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Présentée par :

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Devant le jury composé de :

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Publicly presented and supported

By:

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DEDICATION

to the hopeless years gone by and the unknowns to be arrived

to the one who kneed me with notions and the one whose words rescued me every day

To my Parents: ***Mohamed Adnane & Dalila Benouakhir***

"I love you deeply with all my heart"

To my Brothers: ***Mohamed Riadh & Abelraouf***

"Both of you have been my best cheerleaders"

*To my Sister****: Raouia***

"I love you without measure"

To my Son: ***Ahmed Amine***

"You made me stronger, better and more fulfilled than I could have ever imagined. I love you to the moon "

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Abstract

Artemisia campestris L. and *Fagonia bruguieri* DC. are commonly used in folk medicine due to its antioxidant, anti-diabetic, anti-inflammatory, and neuroprotective properties.

Our study assessed the total phenolics and flavonoids contents, antioxidant, and pharmacological activities of various organic extracts prepared from the aerial parts of *Artemisia campestris* L. and *Fagonia bruguieri* DC. The two species were first collected from the mountainous Hoggar region in Tamanrasset town, southern Algeria. Furthermore, this study focuses on the isolation and structural elucidation of bioactive compounds originating from the polar extracts (n-Butanol) of *Artemisia campestris* L. and *Fagonia bruguieri* DC. aerial parts, as well as their mineral elements and chemical profile were analysed.

Two analytical techniques, namely inductively coupled plasma mass spectrometry (ICP-OES) and instrumental neutron activation analysis (INAA) were applied to determine the concentration of mineral elements. The phytochemical profiling in *A. campestris* L. and *F.bruguieri DC* was characterised using HPLC-UV and LC-MS/MS analysis. A variety of techniques are carried out to isolate secondary metabolites, including maceration, extraction, separation, and purification. Chromatographic strategies such as thin-layer chromatography (TLC), preparative thin-layer chromatography (PTLC), and chromatography column (CC) are utilised, with stationary phases including silica gel, reversed-phase C-18, and Sephadex LH-20. The compounds obtained were structurally identified using 1D and 2D NMR spectroscopic techniques.

An array of assays was conducted to evaluate the antioxidant properties of the samples, including DPPH, ABTS, beta-carotene, GOR free radical, reducing power, cupric reducing antioxidant capacity (CUPRAC), O-Phenanthroline assays and $O_2^{\bullet -}$ free radical scavenging assay.

The present investigation aimed to evaluate the potential inhibitory effects of *A. campestris* L. and *F.bruguieri* DC. extracts in vitro with regard to their anti-cholinesterase, anti-lipase, and anti-diabetic activities. The sun protection factor (SPF) of the plants was quantified to determine its photoprotective effect, whereas its anti-inflammatory potential was assessed through the protein denaturation technique.

Abstract

The findings of our study indicate that *A. campestris* L. and *F. bruguieri* DC.exhibit a notable abundance of essential minerals, encompassing both macro and micro elements (such as Ca, Na, Fe, Mg, Cu, Zn, Li, Mn, Sr, Ti, Ni, Co, Mo, Cd, Pb and Cr), with concentrations that are in close proximity to the recommended values established by the FAO. The results obtained from LC/MS-MS and HPLC-UV analyses indicated that the AcEAE and FbBE extracts are the richest in phenolic acids and flavonoids. The investigation of chemical compounds in AcBE and FbBE led to the identification of three compounds, consisting of two derivatives of phenolic acid and one polysaccharide, all of which were characterised.

AcPEE and FbDE have been discovered as the most potent inhibitors of alpha-amylase. The extracts AcEAE, AcBE, and FbBE exhibited noteworthy inhibitory effects on both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In terms of anti-lipase activity, AcEAE and FbDE exhibited potent inhibition of the pancreatic lipase enzyme. The anti-inflammatory efficacy of AcBE and FbBE was found to be important. *A. campestris* L. and *F.bruguieri* DC.were found to have a robust photoprotective ability, absorbing UV radiations with SPF values ranging from 11.25±0,1 to 43,86±0,12. The findings demonstrate that polar extracts displayed remarkable antioxidant activity across multiple systems, with AcEAE and FbBE exhibiting the most optimal performance, except for the $O_2^{\bullet -}$ free radical scavenging assay, where AcEA and FbAE exhibited boosted antioxidant activity.

Due to their compositions and potent biological properties, *A. campestris* L. and *F.bruguieri* DC. have the potential to prove pharmaceutical formulations, medical research, and health.

Keywords: *A. campestris* L.; *F.bruguieri DC.*; antioxidant activity; Alzheimer's disease; LC-MS/MS, INAA; secondary metabolite.

Résumé

Artemisia campestris L. et *Fagonia bruguieri* DC.sont deux espéces couramment utilisées en médecine traditionnelle en raison de leurs propriétés antioxydantes, antidiabétiques, anti-inflammatoires et neuroprotectrices.

L'objectif principal de ce travail est l'étude phytochimique, pharmacologique des différents extraits des parties aériennes d'*Artemisia campestris* L. et de *Fagonia bruguieri* DC. Les deux espèces ont d'abord été collectées dans la région montagneuse du Hoggar dans la ville de Tamanrasset, au sud de l'Algérie. L'analyse quantitative des extraits de plantes a été évaluée à l'aide des méthodes colorimétriques, ainsi que les activités antioxydantes et pharmacologiques de divers extraits organiques d'*Artemisia campestris* L. et de *Fagonia bruguieri* DC. De plus, cette étude a porté sur l'isolement et l'élucidation structurale de composés bioactifs provenant des extraits polaires (n-Butanol) d'*Artemisia campestris* L. et de *Fagonia bruguieri* DC. ainsi que leur analyse qualitative et quantitative , et la mesure de leurs concentration en éléments minéraux.

Deux techniques analytiques, à savoir la spectrométrie de masse à plasma à couplage inductif (ICP-OES) et l'analyse instrumentale par activation neutronique (INAA) ont été appliquées pour mesurer la concentration des éléments minéraux. Dans le but d'évaluer la composition chimique d'*A. campestris* L. et *F.bruguieri* DC. l'analyse quantitative et qualitative a été caractérisée par l'analyse HPLC-UV et LC-MS/MS.

