oxysporum* are given in Figure II.3 and Figure II.4.

11. Action mechanisms of natural products against *F. oxysporum*

According to most studies cited in this chapter, natural products were tested for their ability to inhibit the growth of *F. oxysporum*. This inhibition was visualized through different evaluation mechanisms (mycelial growth, colony growth, hyphal measurement, and radial growth) to determine the *in vitro* antifungal activity.

The comprehension of inhibition evaluation may explain the mechanism of action. However, the action mechanisms of natural products are not always clear. It has been reported that natural products can act through the following effects: Inhibition of conidial germination (Hsu *et al.*, 2017; Kälvö *et al.*, 2018; Park *et al.*, 2017; Zandvakili *et al.*, 2017), hyphal morphology (Medina-Romero *et al.*, 2017; Sun *et al.*, 2017; M. Zhang *et al.*, 2018), inhibition of spore germination (Mihalache *et al.*, 2018; Wang *et al.*, 2017; Xiong *et al.*, 2017; Yu *et al.*, 2017; Zandvakili *et al.*, 2017; Zhao *et al.*, 2018), cellular leakage effects (Montenegro *et al.*, 2018), cellulases inhibition (Belhi *et al.*, 2020), the effect on respiration (Medina-Romero *et al.*, 2017), Cell Membrane Permeability (Han *et al.*, 2019; Medina-Romero *et al.*, 2017; Ramos *et al.*, 2018; Silva *et al.*, 2017; Sun *et al.*, 2017; M. Zhang *et al.*, 2018), enzymatic inhibition (Mohamed *et al.*, 2017), oxidative stress (Silva *et al.*, 2017), and mycotoxin production (Zhao *et al.*, 2018).

The mechanisms of action of these organisms are not perfectly clear; however, taking into account experimental evidence, some authors suggest that the mechanism is related to vital systems in these fungi (mycelial growth inhibition, enzyme inhibition …). On the other hand, the mechanism in the host plant is generally through stimulation of their defense-related enzymes or metabolites.

If the mechanisms of action are varied, the commercial products based on natural substances will be possible to avert the evolution of resistance in *F. oxysporum.* Thus, modes of action studies are needed to reveal target(s) and understand the mechanism.

12. Conclusion

In the last few years, new or updated control methods have emerged to offer plant growers alternatives to chemicals to manage plant diseases. In this chapter, we have

focused on natural sources that have antifungal properties against *F. oxysporum* f. sp., and thus could be a new source of molecules to be used in chemistry, agronomy, or medicine.

According to our analysis, approximately 50 plants species belonging to different plant families and about 36 compounds have promissory antifungal activity. These substances should be postulated as interesting agents for the control of *F. oxysporum* f. sp.

Many reports of antifungal activity of natural products contrast with the low number of publications with the action mechanisms of antifungal activity. Therefore, the challenge is not only finding potential species for pest control but determining the biochemical mechanisms that these products target. Understanding these mechanisms is important to improve products with higher selectivity.

This work shows that plant extracts could be a good alternative in improvement of potent plant based fungicides, which can be used in organic farming for the management of *F. oxysporum formae speciales*.

As concluded from this analysis:

1) Pathogenic *F. oxysporum* f. sp. are harmful to many vegetables, and ornamental plants, as well as for field crops, only a few publications cooperation with biocontrol of these species have been listed in comparison to other host plants of *Fusarium*. But the limitation of chemical use for environmental and human health concerns has motivated the study of these alternatives.

2) Their antimicrobial properties for other uses have been well known for decades, medicinal plants for the control of pathogenic fungi have been greatly studied. Nevertheless, studies indicate a good potential of these mixtures. In addition, they prove interesting features such as their low danger to the environment and human health and their biodegradability.

3) Among the microorganisms evaluated (bacteria and fungi), a majority has proved efficient in reducing the disease. However, only a few of them are commercialized. Thus, these microorganisms represent good candidates that should be exploited.

4) Many methods and parameters are available to evaluate the antifungal effect competencies of microorganisms and plant extracts. However, *in vivo* tests under

controlled conditions are certainly the best technique. Even *in vivo* tests are more convenient, *in vitro* tests should first be performed to obtain reliable screening results.

5) In this context, research and development should play a key role in the evolution of these products to specify and optimize the parameters of production and use.

Finally, the important number of researches realized on the use of natural products against *Fusarium oxysporum* reflects the big influence of this fungus on human and animal resources and the opportunities present in natural products from different sources to solve this problem. Among all positive sides of natural products use against *Fusarium oxysporum* phytopathogens, many obstacles are present to pass from research to *in vivo* application and commercialization. The application of treatment against soil-borne fungi is difficult and needs more advanced researches on selectivity and technological approaches for in-soil treatment.

CHAPTER III

Materials and Methods

CHAPTER III: Materials and Methods

1. Presentation of the study area

The wilaya of Bechar is located in the Southwest of Algeria (at a distance of 950 km from the capital Algiers), on an area of 161,000 km² before the new administrative divisions in 2019 (Figure III. 1). Bechar is a typical Saharan city with an arid climate. It is subject to a hot desert climate, with high daytime temperatures and very little rainfall. The locals rely on agricultural production, thanks to an expansive area of irrigated land, where primarily dates are grown.

Its relief is made up of five types:

-Mountains: Such as Djebel Antar (1.953 m) and Djebel Grouz (1.835 m).

-The wadis: four main wadis crisscross the wilay: Wadi Bechar, Wadi Zouzfana , Wadi Guire, and wadi Saoura.

-The valleys: the main ones are those of Zouzfana, Abadla, and Saoura.

-The hamadas: the Guire and Saoura hamadas.

-The ergs: these are dune massifs. We distinguish the great Western Erg, Erg Erraoui, Erg El-Atchane, and Erg Iguidi. (Ozenda, 1991)

Figure III. 1: Localization of study area on the map of Algeria

Wilaya of BECHAR

Available at: https://www.nationsonline.org/oneworld/map/algeria_map.htm

2. Plant materials

The aerial parts of studied plants were collected in March 2017 from Bechar (road of Lahmer, Bechar, Algeria). The collected plants were identified by Prof. Abderrazak Marouf (Botanist at Salhi Ahmed University, Naama, Algeria), and voucher specimens were conserved at the herbarium of the Phytochemistry and Organic Synthesis Laboratory of Bechar University (**Table III.1**). The aerial parts were air-dried at room temperature in a shady place and then ground in the blender.

Table III. 1: Plant materials used in this study

Plants	**Family**	**Vernacular names**	**Code**
Acacia raddiana	Fabaceae	Talh	CA00/37
Anabasis aretioides	Chenopodiaceae	Dega'a	CA00/42
Asteriscus graveolens	Asteraceae	Tafs	CA00/14
Calotropis procera	Asclepiadaceae	Kranka	CA04/02
Citrullus colocynthis	Cucurbitaceae	Handhal or Hdaj	CA99/41
Launeae arborescens	Asteraceae	Oum Lbina	CA00/25
Limoniastrum feei	Plumbaginaceae	M'leffet Lkhadem	CA99/14
Nerium oleander	Apocynaceae	Dafla	CA99/26
Pergularia tomentosa	Asclepiadaceae	Kalga or Aneb Dib	CA00/44

These medicinal plants have previously been investigated by our research group (POSL team) for their antibacterial and antifungal properties. They were chosen for initial testing based on a systematic review conducted on promising bioactive plants which highlighted the above species (Belhi *et al.*, 2020; Boulenouar *et al.*, 2009; Boulenouar *et al.*, 2012; Ghazi *et al.*, 2020).

3. Extraction and bioguided fractionation

The dried aerial part plants extracted with 80% ethanol for 18 h using Soxhlet apparatus and then evaporated to dryness by a rotary evaporator (Büchi Rotavapor R-210) at 55 ◦C under reduced pressure. This extract was suspended in distilled water and portioned sequentially with hexane, dichloromethane, ethyl acetate, and n-Butanol. The organic phase was evaporated to dryness under reduced pressure.

TLC was carried out on silica gel 60 F_{254} plates (Merck, Germany). Column chromatography was performed over silica gel 60 (Merck, particle size 290-320 mesh).

The analysis of extracts was achieved by the TLC method using ethyl acetate - petroleum ether (EtOAc-PE) gradient (100:0 – 0:100) and then using a gradient elution of heptane- ethyl acetate (Hep-EtOAc) (100:0–0:100). Compounds were visualized under UV light (254 and 366 nm). R_f values of evaluated spots were recorded.

The ethyl acetate fractions were chromatographed over silica gel open Column chromatography (30 g) using a mobile phase: (ethyl acetate: heptane) with the report in the following volume: (75: 25). The recovered fractions were analyzed again by TLC, fractions with identical spots and R_f values were pooled together for the antifungal evaluation using the antimicrobial assays described below.

Among all extracts and fractions, ethyl acetate fractions exhibited great enzyme inhibition on cellulases of Foa and has been further characterized by chemical methods (TPC, TFC, NMR, and LC-MS analysis).

4. Determination of the Total Phenolic Contents (TPC)

The total phenolic content (TPC) of the extracts was determined by the Folin–Ciocalteu method using a modified procedure of (Slinkard *et al.*, 1977) and (Sengul *et al.*, 2009). Gallic acid was used as the standard phenolic compound. The calibration was plotted by mixing aliquots of 1000; 500; 250; 125; 62.5 and 31.25 ppm of gallic acid solutions with 5 ml of Folin Ciocalteu reagent and 5 ml of crude extract. After 3 min, a solution of sodium carbonate 10 % Na_2CO_3 was added and the mixture was allowed to stand for 1 h with intermittent shaking. The color was developed and absorbance was measured at 760 nm in a Shimadzu UV 1800 Spectrophotometer after 30 min using Gallic acid as a standard. The total phenolic content (TPC) was calculated from the

calibration curve, and the results were expressed as µg of gallic acid equivalent per mg dry weight (µg GA/mg).

5. Determination of the Total Flavonoid Contents (TFC)

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the samples; quercetin was used to make the standard calibration curve (Chandra *et al.*, 2014). 10 mg sample (the organic extract of each plant studied) is weighed and added into 5 ml of methanol. Stock quercetin solution was prepared by dissolving 5 mg quercetin in 1 ml methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol. An amount of 0.5 ml diluted standard quercetin solutions or samples were separately mixed with 0.5 ml of 2 % aluminum chloride solution ($AlCl_3$). After mixing, the mixture was incubated for 40 min at room temperature followed by the measurement of absorbance at 415 nm wavelength against the blank. All the determinations were carried out in triplicate. The outcome data were expressed as µg quercetin equivalents per mg extract. (µg QE/mg)

6.Thin-layer chromatography (TLC)

The extracts of each solvent were subjected to TLC. The used solvent system was ethyl acetate: heptane (75:25). Spots were detected on TLC under UV light.

7. Phytochemical screening on TLC plate

The extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extract. The screening was done according to Pascual, *et al.* (Pascual *et al.*, 2002).

7.1.Test for flavonoids

The TLC plates containing the extracts are sprayed with an ethanolic solution with 5% aluminum chloride ($AlCl_3$). The appearance of the greenish-yellow color, with the revelation by UV 365 ηm, indicates the presence of flavonoids.

7.2.Test for coumarins

The TLC separation plates are sprayed, immediately, with the ethanolic solution of KOH. The appearance of red color, in the visible, indicates the presence of anthraquinones,

or the yellow color, with the revelation by UV 365 ηm, which indicates the presence of anthrones or furanocoumarins.

7.3.Test for tannins

The TLC plates containing the extracts are sprayed with a methanolic solution (80%) of ferric chloride 1 % ($FeCl_3$). The appearance of black-blue color indicates the presence of gallic tannins, or greenish-brown indicates the presence of catechin tannins.

7.4.Test for alkaloids

Formaldehyde solution (10 ml) with 100 ml of sulfuric acid (H_2SO_4) was prepared. The TLC separation plates are sprayed immediately. The color change of the spots indicates the presence of alkaloids or aromatic hydrocarbons.

7.5.Test for saponins

Froth test for saponins (foam) was used. 2 g of the sample was weighed into Erlenmeyer flask in which 100 ml of sterile distilled water was added and boiled for 15 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. The height of the foam was measured in each tube. The formation of a 1 cm layer of foam indicates the presence of saponins.

8. Antifungal screening by direct bioautography

In order to screen for and identify compounds with the antifungal activity present in the plant extracts, direct bioautography was used as described by Boulenouar *et al.* (2011). This method is based on the direct immersion and growing of a fungal spore suspension on a developed TLC chromatogram.

8.1.Fungal strain

The phytopathogenic filamentous fungus (Foa) used in this work was obtained from The Technical Institute for Saharian Agronomy (TISA), Adrar, Algeria. The strain was identified and a voucher specimen was stored at Phytochemistry and Organic Synthesis Laboratory under N° POSL/2011/01.

8.2.Preparation of spore suspension

Spore suspensions of plant pathogens (Foa) were obtained by transferring 7 days old culture of Foa on potatoes dextrose agar medium PDA (consisting of 4 g potatoes extract, 20 g glucose, 15 g agar, distilled water up to 1 liter) to synthetic nutrient-poor agar SNA (consisting of 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$-$7H_2O$, 0.5 KCl, 0.2 g glucose, 0.2 g saccharose, 15 g agar, distilled water up to 1 liter) to induce spores formation. Ten days old culture of Foa on SNA was surface flooded with 10 ml of sterilized water to dislodge fungal spores, then, filtrated to eliminate mycelia fragments. The concentration of Foa spores was adjusted to approximately 10^7 spores/ ml by dilution and counting.

8.3.Developing the chromatogram

80 µg/µl of each extract was applied to the TLC plate of silica gel 60 F_{254} (7 × 1.5 cm). After developing the chromatogram using the suitable eluent for efficient separation and location of the UV absorbing spot, chromatograms were then dried at room temperature under a stream of air overnight to remove the remaining solvent.

8.4.Fungal growth on TLC

The chromatograms were then moved directly into Petri dishes containing 20 ml of spores for 10 seconds, where the concentration is 2×10^7 spores/ml. Bioautograms were examined for fungal growth at regular intervals until TLC plates were completely covered with a lawn of mycelial growth. Then, they are added to other sterile Petri dishes, under humid conditions, containing cotton wet with distilled water to provide moisture. These are incubated for 4 days at 21°C. Control plates spotted with the corresponding organic solvent were run in parallel (Rana *et al.*, 1996).

8.5.Visualization of microbial growth

For the visualization of microbial growth, tetrazolium salts are frequently used. The bioautograms were sprayed with a 2 mg /ml solution of p-iodonitrotetrazolium violet (INT) and reincubated overnight at 21°C (Mahlo *et al*., 2016). Clear white zones against a purple background on the TLC plate indicate antimicrobial activity of the sample (Saxena *et al.*, 1995). R_f zones on the plates were compared to that of reference plates to find the R_f of the active compound.

9. Enzyme inhibitory activity against cellulases by contact bioautography

The use of TLC support to screen for potential plant-derived enzyme inhibitors is a rapid method that is relatively free of disturbances due to the solvent (Pascual *et al.*, 2002). In contact bioautography, antimicrobial compounds diffuse from the chromatogram to the inoculated agar plate. The test by contact bioautography used in this study was described by Choma and Jesionek (Choma *et al.*, 2015) with slight modifications.

9.1.Cellulases production by *Fusarium oxysporum* f. sp. *albedinis*

Fusarium oxysporum f. sp. *albedinis* was cultivated on a liquid medium, containing (g per liter of distilled water): 2.5g cellulose, 2g $NaNo_3$; 1g KH_2PO_4; 1g $MgSO_4.7H_2O$; 0.5 KCl; 0.005 $ZnSO_4.7H_2O$; 0.001 $CuSO_4$; $5H_2O$; 0.001 $MnCl_2$, and was incubated at 21°C for 96 h under moderate agitation. For the inoculation of the cultures, a suspension of spores (4.3×10^5 spores/ml) was used in sterile distilled water added to 2 ml of Tween 80. The filtrate containing cellulases was put in tubes (5 mL in each tube). The tubes were conserved at −18°C until use.

9.2.Preparation of medium with the substrate for the enzymatic activity

Cellulolytic activity of the plant extracts and fractions was determined on a solid medium containing cellulose, which was used as a substrate. In this assay, the test has been realized on cellulose agar medium (2% cellulose, 1.5% agar). Ten milliliters of the medium have been poured into each Petri dish and left to solidify.

9.3. TLC analysis and chromatogram diffusion

Plant extracts were applied to a TLC plate of silica gel 60 F_{254} (5×6 cm). After drying, the chromatogram was developed using a mobile phase. This phase was a mixture of ethyl acetate: heptane (75:25, *v/v*). After the solvent reached the top of the plate, the chromatogram was dried and visualized under visible and UV light.

Each chromatogram (after evaporation of the eluent) was put face down on the cellulose agar plate for 2 hours to promote diffusion. The filtrate containing cellulases previously prepared was poured on the cellulose agar plates after removal of the chromatogram and incubated at 21°C for 2 hours.

9.4.Detection of enzyme inhibition activity

The detection of inhibition zones was done using Gram's iodine. Gram's iodine was used as described by Kasana *et al.* (2008). Plates were flooded with Gram's iodine (3% KI and 2% I_2 in 70 % ethanol) for 5 minutes then washed with distilled water. Gram's iodine formed a dark brown color with cellulose but not with hydrolyzed cellulose, giving a distinct, clear, and prominent zone in 3 to 5 minutes (Kasana *et al.*, 2008). The inhibition zones were visible as dark brown zones. The evaluation of cellulases activity inhibition was expressed as the diameter of inhibition zones (mm) and the R_f values to reflect the inhibitors. The control tests passed all protocols without extracts or filtrate (the filtrate has been replaced by the culture medium). The tests were realized in triplicate (the standard errors were less than 10%).

10. Preliminary evaluation of the antifungal activity

Preliminary analysis of the antifungal activity was performed using the agar-disc diffusion bioassay (Boulenouar *et al.*, 2009) and the agar-well diffusion bioassay (Magaldi *et al.*, 2004) for the evaluation of ethyl acetate fractions. Briefly, Spore suspension of the fungal test microorganism was prepared by transferring 7 days old culture of Foa on potatoes dextrose agar medium PDA to synthetic nutrient-poor agar SNA to induce spores formation.

For the disc diffusion bioassay, sterile discs (6 mm in diameter) of Whatman filter paper No.10 were impregnated with (20, 50, 80, and 100 µl) of each extract. The solvent was left to evaporate at room temperature, and the discs were placed on the surface of the plates previously seeded. Paper discs impregnated with ethyl acetate were used as controls.

For the well-diffusion bioassay, wells were made in the agar using an inverted sterile Pasteur pipette (6 mm in diameter), and (20, 50, 80, and 100 µl) of ethyl acetate extracts were deposited in the wells. Ethyl acetate was used as a control (all manipulations were done in sterile conditions). Plates were incubated at 21°C for 5 days.

Antimicrobial activity was detected by the presence of a growth inhibition zone surrounding the disc or well. The diameter of this zone was measured and recorded. The tests were realized in triplicate (the standard errors were less than 10%).

11. Characterization of isolated compounds

The extracted and purified bioactive compounds from *Asteriscus graveolens* and *Launaea arborescens* were characterized by nuclear magnetic resonance (NMR) techniques: Routine ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Model Avance AMX spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz respectively) in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal reference. The NMR studies were carried out in high-quality 5 mm NMR tubes. Chemical shifts δ and coupling contents *J* are reported in ppm and Hz, respectively. ^{1}H NMR spectra are referenced to residual protiated solvents (δ = 7.26 ppm for $CDCl_3$), ^{13}C NMR chemical shifts are reported relative to deuterated solvents (δ = 77.16 ppm for $CDCl_3$). Mass spectrometry detection was conducted using a Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. Data were acquired by Lab Solutions software (Appendix A). Ethyl acetate fractions of *Asteriscus graveolens* and *Launaea arborescens* were analyzed by the LCMS-8040 system (Shimadzu, Kyoto, Japan). The mobile phase consisted of 100% methanol (solvent A) and acetonitrile (solvent B) (1:1 v/v). The mobile phase flow rate was 0.3 mL/min. The column temperature was fixed at 40°C. Plant compounds were detected by a full scan mode ranged m/z 100 - 1000 amu.

The LC-MS mass spectrometer, ^{13}C NMR, and ^{1}H NMR analyzes were carried out in the laboratory of "Catalysis Research and Application Center" of the University of İnönü, Malatya. Turkey.

CHAPTER IV

Results and Discussion

CHAPTER IV: Results and Discussion

I. Results

1.1.Thin-layer chromatography (TLC)

Thin-layer chromatography was used as a preliminary method for compositional analysis of the crude extracts. Table 1 groups obtained R_f values. According to the chromatograms obtained by TLC, all the parts of the plants contain several chemical compounds (several spots).

Table IV. 1: R_f values of TLC in different extracts

Eluent	Extraction Solvent	R_f					
EtOAc: Hep 75:25	EtOH	0.00	0.57	0.85	0.90	0.95	-
	Hex	0.00	0.35	0.90	-	-	-
	DCM	0.00	0.54	0.81	0.90	0.93	-
	EtOAc	0.00	0.35	0.57	0.85	0.90	0.95
	n-But	0.00	0.57	-	-	-	-

EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol; Hep: heptane; R_f: rapport frontal.

TLC analysis (EtOAc: Hep, 75:25) revealed four constituents in the ethanol extract (yield 1.22%, R_f 0.57, 0.85, 0.90, 0.95), and two constituents in the hexane extract (yield 11.88%, Rf 0.35, 0.90), and four constituents in the methylene chloride (DCM) extract (yield 1.58%, R_f 0.54, 0.81, 0.90, 0.93), five constituents in the ethyl acetate extract (yield 2.83%, R_f 0.35, 0.57, 0.85, 0.90, 0.95), and one constituent in the butanol extract (yield 1.12%, Rf 0.57)

The TLC analyses indicated that the extracts of *L. feei* were composed of a mixture of secondary metabolites. Rahmani *et al.* (2014) reportedly obtained the R_f values of *L.*

feei extract by ethyl acetate in the comparable range of 0.36–0.77, eluted with another solvent system, not as that used in the present study.

1.2.Phytochemical screening of the plant extracts

The characterization reactions revealed several chemical groups in the different extracts. The use of solvents with varying polarity in the extraction procedure is determined for the successful isolation of compounds with different ranges of polarity.

In the present study, phytochemical screening for all extracts of *L. feei* aerial part showed a significant indication of the presence of metabolites. Saponins, flavonoids, tannins, coumarins, and alkaloids, were found to be present in the majority of the plant extracts (**Table IV.2**).

The phytochemical screening of aerial parts of *L. feei* on flavonoids, tannins, and coumarins, shows the presence of flavonoids in ethyl acetate, hexane, and methanol extract and total absence in dichloromethane, but the tannins derivative are present in all parts. It is also observed that the extracts of ethyl acetate are the richest in tannins and coumarins by contribution to other extracts e.g., methanolic and hexane extracts.

Table IV. 2: Phytochemical screening of various extracts.

Plants	Extraction Solvent	Constituents					
		Flavonoids	**Rf**	**Tannins**	**Rf**	**Coumarins**	**Rf**
A. raddiana	EtOH	+	0.00	-	-	-	-
	Hex	+	0.00	+	0.00 0.85	+	0.95
	DCM	-	-	+	0.95	-	-
	EtOAc	+	0.00	+	0.00	-	-
	n-But	-	-	-	-	+	0.98
A. aretioides	EtOH	+	0.00 0.96	-	-	+	0.00
	Hex	+	0.95	-	-	-	-
	DCM	-	-	+	0.95	-	-
	EtOAc	+	0.00	+	0.00	+	0.95
	n-But	+	0.57	-	-	+	0.96

EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol; Rf: rapport frontal; +: positive; -: negative.

Table IV. 2 (continued): Phytochemical screening of various extracts.

Plants	Extraction Solvent	Constituents					
		Flavonoids	**Rf**	**Tannins**	**Rf**	**Coumarins**	**Rf**
A. graveolens	EtOH	+	0.00	-	-	+	0.00
	Hex	-	-	-	-	+	0.94
	DCM	+	0.00	-	-	+	0.00
	EtOAc	+	0.24 0.88	+	0.64	+	0.00
	n-But	-	-	-	-	+	0.95
C. procera	EtOH	+	0.00	-	-	+	0.00
	Hex	-	-	-	-	+	0.00
	DCM	-	-	-	-	-	-
	EtOAc	-	-	-	-	+	0.35
	n-But	-	-	-	-	-	-

EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol; Rf: rapport frontal; +: positive; -: negative.

Table IV. 2 (continued): Phytochemical screening of various extracts.

Plants	Extraction Solvent	Constituents					
		Flavonoids	**Rf**	**Tannins**	**Rf**	**Coumarins**	**Rf**
C. colocynthis	EtOH	+	0.00	+	0.00	+	0.00
	Hex	+	0.00	-	-	+	0.95
	DCM	+	0.00 0.62	-	-	-	-
	EtOAc	+	0.62	+	0.86	-	-
	n-But	+	0.00	-	-	-	-
L. arborescens	EtOH	+	0.96	-	-	+	0.00
	Hex	-	-	-	-	-	-
	DCM	-	-	-	-	+	0.00
	EtOAc	+	0.30 0.96	+	0.00	+	0.00
	n-But	-	-	-	-	-	-

EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol; Rf: rapport frontal; +: positive; -: negative.

Table IV. 2 (continued): Phytochemical screening of various extracts.

Plants	Extraction Solvent	Constituents					
		Flavonoids	**Rf**	**Tannins**	**Rf**	**Coumarins**	**Rf**
L. feei	EtOH	+	0.00 035 0.90	-	-	-	-
	Hex	+	0.95	-	-	-	-
	DCM	-	-	+	0.95	-	-
	EtOAc	+	0.00 0.35	+	0.00 0.85 0.95	+	0.95
	n-But	-	-	-	-	-	-
N. oleander	EtOH	-	-	+	0.00	+	0.00
	Hex	-	-	-	-	-	-
	DCM	-	-	-	-	-	-
	EtOAc	+	00	+	0.00	+	0.86
	n-But	-	-	-	-	-	-

Table IV. 2 (continued): Phytochemical screening of various extracts.

Plants	Extraction Solvent	Constituents					
		Flavonoids	**Rf**	**Tannins**	**Rf**	**Coumarins**	**Rf**
P. tomentosa	EtOH	+	00	+	0.00	+	0.00
	Hex	-	-	-	-	-	-
	DCM	-	-	-	-	-	-
	EtOAc	+	00	+	0.00	-	-
	n-But	-	-	-	-	-	-

EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol; Rf: rapport frontal; +: positive; -: negative

The revelation of the alkaloids on the TLC plate is provided in **Table IV.3**.

Table IV. 3: Characterization of alkaloids on the TLC plate

Extraction Solvent	**Spots number**	**R_f**	**Color**
EtOH	6	00	Brown
		0.35	Brown
		0.57	Green
		0.85	Brick red
		0.90	Light Green
		0.95	Green
Hex	5	00	Light Yellow
		0.57	Light Brown
		0.85	Green
		0.90	Brown
		0.95	Green
DCM	6	00	Brown
		0.35	Brown
		0.57	Green
		0.85	Brick red
		0.90	Light Green
		0.95	Green
EtOAc	6	00	Brown
		0.35	Light Purple
		0.57	Purple
		0.85	Light Brown
		0.90	Brown
		0.95	Green
n-But	3	00	Brown
		0.35	Yellow
		0.90	Green

EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol; R_f: rapport frontal.

According to Table 3, we observe: Colors change in most parts of the spots; we can say that these are alkaloids or aromatic hydrocarbons. The different colors are shown in all the extracts (MeOH, DCM, Hex, and EtOAc).

1.3. Antifungal activity of the plant extracts

1.3.1. Direct bioautography

Frequently, TLC-Direct Bioautography is used as a bio-guiding method to destine to substances with biological activity that can be further analyzed by spectroscopic methods to obtain information on their structure (Choma *et al.*, 2015).

The richness of natural substances reported by Cheriti *et al*. (2007) can explain the antifungal activity in certain extracts of *A. graveolens* (**Table IV.4**).

Table IV. 4: Direct bioautography results of the extracts of *Asteriscus graveolens*

Specie	Eluent	Extraction Solvent	Antifungal effect
Asteriscus graveolens	EtOAc: Hep 75:25	EtOH	++
		Hex	-
		DCM	++
		EtOAc	++
		n-But	+

Hep: heptane; EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol.

In identifying terpenoids of *Launeae arborescens*, Bitam *et al.* (2008) demonstrated the existence of antifungal and antibacterial activity against certain microorganisms. Probably, for this reason, most extracts of *Citrullus colocynthis* gave a positive effect (**Table IV.5**).

Table IV. 5: Direct bioautography results of the extracts of *Launeae arborescens*

Specie	Eluent	Extraction Solvent	Antifungal effect
Launeae arborescens	**EtOAc: Hep 75:25**	EtOH	++
		Hex	-
		DCM	++
		EtOAc	++
		n-But	+

Hep: heptane; EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol.

Strong growth inhibition against *Fusarium oxysporum* f. sp. *albedinis* has been shown by the ethyl acetate extract that was assayed using direct bioautography on the TLC plate (**Table IV.6**).

Table IV. 6: Direct bioautography results of the extracts of *Limoniastrum feei*

Specie	Eluent	Extraction Solvent	Antifungal effect
Limoniastrum feei	**EtOAc: Hep 75:25**	EtOH	++
		Hex	-
		DCM	-
		EtOAc	+++
		n-But	+

Hep: heptane; EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol.

Several studies showed that *L. feei* contains various secondary metabolites with pharmacological properties. Cheriti *et al.* (2005) showed that the methanolic extracts of *L. feei* leaves are active against certain bacteria and fungi.

Citrullus colocynthis is one of the most studied species in the Cucurbitaceae family. Most of the studies have shown that this species is rich in phenolic compounds, and several natural substances (Ghazi *et al*., 2020). Probably, for this reason, four extracts of *Citrullus colocynthis* gave a positive effect (**Table IV.7**).

Table IV. 7: Direct bioautography results of the extracts of *Citrullus colocynthis*

Specie	**Eluent**	**Extraction Solvent**	**Antifungal effect**
Citrullus colocynthis	**EtOAc: Hep 75:25**	EtOH	++
		Hex	-
		DCM	+
		EtOAc	++
		n-But	+

Hep: heptane; EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol.

Table IV. 8: Direct bioautography results of the extracts of *Acacia raddiana, Anabasis aretioides, Calotropis procera, Nerium oleander,* and *Pergularia tomentosa*

Eluent	**Solvent**	**Antifungal effect**				
		Acacia raddiana	*Anabasis aretioides*	*Calotropis procera*	*Nerium oleander*	*Pergularia tomentosa*
EtOAc: Hep 75:25	EtOH	-	-	-	-	-
	Hex	-	-	-	-	-
	DCM	-	-	-	-	-
	EtOAc	-	-	-	-	-
	n-But	-	-	-	-	-

Hep: heptane; EtOH: ethanol; Hex: hexane; DCM: dichloromethane; EtOAc: ethyl acetate; n-But: butanol.

1.3.2. The cellulases inhibition by contact bioautography

The results of the cellulases inhibition are presented in Table IV.9. Various parameters are to be taken into account for good comprehension and evaluation of these results. For the tests carried out by TLC, the evaluation of effects is made by comparison between extracts.

Table IV. 9: Effect of plant ethyl acetate fractions extracts on *Fusarium oxysporum* f. sp. *albedinis* cellulases using contact bioautography.

Eluent	Plants	Number of IZ	Inhibition zone			
			Rf	Ø (mm)	Rf	Ø (mm)
EtOAc: Hep 75:25	*A. raddiana*	0	-	-	-	-
	A. aretioides	1	0.54	1.20	-	-
	A. graveolens	2	0.24	2.70	0.88	2.00
	C. procera	1	0.33	1.00	-	-
	C. colocynthis	2	0.62	1.30	0.86	2.00
	L. arborescens	2	0.30	2.40	0.96	2.40
	L. feei	2	0.00	2.70	0.35	2.00
	N. oleander	2	0.00	1.00	0.86	2.00
	P. tomentosa	0	-	-	-	-

IZ: Inhibition zone; EtOAc: Ethyl acetate; Hep: Heptane; **Ø:** diameter in mm; R_f: rapport frontal.

All extracts using various solvents were evaluated for their enzymatic activity against cellulases. The results indicated that the ethyl acetate extract showed the greatest antifungal activity, obtaining inhibition zones of 2.00 ± 0.1 mm, 2.4 ± 0.1 mm, and 2.7 ± 0.1 mm against *F. oxysporum* f. sp. *albedinis* cellulases.

The inhibition zones reflect either the intensity of cellulosic activity or the concentration of the tested compound.

The fractions obtained with hexane, dichloromethane, and n-Butanol did not show any enzyme inhibition activity on cellulases (**Table IV.10**).

Table IV. 10: Effect of plant ethyl acetate fractions extracts on *Fusarium oxysporum* f. sp. *albedinis* cellulases using contact bioautography.

Solvent	Plants	Number of IZ	Inhibition zone			
			Rf	Ø (mm)	Rf	Ø (mm)
Hex	*A. raddiana*	0	-	-	-	-
	A. aretioides	0	-	-	-	-
	A. graveolens	0	-	-	-	-
	C. procera	0	-	-	-	-
	C. colocynthis	0	-	-	-	-
	L. arborescens	0	-	-	-	-
	L. feei	0	-	-	-	-
	N. oleander	0	-	-	-	-
	P. tomentosa	0	-	-	-	-
DCM	*A. raddiana*	0	-	-	-	-
	A. aretioides	0	-	-	-	-
	A. graveolens	0	-	-	-	-
	C. procera	0	-	-	-	-
	C. colocynthis	0	-	-	-	-
	L. arborescens	0	-	-	-	-
	L. feei	0	-	-	-	-
	N. oleander	0	-	-	-	-
	P. tomentosa	0	-	-	-	-
n-Butanol	*A. raddiana*	0	-	-	-	-
	A. aretioides	0	-	-	-	-
	A. graveolens	0	-	-	-	-
	C. procera	0	-	-	-	-
	C. colocynthis	0	-	-	-	-
	L. arborescens	0	-	-	-	-
	L. feei	0	-	-	-	-
	N. oleander	0	-	-	-	-
	P. tomentosa	0	-	-	-	-

1.4.Phytochemical study of the bioactive extracts/fractions

1.4.1. Extraction and bioguided fractionation

The analysis of extracts is achieved by the TLC method using EtOAc-PE gradient (100:0 – 0:100), and then using a gradient elution of Hep- EtOAc (100:0–0:100) to afford 6 spots for *L. arborescens* and 08 spots for *A. graveolens.*

1.4.2. Total phenolic contents

Phytochemical screening of the crude extracts and ethyl acetate fractions of studied plants was conducted, and both the extracts showed the presence of phenolics. The total phenolic contents were determined using the Folin Ciocalteu method in terms of the Gallic acid equivalent (GAE) in µg GA/mg of the extract. The total phenolic content was calculated with the help of the graph shown in Figure IV.2, and the standard curve equation was $y = 0.00146x + 0.02028$, where $R^2 = 0.99913$. The total phenolic contents (Gallic acid equivalents, µg GA/mg) in the samples were calculated to be 821,699 and 174,291 µg GA/mg in *L. arborescens*; 1144,879 and 366,052 µg GA/mg in *A. graveolens,* respectively (**Table IV.10**).

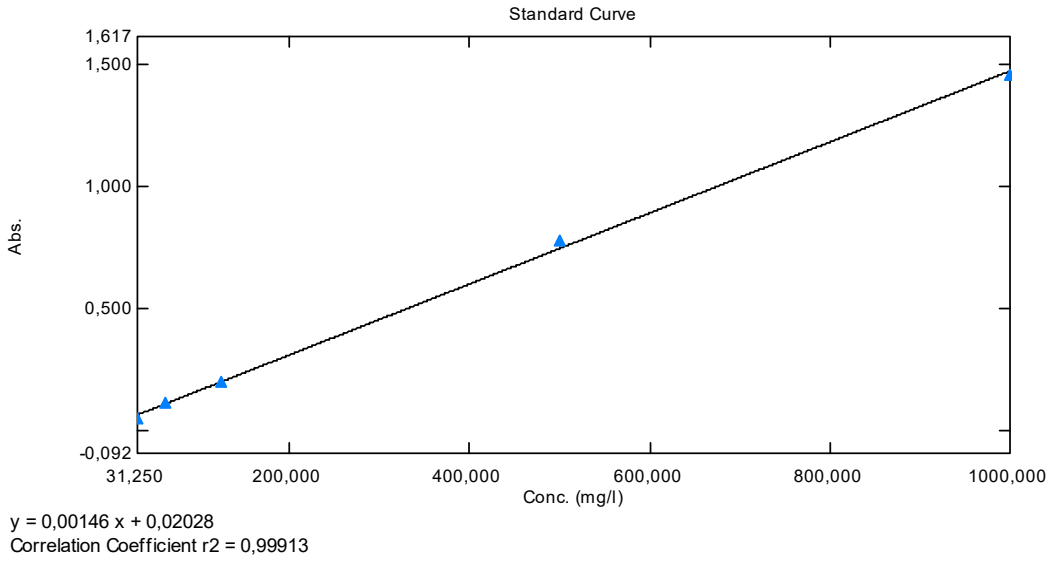


Figure IV. 2: Standard curve of gallic acid.

Figure IV.2 shows the total phenolic content in the samples of aerial parts of *L. arborescens* and *A. graveolens* spontaneous grown in the South-Oust of Algeria. The

highest phenolic content was found in the crude extract of *A. graveolens* (1144, 879 µg GA/mg) in comparison with the crude extract of *L. arborescens* (821,699 µg GA/mg).

The high amount of phenolic compounds from *A. graveolens* was reported by Ramdane *et al.*(2017). On the other side, the phytochemical investigation revealed that *L. arborescens* is rich in phenolic compounds (Bitam *et al.*, 2008).

The variance in total phenolic content could be due to the chemical composition of extract but also to the extreme conditions of growth and arid ecosystem.

1.4.3. Total flavonoid contents

The concentration of total flavonoid contents in the test samples was calculated from the calibration plot (Y=0.00535 – 0.00381; R^2=0.99917) and expressed as µg quercetin equivalents per mg of dry extract. (µg QE/mg).

The total flavonoid contents in different extracts are shown in Figure IV.3 The highest amount of flavonoid content was found in *L. arborescens* extracts (46,418 µg QE/mg) compared to the extracts of *A. graveolens (*13,824 µg QE/mg) (**Table IV.11**).

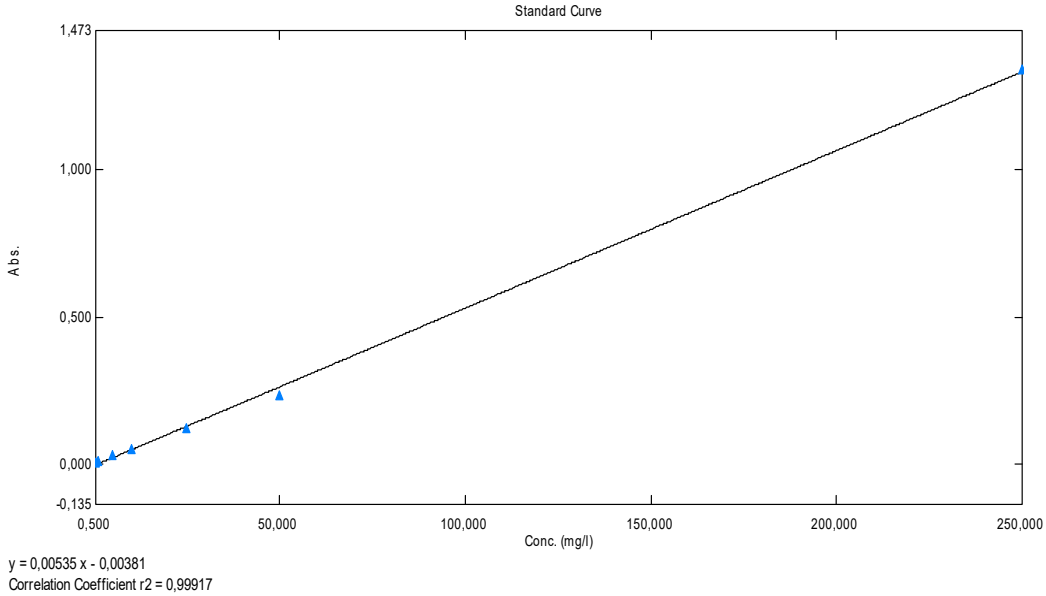


Figure IV. 3: The total flavonoids content in the samples of aerial parts of *Launaea arborescens* and *Asteriscus graveolens*.

Many studies on the phytochemical composition of *A. graveolens and L. arborescens* showed that this plant produced phenolic compounds including flavonoids:

Ahmed *et al*. (1991) have identified kaempferol 3-*O*-β-glucoside, kaempferol 3-*O*-β-galactoside, kaempferol 7-*O*-β-galactoside, quercetin 7-*O*-β-glucoside, luteolin 7-*O*-β-glucoside, and quercetin as major compounds in *A. graveolens* in Egypt.

Eight compounds were isolated and identified in the butanol fraction of *L. arborescens* aerial part, namely: 7-*O*-[α-rhamnopyranosyl 4',5,6-Trihydroxy flavone, 4',5'-Di-Methoxy 7-(5''-Me Hexan)1-oyl flavanone, 3"-isopropyl pyrano [1":7,4":6] 3',4',5',5-Tetrahydroxy flavanone, 5,4',5'-Tri-Hydroxy 7-(3''-Me butan) -yl flavanone, 5,7-Dihydroxy-2',4',5' –trimethoxy-isoflavanone 5,5,6,7,4'-tetrahydroxy flavonol, 7-*O*[α-rhamnopyranosyl-(1->6)-β-glucopyranosyl]- 4',5,7-tri-hydroxy-flavanone 7,7-*O*-[αrhamnopyranosyl-(1->6)-β-glucopyranosyl] 3',5-Dihydroxy 4'-Methoxy flavanone (Sekkoum *et al.*, 2014).

Table IV. 11: Total phenolic and flavonoids contents of crude extracts and ethyl acetate fractions of *L. arborescens* and *A. graveolens*.

Sample Code	TPC (mg GAE/g Dry extract wt)	TFC (mg QE/g dry extract wt)
L. arborescens Crude extract	821,699	46,418
L. arborescens EtOAc fraction	174,291	17,788
A graveolens Crude extract	1144,879	13,824
A graveolens EtOAc fraction	366,052	5,573

TPC: total phenol content; TFC: total flavonoid content; GAE: gallic acid equivalents; QE: quercetin equivalents; wt: weight; EtOH: ethanol; EtOAc: ethyl acetate. (Aryal *et al.*, 2019).

The total phenolic content of the ethyl acetate fractions, calculated from the calibration curve (R^2 = 0.99913), was 174,291 µg GA/mg in *L. arborescens* and 366,052 µg GA/mg in *A. graveolens* and the total flavonoid content (R^2 = 0, 99917) was 17,788 µg QE/mg in *L. arborescens* and 5,573 µg QE/mg in *A. graveolens* (**Table IV.12**).

Recent study on the phytochemical composition of *A. graveolens* (Ramdane *et al.*, 2017) showed that ethyl acetate is the most suitable solvent for the extraction of bioactive compounds from this plant.

1.5. Bioautographic method (Crude extracts)

The antifungal activity against the phytopathogenic strain (*Fusarium oxysporum* f. sp. *albedinis*) was evaluated by direct bioautography in a TLC bioassay.

Table IV. 12: Antifungal activity of plant crude extracts using direct bioautography

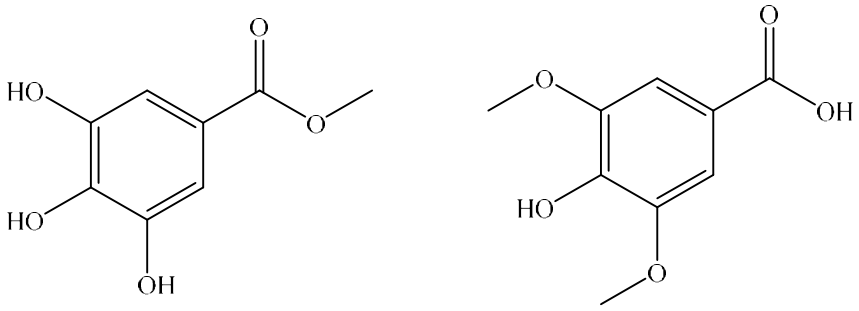
Samples (crude extract)	**Antifungal activity**	**R_f**
A. graveolens	+	0.24
		0.88
L. arborescens	+	0.30
		0.96

The bioautography was performed only for the ethanolic crude extracts using ethyl acetate/pentane 7.5:2.5 to develop silica gel TLC plates. Clear inhibition zones at an R_f of 0. 24 and 0.88 for *A. graveolens* and 0.30 and 0.96 for *L. arborescens* ethanolic extracts were observed against *Fusarium oxysporum* f. sp. *albedinis*, showing that the substances responsible for the antifungal activity are strongly polar.

1.6.Characterization of bioactive compounds by NMR and LC-MS

LC-MS analyses showed that plant extracts were decomposed to previously known. The structures of compounds were elucidated by NMR techniques, mass spectroscopy. The compounds isolated from the ethyl acetate fractions of the species *Asteriscus graveolens* and *Launaea arborescens* display a powerful antifungal effect.

The known compounds were identified as methyl gallate and syringic acid based on comparing their spectral and physical data with the literature (Figure IV.4). Evidently, the ***m***/***z*** value of 185 and 198 corresponded to their molecular weight of 184.15 and 198.17 g/mol respectively, thus validating the output of the mass spectrometer (Appendix A).



116: Methyl gallate **117**: Syringic acid

Figure IV. 4: Compounds isolated and identified in aerial parts of *Launaea arborescens* and *Asteriscus graveolens.*

Identification of molecules by NMR (^{1}H and ^{13}C) spectrophotometry and LC-MS showed the presence of methyl gallate **116** and syringic acid **117** in aerial parts of *L. arborescens* and *A. graveolens* (Appendix B).

NMR spectra of methyl gallate ($C_8H_8O_5$): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): **3.95** (s, 3H, CH_3), **6.91** (s, 2H, C_6H_2), **8.73** (s, 3H, OH). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): **52.08** (CH_3O), **110.8, 123.82, 137.57** and **146.65** (C_6H_2), **166.51** (CO). The molecular mass of isolated methyl gallate was determined as 185 using LC-MS analysis. Results obtained with ^{1}H-NMR; ^{13}C-NMR and LC-mass spectroscopy were identical to published data (Kamatham *et al.*, 2015).

NMR spectra of syringic acid ($C_8H_8O_5$): ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): **3.82** (s, 6H, CH_3), **7.07** (s, 2H, C_6H_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): **56.78** (CH_3O), **106.09, 121.39, 141.78** and **149.19** (C_6H_2), **167.49** (CO). The molecular mass of isolated syringic acid was determined as 198 using LC-MS analysis.

Note: The ^{1}H NMR spectra are referred to the residual protiated solvents (δ = 7.26 ppm for $CDCl_3$), the chemical shifts by ^{13}C NMR are reported relative to the deuterated solvents (δ = 77.16 ppm for $CDCl_3$).

II. Discussion

Bioautography is notably significant to avoid the time-consuming isolation of inactive compounds (Dewanjee *et al.*, 2015). TLC bioautographic methods combine

chromatographic separation and in situ activity determination facilitating the localization and target-directed isolation of active constituents in a mixture (Suleiman *et al.*, 2010).

The bioautography technique is inexpensive, so beneficial for screening large numbers of samples (particularly crude extracts). Although results are not completely quantitative, they can give information about how many and which substances in a mixture showed antifungal activity (Balouiri *et al.*, 2016).