Afin d'isoler les métabolites secondaires, on a procédé à la macération, l'extraction, la séparation et la purification. Des techniques chromatographiques telles que la chromatographie sur couche mince (TLC), la chromatographie préparative sur couche mince (PTLC) et la chromatographie sur colonne (CC) ont t'utilisées, avec des phases stationnaires comprenant du gel de silice, du RP C-18 et du Sephadex LH-20. Les composés isolés ont été structurellement identifiés à l'aide de techniques spectroscopiques RMN 1D et 2D.

Une série des tests a été menée pour évaluer les propriétés antioxydantes des différents extraits, y compris DPPH, ABTS, bêta-carotène, GOR, pouvoir réducteur, CUPRAC, *O*-Phenanthroline et $O_2^{\bullet-}$ par voie électrochimique.

En outre, La présente étude visait à évaluer les effets inhibiteurs potentiels de *A. campestris* L. et *F.bruguieri* DC. in-vitro en ce qui concerne leurs activités Anti-cholinestérase, Anti-lipase et Anti-diabétique. Le facteur de protection solaire (FPS) des plantes a été quantifié

pour déterminer leurs effets photo-protecteur, tandis que leur potentiel anti-inflammatoire a été évalué par la technique de dénaturation des protéines.

Les résultats de notre étude indiquent que *A. campestris* L. et *F. bruguieri* DC.présentent une abondance notable de minéraux essentiels, englobant à la fois des éléments macro et micro-nutritions (tels que Ca, Na, Fe, Mg, Cu, Zn, Li, Mn, Sr, Ti, Ni, Co, Mo, Cd, Pb et Cr), avec des concentrations proches des valeurs recommandées établies par la FAO. Les résultats obtenus à partir des analyses LC/MS-MS et HPLC-UV ont indiqué que les extraits AcEAE et FbBE sont les plus riches en acides phénoliques et en flavonoïdes. L'étude de la composition chimique dans AcBE et FbBE a conduit à l'identification de trois composés, constitués de deux dérivés d'acide phénolique et d'un polysaccharide.

AcPEE et FbDE ont montrés l'effet inhibiteur le plus puissant contre l'alpha-amylase. Les extraits AcEAE, AcBE et FbBE ont révélé des effets inhibiteurs notables sur l'acétylcholinestérase (AChE) et la butyrylcholinestérase (BChE). En termes d'activité anti-lipase, AcEAE et FbDE ont présenté une puissante inhibition de l'enzyme lipase pancréatique. L'efficacité anti-inflammatoire de l'AcBE et du FbBE s'est avérée importante. *A. campestris* L. et *F.bruguieri* DC.se sont avérés avoir une capacité photoprotectrice robuste, absorbant les rayonnements UV avec des valeurs SPF allant de 11,25 ± 0,1 à 43,86 ± 0,12. Les résultats montrent que les extraits polaires présentent une activité antioxydante importante dans plusieurs systèmes, AcEAE et FbBE montrant les performances les plus optimales,except lors du test de piégeage des radicaux libres $O_2^{\bullet-}$, où AcEA et FbAE présentent une activité antioxydante très forte. En raison de la compositions chimiques et de la puissances des propriétés biologiques, d'*A.campestris* L. et *F.bruguieri* DC. ont le potentiel d'améliorer les formulations pharmaceutiques, la recherche médicale et la santé.

Mots clés : *F.bruguieri* DC.; *A. campestris* L.*;* activité antioxydante ; AChE; LC-MS/MS, INAA; métabolite secondaire.

ملخص

تستخدم النبتتان المختارتان *Artemisia campestris* L. و *Fagonia bruguieri* DC. للدراسة بشكل شائع في الطب التقليدي نظرًا لخصائصهما المضادة للأكسدة والسكري والالتهابات والوقاية العصبية.

الهدف من هذا العمل هو الدراسة الكيميائية النباتية والفعالية الدوائية (الفارماكولوجية) و كذلك المحتوى الفينولى و الفلافونويدى للمستخلصات العضوية المختلفة المحضرة من الأجزاء الهوائية للنبتتين من الصحراء الجزائرية , اين تم جمع النوعين لأول مرة من المنطقة الجبلية الهقار لمدينة تمنراست، جنوب الجزائر. علاوة على ذلك، تركز هذه الدراسة على الفصل والكشف عن البني للمركبات الفعالة بيولوجيًا المستخلصة من المستخلصات القطبية (n- بيوتانول) من *Artemisia campestris* L.و *Fagonia bruguieri* DC. بالإضافة الى دراسة التحليل النوعي لمختلف المستخلصات للنبتتين وكذلك العناصر المعدنية والملف الكيميائي.

طبقت تقنيتين تحليليتين ، وهما مقياس الطيف الكتلي للبلازما المقترن بالحث (ICP-OES) وتحليل التنشيط النيوتروني (INAA) لتحديد تركيز العناصر الكيميائية المعدنية. كما اجري التحليل الكمى و النوعى بهدف تحديد التركيب الكيميائي للنبتتين في *A. campestris* و *F.bruguieri* باستخدام HPLC-UV و LC-MS / MS. قمنا بتطبيق العديد من التقنيات الكروماتوغرافية لعزل منتجات الايض الثانوية، بما في ذلك النقع، الاستخلاص، الفصل، والتنقية اين استخدامت تقنيات مختلفة مثل عمود هلام السيليكا، عمود C18 ، وكروماتوغارفيا الطبقة الرقيقة التحليلية والتحضيرية. حددت البنى الكيميائية للمركبات التي تم فصلها و الكشف عنها باستخدام تقنيات طيف الرنين المغناطيسي أحادي وثنائي البعد. اجريت مجموعة من الاختبارات لتقييم الخصائص المضادة للأكسدة للعينات، بما في ذلك β-caroténe , DPPH , ABTS , CUPRAC, GOR, Phenanthroline و $O_2^{\bullet-}$.