Plants were selected for inclusion in this study because their ability to inhibit the respective enzymes and biological activities have already been established in studies published by others (see chapter I) and in previous studies carried out by our research group(Boulenouar *et al*., 2009; Boulenouar *et al*., 2012; Ghazi *et al*., 2020).

Phytochemical analysis was intended to know which metabolite is responsible for the antifungal activity. According to the TLC profiling, we can suppose that inhibition is caused by flavonoids present in ethyl acetate extracts of aerial parts of *A. graveolens* (R_f:0.24, 0.88), *L. arborescens* (R_f:0.30, 0.96), and *L. feei* (R_f: 0.35, 00).

The number of active compounds in the plant extracts was determined using the bioautography method, those compounds were separated with CC had similar R_f values of 0.24, 0.88 in *A. graveolens*, and 0.30, 0.96 in *L. arborescens* ethyl acetate fractions.

Apart from the advantages of rapidly detecting active compounds in mixtures and high sensitivity, the depicted bioautography also points to a potential disadvantage of this diffusion assay. Its applicability is limited to microorganisms that easily grow on TLC plates (Prieto *et al.*, 2013)

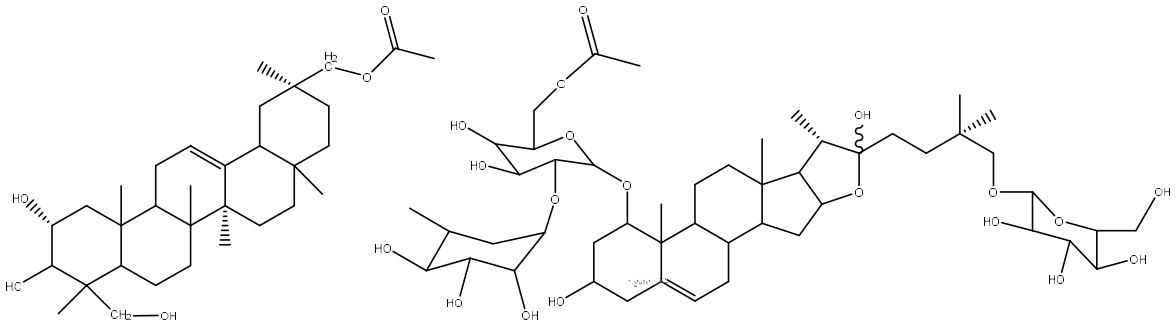
In this study, the evaluation of *L. feei* extracts on the causal agent of Bayoud "*Fusarium oxysporum* f. sp. *albedinis*" (Foa), was realized using new principles added to contact bioautography. Some studies made at the Phytochemistry and Organic Synthesis Laboratory (POSL, Bechar University, Algeria) have demonstrated that this plant contains secondary metabolites with biological activities (Belboukhari *et al.*, 2005; Belboukhari *et al.*, 2007; Belboukhari *et al.*, 2009).

The extracts of *A. graveolens, L. arborescens,* and *L. feei* are revealed active on cellulases with the appearance of the dark zones. The ethyl acetate extract of the aerial part of *L. feei* showed a remarkable result with an inhibition zones diameter greater than 2 mm. Indeed, the hexane, dichloromethane, and n-Butanol extracts showed no antifungal activity on Foa.

In this study, the tests were carried out using crude extracts; then a positive result is the component of two parameters: the intrinsic activity of the active products and their relative quantity in the extract. Among the 8 tests carried out, only one extract presented a good effect on the cellulases of Foa ($Ø \geq 2.00 \pm 0.1$ mm). These results prove the resistance of Foa against the extracts of *L. feei*. Boulenouar *et al.* (2012) determined this resistance against several treatments (Boulenouar *et al*., 2012).

A previous study by Rahmani *et al.* (2014) reported the presence of eleven polyphenol compounds in the extract of the aerial part of *L. feei*. In another study, two molecules of the saponins family were extracted and identified by Belboukhari and Cheriti (Figure IV.5), from the stems of *L. feei* (Belboukhari *et al.*, 2009), namely:

2α,3 β,23-Trihydroxy-30-acetylolean-12-ene **88**($C_{32}H_{52}O_5$) and 1-*O*-[α -L-Rhamnopyranosyl-(1)-6-*O*-acetylβ -D-galactopyranosyl]-1 β,3 β,22 ξ − 26-tetrahydroxyfurost-5(6)-ene-26-*O*β -D-glucopyranosid **89** ($C_{50}H_{82}O_{19}$).



88 **89**

Figure IV. 5: Saponins from the stems of *Limoniastrum. feei.*

El Hassni *et al.* (2007) revealed that the strategies of the fight against Foa are very limited that proves its high resistance (El Hassni *et al.*, 2007). Several studies showed that

the studied plant contains several secondary metabolites very significant with pharmacological properties (Belboukhari *et al.*, 2005; Belboukhari *et al.*, 2009; Boulenouar *et al.*, 2014). Belboukhari and Cheriti (2009) showed by spectroscopic analyses the presence of the saponins in the leaves and the stems *of L. feei.* This plant is rich in phenolic compounds. The antimicrobial activity of *L. feei* extract was studied by Belboukhari and Cheriti (2005); the methanolic extracts of *L. feei* leaves were revealed active against certain bacteria and two yeasts. By comparing these findings with the obtained results, the methanolic extracts of the aerial part gave a remarkable result ($Ø \geq 2.00 \pm 0.1$ mm) and confirm the presence of antimicrobial compounds in *L. feei*.

The analytical investigations reveal that the ethyl acetate extract of the aerial part of *L. feei* showed the highest phenolic compounds. In addition, the presence of flavonoids in *L. feei* (Belboukhari *et al.*, 2007; Rahmani *et al.*, 2014) may be responsible for the antifungal activity.

Belboukhari and Cheriti (2005) revealed no effect of ethyl acetate extract from leaves and twigs of *L. feei* on two fungi: *Candida albicans* and *Saccharomyces cerevisiae*. The difference with our results is possibly due to the difference between species biology (*Foa, C. albicans*, and *S. cerevisiae*) and/or to the difference between plant parts used for extraction.

The effect of the extracts used in this study was shown by Boulenouar *et al.* (2014) but by the disc diffusion technique. The results of the effect against Foa showed that these extracts have a detectable effect at least in two tests (Boulenouar *et al.*, 2014). Confirming the presence of antifungal substances despite the difference between the techniques used. The significant effect of the various parts of the plant can be related to the difference in the components present in the various parts. This difference can depend on the chemical nature of the substances or the mechanism of action. There are substances with antifungal activity against Foa but not on its cellulases, this reflects the complexity of the pathogenicity mechanism. On the other hand, substances inhibiting cellulases play a crucial role in antifungal activity against Foa because of the importance of these enzymes in invading date palm through roots.

By comparing the results obtained using contact bioautography on the cellulases of Foa and the other results using other techniques. Inhibition of cellulase activity is not always related to the inhibition of growth. Foa produces several toxins; these mycotoxins play a very significant role in the pathogenicity of this fungus. Therefore the different researches carried out previously against Foa reveals active substances that influence one or more of these mycotoxins by the modification of their metabolism or their effects (Boulenouar *et al.*, 2009; El Hadrami *et al.*, 2005).

Belboukhari and Cheriti (2007) contributed to the study of the characterization of flavonoids extracted from the stems of *L. feei*; they identified four flavonoids. We can suppose that one or more of these four flavonoids have antifungal activity against Foa and its cellulases.

From the results obtained by El Haci *et al.* (2017), polar organic extracts: methanol, ethanol, and acetone of *L. feei* presented considerable levels of phenols and flavonoids. These levels ranged from 127.07 to 262.11 mg GAE/g and 84 to 157.88 mg CEQ/g, respectively (El Haci *et al.*, 2017).

The flavonoids are a group of substances, which are responsible for the inhibition of many enzymes. The phytochemical screening showed a variety of phytoconstituents with the prevalence of phenolic compounds (El Haci *et al.*, 2017). Various studies were carried out on the structure-activity relationship of different polyphenols in a glance with their antifungal activity, but despite their great number, this relationship is not clear (Munari, 2006).

The absence of an effect for an extract tested on a given biological target always does not exclude the presence of active substances (case of synergy). In certain cases also, the concentration of these substances can be very weak so that we can detect their activity on TLC plates.

Recently, the fungicidal activities of plant extracts have been extensively reported (Balouiri *et al.*, 2016). However, little research has been performed on the fungicidal activity of *L. feei* extracts on the pathogen *F. oxysporum*, which poses the greatest threat to

date palm by causing *Fusarium* wilt. The potential medicinal uses of *L. feei* are supported by the presence of the above-mentioned phenolics and flavonoids activities.

Plants synthesize a wide range of metabolites for their survival, growth, development, and protection from a broad spectrum of bacterial, fungal, and viral pathogens. In this study, we isolated methyl gallate (MG) and syringic acid (SA) as major metabolites with antifungal activity from the aerial parts of *L. arborescens* and *A. graveolens*.

The LC-MS chromatogram data of EtOAc extract revealed a group of peaks that were fractionated from one to seven by open silica column chromatography. Among all the fractions, only 5 and 6 fractions significantly inhibited the enzyme activity (Foa cellulases). Only 5 and 6 chromatographed fractions that inhibited the Foa cellulases were further subjected to active compound isolation. The active compounds of fractions 5 and 6 were purified and were identified as MG and SA using NMR and LC-MS analysis. MG and SA are natural constituents isolated from different plants (Abou-Zaid *et al.*, 2009; Kamatham *et al.*, 2015). *In vitro* studies on the antifungal activity of SA were done by Chong *et al.* using concentrations ranging from 50 to 110 mL μg^{-1}, those typically recorded in oil palm roots. SA was found to be antifungal against *G. boninense* (Chong *et al.*, 2012; Chong *et al.*, 2009).

Phenylpropanoid metabolism produces an enormous array of secondary metabolites. The biosynthesis of GA and its derivative MG takes place via phenylpropanoid metabolism (Vogt, 2010).

III. Conclusion

Extracts obtained from studied plants presented antifungal activities against Foa and its cellulases. Regarding the antifungal activity by bioautography techniques, the extracts from *A. graveolens, L. arborescens,* and *L. feei* provided the best inhibitions, which indicate these extracts are useful agents to control agricultural diseases caused by this phytopathogenic fungi. In addition, we isolated methyl gallate and syringic acid as major metabolites with antifungal activity from the aerial parts of *L. arborescens* and *A. graveolens*.

Therefore, our study will help in the discovery of novel eco-friendly antifungal agents used in the agriculture field.

General conclusion

General conclusion

Natural substances have always been interested by scientists for their potential as therapeutic agents, the originality of their structures, but also their ecological role in nature. The use of plants for medicinal purposes is a therapeutic alternative for populations in the treatment, because of the high cost of drugs, and because experience has shown therapeutic efficacy. The vegetation of the Southwestern region of Algeria (Saoura) is a good example of the study of plant diversity. The Saoura is one of the richest in Algeria is characterized by a set of endemic taxa and has many plants used in traditional medicine. Among them, nine plants were the subject of this study: *Acacia raddiana, Anabasis aretioides, Asteriscus graveolens, Calotropis procera, Citrullus colocynthis, Launeae arborescens, Limoniastrum feei, Nerium oleander,* and *Pergularia tomentosa.* Our choice for these species is justified by the endemism of a few and their richness in secondary metabolites with various biological activities.

The date palm (*Phoenix dactylifera* L.) is a "tree" of great ecological and socio-economic importance in oases in desert regions. This plant is exposed to fungal contamination, mainly, by a vascular disease called Bayoudh and caused by *Fusarium oxysporum* f. sp. *albedinis* (Fao).

This study is part of the research work of the Phytochemistry and Organic Synthesis Laboratory (POSL) team, University of Bechar. The objective was to search for substances with an inhibitory effect on enzymatic activity. This current work targeted the host plant cell wall degradation enzyme (*Phoenix dactylifera* L.), specifically the cellulases of *Fusarium oxysporum* f. sp. *albedinis* (Foa).

A simple experimental protocol was therefore developed. The tests for cellulases activity were performed by the technique of contact bioautography. The main advantage of bioautography is that it provides information about the antimicrobial activities of substances separated from a mixture.

The richness in natural substances was observed in the extracts of the plants studied, but it should be noted that the activity of these crude extracts depends on the intrinsic activity of the active products and their relative amount in the extract.

The study of the cellulases activity of Foa showed that certain extracts of selected Saharan plants show a strong effect on the cellulases of *Fusarium oxysporum* f. sp. *albedinis.* The results indicated that the ethyl acetate extract showed the greatest antifungal activity, obtaining inhibition zones of 2.00 ± 0.1 mm, 2.4 ± 0.1 mm, and 2.7 ± 0.1 mm against *F. oxysporum* f. sp. *albedinis* cellulases. The fractions obtained with hexane, dichloromethane, and n-Butanol showed no antifungal activity against strains tested.

The results of the experimental studies made it possible to better understand the inhibitory capacity and the phytochemical profile of the plants tested. The antifungal activity was undertaken to evaluate the inhibition of Foa cellulases and therefore, to predict the therapeutic potential of these plants for use as a source of bioactive substances against Foa.

This work made it possible, on the one hand, to scientifically validate the medicinal and/or toxic plants selected, and on the other hand, to determine the active natural substances from extracts of these plants, the isolation of the active principle (s) responsible for the activity. Based on the results obtained in this study, it is possible to consider plants with significant inhibitory activity as potential sources of natural antifungal agents that can be used for therapeutic purposes.

To get a more precise idea of their mode of action, this work should be continued with split extracts. It would therefore be judicious to continue this work by the biological evaluation *in vivo* against Bayoudh with the isolated active compounds.

References

References

Abou-Zaid, M. M., Lombardo, D. A., and Nozzolillo, C. (2009). Methyl gallate is a natural constituent of maple (Genus Acer) leaves. *Natural product research, 23*(15), 1373-1377.

Achoub, H., Mencherini, T., Esposito, T., Luca, R., Aquino, R., Gazzerro, P., Zaiter, L., Benayache, F., and Benayache, S. (2019). New sesquiterpenes from Asteriscus graveolens. *Natural product research*, 1-9. doi:10.1080/14786419.2019.1666390

Adnan, M., Alshammari, E., Ashraf, S. A., Patel, K., Lad, K., and Patel, M. (2018). Physiological and molecular characterization of biosurfactant producing endophytic fungi Xylaria regalis from the cones of Thuja plicata as a potent plant growth promoter with its potential application. *BioMed research international, 2018*. doi:10.1155/2018/7362148

Agarwal, M., Dheeman, S., Dubey, R. C., Kumar, P., Maheshwari, D. K., and Bajpai, V. K. (2017). Differential antagonistic responses of Bacillus pumilus MSUA3 against Rhizoctonia solani and Fusarium oxysporum causing fungal diseases in Fagopyrum esculentum Moench. *Microbiological research, 205*, 40-47. doi:10.1016/j.micres.2017.08.012

Agrios, G. N. (2005). Plant Pathology. Fifth edition. *Ed Elsevier Academia Press. San Diego Calf. USA*.

Ahmad, Z., Wu, J., Chen, L., and Dong, W. (2017). Isolated Bacillus subtilis strain 330-2 and its antagonistic genes identified by the removing PCR. *Scientific Reports, 7*(1), 1777. doi:10.1038/s41598-017-01940-9

Ait Kettout, T., and Rahmania, F. (2010). Identification par CG-SM de l'acide phénylacétique produit par Fusarium oxysporum f. sp. albedinis, agent causal du bayoud. *Comptes Rendus Biologies, 333*(11), 808-813. doi:10.1016/j.crvi.2010.10.001

Akhtar, R., Javaid, A., and Qureshi, M. (2020). Bioactive Constituents of Shoot Extracts of Sisymbrium irio L. Against Fusarium oxysporum f. sp. cepae. *Planta Daninha, 38*.

Akssira, M., Mellouki, F., Salhi, A., Alilou, H., Saouf, A., El Hanbali, F., Arteaga, J. F., and Barrero, A. F. (2006). Naupliolide, a sesquiterpene lactone with a novel

tetracyclic skeleton from Nauplius graveolens subsp. odorus. *Tetrahedron letters, 47*(38), 6719-6721. doi:10.1016/j.tetlet.2006.07.094

Al-Asmari, A. K., Alamri, M. A., Almasoudi, A. S., Abbasmanthiri, R., and Mahfoud, M. (2017). Evaluation of the in vitro antimicrobial activity of selected Saudi scorpion venoms tested against multidrug-resistant micro-organisms. *Journal of global antimicrobial resistance, 10*, 14-18. doi:10.1016/j.jgar.2017.03.008

Al-Asmari, F., Mereddy, R., and Sultanbawa, Y. (2017). A novel photosensitization treatment for the inactivation of fungal spores and cells mediated by curcumin. *Journal of Photochemistry and Photobiology B: Biology, 173*, 301-306. doi:10.1016/j.jphotobiol.2017.06.009

Al-Ghaithi, F., El-Ridi, M. R., Adeghate, E., and Amiri, M. H. (2004). Biochemical effects of Citrullus colocynthis in normal and diabetic rats. *Molecular and Cellular Biochemistry, 261*(1), 143-149. doi:10.1023/B:MCBI.0000028749.63101.cc

Al-Mekhlafi, N. A., and Masoud, A. (2017). Phytochemical and pharmacological activities of pergularia tomentosa L. - A review. . *Indo American Journal of Pharmaceutical sciences, 4*(11), 4558-4565.

Alabouvette, C., Olivain, C., Migheli, Q., and Steinberg, C. (2009). Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing Fusarium oxysporum. *New Phytologist, 184*(3), 529-544. doi:10.1111/j.1469-8137.2009.03014.x

Alajmi, R., Al-ghamdi, S., Barakat, I., Mahmoud, A., Abdon, N., Al-Ahidib, M., and Abdel-Gaber, R. (2019). Antimicrobial Activity of Two Novel Venoms from Saudi Arabian Scorpions (Leiurus quinquestriatus and Androctonus crassicauda). *International Journal of Peptide Research and Therapeutics*. doi:10.1007/s10989-019-09816-4

Alghanem, S. M., and El-Amier, Y. A. (2017). Phytochemical and biological evaluation of Pergularia tomentosa L.(Solanaceae) naturally growing in arid ecosystem. *International Journal of Plant Science and Ecology, 3*, 7-15.

Alilou, H., Bencharki, B., Hassani, L. I., and Barka, N. (2014). Screening phytochimique et identification spectroscopique des flavonoïdes d'Asteriscus graveolens subsp. odorus. *Afrique Science: Revue Internationale des Sciences et Technologie, 10*(3).

References

ALrawashdeh, I. N., Qaralleh, H., Al-limoun, M. O., and Khleifat, K. M. (2019). Antibactrial Activity of Asteriscus graveolens Methanolic Extract: Synergistic Effect with Fungal Mediated Nanoparticles against Some Enteric Bacterial Human Pathogens. *arXiv preprint arXiv:1911.02245*.

Amari, A., Cheriti, A., Belboukhari, N., Kandappa H., R., and Patrick, G. (2015). Antimicrobial Activity of Aerial Part Crude Extracts from the Saharan plant Anabasis aretioides *PhytoChem & BioSub Journal, 9*, 14-17.

Anadón, A., Martínez-Larrañaga, M. R., Ares, I., and Martínez, M. A. (2018). Poisonous Plants of the Europe. In *Veterinary Toxicology* (pp. 891-909): Elsevier.

Aouissi, H., Gourine, N., Wang, H., Chen, X., Bombarda, I., Boudjeniba, M., and Yousfi, M. (2018). Chemical composition, antioxidative, antimicrobial and anti-cancer activities of Asteriscus graveolens (Forssk) essential oil. *Oriental Pharmacy and Experimental Medicine, 18*(3), 217-223. doi:10.1007/s13596-018-0315-0

Armitage, A. D., Taylor, A., Sobczyk, M. K., Baxter, L., Greenfield, B. P. J., Bates, H. J., Wilson, F., Jackson, A. C., Ott, S., Harrison, R. J., and Clarkson, J. P. (2018). Characterisation of pathogen-specific regions and novel effector candidates in Fusarium oxysporum f. sp. cepae. *Scientific Reports, 8*(1), 13530. doi:10.1038/s41598-018-30335-7

Armstrong, G., and Armstrong, J. K. (1981). Another approach to race classification of Fusarium oxysporum f. sp. pisi. *Phytopathology, 71*, 474-478.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., and Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western nepal. *Plants, 8*(4), 96. doi:10.3390/plants8040096

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E.-M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., and Heiss, E. H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology advances, 33*(8), 1582-1614.

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71-79. doi:10.1016/j.jpha.2015.11.005

References

Bandara, V., Weinstein, S. A., White, J., and Eddleston, M. (2010). A review of the natural history, toxinology, diagnosis and clinical management of Nerium oleander (common oleander) and Thevetia peruviana (yellow oleander) poisoning. *Toxicon, 56*(3), 273-281.

Bartmańska, A., Wałecka-Zacharska, E., Tronina, T., Popłoński, J., Sordon, S., Brzezowska, E., Bania, J., and Huszcza, E. (2018). Antimicrobial Properties of Spent Hops Extracts, Flavonoids Isolated Therefrom, and Their Derivatives. *Molecules, 23*(8), 2059. doi:10.3390/molecules23082059

Bashir, M. R., Atiq, M., Sajid, M., Mohsan, M., Abbas, W., Alam, M. W., and Bashair, M. (2018). Antifungal exploitation of fungicides against Fusarium oxysporum f. sp. capsici causing Fusarium wilt of chilli pepper in Pakistan. *Environmental Science and Pollution Research, 25*(7), 6797-6801. doi:10.1007/s11356-017-1032-9

Belboukhari, N., and Cheriti, A. (2005). Antimicrobial activity of aerial part crude extracts from Limoniastrum feei. *Asian Journal of plant sciences, 4*(5), 496-498. doi:10.3923/ajps.2005.496.498

Belboukhari, N., and Cheriti, A. (2006). Phytochemical investigation of the bioactive extract from Launeae arbrescens. *Pakistan Journal of Biological Sciences, 9*(15), 2930-2932. doi:10.3923/pjbs.2006.2930.2932

Belboukhari, N., and Cheriti, A. (2007). Flavonoids of Limoniastrum feei. *Research Journal of Phytochemistry, 1*(2), 74-78. doi: 10.3923/rjphyto.2007.74.78

Belboukhari, N., and Cheriti, A. (2009). Analysis and isolation of saponins from Limoniastrum feei by LC-UV. *Chemistry of natural compounds, 45*(5), 756.

Belboukhari, N., and Cheriti, A. (2013). دراسة فيتو كيميائية أولية لنبات الدقع Anabasis aretioides. *Annales de l'Université de Bechar, 13*, 201-209.

Belboukhari, N., Cheriti, A., Roussel, C., and Vanthuyne, N. (2010). Chiral separation of hesperidin and naringin and its analysis in a butanol extract of Launeae arborescens. *Natural product research, 24*(7), 669-681.

Belboukhari, N., Cheriti, A., Sekkoum, K., Rahmani, S., and Habbab, A. (2016). Triterpenoid and flavonoid from aerial part of Launaea arborescens *Der Pharma Chemica, 8*(2), 17-21.

References

Belhadjadji, Y., Belboukhari, N., and Cheriti, A. (2010). Acacia Raddiana in Algerian Sahara Eco-boctanical data & phytochemical screening *Annales de l'Université de Bechar, 7*, 5-17.

Belhi, Z. (2012). *Effet des extraits de quelques plantes sahariennes sur les cellulases de Fusarium oxysporum f. sp. albedinis* (Magister's thesis), Tahri Mohamed University, Bechar, Algeria.,

Belhi, Z., Boulenouar, N., Cheriti, A., and Marouf, A. (2020). Antifungal and anti-cellulases activity of Limoniastrum feei extracts to promote Bayoud disease treatment using bioautography. *Cogent Food & Agriculture, 6*(1), 1726083. doi:10.1080/23311932.2020.1726083

Benabdelkader, M., Malek, A., and Draoui, B. (2011). Perspective du pompage éolien appliqué à l'irrigation du palmier dattier dans la région de Béchar. *Revue des Energies Renouvelables, 14*(3), 381-395.

Bending, G. D. (2017). The Rhizopshere and Its Microorganisms. In B. Thomas, B. G. Murray, & D. J. Murphy (Eds.), *Encyclopedia of Applied Plant Sciences (Second Edition)* (pp. 347-351). Oxford: Academic Press.

Benhouhou, S. (2005). *A guide to medicinal plants in North Africa*: IUCN.

Bentabet, N., Boucherit-Otmani, Z., and Boucherit, K. (2014). Composition chimique et activité antioxydante d'extraits organiques des racines de Fredolia aretioides de la région de Béchar en Algérie. *Phytothérapie, 12*(6), 364-371.

Bentrad, N., Gaceb-Terrak, R., Benmalek, Y., and Rahmania, F. (2017). Studies on chemical composition and antimicrobial activities of bioactive molecules from date palm (Phoenix dactylifera L.) pollens and seeds. *African Journal of Traditional, Complementary and Alternative Medicines, 14*(3), 242-256. doi:10.21010/ajtcam.v14i3.26

Berrani, A., Benassaoui, H., Zouarhi, M., Alrhorfi, A., Hajjaji, N., and Bengueddour, R. (2018). Evaluation of inhibitory effect of the methanolic extract of the two parts from anabasis aretioides plant against the corrosion of E 24 steel in a neutral solution NaCl 3.5%. *Anal. and Bioanal. Electrochem, 10*, 1299-1316.

Berrani, A., Marmouzi, I., Kharbach, M., Bouyahya, A., El Hamdani, M., El Jemli, M., Lrhorfi, A., Benassaoui, H., Zouarhi, M., and Larbi, O. M. (2018). Anabasis

aretioides Coss. & Moq. phenolic compounds exhibit in vitro hypoglycemic, antioxidant and antipathogenic properties. *Journal of basic and clinical physiology and pharmacology, 30*(2), 251-257.

Bhuvaneshwari, L., Arthy, E., Anitha, C., Dhanabalan, K., and Meena, M. (2007). Phytochemical analysis & Antibacterial activity of Nerium oleander. *Ancient science of life, 26*(4), 24.

Bitam, F., Ciavatta, M. L., Manzo, E., Dibi, A., and Gavagnin, M. (2008). Chemical characterisation of the terpenoid constituents of the Algerian plant Launaea arborescens. *Phytochemistry, 69*(17), 2984-2992. doi:10.1016/j.phytochem.2008.09.025

Bouayad Alam, S., Dib, M. E. A., Djabou, N., Tabti, B., Gaouar Benyelles, N., Costa, J., and Muselli, A. (2017). Essential oils as biocides for the control of fungal infections and devastating pest (Tuta absoluta) of tomato (Lycopersicon esculentum Mill.). *Chemistry & biodiversity, 14*(7), e1700065. doi:10.1002/cbdv.201700065

Bouchal, B., Errahhali, M. E., Errahhali, M. E., Boulouiz, R., Ouarzane, M., Tajir, M., Matsunami, K., and Bellaoui, M. (2019). Exploring the Antimicrobial Properties Against Human Pathogens and Mode of Action of Various Extracts from Fredolia aretioides, an Endemic Medicinal Plant of Morocco and Algeria. *The Natural Products Journal, 9*(4), 321-329.

Boulenouar, N., Marouf, A., and Cheriti, A. (2009). Effect of some poisonous plants extracts on Fusarium oxysporum f. sp. albedinis. *Journal of Biological Sciences, 9*(6), 594-600.

Boulenouar, N., Marouf, A., and Cheriti, A. (2011). Antifungal activity and phytochemical screening of extracts from Phoenix dactylifera L. cultivars. *Natural product research, 25*(20), 1999-2002.

Boulenouar, N., Marouf, A., and Cheriti, A. (2014). Direct Bioautography for Antifungal. Measurement “Case of Bayoud Disease”. . *PhytoChem & BioSub Journal, 8*, 33-37. .

Boulenouar, N., Marouf, A., Cheriti, A., and Belboukhari, N. (2012). Medicinal Plants Extracts as Source of Antifungal Agents against Fusarium oxysporum f. sp. albedinis. *Journal of agricultural science and Technology, 14*, 659-669.

References

Bouzoumita, A., Metoui, M., Jemni, M., Kabaeir, N., Belhouchette, K., and Ferchichi, A. (2020). The Efficacy of Various Bacterial Organisms for Biocontrol of Fusarium Root Rot of Olive in Tunisia. *Polish Journal of Environmental Studies, 29*(1).

Caesar, L. K., and Cech, N. B. (2019). Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. *Natural Product Reports, 36*(6), 869-888. doi:10.1039/C9NP00011A

Cao, Y.-L., Zhang, M.-H., Lu, Y.-F., Li, C.-Y., Tang, J.-S., and Jiang, M.-M. (2018). Cardenolides from the leaves of Nerium oleander. *Fitoterapia, 127*, 293-300.

Carvalho, C. d., Ferreira-D'Silva, A., Wedge, D., Cantrell, C., and Rosa, L. (2018). Antifungal activities of cytochalasins produced by Diaporthe miriciae, an endophytic fungus associated with tropical medicinal plants. *Canadian journal of microbiology, 64*(11), 835-843. doi:10.1139/cjm-2018-0131

Chaabi, M. (2008). *Étude phytochimique et biologique d'espèces végétales africaines: Euphorbia stenoclada Baill.(Euphorbiaceae), Anogeissus leiocarpus Guill. & Perr.(Combretaceae), Limoniastrum feei (Girard) Batt.(Plumbaginaceae).* Strasbourg 1, Retrieved from https://www.theses.fr/2008STR13039

Chaabi, M., Beghidja, N., Benayache, S., and Lobstein, A. (2008). Activity-guided isolation of antioxidant principles from Limoniastrum feei (Girard) Batt. *Zeitschrift für Naturforschung C, 63*(11-12), 801-807.

Chaib, F., Allali, H., Bennaceur, M., and Flamini, G. (2017). Chemical composition and antimicrobial activity of essential oils from the aerial parts of Asteriscus graveolens (Forssk.) Less. and Pulicaria incisa (Lam.) DC.: Two Asteraceae herbs growing wild in the Hoggar. *Chemistry & biodiversity, 14*(8), e1700092.

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., and Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evidence-based complementary and alternative medicine, 2014*.

Chen, X., Hu, L.-F., Huang, X.-S., Zhao, L.-X., Miao, C.-P., Chen, Y.-W., Xu, L.-H., Han, L., and Li, Y.-Q. (2019). Isolation and Characterization of New Phenazine Metabolites with Antifungal Activity against Root-Rot Pathogens of Panax

notoginseng from Streptomyces. *Journal of agricultural and food chemistry, 67*(41), 11403-11407. doi:10.1021/acs.jafc.9b04191

Chen, Y., Tan, W., Li, Q., Dong, F., Gu, G., and Guo, Z. (2018). Synthesis of inulin derivatives with quaternary phosphonium salts and their antifungal activity. *International journal of biological macromolecules, 113*, 1273-1278. doi:10.1016/j.ijbiomac.2018.03.055

Cheriti, A. (2019). Limoniastrum feei from the Algerian Sahara: Ethnopharmacology, Phytochemistry and Pharmacological importance. In I. Ryan (Ed.), *Phytochemicals: Plant Sources and Potential Health Benefits*. New York, USA: Nova Science Publishers.

Cheriti, A., Belboukhari, M., Belboukhari, N., and Djeradi, H. (2012). Phytochemical and biological studies on Launaea Cass. genus (Asteraceae) from Algerian Sahara. *Phytochemistry, 11*.

Cheriti, A., Saad, A., Belboukhari, N., and Ghezali, S. (2007). The essential oil composition of Bubonium graveolens (Forssk.) Maire from the Algerian Sahara. *Flavour and fragrance journal, 22*(4), 286-288. doi:10.1002/ffj.1794

Choma, I., and Jesionek, W. (2015). TLC-direct bioautography as a high throughput method for detection of antimicrobials in plants. *Chromatography, 2*(2), 225-238. doi:10.3390/chromatography2020225

Chong, K., Atong, M., and Rossall, S. (2012). The role of syringic acid in the interaction between oil palm and Ganoderma boninense, the causal agent of basal stem rot. *Plant Pathology, 61*(5), 953-963.

Chong, K. P., Rossall, S., and Atong, M. (2009). In vitro antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against Ganoderma boninense. *Journal of Agricultural Science, 1*(2), 15.

Christakopoulos, P., Kekos, D., Kolisis, F. N., and Macris, B. J. (1995). Controlling simultaneous production of endoglucanase and beta-glucosidase by Fusarium oxysporum in submerged culture. *Biotechnology Letters, 17*(8), 883-888. doi:10.1007/BF00129023

Confortin, T. C., Todero, I., Soares, J. F., Luft, L., Brun, T., Rabuske, J. E., Nogueira, C. U., Mazutti, M. A., Zabot, G. L., and Tres, M. V. (2019). Extracts from Lupinus

albescens: antioxidant power and antifungal activity in vitro against phytopathogenic fungi. *Environmental technology, 40*(13), 1668-1675. doi:10.1080/09593330.2018.1427800

Cos, P., Vlietinck, A. J., Berghe, D. V., and Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *Journal of ethnopharmacology, 106*(3), 290-302. doi:10.1016/j.jep.2006.04.003

Cristofari, G., Znini, M., Majidi, L., Mazouz, H., Tomi, P., Costa, J., and Paolini, J. (2012). Chemical Diversity of Essential Oils from Asteriscus graveolens (Forssk.) Less.: Identification of cis-8-Acetoxychrysanthenyl Acetate as a New Natural Component. *Chemistry & biodiversity, 9*(4), 727-738.

Davari, M., and Ezazi, R. (2017). Chemical composition and antifungal activity of the essential oil of Zhumeria majdae, Heracleum persicum and Eucalyptus sp. against some important phytopathogenic fungi. *Journal de Mycologie Médicale, 27*(4), 463-468. doi:10.1016/j.mycmed.2017.06.001

de Lima, J. M., de Freitas, F. J. C., Amorim, R. N. L., Câmara, A. C. L., Batista, J. S., and Soto-Blanco, B. (2011). Clinical and pathological effects of Calotropis procera exposure in sheep and rats. *Toxicon, 57*(1), 183-185. doi:10.1016/j.toxicon.2010.11.007

Dewanjee, S., Gangopadhyay, M., Bhattacharya, N., Khanra, R., and Dua, T. K. (2015). Bioautography and its scope in the field of natural product chemistry. *Journal of Pharmaceutical Analysis, 5*(2), 75-84. doi:10.1016/j.jpha.2014.06.002

Dhinesh, B., and Annamalai, M. (2018). A study on performance, combustion and emission behaviour of diesel engine powered by novel nano nerium oleander biofuel. *Journal of cleaner production, 196*, 74-83.

Djelaila, S. (2007). *Contribution à l'étude phytochimique du Bubonium graveolens-Astraceae.* (Magister's thesis), Bechar University Center, Algeria

Edel-Hermann, V., and Lecomte, C. (2019). Current Status of Fusarium oxysporum Formae Speciales and Races. *Phytopathology, 109*(4), 512-530.

El-Haci, I. A., Bekkara, F. A., Mazari, W., and Gherib, M. (2013). Phenolics content and antioxidant activity of some organic extracts of endemic medicinal plant Anabasis

aretioides Coss. & Moq. from Algerian Sahara. *Pharmacognosy Journal, 5*(3), 108-112. doi:10.1016/j.phcgj.2013.05.004

El-Mousallamy, A. M. D., Barakat, H. H., Souleman, A. M. A., and Awadallah, S. (1991). Polyphenols ofAcacia Raddiana. *Phytochemistry, 30*(11), 3767-3768. doi:10.1016/0031-9422(91)80106-B

El Haci, I. A., Mazari, W., Atik-Bekkara, F., Hassani, F., and Gherib, M. (2017). Assessment of the cytotoxic activity and the reduction power of Limoniastrum feei Girard (Batt.): a medicinal plant from Algerian Sahara. *Oriental Pharmacy and Experimental Medicine, 17*(2), 143-150. doi:10.1007/s13596-017-0270-1

El Hadrami, A., El Idrissi-Tourane, A., El Hassni, M., Daayf, F., and El Hadrami, I. (2005). Toxin-based in-vitro selection and its potential application to date palm for resistance to the bayoud Fusarium wilt. *Comptes Rendus Biologies, 328*(8), 732-744.

El Hadrami, I., El Bellaj, M., El Idrissi, A., J'Aiti, F., El Jaafari, S., and Daayf, F. (1998). Biotechnologies végétales et amélioration du palmier dattier (Phoenix dactylifera L.), pivot de l'agriculture oasienne marocaine. *Cahiers Agricultures, 7*(6), 463-468 (461).

El Hassni, M., El Hadrami, A., Daayf, F., Chérif, M., Barka, E. A., and El Hadrami, I. (2007). Biological control of bayoud disease in date palm: Selection of microorganisms inhibiting the causal agent and inducing defense reactions. *Environmental and Experimental Botany, 59*(2), 224-234.

El Modafar, C., Tantaoui, A., and El Boustani, E. (2000). Effet de L'acide Caféoylshikimique des Racines du Palmier Dattier sur L'activité et la Production des Enzymes Hydrolytiques de Fusarium oxysporum f. sp. albedinis. *Phytopathologische Zeitschrift, 148* (2), 101–108.

Embaby, H. E., and Rayan, A. M. (2016). Chemical composition and nutritional evaluation of the seeds of Acacia tortilis (Forssk.) Hayne ssp. raddiana. *Food Chemistry, 200*, 62-68. doi:10.1016/j.foodchem.2016.01.019

Fakchich, J., and Elachouri, M. (2014). Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. *Journal of ethnopharmacology, 154*(1), 76-87.

References

Farid, O., Hajji, L., and Eddouks, M. (2018). Aqueous Extract of Anabasis aretioides Ameliorates Streptozotocininduced Diabetes Mellitus in Rats. *The Natural Products Journal, 8*(2), 139-146.

Farid, O., Khallouki, F., Akdad, M., Breuer, A., Owen, R. W., and Eddouks, M. (2018). Phytochemical characterization of polyphenolic compounds with HPLC–DAD–ESI–MS and evaluation of lipid-lowering capacity of aqueous extracts from Saharan plant Anabasis aretioides (Coss & Moq.) in normal and streptozotocin-induced diabetic rats. *Journal of Integrative Medicine, 16*(3), 185-191. doi:10.1016/j.joim.2018.03.003

Freitas, C. D. T., Oliveira, J. S., Miranda, M. R. A., Macedo, N. M. R., Sales, M. P., Villas-Boas, L. A., and Ramos, M. V. (2007). Enzymatic activities and protein profile of latex from Calotropis procera. *Plant Physiology and Biochemistry, 45*(10), 781-789. doi:10.1016/j.plaphy.2007.07.020

Freitas, C. D. T., Silva, R. O., Ramos, M. V., Porfírio, C. T. M. N., Farias, D. F., Sousa, J. S., Oliveira, J. P. B., Souza, P. F. N., Dias, L. P., and Grangeiro, T. B. (2020). Identification, characterization, and antifungal activity of cysteine peptidases from Calotropis procera latex. *Phytochemistry, 169*, 112163. doi:10.1016/j.phytochem.2019.112163

Galletti, J., Tobaldini-Valerio, F. K., Silva, S., Kioshima, É. S., Trierveiler-Pereira, L., Bruschi, M., Negri, M., and Estivalet Svidzinski, T. I. (2017). Antibiofilm activity of propolis extract on Fusarium species from onychomycosis. *Future microbiology, 12*(14), 1311-1321.

Gao, L., Han, J., Liu, H., Qu, X., Lu, Z., and Bie, X. (2017). Plipastatin and surfactin coproduction by Bacillus subtilis pB2-L and their effects on microorganisms. *Antonie Van Leeuwenhoek, 110*(8), 1007-1018. doi:10.1007/s10482-017-0874-y

Ghazi, R., Boulenouar, N., Cheriti, A., Reddy, K., and Govender, P. (2020). Bioguided Fractionation of Citrullus colocynthis Extracts and Antifungal Activity Against Fusarium oxysporum f.sp. albedinis. *Current Bioactive Compounds, 16*(3), 302-307. doi:10.2174/1573407214666181001124737

Gnanathasan, C. A. (2015). Oleander Poisoning. In P. Gopalakrishnakone, C. R. Carlini, & R. Ligabue-Braun (Eds.), *Plant Toxins* (pp. 1-20). Dordrecht: Springer Netherlands.

References

Hamza, N., Berke, B., Umar, A., Cheze, C., Gin, H., and Moore, N. (2019). A review of Algerian medicinal plants used in the treatment of diabetes. *Journal of ethnopharmacology, 238*, 111841. doi:10.1016/j.jep.2019.111841

Han, Y., Zhao, J., Zhang, B., Shen, Q., Shang, Q., and Li, P. (2019). Effect of a novel antifungal peptide P852 on cell morphology and membrane permeability of Fusarium oxysporum. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1861*(2), 532-539. doi:10.1016/j.bbamem.2018.10.018

Hase, J., Desmukh, K., Pokharkar, R. D., Gaje, T. R., and Phatanagre, N. D. (2017). Phytochemical Studies on Nerium oleander L. using GC-MS. *Int. J. Pharmacogn. Phytochem. Res, 9*(6), 885-891.

Hassan, L. M., Galal, T. M., Farahat, E. A., and El-Midany, M. M. (2015). The biology of Calotropis procera (Aiton) WT. *Trees, 29*(2), 311-320.

Hassan, S., Umar, R., Ladan, M., Nyemike, P., Wasagu, R., Lawal, M., and Ebbo, A. (2007). Nutritive value, phytochemical and antifungal properties of Pergularia tomentosa L.(Asclepiadaceae). *International Journal of Pharmacology, 3*(4), 334-340. doi:10.3923/ijp.2007.334.340

Hsu, L.-H., Wang, H.-F., Sun, P.-L., Hu, F.-R., and Chen, Y.-L. (2017). The antibiotic polymyxin B exhibits novel antifungal activity against Fusarium species. *International journal of antimicrobial agents, 49*(6), 740-748. doi:10.1016/j.ijantimicag.2017.01.029

Huang, N., Wang, W., Yao, Y., Zhu, F., Wang, W., and Chang, X. (2017). The influence of different concentrations of bio-organic fertilizer on cucumber Fusarium wilt and soil microflora alterations. *PloS one, 12*(2), e0171490. doi:10.1371/journal.pone.0171490

Hussain, A. I., Rathore, H. A., Sattar, M. Z. A., Chatha, S. A. S., Sarker, S. D., and Gilani, A. H. (2014). Citrullus colocynthis (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses and nutritional potential. *Journal of ethnopharmacology, 155*(1), 54-66. doi:10.1016/j.jep.2014.06.011

Kalleli, F., Ghassen, A., Ben Salem, I., Boughalleb-M'hamdi, N., and M 'hamdi, M. (2020). Essential oil from fennel seeds (Foeniculum vulgare) reduces Fusarium wilt of tomato (Solanum lycopersicon). *Phytopathologia Mediterranea, 59*(1), 63-76.

Kälvö, D., Menkis, A., and Broberg, A. (2018). Secondary Metabolites from the Root Rot Biocontrol Fungus Phlebiopsis gigantea. *Molecules, 23*(6), 1417. doi:10.3390/molecules23061417

Kamatham, S., Kumar, N., and Gudipalli, P. (2015). Isolation and characterization of gallic acid and methyl gallate from the seed coats of Givotia rottleriformis Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells. *Toxicology Reports, 2*, 520-529. doi:10.1016/j.toxrep.2015.03.001

Kamel, N. M., Abdel-Motaal, F. F., and El-Zayat, S. A. (2020). Endophytic fungi from the medicinal herb Euphorbia geniculata as a potential source for bioactive metabolites. *Archives of Microbiology, 202*(2), 247-255.

Kasana, R. C., Salwan, R., Dhar, H., Dutt, S., and Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Current microbiology, 57*(5), 503-507.

Kazeem, M. I., Mayaki, A. M., Ogungbe, B. F., and Ojekale, A. B. (2016). In-vitro Studies on Calotropis procera Leaf Extracts as Inhibitors of Key Enzymes Linked to Diabetes Mellitus. *Iranian journal of pharmaceutical research : IJPR, 15*(Suppl), 37-44.

Keffous, F., Belboukhari, N., Sekkoum, K., Djeradi, H., Cheriti, A., and Aboul-Enein, H. Y. (2016). Determination of the antioxidant activity of Limoniastrum feei aqueous extract by chemical and electrochemical methods. *Cogent Chemistry, 2*(1), 1186141. doi:10.1080/23312009.2016.1186141

Khairnar, A. K., Bhamare, S., and Bhamare, H. (2012). Calotropis procera: an ethnopharmacological update. *Advance Research in Pharmaceuticals and Biologicals, 2*(2), 142-156.

Kim, D.-R., Jeon, C.-W., Shin, J.-H., Weller, D. M., Thomashow, L., and Kwak, Y.-S. (2019). Function and Distribution of a Lantipeptide in Strawberry Fusarium Wilt Disease–Suppressive Soils. *Molecular plant-microbe interactions, 32*(3), 306-312.

Kumar, S., and Anand, G. R. (2010). Evaluation of anti-inflammatory activity of Nerium oleander. *Pharmacia, 1*, 33-36.

Lahmar, I., Belghith, H., Ben Abdallah, F., and Belghith, K. (2017). Nutritional composition and phytochemical, antioxidative, and antifungal activities of

Pergularia tomentosa L. *BioMed research international, 2017*. doi:10.1155/2017/6903817

Lastochkina, O., Baymiev, A., Shayahmetova, A., Garshina, D., Koryakov, I., Shpirnaya, I., Pusenkova, L., Mardanshin, I. d., Kasnak, C., and Palamutoglu, R. (2020). Effects of Endophytic Bacillus Subtilis and Salicylic Acid on Postharvest Diseases (Phytophthora infestans, Fusarium oxysporum) Development in Stored Potato Tubers. *Plants, 9*(1), 76.

Li, W., Xiong, P., Zheng, W., Zhu, X., She, Z., Ding, W., and Li, C. (2017). Identification and antifungal activity of compounds from the mangrove endophytic fungus Aspergillus clavatus R7. *Marine drugs, 15*(8), 259. doi:10.3390/md15080259

Limbadri, S., Luo, X., Lin, X., Liao, S., Wang, J., Zhou, X., Yang, B., and Liu, Y. (2018). Bioactive Novel Indole Alkaloids and Steroids from Deep Sea-Derived Fungus Aspergillus fumigatus SCSIO 41012. *Molecules, 23*(9), 2379. doi:10.3390/molecules23092379

López-Seijas, J., García-Fraga, B., da Silva, A. F., and Sieiro, C. (2020). Wine Lactic Acid Bacteria with Antimicrobial Activity as Potential Biocontrol Agents against Fusarium oxysporum f. sp. lycopersici. *Agronomy, 10*(1), 31. doi:10.3390/agronomy10010031

Magaldi, S., Mata-Essayag, S., De Capriles, C. H., Perez, C., Colella, M., Olaizola, C., and Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International journal of infectious diseases, 8*(1), 39-45. doi:10.1016/j.ijid.2003.03.002

Mahlo, S. M., Chauke, H. R., McGaw, L., and Eloff, J. (2016). Antioxidant and antifungal activity of selected medicinal plant extracts against phytopathogenic fungi. *African Journal of Traditional, Complementary and Alternative Medicines, 13*(4), 216-222. doi:10.21010/ajtcam.v13i4.28

Mani Chandrika, K. V. S., and Sharma, S. (2020). Promising antifungal agents: A minireview. *Bioorganic & medicinal chemistry, 28*(7), 115398. doi:10.1016/j.bmc.2020.115398

Marzouk, B., Marzouk, Z., Haloui, E., Fenina, N., Bouraoui, A., and Aouni, M. (2010). Screening of analgesic and anti-inflammatory activities of Citrullus colocynthis

from southern Tunisia. *Journal of ethnopharmacology, 128*(1), 15-19. doi:10.1016/j.jep.2009.11.027

Medina-Romero, Y. M., Roque-Flores, G., and Macías-Rubalcava, M. L. (2017). Volatile organic compounds from endophytic fungi as innovative postharvest control of Fusarium oxysporum in cherry tomato fruits. *Applied microbiology and biotechnology, 101*(22), 8209-8222. doi:10.1007/s00253-017-8542-8

Meena, A. K., Yadav, A. K., Niranjan, U. S., Singh, B., Nagariya, A. K., Sharma, K., Gaurav, A., Sharma, S., and Rao, M. (2010). A review on Calotropis procera Linn and its ethnobotany, phytochemical, pharmacological profile. *Drug Invention Today, 2*(2), 185-190.