هدفت هذه الدراسة ايضا إلى تقييم التأثيرات المثبطة المحتملة لمستخلصات *A. campestris* L. و *F.bruguieri DC.* فيما يتعلق بأنشطتها Anti-cholinesterase, Anti-lipase ومضاد السكري. بالإضافة إلى ذلك، تطرقنا الى قياس عامل الحماية من الشمس (SPF) للنباتات لتحديد تأثيرها الواقي من الضوء، في حين قمنا بتقييم إمكاناتها المضادة للالتهابات من خلال تقنية تمسخ البروتين.

سمحت الدراسة فيما يخص المحتوى المعدني للنبتتين التي تم تحديدها بطريقتان هما :INAA وICP-OES ، أنهما غنيتان بالمعادن الضرورية للكائن الحى، (مثل Ca و Na و Fe و Mg و Cu و Zn و Li و Mn و Sr و Ti و Ni و Co و Mo و Cd و Pb و Cr) .بالإضافة إلى ذلك، كان تركيز العناصر السامة أقل بكثير من القيم المرجعية للسمية المحددة من طرف المنظمة للصحة العالمية. الدراسة الفيتو كيميائية للمستخلصين AcBE و FbBE باستخدام مختلف الطرق الكروماتوغارفيا، مكنتنا من تحديد ثلاث مركبات ، تتكون من 2 مركبات فينولية و 1 متعدد السكاريات.

اظهر كل من المستخلصين AcPEE و FbDE على أنهما أكثر فاعلية اتجاه تثبيط ألفا أميلاز. كما تميزت المستخلصات AcBE AcEAE و FbBE بتأثيرات مثبطة على كل من Acetylcholinesterase (AChE) و Butyrylcholinesterase (BChE). فيما يخص النشاط المضاد لليباز، أظهر AcEAE و FbDE تثبيطًا قويًا لإنزيم الليباز البنكرياس. اما المستخلصات AcBE و FbBE أثبتت فعالية جد مهمة كمضادات للالتهابات. للنبتتين قدرة قوية للحماية من الأشعة فوق البنفسجية بقيم SPF تتراوح من 0.1±11.25 إلى0.12± 43،86. واوضحت النتائج

ملخص

للمستخلصات القطبية نشاطًا مضادًا للأكسدة ملحوظًا من خلال استعمال أنظمة متعددة، حيث أظهر AcEAE و FbBE فعالية كبيرة، باستثناء $O_2^{\bullet-}$ حيث أظهر AcEA و FbAE نشاطًا مضادًا للأكسدة عالى.

نظرًا للنتائج المتحصل عليها والخصائص البيولوجية القوية و التى اثبتت ان للنبتتين: *A. campestris* و *F.bruguieri* القدرة على تحسين التركيبات الصيدلانية ، والبحوث الطبية ، والصحة.

الكلمات المفتاحية: *A. campestris L; F.bruguieri DC.* ;نشاط مضاد للأكسدة ;;AchE; LC-MS / MS ; ;INAA; منتجات الايض الثانوية.

Chapter III: Extraction, Chemical Composition, Purification and Separation

Chapter IV : Biological Activities

ABBREVIATIONS AND SYMBOLS

1D	one-dimensional
2D	two-dimensional
ABTS	2,2`-azinobis-(3-ethylbenzothiazolin-6-sulfonic)
AcAE	*Artemisa campestris* Aqueous Extract
AcBE	*Artemisa campestris* Butanol Extract
AcDE	*Artemisa campestris* Dichloromethane Extract
AcEAE	*Artemisa campestris* Ethyl Acetate Extract
AchE	Acetylcholine
AcME	*Artemisa campestris* Methanol Extract
AcPEE	*Artemisa campestris* Petroleum ether Extract
BchE	Butyrylcholinesterase
BHA	ButylHydroxyAnisol
BHT	ButylHydroxyToluen
BSA	Bovine serum albumin
COSY	correlated spectroscopy
CUPRAC	Cupric Reducing Antioxidant Capacity
d	Doublet
dd	Doublet of Doublet
DEPT	Distortionless Enhanced Polarization Transfer
DPPH	1,1-Diphényl-2-PicrylHydrazyl
DTNBA	5,5'-dithio-bis(2-nitrobenzoic) acid
FbAE	*Fagonia bruguieri* Aqueous Extract
FbBE	*Fagonia bruguieri* Butanol Extract
FbDE	*Fagonia bruguieri* Dichloromethane Extract
FbEAE	*Fagonia bruguieri* Ethyl Acetate Extract
FbPEE	*Fagonia bruguieri* Petroleum Ether Extract
HMBC	Heteronuclear Multiple Bond Coherence
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	Concentration Inhibitory at 50%
ICP-OES	Inductively coupled plasma - optical emission spectrometry
INAA	Instrumental neutron activation analysis
J (Hz)	Coupling constant (Hertz)

LC-MS/MS	Liquid Chromatography-Mass/Mass Spectrometry
m	Multiplet
m/z	Mass to charge ratio
NMR	Nuclear Magnetic Resonance
RP C-18	Reversed-phase C-18 column
SPF	Sun Protective Factor
TLC	Thin-layer chromatography
WHO	World health organization
δ (ppm)	Chemical shift
U	Unit
SF	Sub-fractions

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GENERAL INTRODUCTION

Introduction

In recent years, multiple fields, including the valorisation of the use of plants in natural remedies and scientific research that enriches medical knowledge, have contributed to the allure of returning to nature. In fact, the treatment with plants, which had lost the upper hand in the therapeutic arsenal since the dawn of the pharmaceutical industry, is currently being enhanced due to recent advances in chemical research.

Only half of the world's 500,000 plant species have been identified by botanists. The World Health Organisation (WHO) has catalogued 22,000 distinct plant species that are traditionally utilised in medical treatment. Only 3,000 have been subjected to scientific assessment [1]. According to the WHO, nearly 6,377 plant species are utilised in Africa, of which over 400 are medicinal plants that account for 90% of traditional medicine [2].

Medicinal plants contain a vast array of biomolecules that are of significant interest for the development of pharmaceuticals against a variety of pharmacological targets. For millennia, humans have used substances derived from plants to prevent or treat many kinds of illnesses. A broad spectrum of compounds derived from plants is relevant to a wide range of disorders affecting humans, including analgesics (aspirin, morphine extracted from opium, codeine, etc.), anti-cancer drugs (vinblastine, taxol, etc.), anaesthetics (tropane-ring alkaloids including cocaine), cardio-stimulants (digitalis), psychostimulants (caffeine, theophylline), antimalarial drugs (quinine, artemisinin, etc.), and immunosuppressants (cyclosporine, etc.) etc… [3]. Actually, secondary metabolites have been and will continue to be the focus of a great deal of in vitro and in vivo study, which includes searching for novel naturally occurring components such as phenolic compounds [4].

The Algerian Sahara is known for its extended floral variation. There are 650 species in the Algerian Sahara, 162 of which are endemic; endemism constitutes about 25% of the species. This vast floristic richness has helped advance scientific study in Algeria in a variety of fields, including ethnomedicine, traditional pharmacopoeia, and the valorisation of natural substances. Species in this environment usually offer molecules with intriguing biological activities [5].

Within this context, and in continuation with our axis's research program on Algerian Saharan plants, this work aims to research and characterize novel compounds with biological activity. Thus, two plants native to Algerian Sahara, one belonging to the family Asteraceae named *Artemisia campestris* L. and the other belonging to the family Zygophyllaceae named

Fagonia bruguieri DC. which were chosen for the purpose of researching their respective chemical compositions and the biological activities they engage in and determining the concentrations of different mineral elements in the studied plant's. In addition, to isolating and identifying their secondary metabolites. The selection of both species was selected for their respective endemism and pharmacopoeia interest. Interestingly, *Fagonia bruguieri* DC. has not been examined in terms of its chemical composition, pharmacological effects (anti-cholinesterase, anti-lipase, BSA denaturation, and dermatoprotector), as well as mineral profile. *Artemisia campestris* L., on the other hand, has yet to be previously investigated for its numerous pharmacological activities (lipase inhibitory and dermatoprotective) and mineral composition.

This present manuscript describes our work in three distinct parts:

PART 1: Bibliographical part contains two chapters

- Chapter I: Literature studies on the plants studies (family, genus, and species). It overviews their chemical composition, pharmacological uses, and phytotherapeutic uses.
- Chapter II: An overview of secondary metabolites and their pharmacological activity.

PART 2: Experimental part contains two chapters

- Chapter III: This chapter planned to include the phytochemical and chemical study of *Artemisia campestris* L. and *Fagonia bruguieri* DC.by presenting all experimental steps, starting with maceration, separation, and purification of isolated compounds using different chromatographic techniques, as well as protocols used for mineral and chromatographic analysis (LC-MS/MS, HPLC-UV, ICP-OES, INAA).
- Chapter IV: Protocols of the experimental biological evaluation (antioxidant, anti-lipase, anti-inflammatory, anti-cholinesterase, anti-alpha amylase, and dermatoprotector activities) and quantified the total phenol and flavonoid content of different extracts from two species studied.

PART 3: Results & Discussion contains two chapters

- Chapter V: The results and discussions in this chapter focus on the structural elucidation of isolated compounds using NMR 1D and 2D (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC) and chemical composition (Chromatographic and Mineral Analyses).
- Chapter VI: Results & Discussion of Quantitative analysis and biological activities.

PART 1:

BIBLIOGRAPHIC

PART

Chapter I: Literature Overview

I.1. Asteraceae

Asteraceae, commonly known as Compositae, the scientific name for this family is the Asteraceae Martynov (1820); it is also referred to as the Compositae Giseke(1792). It is one of the most prominent dicotyledonous angiospermic plant families based on the number of species (1500 genus and 13000 species) that comprise this cosmopolitan plant family [6].

Asteraceae, which includes approximately 10 % of the world's flowering plants, is typically divided into 12 subfamilies. About 109 genus of plants and more than 408 species promote plant growth in Algeria [7]. It includes edible, medicinal, harmful, invasive, and endangered species [6].

I.1.1 Botany of the family Asteraceae

A wide variety of morphological characteristics can be found in this family of Asteraceae, including evergreen shrubs, subshrubs, perennial herbs (rhizomatous and tuberous), annual herbs or biennials, giant herbs, epiphytes, and aquatic plants that are in extreme cases [8].

Vegetative apparatus

- ✓ **Roots:** typically pivotal and sometimes fibrous.
- ✓ **Stems**: generally straight but sometimes droop on rising. Some species have underground stems in the form of rhizomes; these can be fleshy or woody, depending on the species. In Asteraceae, there are different morphological variations of the stem:

  - Cylindrical stems like the Arnica of the Mountains, *Arnica montana*.

  - Angular stems as for *Jacobean Seneçon, Senecio jacobaea*.

  - Winged stems, with small wings on each side as for Black Thistle, *Carduus nigrescens*.

  - Fistulous stems, meaning hollow stems [9].

- ✓ **Leaves**: the arrangement is alternate, often appearing in a basal rosette formation, and the leaves are devoid of stipules, sometimes opposite, simple, pinnate or palmate, sessile cut, laciniated, and sometimes they can be succulent [10].

Producing apparatus

✓ **Flowers**: The arrangement of flowers unique to this plant family, with a single flower, called a capitulum, that looks like a head. The morphology related to the reproduction of tiny flowers clustered together in tight heads, called capitula, that give the appearance of single, enormous flowers. Flowers can be regular (actinomorphic), or they can be severely actinomorphic, in which case the corolla tube will be extended into a long, strap-shaped extension known as a ligula. There are three basic types of composite heads [11]:

- The flowers will be tube-shaped and regular (Figure.I.1).

- Exceptionally bilabiate, the flower will have two lips formed by the petals.

- With only one lip, the flowers are ligulated (Figure.I.1).