Melekmi, N., Saad, A., Belboukhari, N., and Cheriti, A. (2006). Antimicrobial activity of the essential oil of Bubonium graveolens. *Annales de l'Université de Bechar, 2*, 22-26.

Messaoudi, R., Cheriti, A., and Bourmita, Y. (2018). Bioassay-guided isolation of the major compound with antioxidant activity from the algerian medicinal plant Bubonium graveolens. *Asian Journal of Pharmaceutical and Clinical Research, 11*(11), 424-426. doi:10.22159/ajpcr.2018.v11i11.28514

Mihalache, G., Balaes, T., Gostin, I., Stefan, M., Coutte, F., and Krier, F. (2018). Lipopeptides produced by Bacillus subtilis as new biocontrol products against fusariosis in ornamental plants. *Environmental Science and Pollution Research, 25*(30), 29784-29793. doi:10.1007/s11356-017-9162-7

Mohamed, M. S., Saleh, A. M., Abdel-Farid, I. B., and El-Naggar, S. A. (2017). Growth, hydrolases and ultrastructure of Fusarium oxysporum as affected by phenolic rich extracts from several xerophytic plants. *Pesticide biochemistry and physiology, 141*, 57-64. doi:10.1016/j.pestbp.2016.11.007

Montenegro, I., Madrid, A., Cuellar, M., Seeger, M., Alfaro, J., Besoain, X., Martínez, J., Ramirez, I., Olguín, Y., and Valenzuela, M. (2018). Biopesticide activity from drimanic compounds to control tomato pathogens. *Molecules, 23*(8), 2053. doi:10.3390/molecules23082053

References

Mouhcine, M., Amin, L., Saaid, A., Khalil, H., and Laila, B. (2019). Cytotoxic, antioxidant and antimicrobial activities of Nerium oleander collected in Morocco. *Asian Pacific Journal of Tropical Medicine, 12*(1), 32.

Munari, C. (2006). *Investigation phytochimique de plantes alpines: Etude d'espèces du genre Oxytropis (Fabaceae) et isolement de composés antifongiques et antiradicalaires à partir d'Oxytropis fetida (Vill.) DC., Potentilla grandiflora L. (Rosaceae) et Vaccinium uliginosum ssp.*, University of Geneva, Retrieved from https://nbn-resolving.org/urn:nbn:ch:unige-33548

Namian, P., Talebi, T., Germi, K. G., and Shabani, F. (2013). Screening of biological activities (antioxidant, antibacterial and antitumor) of Nerium oleander leaf and flower extracts. *vacuum, 10*(11).

Nesrine, B., Zahia, B.-O., and Kamila, B. K. G. (2014). Preliminary phytochemical study of leaves and roots of Fredolia aretioides, endemic plant of Algeria.

Nirmaladevi, D., Venkataramana, M., Srivastava, R. K., Uppalapati, S. R., Gupta, V. K., Yli-Mattila, T., Clement Tsui, K. M., Srinivas, C., Niranjana, S. R., and Chandra, N. S. (2016). Molecular phylogeny, pathogenicity and toxigenicity of Fusarium oxysporum f. sp. lycopersici. *Scientific Reports, 6*, 21367. doi:10.1038/srep21367

Ozenda, P. (1991). Les relations biogéographiques des montagnes sahariennes avec la région méditerranéenne. *Revue de géographie alpine, 79*(1), 43-53.

Panagiotou, G., Kekos, D., Macris, B. J., and Christakopoulos, P. (2003). Production of cellulolytic and xylanolytic enzymes by Fusarium oxysporum grown on corn stover in solid state fermentation. *Industrial Crops and Products, 18*(1), 37-45. doi:10.1016/S0926-6690(03)00018-9

Park, J. Y., Kim, S. H., Kim, N. H., Lee, S. W., Jeun, Y.-C., and Hong, J. K. (2017). Differential inhibitory activities of four plant essential oils on in vitro growth of Fusarium oxysporum f. sp. fragariae causing Fusarium wilt in strawberry plants. *The plant pathology journal, 33*(6), 582. doi:10.5423/PPJ.OA.06.2017.0133

Pascual, M., Carretero, M., Slowing, K., and Villar, A. (2002). Simplified screening by TLC of plant drugs. *Pharmaceutical biology, 40*(2), 139-143. doi:10.1076/phbi.40.2.139.5849

Patiño, B., Vázquez, C., Manning, J. M., Roncero, M. I. G., Córdoba-Cañero, D., Di Pietro, A., and Martínez-del-Pozo, Á. (2018). Characterization of a novel cysteine-rich antifungal protein from Fusarium graminearum with activity against maize fungal pathogens. *International journal of food microbiology, 283*, 45-51. doi:10.1016/j.ijfoodmicro.2018.06.017

Prieto, J. A., Patiño, O. J., Plazas, E. A., Pabón, L. C., Ávila, M. C., Guzmán, J. D., Delgado, W. A., and Cuca, L. E. (2013). Natural products from plants as potential source agents for controlling fusarium. *Fungicides-Showcases of Integrated Plant Disease Management from Around the World. Croacia: Intech*, 233-278. doi:10.5772/52338

Quezel, P., and Santa, S. (1963). *Nouvelle flore de l'Algérie et des régions désertiques méridionales*. Retrieved from

Rached, W., Benamar, H., Bennaceur, M., and Marouf, A. (2010). Screening of the antioxidant potential of some Algerian indigenous plants. *J. Biol. Sci, 10*(4), 316-324.

Rahmani, S., Belboukhari, N., and Cheriti, A. (2014). Phytochemical investigation of bioactive extract from endemic medicinal plant Limoniastrum feei (Girard) Batt (Plumbaginaceae). *Asian Journal of Chemistry, 26*(2), 365. doi:10.14233/ajchem.2014.15395

Rahmani, S., Belboukhari, N., Cheriti, A., Bourmita, Y., and Ould El Hadj, M. (2013). Glucoside flavonoids from the antimicriobial extract of the Saharan medicinal plant Limoniastrum feei. *Biochemistry An Indian Journal, 7*(4), 137-140.

Rahmani, S., Belboukhari, N., Sekkoum, K., and Cheriti, A. (2016). Evaluation de l'activité anti-inflammatoire d'extraits aqueux de feuilles Limoniastrum feei (PLUMBAGINACEA). *Algerian Journal of Arid Environment "AJAE", 6*(1).

Rahmani, S., Ziane, L., Belboukhari, N., and Cheriti, A. (2012). The Saharan medicinal plant Limoniastrum feei: Ethnomedical survey and prelimnary phytochemical screening of antibacterial extracts. *PhytoChem & BioSub Journal, 6*(2), 83-87.

Ramdane, F., Essid, R., Mkadmini, K., Hammami, M., Fares, N., Mahammed, M. H., El Ouassis, D., Tabbene, O., Limam, F., and Hadj, M. D. O. (2017). Phytochemical

composition and biological activities of Asteriscus graveolens (Forssk) extracts. *Process Biochemistry, 56*, 186-192. doi:10.1016/j.procbio.2017.03.004

Ramos, M. V., Brito, D., Freitas, C. D., Gonçalves, J. F. C., Porfirio, C. T., Lobo, M. D., Monteiro-Moreira, A. C. O., Souza, L. A., and Fernandes, A. V. (2018). Proteomic identification and purification of seed proteins from native Amazonian species displaying antifungal activity. *Planta, 248*(1), 197-209. doi:10.1007/s00425-018-2893-y

Rana, B., Singh, U., and Taneja, V. (1996). A rapid method for detecting fungi-toxic substances. *World Journal of Microbiology and Biotechnology, 12*(3), 301-302. doi:10.1007/BF00360934

Rasik, A. M., Raghubir, R., Gupta, A., Shukla, A., Dubey, M. P., Srivastava, S., Jain, H. K., and Kulshrestha, D. K. (1999). Healing potential of Calotropis procera on dermal wounds in Guinea pigs. *Journal of ethnopharmacology, 68*(1), 261-266. doi:10.1016/S0378-8741(99)00118-X

Rodríguez-Ortiz, R., Michielse, C., Rep, M., Limón, M. C., and Avalos, J. (2012). Genetic basis of carotenoid overproduction in Fusarium oxysporum. *Fungal Genetics and Biology, 49*(9), 684-696. doi:10.1016/j.fgb.2012.06.007

Roşca-Casian, O., Mircea, C., Vlase, L., Gheldiu, A.-M., Teuca, D. T., and Pârvu, M. (2017). Chemical composition and antifungal activity of Hedera helix leaf ethanolic extract. *Acta Biologica Hungarica, 68*(2), 196-207. doi: 10.1556/018.68.2017.2.7

Rubini, S., Rossi, S. S., Mestria, S., Odoardi, S., Chendi, S., Poli, A., Merialdi, G., Andreoli, G., Frisoni, P., and Gaudio, R. M. (2019). A Probable Fatal Case of Oleander (Nerium oleander) Poisoning on a Cattle Farm: A New Method of Detection and Quantification of the Oleandrin Toxin in Rumen. *Toxins, 11*(8), 442.

Saad, A. (2006). *Caractérisation chimique des huiles essentielles de trois plantes médicinales endémique du Sud-ouest Algérien: Bubonium gravoelens, Launeae arborescenset, Limoniastrium feei.* (Magister's thesis), Bechar University Center, Algeria. ,

Said, M. E. A., Bombarda, I., Naubron, J. V., Vanloot, P., Jean, M., Cheriti, A., Dupuy, N., and Roussel, C. (2017). Isolation of the major chiral compounds from Bubonium

graveolens essential oil by HPLC and absolute configuration determination by VCD. *Chirality, 29*(2), 70-79. doi:10.1002/chir.22672

Santhi, R. (2011). Phytochemical screening of Nerium oleander leaves. *International research journal of pharmacy, 2*(1), 131-135.

Sarwar, A., Brader, G., Corretto, E., Aleti, G., Abaidullah, M., Sessitsch, A., and Hafeez, F. Y. (2018). Qualitative analysis of biosurfactants from Bacillus species exhibiting antifungal activity. *PloS one, 13*(6), e0198107. doi:10.1371/journal.pone.0198107

Sarwar, A., Hassan, M. N., Imran, M., Iqbal, M., Majeed, S., Brader, G., Sessitsch, A., and Hafeez, F. Y. (2018). Biocontrol activity of surfactin A purified from Bacillus NH-100 and NH-217 against rice bakanae disease. *Microbiological research, 209*, 1-13. doi:10.1016/j.micres.2018.01.006

Saxena, G., Farmer, S., Towers, G., and Hancock, R. (1995). Use of specific dyes in the detection of antimicrobial compounds from crude plant extracts using a thin layer chromatography agar overlay technique. *Phytochemical analysis, 6*(3), 125-129. doi:10.1002/pca.2800060303

Schalchli, H., Hormazábal, E., Rubilar, O., Briceño, G., Mutis, A., Zocolo, G., and Diez, M. (2017). Production of ligninolytic enzymes and some diffusible antifungal compounds by white-rot fungi using potato solid wastes as the sole nutrient source. *Journal of applied microbiology, 123*(4), 886-895. doi:10.1111/jam.13542

Sekkoum, K., Belboukhari, N., and Cheriti, A. (2014). New flavonoids from bioactive extract of algerian medicinal plant Launeae Arborescens. *Asian Pacific Journal of Tropical Biomedicine, 4*(4), 267-271.

Sen, S., Borah, S. N., Bora, A., and Deka, S. (2017). Production, characterization, and antifungal activity of a biosurfactant produced by Rhodotorula babjevae YS3. *Microbial cell factories, 16*(1), 95. doi:10.1186/s12934-017-0711-z

Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z., and Ercisli, S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pakistan Journal of Pharmaceutical Sciences, 22*(1).

Senhaji, S., Lamchouri, F., and Toufik, H. (2020). Phytochemical Content, Antibacterial and Antioxidant Potential of Endemic Plant Anabasis aretioïdes Coss. & Moq.(Chenopodiaceae). *BioMed research international, 2020*.

References

Shaker, K. H., Morsy, N., Zinecker, H., Imhoff, J. F., and Schneider, B. (2010). Secondary metabolites from Calotropis procera (Aiton). *Phytochemistry Letters, 3*(4), 212-216. doi:10.1016/j.phytol.2010.07.009

Shobowale, O. O., Ogbulie, N. J., Itoandon, E. E., Oresegun, M. O., and Olatope, S. O. A. (2013). Phytochemical and Antimicrobial Evaluation of Aqueous and Organic Extracts of Calotropis procera Ait Leaf and Latex. *Nigerian Food Journal, 31*(1), 77-82. doi:10.1016/S0189-7241(15)30059-X

Siddiqui, B. S., Khatoon, N., Begum, S., Farooq, A. D., Qamar, K., Bhatti, H. A., and Ali, S. K. (2012). Flavonoid and cardenolide glycosides and a pentacyclic triterpene from the leaves of Nerium oleander and evaluation of cytotoxicity. *Phytochemistry, 77*, 238-244.

Silva, M. S., Ribeiro, S. F., Taveira, G. B., Rodrigues, R., Fernandes, K. V., Carvalho, A. O., Vasconcelos, I. M., Mello, E. O., and Gomes, V. M. (2017). Application and bioactive properties of CaTI, a trypsin inhibitor from Capsicum annuum seeds: membrane permeabilization, oxidative stress and intracellular target in phytopathogenic fungi cells. *Journal of the science of food and agriculture, 97*(11), 3790-3801. doi:10.1002/jsfa.8243

Singh, M. K., Pandey, A., Sawarkar, H., Gupta, A., Gidwani, B., Dhongade, H., and Tripathi, D. K. (2017). Methanolic extract of Plumbago Zeylanica-a remarkable antibacterial agent against many human and agricultural pathogens. *Journal of pharmacopuncture, 20*(1), 18. doi:10.3831/KPI.2017.20.004

Singh, S., Shenoy, S., Nehete, P. N., Yang, P., Nehete, B., Fontenot, D., Yang, G., Newman, R. A., and Sastry, K. J. (2013). Nerium oleander derived cardiac glycoside oleandrin is a novel inhibitor of HIV infectivity. *Fitoterapia, 84*, 32-39.

Slinkard, K., and Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture, 28*(1), 49-55.

Smith, S. (2007). An overview of ecological and habitat aspects in the genus Fusarium with special emphasis on the soil-borne pathogenic forms. *Plant Pathology Bulletin, 16*(3), 97-120.

References

Sofowora, A. (2010). *Plantes médicinales et médecine traditionnelle d'Afrique*. Paris, France: KARTHALA Editions.

Srivastava, A. K., and Pandey, L. K. (2019). Synthesis of Chalcones and Nucleosides Incorporating [1, 3, 4] Oxadiazolenone Core and Evaluation of their Antifungal and Antibacterial Activities. *Current Bioactive Compounds, 15*(6), 665-679.

Subhan, N., Burrows, G. E., Kerr, P. G., and Obied, H. K. (2018). Chapter 9 - Phytochemistry, Ethnomedicine, and Pharmacology of Acacia. In R. Atta ur (Ed.), *Studies in Natural Products Chemistry* (Vol. 57, pp. 247-326): Elsevier.

Suleiman, M. M., McGaw, L., Naidoo, V., and Eloff, J. (2010). Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *African Journal of Traditional, Complementary and Alternative Medicines, 7*(1).

Sun, Y., Wang, Y., Han, L., Zhang, X., and Feng, J. (2017). Antifungal activity and action mode of cuminic acid from the seeds of Cuminum cyminum L. against Fusarium oxysporum f. sp. niveum (FON) Causing Fusarium Wilt on Watermelon. *Molecules, 22*(12), 2053. doi:10.3390/molecules22122053

Talhi, M. F., Cheriti, A., Belboukhari, N., Agha, L., and Roussel, C. (2010). Biosorption of copper ions from aqueous solutions using the desert tree Acacia raddiana. *Desalination and Water Treatment, 21*(1-3), 323-327. doi:10.5004/dwt.2010.1649

Tan, W., Li, Q., Dong, F., Chen, Q., and Guo, Z. (2017). Preparation and characterization of novel cationic chitosan derivatives bearing quaternary ammonium and phosphonium salts and assessment of their antifungal properties. *Molecules, 22*(9), 1438. doi:10.3390/molecules22091438

Tan, W., Zhang, J., Luan, F., Wei, L., Chen, Y., Dong, F., Li, Q., and Guo, Z. (2017). Design, synthesis of novel chitosan derivatives bearing quaternary phosphonium salts and evaluation of antifungal activity. *International journal of biological macromolecules, 102*, 704-711. doi:10.1016/j.ijbiomac.2017.04.073

Tan, W., Zhang, J., Mi, Y., Dong, F., Li, Q., and Guo, Z. (2018). Synthesis, characterization, and evaluation of antifungal and antioxidant properties of cationic chitosan derivative via azide-alkyne click reaction. *International journal of biological macromolecules, 120*, 318-324. doi:10.1016/j.ijbiomac.2018.08.111

References

Tao, P., Wu, C., Hao, J., Gao, Y., He, X., Li, J., Shang, S., Song, Z., and Song, J. (2020). Antifungal Application of Rosin Derivatives from Renewable Pine Resin in Crop Protection. *Journal of agricultural and food chemistry, 68*(14), 4144-4154. doi:10.1021/acs.jafc.0c00562

Tarhriz, V., Eyvazi, S., Shakeri, E., Hejazi, M. S., and Dilmaghani, A. (2020). Antibacterial and Antifungal Activity of Novel Freshwater Bacterium Tabrizicola aquatica as a Prominent Natural Antibiotic Available in Qurugol Lake. *Pharmaceutical Sciences, 26*(1), 88-92.

Thrane, U. (1999). FUSARIUM. In R. K. Robinson (Ed.), *Encyclopedia of Food Microbiology* (pp. 901-906). Oxford: Elsevier.

Thrane, U. (2014). Fusarium. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology (Second Edition)* (pp. 76-81). Oxford: Academic Press.

Triana, J., Eiroa, J. L., Morales, M., Perez, F. J., Brouard, I., Quintana, J., Ruiz-Estévez, M., Estévez, F., and León, F. (2016). Sesquiterpenoids isolated from two species of the Asteriscus alliance. *Journal of Natural Products, 79*(5), 1292-1297. doi:10.1021/acs.jnatprod.5b01013

Tsala, D. E., Nga, N., Thiery, B. N. M., Bienvenue, M. T., and Theophile, D. (2015). Evaluation of the antioxidant activity and the healing action of the ethanol extract of Calotropis procera bark against surgical wounds. *Journal of intercultural ethnopharmacology, 4*(1), 64. doi:10.5455/jice.20141211071136

Tsimogiannis, D., and Oreopoulou, V. (2019). Chapter 16 - Classification of Phenolic Compounds in Plants. In R. R. Watson (Ed.), *Polyphenols in Plants (Second Edition)* (pp. 263-284): Academic Press.

Van Dijck, P., Sjollema, J., Cammue, B. P., Lagrou, K., Berman, J., d'Enfert, C., Andes, D. R., Arendrup, M. C., Brakhage, A. A., and Calderone, R. (2018). Methodologies for in vitro and in vivo evaluation of efficacy of antifungal and antibiofilm agents and surface coatings against fungal biofilms. *Microbial Cell, 5*(7), 300.

Vogt, T. (2010). Phenylpropanoid Biosynthesis. *Molecular Plant, 3*(1), 2-20. doi:10.1093/mp/ssp106

Wang, Y., Zhang, J., Sun, Y., Feng, J., and Zhang, X. (2017). Evaluating the potential value of natural product cuminic acid against plant pathogenic fungi in cucumber. *Molecules, 22*(11), 1914. doi:10.3390/molecules22111914

Wu, H., Liu, T., Lian, Y., and Wang, W. (2019). Two new eremophilenolides from the roots of Ligulariopsis shichuana and their anti-phytopathogenic fungal and antifeedant activities. *Natural product research, 33*(10), 1442-1448. doi: 10.1080/14786419.2017.1419240

Wu, X., Pang, X.-J., Xu, L.-L., Zhao, T., Long, X.-Y., Zhang, Q.-Y., Qin, H.-L., Yang, D.-F., and Yang, X.-L. (2018). Two new alkylated furan derivatives with antifungal and antibacterial activities from the plant endophytic fungus Emericella sp. XL029. *Natural product research, 32*(22), 2625-2631. doi: 10.1080/14786419.2017.1374269

Wu, Z., Xie, Z., Wu, M., Li, X., Li, W., Ding, W., She, Z., and Li, C. (2018). New Antimicrobial Cyclopentenones from Nigrospora sphaerica ZMT05, a Fungus Derived from Oxya chinensis Thunber. *Journal of agricultural and food chemistry, 66*(21), 5368-5372.

Xego, S., Kambizi, L., and Nchu, F. (2017). Effects of different hydroponic substrate combinations and watering regimes on physiological and anti-fungal properties of Siphonochilus aethiopicus. *African Journal of Traditional, Complementary and Alternative Medicines, 14*(3), 89-104. doi:10.21010/ajtcam. v14i3.10

Xiong, C., Li, Q., Li, S., Chen, C., Chen, Z., and Huang, W. (2017). In vitro Antimicrobial Activities and Mechanism of 1-Octen-3-ol against Food-related Bacteria and Pathogenic Fungi. *Journal of oleo science*, ess16196. doi:10.5650/jos.ess16196

Xu, X., Han, L., Zhao, L., Chen, X., Miao, C., Hu, L., Huang, X., Chen, Y., and Li, Y. (2019). Echinosporin antibiotics isolated from Amycolatopsis strain and their antifungal activity against root-rot pathogens of the Panax notoginseng. *Folia Microbiologica, 64*(2), 171-175. doi:10.1007/s12223-018-0642-z

Yoo, S.-J., Weon, H.-Y., Song, J., and Sang, M. K. (2020). Effects of Chryseobacterium soldanellicola T16E-39 and Bacillus siamensis T20E-257 on Biocontrol against Phytophthora Blight and Bacterial Wilt and Growth Promotion in Tomato Plants.

INTERNATIONAL JOURNAL OF AGRICULTURE AND BIOLOGY, 23(3), 534-540. doi:10.17957/IJAB/15.1320

Yoshida, N., Fukushima, T., Saito, H., Shimosaka, M., and Okazaki, M. (1989). Cellulose and xylan degrading enzymes of the plant pathogenic fungus, Fusarium oxysporum SUF850. *Agricultural and biological chemistry, 53*(7), 1829-1836.