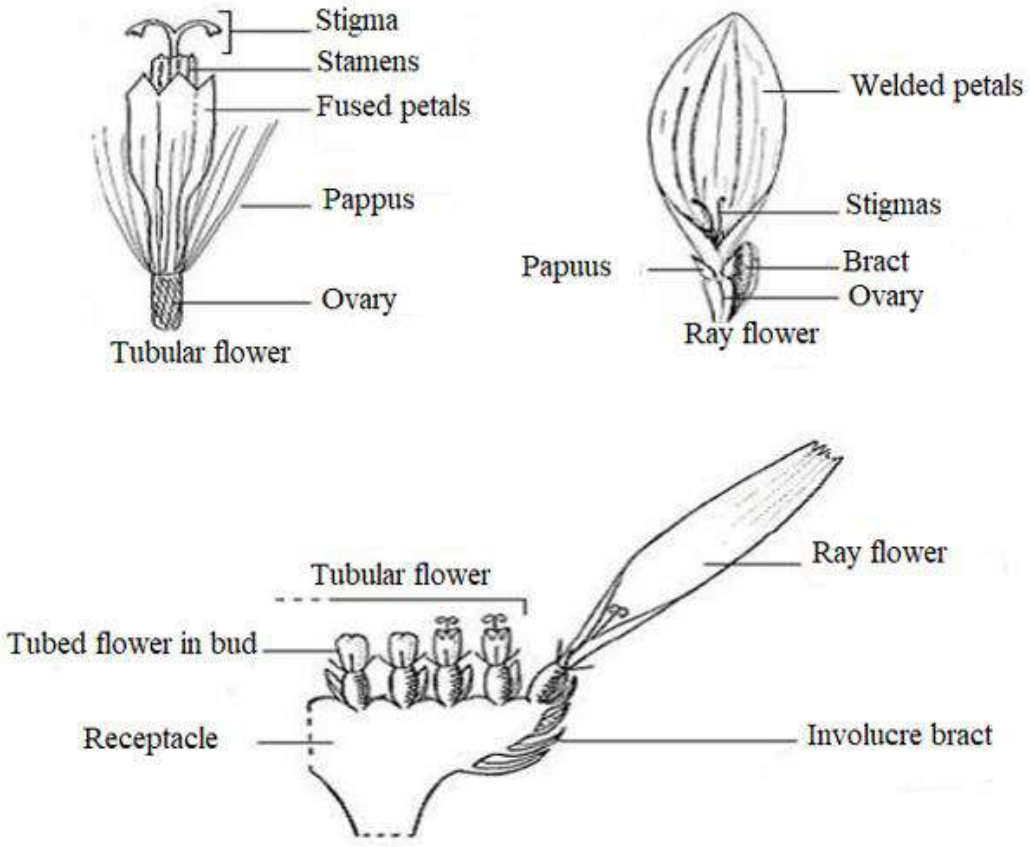


Figure.I.1. Different of some types of flowers [11].

✓ **Fruits:** Achenes are the most common type of Asteraceae fruit. It is considered that the particular design of the flowers and inflorescence promotes fertilization and the process of cross-pollination. Pappus, which are bristles or scaly structures, typically take the

role of calyx in this family. The pappus is well-known for its role in the wind-borne dissemination of ripe seeds throughout an area [12].

I.1.2. Geographic distribution of the family Asteraceae

The plants of the family Asteraceae are found on the entire surface of the earth (Figure.I.2). It is a cosmopolitan family with a greater diversification at the level of dry regions and responds well to semi-arid tropical and subtropical zones; for example, in the Mediterranean basin, southern Africa, Mexico and South America, as well as the southwestern United States. They can be found in meadows, valleys, grassy plains, rolling plateaus, and mountain slopes [13].

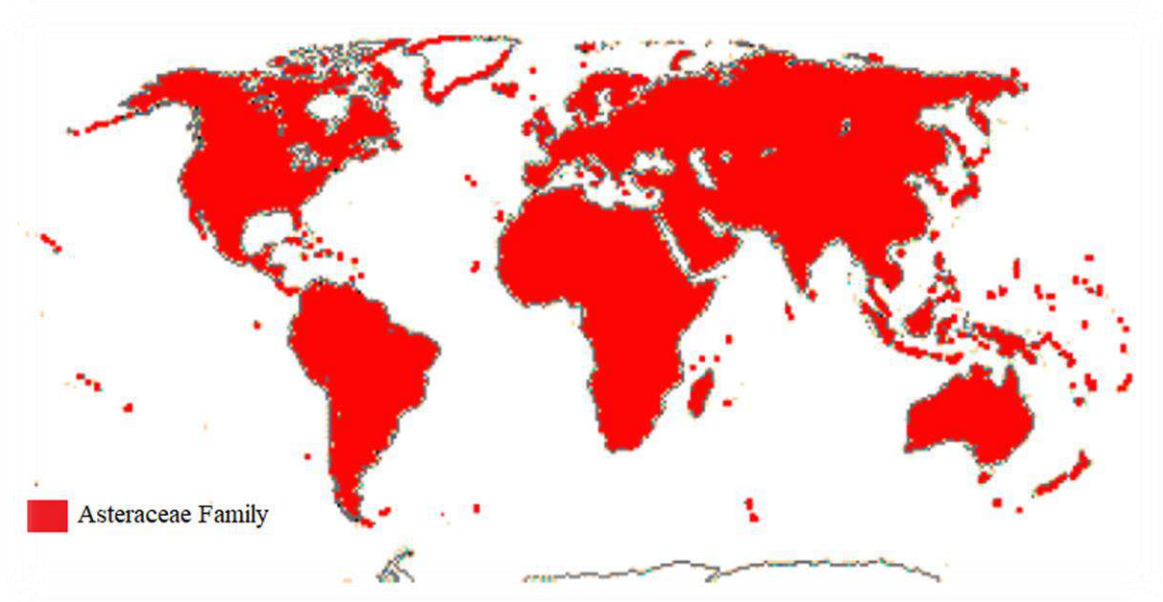


Figure.I.2. Geographic distribution of the family Asteraceae [13].

I.1.3. Folk-medicinal and Pharmacology of the family Asteraceae

The Asteraceae plant family is one of the most diverse and complex in the plant kingdom, with almost 1500 genera and approximately 13000 species. Around 7,000 chemicals were isolated and identified from 5,000 species reported by ZDERO and BOHLMANN [14]. A substantial amount of bioactivity complements this astonishing variety. It has been established that the plants and the secondary metabolites possess diverse pharmacological properties. The aforementioned phenolic compounds and saponins were isolated from the aerial parts of *Centaurea melitnetis* L., making it an acknowledged source; anti-inflammatory [15], antimicrobial [16, 17], antioxidant [17], antidiabetic, and neuroprotective effects are among these compound's numerous known biological activities [17]. Several biological activities of species belonging to the *Lychnophorinae* subtribe have been investigated, the most frequently reported activities are antibacterial, analgesic, antinociceptive, anti-inflammatory, and poisonous [18]. Traditional medicinal practices significantly use the plants that belong to this

family, In traditional Chinese medicine, *Bidens pilosa* L. is used as antirheumatismal, a whole plant decoction that is claimed to be effective once taken orally as a remedy for Snake bites, Pulmonary tuberculosis [19], Cuts, burns, skin problems [20], and Renal infection [21]. Even in folk medicine, a decoction prepared from the seeds of *Helianthus annuus* has been used to decrease the severity and frequency of asthma attacks and migraines, as well as the danger of heart attack [22]. This practise originated in traditional indian medicine.

Perennial *Arnica montana* grows wild in Europe, Russia's Alps, and the Caucasus Mountains, european pharmacopoeias define it as a therapy for minor injuries such as hematomas, which it has been used to treat. In traditional herbal medicine, this plant is used to help relieve pain and swelling in muscles and joints (such as from sprains, bruises, and joint pain) [22, 23]

In China, *Inula Britannica* L. is recommended for the treatment of asthma, whereas *Inula Grantioide* L. is used for treating bronchitis and other inflammatory disorders [24].

Tanacetum Parthenium's dry leaves are made into fresh healthcare used to treat arthritis and migraine [25]. It has been shown that *Ambrosia Maritima* L. is utilised as an antispasmodic diuretic to treat and prevent bilharzia [26]. Whereas the aqueous and alcoholic extracts of the plant *Marticaria Chamomilla* L. have been used orally and topically for wound healing and anti-inflammatory properties, notably that *Matricaria Chamomilla* L. is one of the oldest medicinal herbs [27].

I.1.4. Chemistry of the family Asteraceae

In regards to their contribution to phytochemistry, the species of the Asteraceae family are abundantly present with secondary metabolites, specifically Flavonoids [28, 29], phenolic compounds [30, 31], diterpenes [32], sesquiterpenes [33], coumarins [29], Lignans and alkaloids [34] and other chemicals isolated from Asteraceae have all been the focus of research due to their potential biological activity.

I.1.5. The *Artemisia* genus

Artemisia is among the largest and most extensively distributed genus in the Asteraceae family (Compositae); it is a heterogeneous genus with approximately 500 species, mainly widespread in all of the world's half-northern [35]. In the Algerian flora, there are 11 different species of *Artemisia*: *A. herba-alba , A. campestris ssp. eu-campestris , A. campestris ssp. gIutinosa, A. Absinthium, A. atlantica, A. ar-borescens* L., *A. judaica* L., *A. atlantica, A. Verlotorum, A. vulgaris* L., *and A. alba* [7]. The genus can be divided into the *Artemisia* and Dracunculus

categories. Species that belong to the genus *Artemisia* have therapeutic properties; they are not only used in traditional medicine but also in industry food and pharmaceuticals [36].

I.1.5.1. Botany of the genus *Artemisia*

Vegetative apparatus

Roots: *Artemisia* has two types of fibrous roots [37]:

- Coarse lateral roots that grow near the soil surface and use surface moisture
- Deep tap roots that can grow quickly to large depths

Leaves: The *Artemisia*'s leaves are scattered or arranged in pairs. With a few notable outliers like *A. dracunculus* L., they can be found in a broad variety of sizes, forms, and textures, some of which are shown in Figure.I.3 .

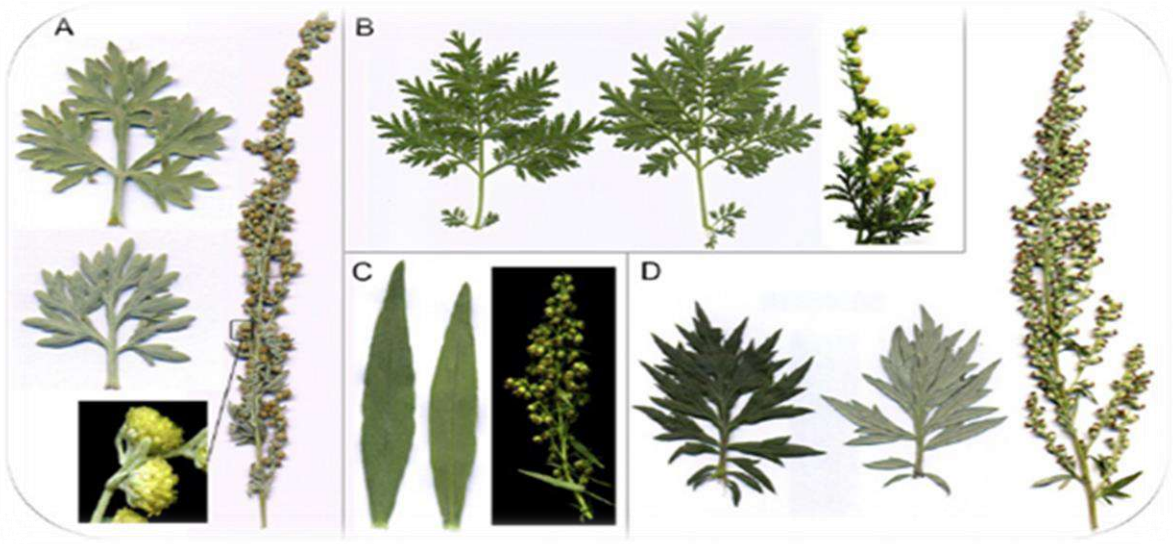


Figure.I.3. The synflorescences and leaves of different *Artemisia* species; **A**: *A. Absinthium*, **B**: *A. annua*, **C**: *A.dracunculus*, **D**: *A.vulgaris* [38].