Yu, S., Teng, C., Liang, J., Song, T., Dong, L., Bai, X., Jin, Y., and Qu, J. (2017). Characterization of siderophore produced by Pseudomonas syringae BAF. 1 and its inhibitory effects on spore germination and mycelium morphology of Fusarium oxysporum. *Journal of Microbiology, 55*(11), 877-884. doi:10.1007/s12275-017-7191-z

Yuan, C., Ding, G., Wang, H.-Y., Guo, Y.-H., Shang, H., Ma, X.-J., and Zou, Z.-M. (2017). Polyketide-Terpene Hybrid Metabolites from an Endolichenic Fungus Pestalotiopsis sp. *BioMed research international, 2017*. doi:10.1155/2017/6961928

Zandvakili, N., Zamani, M., Motallebi, M., and Jahromi, Z. M. (2017). Cloning, Overexpression and in vitro Antifungal Activity of Zea Mays PR10 Protein. *Iranian journal of biotechnology, 15*(1), 42. doi:10.15171/ijb.1357

Zehra, A., Meena, M., Dubey, M. K., Aamir, M., and Upadhyay, R. (2017). Synergistic effects of plant defense elicitors and Trichoderma harzianum on enhanced induction of antioxidant defense system in tomato against Fusarium wilt disease. *Botanical studies, 58*(1), 44. doi:10.1186/s40529-017-0198-2

Zhang, J., Tan, W., Zhang, Z., Song, Y., Li, Q., Dong, F., and Guo, Z. (2018). Synthesis, characterization, and the antifungal activity of chitosan derivatives containing urea groups. *International journal of biological macromolecules, 109*, 1061-1067. doi:10.1016/j.ijbiomac.2017.11.092

Zhang, M., Ge, J., and Yu, X. (2018). Transcriptome Analysis Reveals the Mechanism of Fungicidal of Thymol Against Fusarium oxysporum f. sp. niveum. *Current microbiology, 75*(4), 410-419. doi:10.1007/s00284-017-1396-6

Zhao, Q., Chen, L., Dong, K., Dong, Y., and Xiao, J. (2018). Cinnamic Acid Inhibited Growth of Faba Bean and Promoted the Incidence of Fusarium Wilt. *Plants, 7*(4), 84. doi:10.3390/plants7040084

References

Zhao, Y.-M., Cheng, Y.-X., Ma, Y.-N., Chen, C.-J., Xu, F.-R., and Dong, X. (2018). Role of phenolic acids from the rhizosphere soils of Panax notoginseng as a double-edge sword in the occurrence of root-rot disease. *Molecules, 23*(4), 819. doi:10.3390/molecules23040819

Zheng, G., Luo, S., Li, S., Hua, J., Li, W., and Li, S. (2018). Specialized metabolites from Ageratina adenophora and their inhibitory activities against pathogenic fungi. *Phytochemistry, 148*, 57-62. doi:10.1016/j.phytochem.2018.01.013

Zheng, Y.-K., Miao, C.-P., Chen, H.-H., Huang, F.-F., Xia, Y.-M., Chen, Y.-W., and Zhao, L.-X. (2017). Endophytic fungi harbored in Panax notoginseng: diversity and potential as biological control agents against host plant pathogens of root-rot disease. *Journal of ginseng research, 41*(3), 353-360. doi:10.1016/j.jgr.2016.07.005

Zhu, X., Zhong, Y., Xie, Z., Wu, M., Hu, Z., Ding, W., and Li, C. (2018). Fusarihexins A and B: Novel cyclic hexadepsipeptides from the mangrove endophytic fungus Fusarium sp. R5 with antifungal activities. *Planta medica, 84*(18), 1355-1362. doi:10.1055/a-0647-7048

Znini, M., Cristofari, G., Majidi, L., Mazouz, H., Tomi, P., Paolini, J., and Costa, J. (2011). Antifungal activity of essential oil from Asteriscus graveolens against postharvest phytopathogenic fungi in apples. *Natural product communications, 6*(11). doi:10.1177/1934578X1100601147

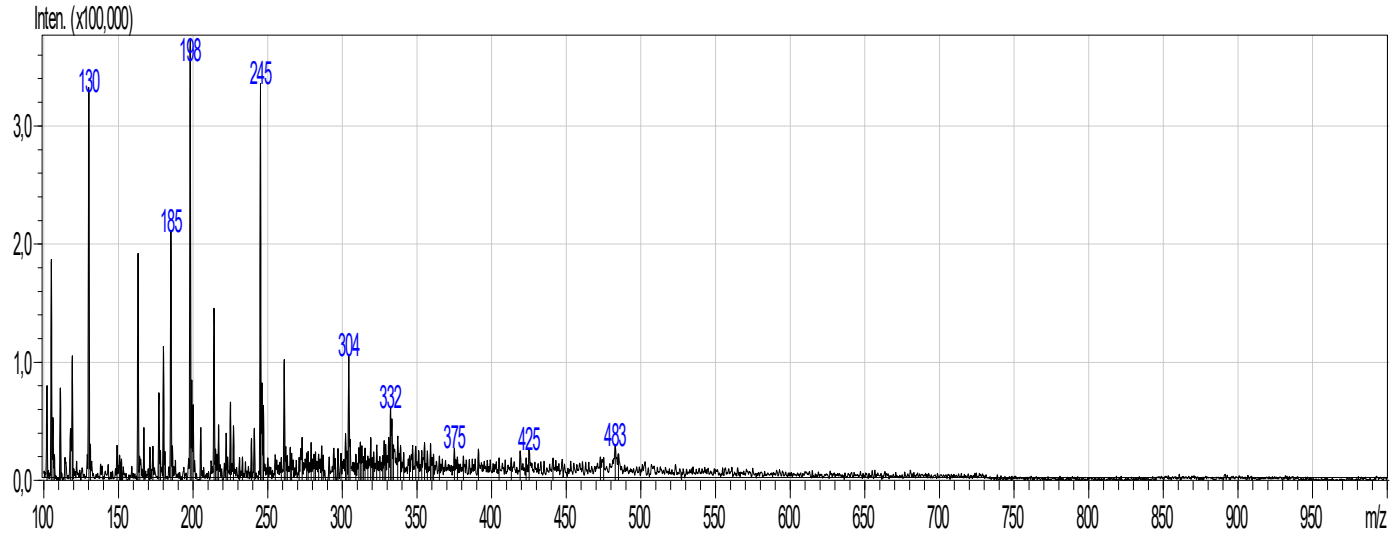
Appendices

Appendices

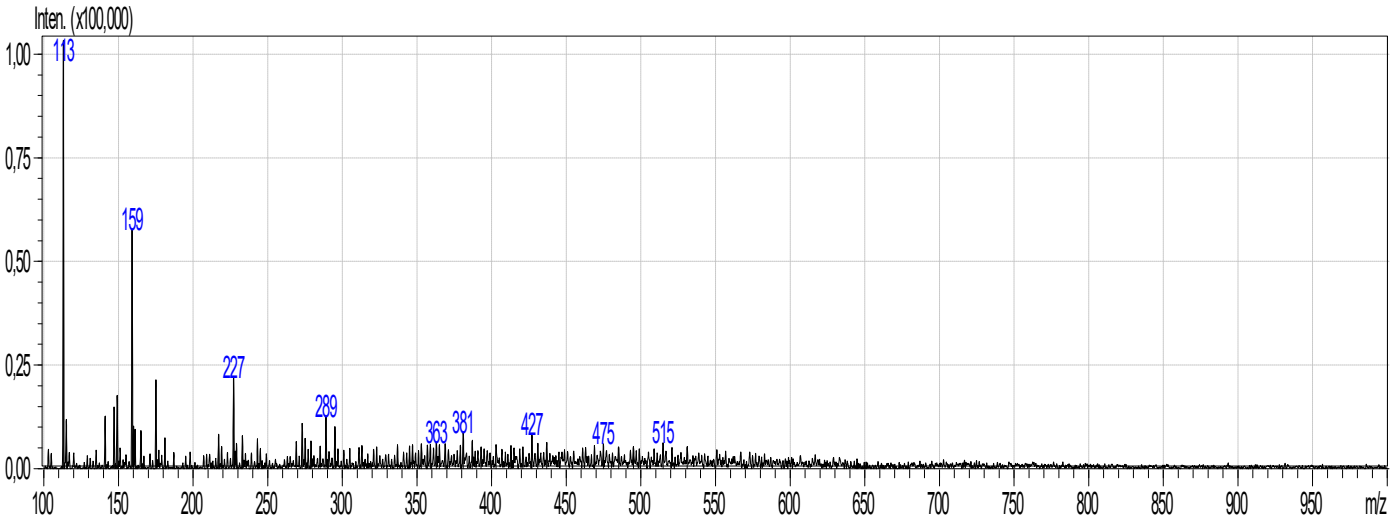
Appendix A: LC-MS analysis ion mass spectra

1-*La*-d

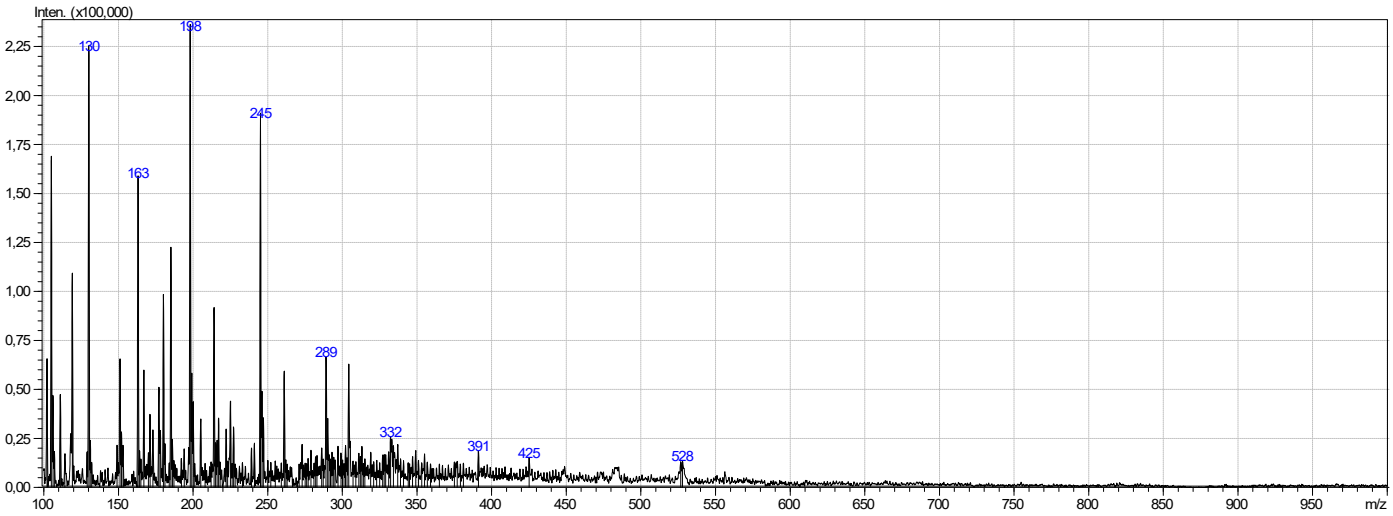
Positive ionization *q3 scan*



Negative ionization *q3 scan*

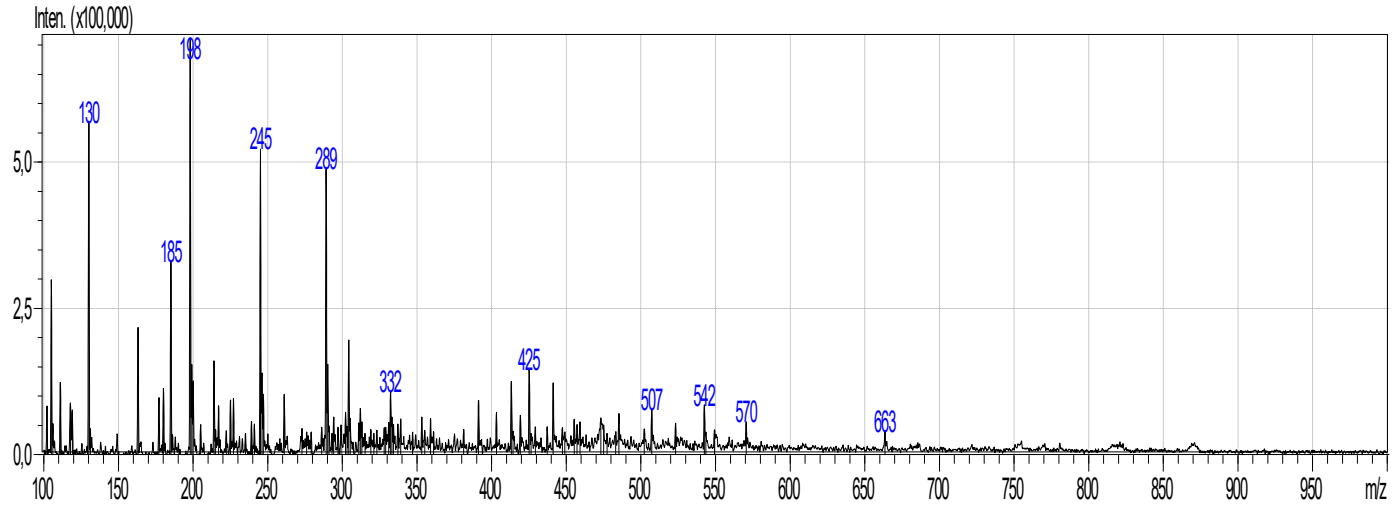


Positive ionization *q3 scan (*detail*)*

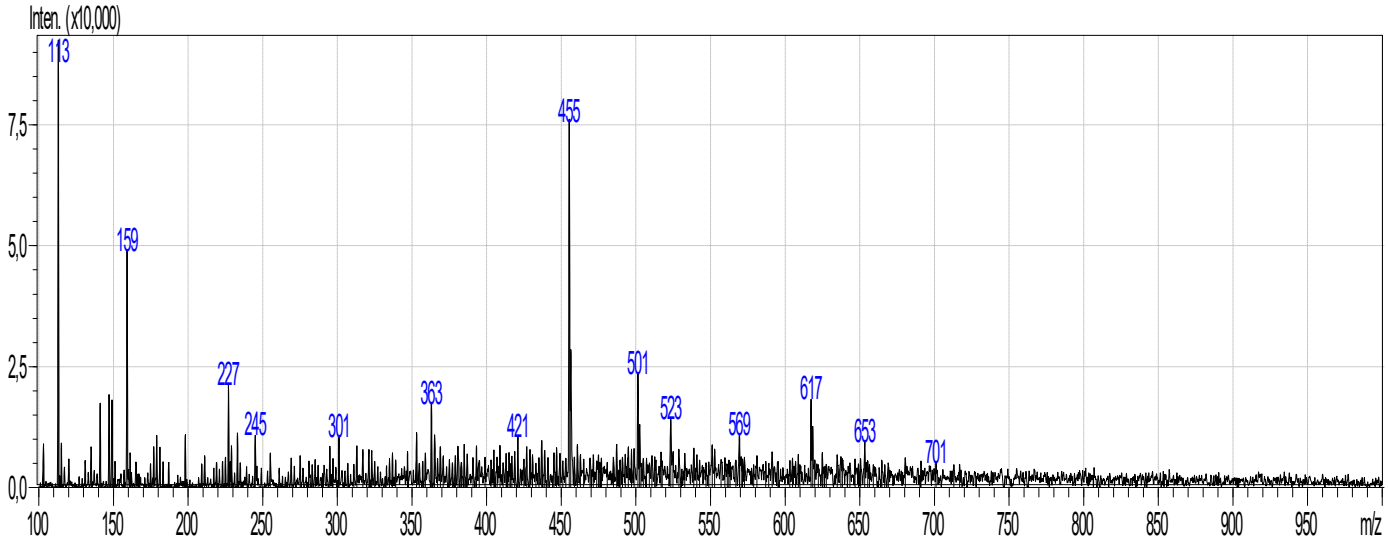


2-*Ag*-c

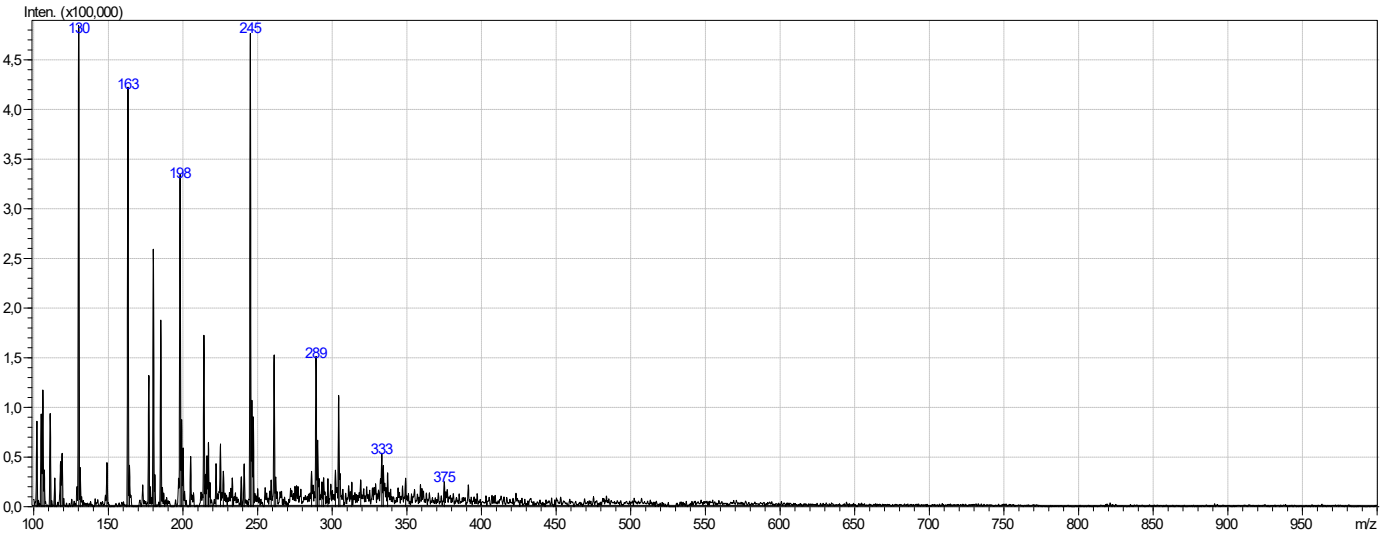
Positive ionization *q3 scan*



Negative ionization *q3 scan*



Positive ionization *q3 scan (*detail*)*



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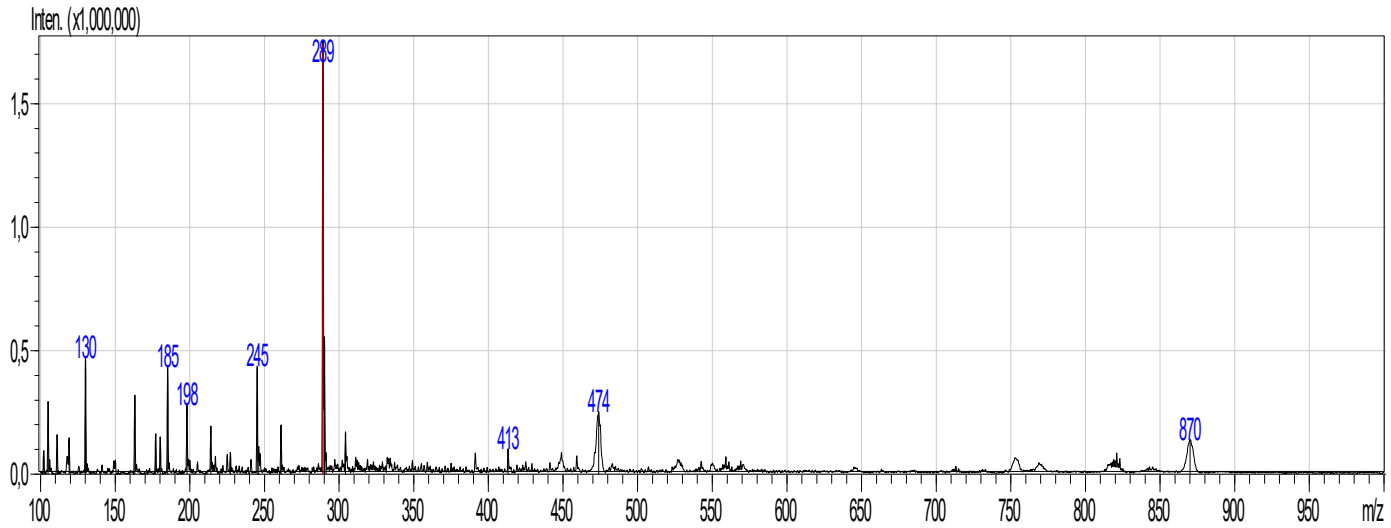
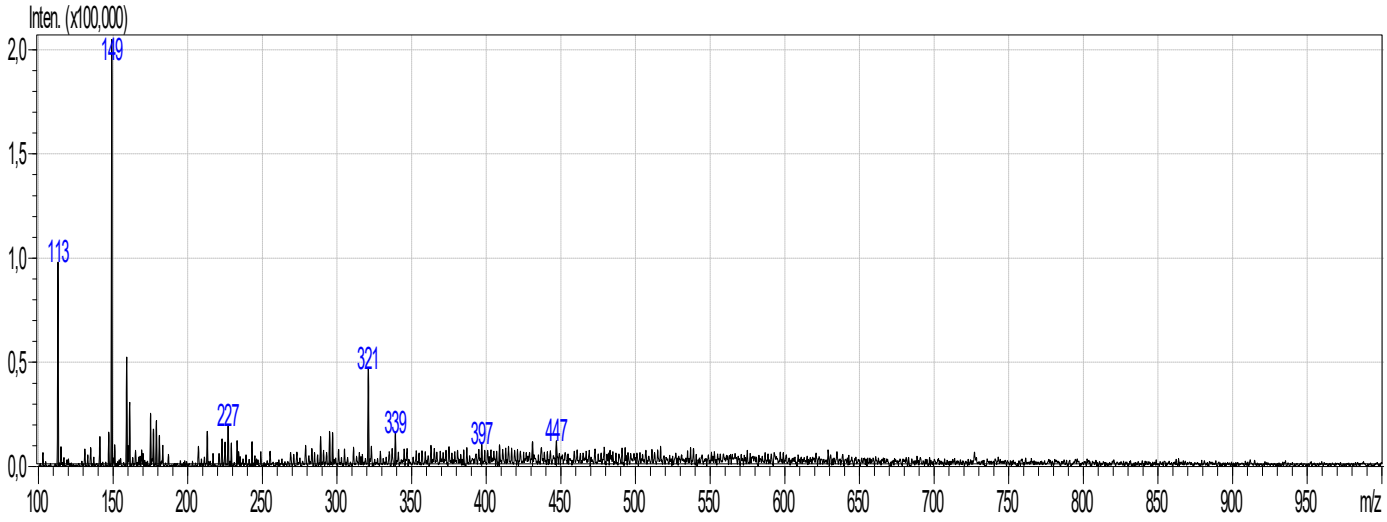
1-*La*-c

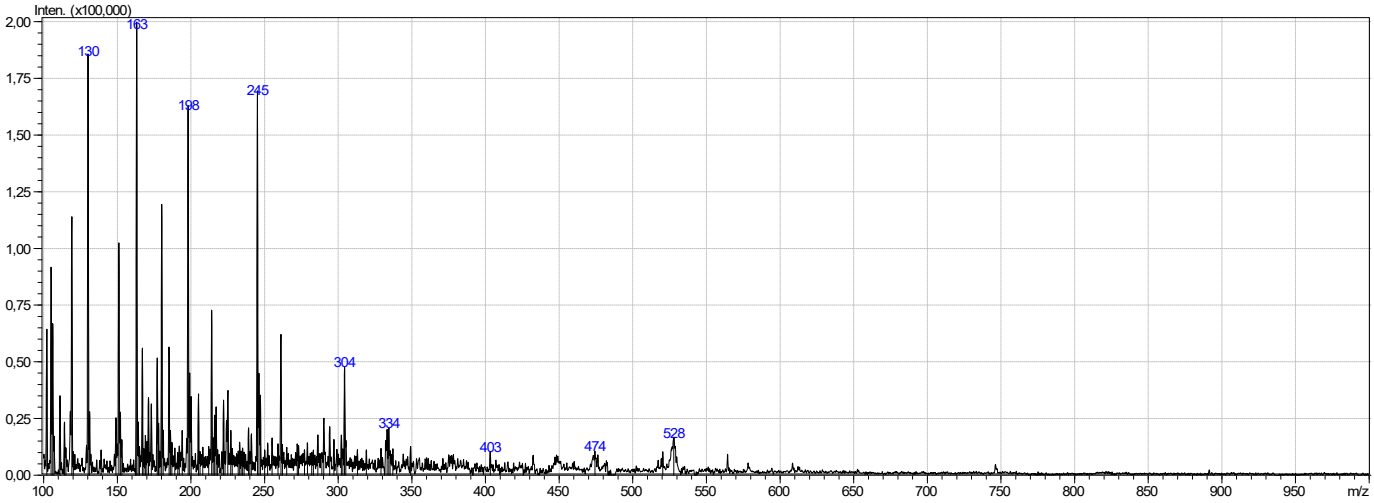
Positive ionization *q3 scan*



Negative ionization *q3 scan*



Positive ionization q3 scan (detail)



Appendix B: NMR specters of methyl gallate

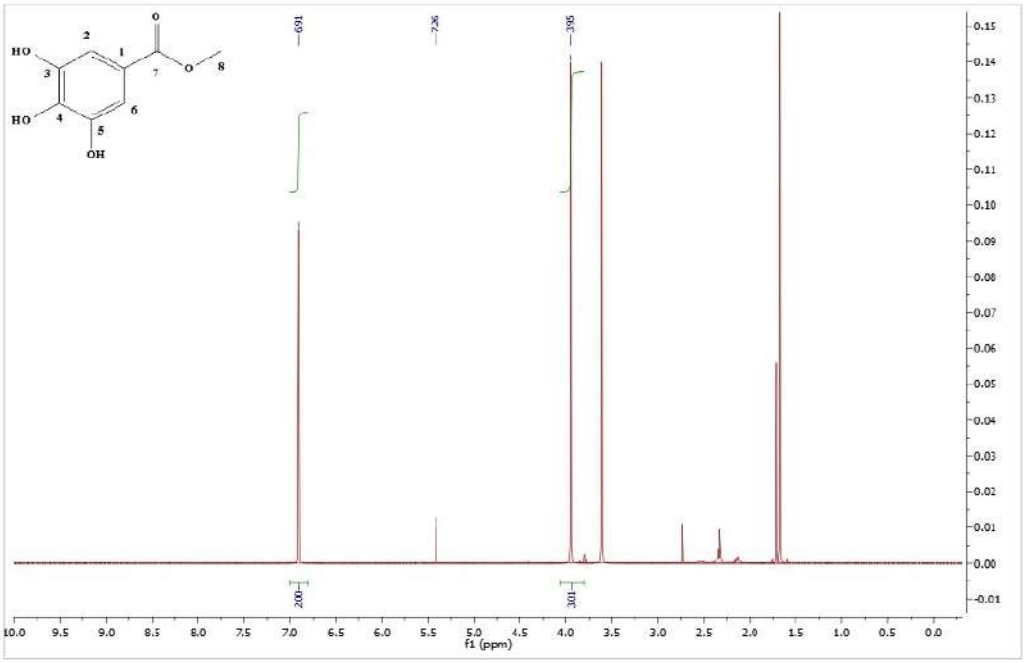


Figure B1: Isolated methyl gallate ^{1}H NMR (δppm): 6.91 (S, 2H), 3.95(S, 3H).