Producing apparatus

- ✓ **Flowers**: The flowers heads, also known as capitula, are some of the most distinctive features of this genus. These capitula are small, spheroidal, ovoid, or cylindrical in shape, and they contain only flosculose florets that are implanted on a tomentose or glabrous receptacle. The receptacle is protected by an involucre that contains herbaceous or partly scarious bracts. The corollas are pale, yellow, or purple and not very showy, and the number of florets per capitulum varies from 4-7 to more than 40 [38].

- ✓ **Fruits:** Most of the fruits in this genus are achenes (also called cypselas) that lack a pappus and are obovoid, laterally compressed, very tiny, and light. These traits are a direct result of anemochory, the most prevalent dispersion process [39].
- ✓ **Grains:** The microechinate exine ornamentation on the pollen grain sculpture is very typical of the genus and has been used as a systemic marker in front of most Anthemideae representatives. Indeed, *Artemisia* and the taxa that are more strongly linked to it are distinguished from one another by their pollen grains, which constitute the *Artemisia* pollen type and are adorned with a simple pattern . In this regard, we assume that the term microechinate is more appropriate than the term psilate. whereas *Artemisia* pollen grains exhibit microechini when viewed through a light microscope [38, 40].

I.1.5.2. Geographic distribution of the genus *Artemisia*

Artemisia is one of the most extensive and widely distributed genus in the Astraceae (Compositae) family. It is a heterogeneous genus with approximately 500 species mainly distributed in the subtropical and tropical regions of Europe, Asia, North America (Mexico, United States, and Canada), Africa, and Australia (Figure.I.4)

Figure.I.4. Geographical distribution of *Artemisia* species [41].

I.1.5.3. Folk-medicinal of the genus *Artemisia*

Different parts of *Artemisia* species, such as seeds, fruit, leaves, and roots, have been used for a wide range of uses. These applications cover a broad spectrum of realms. *Artemisia*, in particular, has a long history of effective treatment for various illnesses related to the digestive

tract and intestinal parasites, asthma, anthelmintic, and antispasmodic [42-44]. Treatment for malaria in Europe and Africa is successful by using several different species of *Artemisia* [45-47] (Table.I.1).

Table.I.1. Traditional medicinal uses of *Artemisia* species.

Region	Plant/ part used	Ethnopharmacology reports	Ref
Kazakhstan	*A.cina* / Leaves	Sudden cardiac arrests, esophagus, duodenum and cancers of the stomach,	[48]
	A.cina/ Seeds	Inflammation and bronchitis diseases, treatment of asthma	[48]
	A. cina/ (seeds,raisins)	Lung illnesses	[49]
	A.tincture / Aerial part	Epilepsy, unpleasant odors of mouth, caecum, hemorrhoids, and stomach ulcers	[50]
Iran	*A. vulgaris* / aerial parts	Cervicitis	[51]
Iran	*A.dracunculus* / Leaves	Anticoagulant and antidiabetic	[52]
South Africa	*A. afra* / (leaves, roots)	Medication for diabetes	[53]
Russia	*A.juice*/ (leaves, roots)	Renal stones, anti-inflammatory drug, and insomnia	[42]
England	*A. absinthium* / Leaves	Improve memory, mental function, and liver inflammation	[54]
Italy	*A.absinthium* / Leaves	Lack of appetite, helminthiasis	[55]
China	*A. annua* / Aeaves	Malaria, various types of fevers	[56]
China	*A. vestita* / (leaves,resins)	Inflammatory illnesses	[57]
Korea	*A. asiatica* / Aerial parts	Infections ulcerogenic, cancer and inflammatory illnesses	[58]
India	*A. vulgaris* / Leaves	Skin diseases, intestinal issues, dysentery, Urinary issues, and haemorrhage	[59]
Egypt	*A. judaica* / Aerial part	Gastrointestinal diseases	[60]
Italy	*A. verlotorum* / Leaves	Hypertension	[61]
Pakistan	*A.vulgaris* / Aerial parts	Liver illnesses , analgesic, antispasmodic and anti-inflammatory	[62, 63]

I.1.5.4. Pharmacology of the genus *Artemisia*

Artemisia species are excellent sources of a wide variety of biologically active chemicals, which are responsible for a wide range of pharmacological effects (Figure.I.5). *Artemisia* has a broad bioactivity spectrum due to its many active components or secondary metabolites, which function through diverse modes of activity.

Artemisia species are often used to treat diseases like malaria, inflammation, wounds, arterial hypertension, hepatitis, and free radical damage [64]. Table.I.2 provides a comprehensive summary of some of the biological activities that are carried out by different species of *Artemisia*.