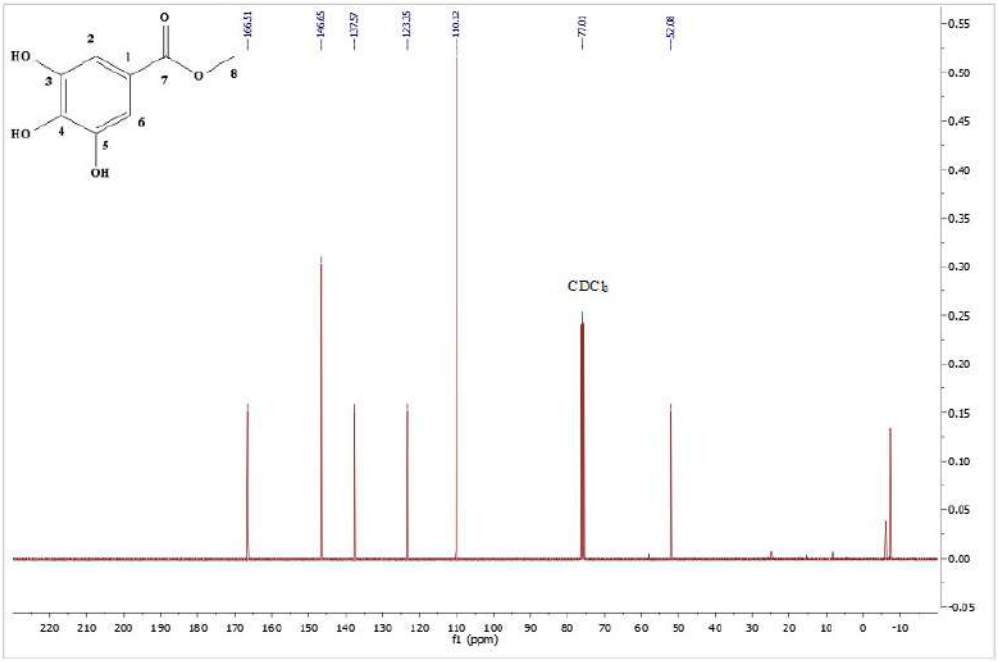


Figure B2. Isolated methyl gallate ^{13}C NMR (δppm): 166.51(C7 =O) 146.65(C3, C5), 137.57(C4), 123.35 (C1) 110.12 (C2, C6) 52.08 (C8).

Appendix C: NMR specters of syringic acid

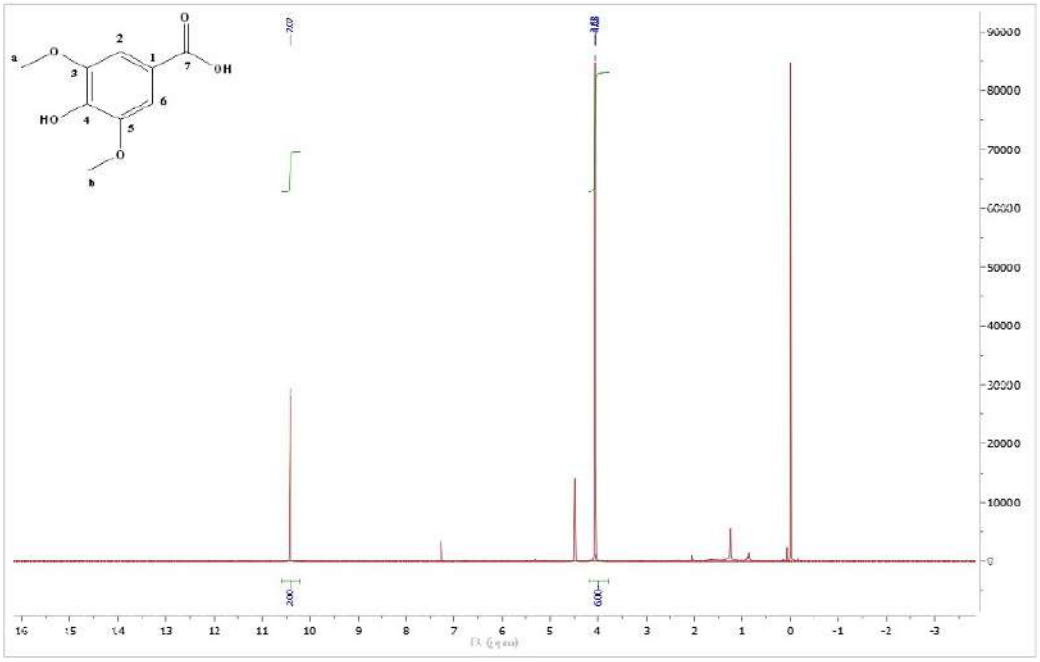


Figure C1: Isolated syringic acid ^{1}H NMR (δppm): 7.07 (S, 2H), 3.82(S, 6H).

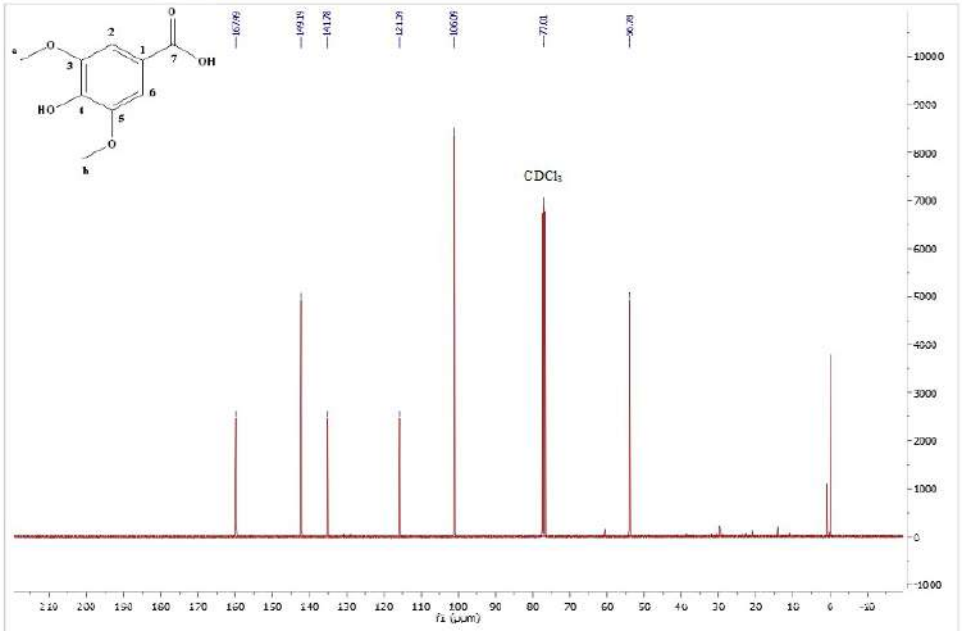


Figure C2. Isolated syringic acid ^{13}C NMR (δppm): 167.49(C7 =O) 149.19(C3, C5), 141.78(C4), 121.39 (C1) 106.09(C2, C6) 56.78 (Ca et Cb).

Appendices

PUBLICATIONS AND COMMUNICATIONS RELATED TO THE THESIS

BELHI, Z., BOULENOUAR, N., CHERITI, A., & MAROUF, A. (2020). Antifungal and anti-cellulases activity of Limoniastrum feei extracts to promote Bayoud disease treatment using bioautography. *Cogent Food & Agriculture, 6* (1), 1726083. DOI:10.1080/23311932.2020.1726083

BELHI, Z., BOULENOUAR, N., & CHERITI, A. (2020). The Use of Natural Products Against Fusarium Oxysporum: A Review. *Natural Products Journal*, 10 (1), 1726083.
DOI: 10.2174/2210315510999200811151154

BELHI, Z., BOULENOUAR, N., & CHERITI, A. (2020). *Enzyme inhibition activity of a phytopathogenic fungus by extracts of some plants growing wild in Algeria.* Paper presented at the Deuxièmes Journées Internationales de Chimie Hétérocyclique et ses Applications, JICHA 2-2020.Tunisia.

BELHI, Z., BOULENOUAR, N., MAROUF, A., & CHERITI, A. (2017). *Antifungal effect of some plants crude extracts against the causative agent of Bayoud disease* Paper presented at the International Conference on Chemical and Biochemical Engineering (ICCBE-2017). Turkey.

BELHI, Z., BOULENOUAR, N., & CHERITI, A. (2016). *Inhibition of the cellulolytic activity of phytopathogenic fungus by plant extracts of selected plants growing wild in Algeria*. Paper presented at the 3rd International Conference on Waste Management, Ecology and Biological Sciences (WMEBS-2016).Turkey.

BELHI, Z., BOULENOUAR, N., & CHERITI, A. (2015). *Effect of the extracts of some Saharan plants on the cellulases of Fusarium oxysporum f. sp. albedinis.* Paper presented at the 2nd Mediterranean Symposium on Medicinal and Aromatic Plants, MESMAP-2. Turkey.

BELHI, Z., BOULENOUAR, N., & CHERITI, A. (2015). *Etude de l'activité antifongique des extraits d'Asteriscus graveolens sur Fusarium oxysporum f. sp. albedinis*. Paper presented at the 1st Symposium of Biomolecules & their Applications: Biomolecules saving life SBA-1.Lebanon.

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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Antifungal and anti-cellulases activity of *Limoniastrum feei* extracts to promote Bayoud disease treatment using bioautography

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Additional information is available at the end of the article

Abstract: In this study, *Limoniastrum feei* extracts were evaluated for management of *Fusarium oxysporum* f. sp. *albedinis* (Foa) through antifungal tests and cellulases inhibition (cellulases are cell wall degrading enzymes "CWDE"). Antifungal activity evaluation was realized by direct bioautography. Cellulases inhibition test was realized by contact bioautography. In addition, a phytochemical screening has been done for these extracts on TLC plates (extraction with solvents of increasing polarity: hexane, ethyl acetate, dichloromethane, and methanol). Phytochemical screening was intended to know which metabolite is responsible for the antifungal activity through correlation with antifungal and anti-cellulases effect. The phytochemical study revealed the presence of saponins, flavonoids, tannins, coumarins, and alkaloids in these extracts. The different extracts showed high variability in their phenolic and flavonoids contents as well as antifungal capacity, as the case of ethyl acetate extract. Detection of inhibition zones was revealed with ethanolic Gram's iodine. Inhibition zones were determined as dark brown spots. The results of enzymatic activity -by contact bioautography technique-showed that certain extracts of *L. feei* present a significant effect on cellulases of Foa. The best effect was presented by methanolic extract of aerial part (inhibition zone diameter in chromatogram is

Abdelkrim Cheriti

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Abdelkrim CHERITI, Born in El-Bayadh (Algeria), 25 November 1963. Received his Ph.D. in the chemistry of natural products from ENSSPICAM & Pharmacy faculty of Marseille (France) in 1992. Professor of Chemistry (Algeria). Contributor in numerous papers in international and national journals; Editor in chief of Phytochem & BioSub Journal, Associate Editor of Annales of TMB University and Director of Al Ouloum collection. Coordinator of various research projects in the field of natural products, bioactive and pharmaceutical substances from Saharan medicinal plants. Founder and Director of the Phytochemistry and Organic Synthesis Laboratory (POSL). The main purpose of POSL is scientific research and development of knowledge and techniques concerning the field of Phytochemistry and the environment. Our present study is a continuation of our research efforts in the context of medicinal plants valorization and to explore the natural compounds for their antifungal activity.

PUBLIC INTEREST STATEMENT

Date palm (*Phoenix dactylifera* L.) constitutes importance in the social and economic life of the Algerian Sahara. It represents the food, shade, garden, and refuge for the Saharan people. In South-West of Algeria, the cultivation of the date palm development and the safeguarding of the already existing date palms constitute a major concern of these oases'farmers while its cultivation is threatened by several diseases such as Fusariosis or Fusarium wilt disease. The vascular wilt of the date palm is caused by a soil fungus: *Fusarium oxysporum* f. sp. *albedinis*.

In this work, we evaluated the effect of *Limoniastrum feei* extracts (an endemic medicinal plant) against the fungus causing vascular disease by using the bioautography technique. To our knowledge, there are few studies used this technique in the antifungal tests. The results showed that certain extracts of *Limoniastrum feei* present a significant effect against *Fusarium oxysporum* f. sp. *albedinis*.

higher than 2 mm). Best antifungal effect was presented by compounds with high probability to be phenolic compounds.

Subjects: Agriculture & Environmental Sciences; Plant & Animal Ecology; Chemical Engineering

Keywords: Limoniastrum feei; Antifungal activity; Fusarium oxysporum f. sp. albedinis; Cellulases; Bioautography

1. Introduction

Due to the notable medicinal value of *Limoniastrum feei*, it was considered of interest to carry out phytochemical and antimicrobial investigations of this species (Belboukhari & Cheriti, 2005). The other uses of *L. feei* are as an antibacterial, for treatment of bronchitis and stomach infection (Belboukhari & Cheriti, 2007). Also, antioxidant and antiradical-scavenging activity were evaluated (Keffous et al., 2016). A previous investigation revealed that methanol extract from *L. feei* leaves contained potential antifungal activity against *Candida albicans* and antibacterial effects against *E. coli* (Belboukhari & Cheriti, 2009). These results prompted us to test *in vitro* several extracts of *L. feei* against *Fusarium oxysporum* f. sp. *albedinis*.

In South-West of Algeria, the culture of the date palm development and the safeguarding of the already existing date palms constitute a major concern of the farmers of these oases (Benabdelkader, Malek, & Draoui, 2011). However, its culture is threatened by several pests and diseases such as vascular fusariosis. The vascular wilt of the date palm (*Phoenix dactylifera* L.), locally known as Bayoud disease, is caused by a soil fungus: *Fusarium oxysporum* f. sp. *albedinis* (Killian and Maire) Gordon (Kettout & Rahmania, 2010).

Many *Fusarium* species are serious plant pathogens, causing symptoms such as necrotic lesions, rot, and wilt. Since its first signal before 1870, Bayoud disease has killed approximately 20 million date palm trees in Morocco and Algeria (Boulenouar, Marouf, & Cheriti, 2014). The only way to fight this disease is to prevent its propagation to other date-growing areas in the region and farther fields (Boulenouar, Marouf, & Cheriti, 2011). It is known that the appearance of a disease is the result of several factors called "factor of pathogenicity ". These factors are mainly enzymes and toxins (Freeman & Maymon, 2000). The most significant enzymes from the pathogenicity point of view of *Fusarium oxysporum* f. sp. *albedinis* are the CWDE (Cell Wall Degrading Enzymes). Among these enzymes, the cellulases, which hydrolyze the cellulose (El Modafar, Tantaoui, & El Boustani, 2000).

In this context, the cellulases can be the target of certain factors and/or substances. Therefore, the objective of this study was to evaluate the effect of *L. feei* extracts on the *Fusarium oxysporum* f. sp. *albedinis* cellulases, degradation enzymes of walls of the host plant (*Phoenix dactylifera* L). The plant was used to evaluate their extracts with four solvents (hexane, ethyl acetate, dichloromethane, and methanol), using the bioautography technique.

2. Materials and methods

2.1. Plant materials and extract preparation

The aerial parts and roots of *L. feei* were collected from Taghit, in Bechar, South-West of Algeria. Plant material was identified and a voucher specimen was conserved at the phytochemical herbarium of Phytochemistry and Organic Synthesis Laboratory under accession No CA99/14. The plant material was dried in a shaded area. After grinding, the material was stored at room temperature. The extraction was prepared by 10 g of the plant parts in 100 ml of solvents (ethyl acetate, dichloromethane, hexane or methanol) during two (02) hours (using heat reflux extraction). The residues are weighed (after filtration and evaporation). The extract was stored at 4°C until use.

2.2. Thin layer chromatography (TLC)

The extracts of each solvent were subjected to TLC. The used solvent system was ethyl acetate: heptane (75:25). Spots were detected on TLC under UV light.

2.3. Phytochemical screening on TLC plate

The extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extract. The screening was done according to Pascual, Carretero, Slowing, and Villar (2002).

2.3.1. Test for flavonoids

The TLC plates containing the extracts are sprayed with an ethanolic solution with 5% aluminum chloride ($AlCl_3$). The appearance of the greenish-yellow color, with the revelation by UV 365 ηm, indicates the presence of flavonoids.

2.3.2. Test for coumarins

The TLC separation plates are sprayed, immediately, with the ethanolic solution of KOH. The appearance of red color, in the visible, indicates the presence of anthraquinones, or the yellow color, with the revelation by UV 365 ηm, which indicates the presence of anthrones or furanocoumarins.

2.3.3. Test for tannins

The TLC plates containing the extracts are sprayed with a methanolic solution (80%) of ferric chloride 1 % ($FeCl_3$). The appearance of black-blue color indicates the presence of gallic tannins, or greenish-brown indicates the presence of catechin tannins.

2.3.4. Test for alkaloids: "marquis reagent"

Formaldehyde solution (10 ml) with 100 ml of sulfuric acid (H_2SO_4) was prepared. The TLC separation plates are sprayed immediately. The color change of the spots indicates the presence of alkaloids or aromatic hydrocarbons.

2.3.5. Test for saponins

Froth test for saponins (foam) was used. 2g of the sample was weighed into Erlenmeyer flask in which 100 ml of sterile distilled water was added and boiled for 15 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube.

The test tube was shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. The height of the foam was measured in each tube. The formation of a 1 cm layer of foam indicates the presence of saponins.

2.4. Antifungal screening by direct bioautography (thin-layer chromatography-direct bioautography)

In order to screen for and identify compounds with the antifungal activity present in the plant extracts, direct bioautography was used as described by Boulenouar et al. (2011). This method, based on the direct immersed and growing of a fungal spore suspension on a developed TLC chromatogram. 80µg/µl of each extract was applied to the TLC plate of silica gel 60 F_{254} (7 × 1.5 cm). After developing the chromatogram using a suitable eluent for efficient separation and location of the UV absorbing spot, chromatograms were then moved directly into Petri dishes containing 20 ml of spores for 10 seconds, where the concentration is 2.10^7 spores/ml. Bioautograms were examined for fungal growth at regular intervals until TLC plates were completely covered with a lawn of mycelial growth. Then, they are added to other sterile Petri dishes containing cotton wet with distilled water to provide moisture. These are incubated for 4 days at 21°C. The plates were immersed in a 2 mg/ml solution of p-iodonitrotetrazolium violet (INT) and further incubated for 1 hour at 21°C. R_f zones on the plates were compared to that of reference plates to find the R_f of the active compound.

2.5. Cellulases production by Fusarium oxysporum f. sp. albedinis

Fusarium oxysporum f. sp. *albedinis* was cultivated on a liquid medium at 21°C and incubated for 96 h under moderate agitation. For the inoculation of the cultures, a suspension of spores (4.3 X 10^5 spores/ml) was used in sterile distilled water added to 2 ml of Tween 80. The filtrate containing cellulases was put in tubes (5 mL in each tube). The tubes were conserved at −18°C until use.

2.6. Contact bioautography

The use of a TLC support to screen for potential plant-derived enzyme inhibitors is a rapid method that is relatively free of disturbances due to the solvent (Pascual et al., 2002). In contact bioautography, antimicrobials compound diffuse from the chromatogram to the inoculated agar plate. The test by contact bioautography used in this study was described by Choma and Jesionek (2015) with slight modifications. The test has been realized on cellulose agar medium (2% cellulose, 1.5% agar). Ten milliliters of the medium have been poured in each Petri dish and left to solidify. The plant's extracts have undergone TLC. Each chromatogram—after evaporation of the eluent- was put face down on the cellulose agar plate for 2 hours to promote diffusion. The filtrate containing cellulases previously prepared was poured on the cellulose agar plates after removal of the chromatogram and incubated at 21°C for 2 hours. The detection of inhibition zones was done using Gram's iodine. Gram's iodine was used as described by Kasana, Salwan, Dhar, Dutt, and Gulati (2008). Plates were flooded with Gram's iodine (3% KI and 2% I_2 in 70 % ethanol) for 5 minutes then washed with distilled water. Gram's iodine formed a dark brown color with cellulose but not with hydrolyzed cellulose, giving a distinct, clear, and prominent zone in 3 to 5 minutes (Kasana et al., 2008). The inhibition zones were visible as dark brown zones. The evaluation of cellulases activity inhibition was expressed as the diameter of inhibition zones (mm) and the R_f values to reflect the inhibitors. The control tests passed all the protocol without extracts or filtrate (the filtrate has been replaced by the culture medium). The tests were realized in triplicate (the standard errors were less than 10%).

3. Results

3.1. Thin-layer chromatography (TLC)

Thin-layer chromatography was used as a preliminary method for compositional analysis of the crude extracts. Table 1 groups obtained Rf values. According to the chromatograms obtained by TLC, all the parts of the plant contain several chemical compounds (several spots).

TLC analysis (EtOAc: Hep, 75:25) revealed four constituents in the hexane extract (yield 1.22%, Rf 0.57, 0.85, 0.90, 0.95), five constituents in the ethyl acetate extract (yield 2.83%, Rf 0.35, 0.57, 0.85, 0.90, 0.95), and five constituents in the methylene chloride (DCM) extract (yield 1.58%, Rf 0.35, 0.57, 0.85, 0.90, 0.93), and two constituents in the methanolic extract (yield 11.88%, Rf 0.35, 0.90).

The TLC analyses indicated that the extracts of *L. feei* were composed of a mixture of secondary metabolites. Rahmani, Belboukhari, and Cheriti (2014) reportedly obtained the Rf values of *L. feei* extract by ethyl acetate in the comparable range of 0.36–0.77, eluted with another solvent system, not as that used in the present study.

3.2. Phytochemical screening of the plant extracts

The characterization reactions revealed several chemical groups in the different extracts. The use of solvents with varying polarity in the extraction procedure is determined for the successful isolation of compounds with different ranges of polarity.

In the present study, phytochemical screening for all extracts of *L. feei* aerial part and root showed a significant indication of the presence of metabolites. Saponins, flavonoids, tannins, coumarins, and alkaloids, were found to be present in the majority of the plant extracts (Table 2).

Table 1. Rf values of TLC in different extracts

Eluent	Extraction Solvent	Parts	R_f					
EtOAc: Hep 75:25	Hex	Aerial part	0.00	0.57	0.85	0.90	0.95	-
		Roots	0.00	0.54	0.81	0.90	0.93	-
	EtOAc	Aerial part	0.00	0.35	0.57	0.85	0.90	0.95
		Roots	0.00	0.6	0.81	0.90	0.96	-
	DCM	Aerial part	0.00	0.35	0.57	0.85	0.90	0.95
		Roots	0.00	0.54	0.81	0.90	0.93	-
	MeOH	Aerial part	0.00	0.35	0.90	-	-	-
		Roots	0.00	0.81	0.93	-	-	-

Hex: hexane; EtOAc: ethyl acetate; DCM: dichloromethane; MeOH: methanol; Hep: heptane.

Table 2. Phytochemical screening of various extracts of *Limoniastrum feei*

Extraction Solvent	Parts	Constituents					
		Flavonoids	Rf	Tannins	Rf	Coumarins	Rf
Hex	Aerial part	+	0.95	-	-	-	-
	Roots	+	0.00 0.54	-	-	-	-
EtOAc	Aerial part	+	0.00 0.35	+	0.00 0.85 0.95	+	0.95
	Roots	+	0.00 0.6	+	0.00 0.81	+	0.00
DCM	Aerial part	-	-	+	0.95	-	-
	Roots	-	-	+	0.00 0.54	-	-
MeOH	Aerial part	+	0.00 0.35 0.90	-	-	-	-
	Roots	+	0.81 0.93	-	-	-	-

Hex: hexane; EtOAc: ethyl acetate; DCM: dichloromethane; MeOH: methanol; Hep: heptane; Rf: rapport frontal; +: positive; -: negative

The phytochemical screening of two parts of *L. feei* on flavonoids, tannins, and coumarins, show the presence of flavonoids in ethyl acetate, hexane and methanol extract and total absence in dichloromethane, but the tannins derivative are present in all parts. It is also observed that the extracts of ethyl acetate are the richest in tannins and coumarins by contribution to other extracts e.g., methanolic and hexane extracts.

The revelation of the alkaloids on the TLC plate provided Table 3.

According to Table 3, we observe: Colors change in most parts of the spots; we can say that these are alkaloids or aromatic hydrocarbons. The different colors are shown in all the extracts (MeOH, DCM, Hex, and EtOAc).

Table 3. Characterization of alkaloids on the TLC plate

Extraction Solvent	Parts	Spots number	Rf	Color
Hex	Aerial part	5	00	Light Yellow
			0.57	Light Brown
			0.85	Green
			0.90	Brown
			0.95	Green
	Roots	5	00	Yellow
			0.54	Purple
			0.81	Brown
			0.90	Purple
			0.93	Light Brown
EtOAc	Aerial part	6	00	Brown
			0.35	Light Purple
			0.57	Purple
			0.85	Light Brown
			0.90	Brown
			0.95	Green
	Roots	5	00	Brown
			0.60	Light Brown
			0.81	Dark Purple
			0.90	Dark Brown
			0.96	Light Brown
DCM	Aerial part	6	00	Brown
			0.35	Brown
			0.57	Green
			0.85	Brick red
			0.90	Light Green
			0.95	Green
	Roots	5	00	Brown
			0.54	Light Brown
			0.81	Light Purple
			0.90	Brown
			0.93	Brown
MeOH	Aerial part	3	00	Brown
			0.35	Yellow
			0.90	Green
	Roots	3	00	Pink
			0.81	Light Pink
			0.93	Yellow

Hex: hexane; EtOAc: ethyl acetate; DCM: dichloromethane; MeOH: methanol; Rf: rapport frontal.

A previous study by Rahmani et al. (2014) reported the presence of eleven polyphenol compounds in the extract of the aerial part of *L. feei*. In another study, two molecules of the saponins family were extracted and identified by Belboukhari and Cheriti (2009) (Figure 1), from the stems of *L. feei*, namely:

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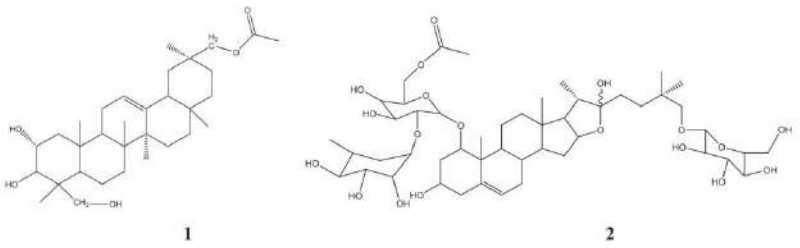

Figure 1. Saponins from the stems of *L. feei* (1 and 2) (Belboukhari & Cheriti, 2009).
1: 2α,3 β,23-Trihydroxy-30-acetylolean-12-ene ($C_{32}H_{52}O_5$)
2:1-O-[α-L-Rhamnopyranosyl-(1)-6-O-acetylβ -D-galactopyranosyl]-1 β,3 β,22 ξ − 26-tetrahydroxyfurost-5(6)-ene-26-Oβ -D-glucopyranosid($C_{50}H_{82}O_{19}$).

2α,3 β,23-Trihydroxy-30-acetylolean-12-ene (1) ($C_{32}H_{52}O_5$) and 1-O-[α -L-Rhamnopyranosyl-(1)-6-O-acetylβ -D-galactopyranosyl]-1 β,3 β,22 ξ − 26-tetrahydroxyfurost-5(6)-ene-26-Oβ -D-glucopyranosid (2) ($C_{50}H_{82}O_{19}$).

3.3. Antifungal activity of the plant extracts

3.3.1. Direct bioautography

Frequently, TLC-Direct Bioautography is used as a bio-guiding method to destine to substances with biological activity that can be further analyzed by spectroscopic methods to obtain information on their structure (Choma & Jesionek, 2015).

Strong growth inhibition against *Fusarium oxysporum* f. sp. *albedinis* has been shown by a methanolic extract that was assayed using direct bioautography on the TLC plate (Table 4).

3.3.2. The cellulases inhibition by contact bioautography

The results of the cellulases inhibition are presented in Table 5. Various parameters are to be taken into account for good comprehension and evaluation of these results. For the tests carried out by TLC, the evaluation of effects is made by comparison between extracts.

4. Discussion

Bioautography is notably significant to avoid the time-consuming isolation of inactive compounds (Dewanjee, Gangopadhyay, Bhattacharya, Khanra, & Dua, 2015). TLC bioautographic methods combine chromatographic separation and in situ activity determination facilitating the localization and target-directed isolation of active constituents in a mixture (Suleiman, McGaw, Naidoo, & Eloff, 2010). In this study, the evaluation of *L. feei* extracts on the causal agent of Bayoud "*Fusarium oxysporum* f. sp. *albedinis*" (Foa), was realized using new principles added to contact bioautography. Some studies made at the Phytochemistry and Organic Synthesis Laboratory (POSL, Bechar

Table 4. Result of the direct bioautography of the plant extracts

Eluent	Extraction Solvent	Parts	Antifungal effect
EtOAc: Hep 75:25	Hex	Aerial part	-
		Roots	+
	EtOAc	Aerial part	+
		Roots	+
	DCM	Aerial part	-
		Roots	-
	MeOH	Aerial part	+
		Roots	+

Hex: hexane; EtOAc: ethyl acetate; DCM: dichloromethane; MeOH: methanol; Hep: heptane; Rf: rapport frontal.

Table 5. Effect of *L. feei* extracts on *Fusarium oxysporum* f. sp. *albedinis* cellulases using contact bioautography

Eluent	Extraction Solvent	Parts	Number of IZ	Inhibition zone	
				Rf	Ø
EtOAc: Hep 75:25	Hex	Aerial part	00	-	-
		Roots	01	0.54	2
	EtOAc	Aerial part	01	0.35	2
		Roots	00	-	-
	DCM	Aerial part	00	-	-
		Roots	00	-	-
	MeOH	Aerial part	01	00	5
		Roots	00	-	-

IZ: Inhibition zone; Hex: Hexane, EtOAc: Ethyl acetate, DCM: Dichloromethane, MeOH: Methanol; Hep: Heptane; **Ø**: diameter in mm

University, Algeria) have demonstrated that this plant contains secondary metabolites with biological activities (Belboukhari & Cheriti, 2005, 2007, 2009).

Three extracts of *L. feei* are revealed active on cellulases with the appearance of the dark zones. The methanol extract of the aerial part of *L. feei* showed a remarkable result with inhibition zone diameter greater than 2 mm. Indeed, the dichloromethanic extracts showed no antifungal activity on Foa.

In this study, the tests were carried out using crude extracts; then a positive result is the component of two parameters: the intrinsic activity of the active products and their relative quantity in the extract. Among the 8 tests carried out, only one extract presented a good effect on the cellulases of Foa (Ø > 2mm). These results prove the resistance of Foa against the extracts of *L. feei*. Boulenouar, Marouf, Cheriti, and Belboukhari (2012) determined this resistance against several treatments (Boulenouar et al., 2012).

El Hassni et al. (2007) revealed that the strategies of the fight against Foa are very limited that proves its high resistance (El Hassni et al., 2007). Several studies showed that the studied plant contains several secondary metabolites very significant with pharmacological properties (Belboukhari & Cheriti, 2005, 2009; Boulenouar et al., 2014). Belboukhari and Cheriti (2009) showed by spectroscopic analyses the presence of the saponins in the leaves and the stems of *L. feei*. This plant is rich in phenolic compounds. The antimicrobial activity of *L. feei* extract was studied by Belboukhari and Cheriti (2005); the methanolic extracts of *L. feei* leaves were revealed active against certain bacteria and two yeasts. By comparing these findings with the obtained results, the methanolic extracts of the aerial part gave a remarkable result (Ø > 2 mm) and confirm the presence of antimicrobial compounds in *L. feei*.

The analytical investigations reveal that the ethyl acetate extract of the aerial part of *L. feei* showed the highest phenolic compounds. In addition, the presence of flavonoids in *L. feei* (Belboukhari & Cheriti, 2007; Rahmani et al., 2014) may be responsible for the antifungal activity.

Belboukhari and Cheriti (2005) revealed no effect of ethyl acetate extract from leaves and twigs of *L. feei* on two fungi: *Candida albicans* and *Saccharomyces cerevisiae*. The difference with our results is possibly due to the difference between species biology (*Foa, C. albicans* and *S. cerevisiae*) and/or to the difference between plants parts used for extraction.

The effect of the extracts used in this study was shown by Boulenouar et al. (2014) but by the disc diffusion technique. The results of the effect against Foa showed that these extracts have a detectable effect at least in two tests (Boulenouar et al., 2014). Confirming the presence of

antifungal substances despite the difference between the techniques used. The significant effect of the various parts of the plant can be related to the difference in the components present in the various parts. This difference can depend on the chemical nature of the substances or on the mechanism of action. There are substances with antifungal activity against Foa but not on its cellulases, this reflects the complexity of pathogenicity mechanism. On the other hand, substances inhibiting cellulases play a crucial role in antifungal activity against Foa because of importance of these enzymes in invading date palm through roots.

By comparing the results obtained using contact bioautography on the cellulases of Foa and the other results using other techniques. Inhibition of cellulases activity is not always related to the inhibition of the growth. Foa produces several toxins; these mycotoxins play a very significant role in the pathogenicity of this fungus. Therefore the different researches carried out previously against Foa reveals active substances that influence one or more of these mycotoxins by the modification of their metabolism or their effects (Boulenouar, Marouf, & Cheriti, 2009; El Hadrami, El Idrissi-Tourane, El Hassni, Daayf, & El Hadrami, 2005).

Phytochemical analysis was intended to know which metabolite is responsible for the antifungal activity. According to the TLC profiling, we can suppose that inhibition is caused by flavonoids present in ethyl acetate and methanolic extracts of aerial parts of *L. feei* (Rf: 0.35, 00) respectively and in hexanic extract of roots of *L. feei* (Rf: 0.54).

Belboukhari and Cheriti (2007) contributed to the study of the characterization of flavonoids extracted from the stems of *L. feei*; they identified four flavonoids (Figure 2). We can suppose that one or more of these four flavonoids have antifungal activity against Foa and its cellulases.

From the results obtained by El Haci, Mazari, Atik-Bekkara, Hassani, and Gherib (2017), polar organic extracts: methanol, ethanol, and acetone of *L. feei* presented considerable levels of phenols and flavonoids. These levels ranged from 127.07 to 262.11 mg GAE/g and 84 to 157.88 mg CEQ/g, respectively (El Haci et al., 2017).

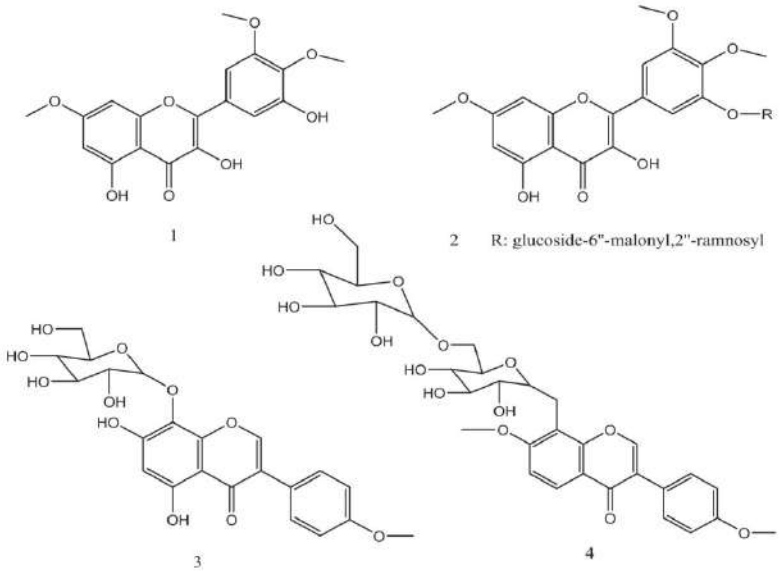

Figure 2. Flavonoids from the stems of *L. feei* (1–4) (Belboukhari & Cheriti, 2007).
(1): 6, 3′, 4′-Tri-methoxy 3, 5, 5′-trihydroxy flavonol.
(2): 3-(6"-malonyl 2"-ramnosyl glucosil) 6, 3', 4'-tri-methoxy 5, 5'-dihydroxy flavonol.
(3): Tetraacetate 7-dihydroxy-4'-Methoxy 8-O-β-glucopyranoside isoflavone.
(4): Tetraacetate 7, 4'-diMethoxy 8-O-β-glucopyranoside isoflavone.

The flavonoids are a group of substances, which are responsible for the inhibition of many enzymes. The phytochemical screening showed a variety of phytoconstituents with the prevalence of phenolic compounds (El Haci et al., 2017). Various studies were carried out on the structure-activity relationship of different polyphenols in a glance with their antifungal activity, but in spite of their great number, this relationship is not really clear (Munari, 2006).

The absence of an effect for an extract tested on a given biological target always does not exclude the presence of active substances (case of synergy). In certain cases also, the concentration of these substances can be very weak so that we can detect their activity on TLC plates.

Recently, the fungicidal activities of plant extracts have been extensively reported (Balouiri, Sadiki, & Ibnsouda, 2016). However, little research has been performed on the fungicidal activity of *L. feei* extracts on the pathogen *F. oxysporum*, which poses the greatest threat to date palm by causing Fusarium wilt.

The potential medicinal uses of *L. feei* are supported by the presence of the above-mentioned phenolics and flavonoids activities.

5. Conclusions

Based on our study, *L. feei* could be used as a source of fungicides for the control of Foa. This study indicated that the extracts of *L. feei* not only demonstrated antifungal activity against *Fusarium oxysporum* f. sp. *albedinis*, but also could inhibit the cellulases enzymes of this phytopathogen. The results obtained shed light on the possibility to use some of these extracts (representing the best effects) against Foa by proceeding with further advanced studies. The antifungal activity of *L. feei* is strongly correlated with the qualitative and quantitative variations in their phenolic constituents.

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Competing Interests
The authors declares no competing interests.

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References

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. doi:10.1016/j.jpha.2015.11.005

Belboukhari, N., & Cheriti, A. (2005). Antimicrobial activity of aerial part crude extracts from Limoniastrum feei. *Asian Journal of Plant Sciences*, *4*(5), 496–498. doi:10.3923/ajps.2005.496.498

Belboukhari, N., & Cheriti, A. (2007). Flavonoids of Limoniastrum feei. *Research Journal of Phytochemistry*, *1*(2), 74–78. doi:10.3923/rjphyto.2007.74.78

Belboukhari, N., & Cheriti, A. (2009). Analysis and isolation of saponins from Limoniastrum feei by LC-UV. *Chemistry of Natural Compounds*, *45*(5), 756. doi:10.1007/s10600-009-9455-2

Benabdelkader, M., Malek, A., & Draoui, B. (2011). Perspective du pompage éolien appliqué à l'irrigation du palmier dattier dans la région de Béchar. *Revue des Energies Renouvelables*, *14*(3), 381–395.

Boulenouar, N., Marouf, A., & Cheriti, A. (2009). Effect of some poisonous plants extracts on Fusarium oxysporum f. sp. albedinis. *Journal of Biological Sciences*, *9*(6), 594–600. doi:10.3923/jbs.2009.594.600

Boulenouar, N., Marouf, A., & Cheriti, A. (2011). Antifungal activity and phytochemical screening of extracts from Phoenix dactylifera L. cultivars. *Natural Product Research*, *25*(20), 1999–2002. doi:10.1080/14786419.2010.536765

Boulenouar, N., Marouf, A., & Cheriti, A. (2014). Direct bioautography for antifungal. Measurement "case of bayoud disease. *PhytoChem & BioSub Journal*, *8*, 33–37.

Boulenouar, N., Marouf, A., Cheriti, A., & Belboukhari, N. (2012). Medicinal plants extracts as source of antifungal agents against fusarium oxysporum f. sp. albedinis. *Journal of Agricultural Science and Technology*, *14*, 659–669.

Choma, I., & Jesionek, W. (2015). TLC-direct bioautography as a high throughput method for detection of antimicrobials in plants. *Chromatography*, *2*(2), 225–238. doi:10.3390/chromatography2020225

Dewanjee, S., Gangopadhyay, M., Bhattacharya, N., Khanra, R., & Dua, T. K. (2015). Bioautography and its scope in the field of natural product chemistry. *Journal of Pharmaceutical Analysis*, *5*(2), 75–84. doi:10.1016/j.jpha.2014.06.002

El Haci, I. A., Mazari, W., Atik-Bekkara, F., Hassani, F., & Gherib, M. (2017). Assessment of the cytotoxic activity and the reduction power of limoniastrum feei girard (Batt.): A medicinal plant from Algerian Sahara. *Oriental Pharmacy and Experimental Medicine*, *17*(2), 143–150. doi:10.1007/s13596-017-0270-1

El Hadrami, A., El Idrissi-Tourane, A., El Hassni, M., Daayf, F., & El Hadrami, I. (2005). Toxin-based in-vitro selection and its potential application to date palm for resistance to the bayoud Fusarium wilt. *Comptes Rendus Biologies*, *328*(8), 732–744. doi:10.1016/j.crvi.2005.05.007

El Hassni, M., El Hadrami, A., Daayf, F., Chérif, M., Barka, E. A., & El Hadrami, I. (2007). Biological control of bayoud disease in date palm: Selection of microorganisms inhibiting the causal agent and inducing defense reactions. *Environmental and Experimental Botany*, *59*(2), 224–234. doi:10.1016/j.envexpbot.2005.12.008

El Modafar, C., Tantaoui, A., & El Boustani, E. (2000). Effet de L'acide Caféoylshikimique des Racines du Palmier Dattier sur L'activité et la Production des. *Phytopathologische Zeitschrift*, *148*(2), 101–108. doi:10.1046/j.1439-0434.2000.00472.x

Freeman, S., & Maymon, M. (2000). Reliable detection of the fungal pathogenfusarium oxysporum f. sp. albedinis, causal agent of bayoud disease of date palm, using molecular techniques. *Phytoparasitica*, *28*(4), 341–348. doi:10.1007/BF02981829

Kasana, R. C., Salwan, R., Dhar, H., Dutt, S., & Gulati, A. (2008). A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Current Microbiology*, *57*(5), 503–507. doi:10.1007/s00284-008-9276-8

Keffous, F., Belboukhari, N., Sekkoum, K., Djeradi, H., Cheriti, A., & Aboul-Enein, H. Y. (2016). Determination of the antioxidant activity of Limoniastrum feei aqueous extract by chemical and electrochemical methods. *Cogent Chemistry*, *2*(1), 1186141. doi:10.1080/23312009.2016.1186141

Kettout, T. A., & Rahmania, F. (2010). Identification par CG-SM de l'acide phénylacétique produit par Fusarium oxysporum f. sp. albedinis, agent causal du bayoud. *Comptes Rendus Biologies*, *333*(11–12), 808–813. doi:10.1016/j.crvi.2010.10.001

Munari, C. (2006). *Investigation phytochimique de plantes alpines: Etude d'espèces du genre Oxytropis (Fabaceae) et isolement de composés antifongiques et antiradicalaires à partir d'Oxytropis fetida (Vill.) DC., Potentilla grandiflora L. (Rosaceae) et Vaccinium uliginosum ssp.* University of Geneva. Retrieved from https://nbn-resolving.org/urn:nbn:ch:unige-33548

Pascual, M., Carretero, M., Slowing, K., & Villar, A. (2002). Simplified screening by TLC of plant drugs. *Pharmaceutical Biology*, *40*(2), 139–143. doi:10.1076/phbi.40.2.139.5849

Rahmani, S., Belboukhari, N., & Cheriti, A. (2014). Phytochemical investigation of bioactive extract from endemic medicinal plant Limoniastrum feei (Girard) Batt (Plumbaginaceae). *Asian Journal of Chemistry*, *26*(2), 365. doi:10.14233/ajchem.2014.15395

Suleiman, M. M., McGaw, L., Naidoo, V., & Eloff, J. (2010). Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *African Journal of Traditional, Complementary and Alternative Medicines*, *7*, 1. doi:10.4314/ajtcam.v7i1.57269

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REVIEW ARTICLE

The Use of Natural Products against *Fusarium oxysporum*: A Review

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Abstract: Currently, numerous researchers have focused their attention on the use of natural products from plants and microorganisms, or compounds derived from these organisms to protect crops from various diseases, thus reducing the negative effects on human health and environmental safety.

Fungal diseases cause a considerable loss of crop yields in agricultural industries worldwide. Fusarium oxysporum comprises a multitude of *formae speciales* that cause vascular wilt diseases of economically important crops.

This review presents an overview of researches realized on natural products tested against *Fusarium oxysporum formae speciales* phytopathogens for the period (2017-2020).

This review aims to collect major research works of the antifungal compounds against these fungi and up-dates information on their developments and approaches that have been rapidly taking place in recent years so that further novel researches can be envisaged.

This review discussed these studies by analyzing different sources for antifusariosis treatment, evaluation of testing methods, and information on their advantages and limitations and to determine those with real efficacy. Despite the important number of natural products with remarkable *in vitro* efficiency, the limiting point is their *in vivo* application for soil microorganisms (in this case: *Fusarium oxysporum*). Therefore, more advanced researches are needed to solve this problem.

Keywords: Antifungal activity, *Fusarium oxysporum,* natural products, bioactive compounds, antifungal techniques, plant disease, crops.

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