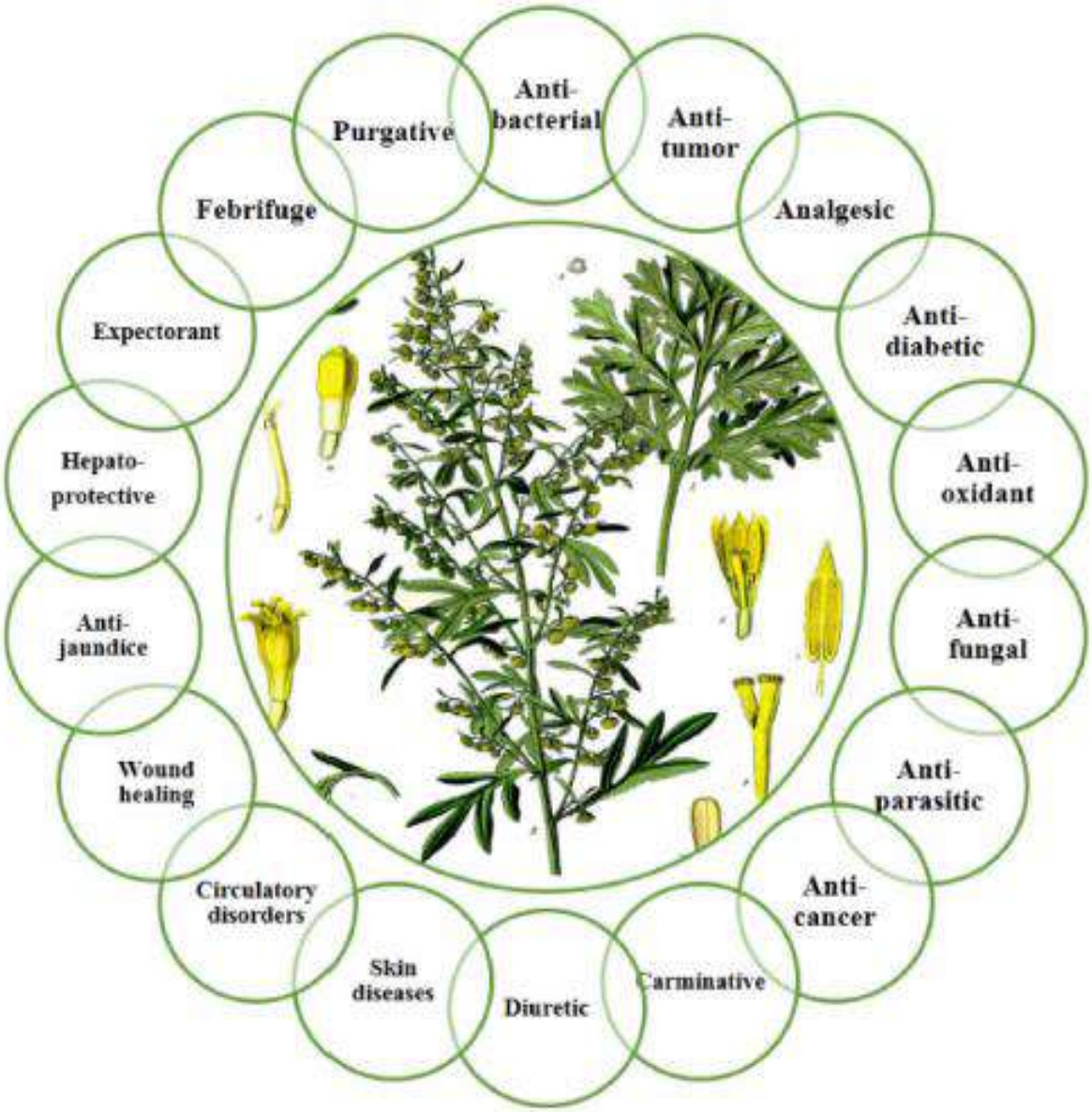


Figure.I.5. *Artemisia* genus medicinal benefits [48].

Table.I.2. Pharmacological studies of *Artemisia* species

Plant / Tested sample	Model	Dose / concentration	Results	Ref
		Anthelmintic activity		
***A. absinthium* / crude aqueous , crude ethanol extracts**	-In-vitro /Haemonchus contortus -In-vivo /Effect on faecal egg count	25 mg/mL	Ethanolic extracts and Crude aqueous exhibited in-vivo and in-vitro anthelmintic effectiveness against ovine nematodes.	[65]
		Neuroprotective effects		
***A. absinthium* / Methanol extract**	In vivo / Middle cerebral artery occlusion (MCAO) for 90 minutes was followed by reperfusion for 24 hours to generate focal ischemia and reperfusion.	100 - 200 mg/kg	Pretreatment with the methanol extract of *A.absinthium* significantly reduced oxidative stress and damage in the brain, as well as cognitive abnormalities.	[66]
***A. judaica* / Ethanol extract**	In-vivo / High-fat diet (HFD) and streptozotocin (STZ, 30 mg/Kg) in rats produce neuronal impairments in a diabetes modeL.	300 mg kg^{-1} day^{-1}	Cognitive abilities in diabetic rats was significantly enhanced by administration with *A.judaica*.	[67]
		Antimalarials effect		
***A. annua* / artemisinin**	In vitro/ Artemisinin's interaction with Hb and Mb, both free and protein-bound.	200 Mm	Artemisinin predominantly reacts with haem in Hb instead of free haem.	[68]
		Antiulcerogenic activity		
***A. annua* / Dihydro-epideoxyarteannuin B, deoxyartemisinin**	In vivo / Models of ulceration in rats produced by ethanol and indomethacin.	100 mg /Kg	Based on the dose-response analysis performed on the ethanol-induced ulcer model, it was determined that dihydro-epideoxyartemisinin B is more effective than deoxyartemisinin.	[69]
		Toxicity of artemisinin		
***A. annua* / artemisinin**	In vivo/ The toxicity of artemisinin on development and reproduction in Wistar rats treated during	35-75 mg/kg	Impact after pregnancy and post-implantation development in rats.	[70]

Table.I.2 continued….

	gestational days (GD) 7–13 and 14–20.			
		Antiviral activity		
***A.arborescens* / Essential oil**	*in vitro* / Inhibition of HSV-1 and HSV-2 plaque development, attachment, penetration, and post-attachment virus neutralizing experiments	100 – 6.25 µg/mL	*A. arborescens* essential oil inhibits the lateral diffusion of both HSV-1 and HSV-2, as indicated by an inhibition of plaque development test. The IC50 values for HSV1 and HSV-2 were 2,4 and 4,1 µg/ml, respectively.	[71]
***A. capillaris* Tablets**	*in vitro* / Hepatitis B virus replication	3.75-600 mg / mL	The levels of HBsAg and HBeAg in 2.2.15 cells that had been treated with A. capillaris Tablets were significantly lower compared to the controL.	[72]
		Antimicrobial activity		
***A.douglasiana* / leaves**	In vitro/ -*B.cereus* -*S. aureus* -*E.coli* -*P.aeruginosa* -*C.albicans* -*A.niger*	100 µg/mL	Neither *A. douglasiana* leaves oil nor its components protected bladder cells against *P. aeruginosa.*	[73]
***A.annua* / Essential oil**	In-vitro / -*E. coli* -*P. aeruginosa* -*S.cerevisiae* -*C. albicans*	2-32 mg/ mL	The oil had antimicrobial effect against all of the microorganisms that were examined, apart from *Pseudomonas aeruginosa.*	[74]
		Inflammatory activity		
***A. asiatica* / ethanol extract**	In-vitro/ Nitric oxide generation, as well as the expression of inducible nitric oxide synthase and inflammatory cytokines	0.5–1 µg/ mL	A. asiatica extract inhibits the inflammatory activation of microglia and preserves PC12 cells against microglial neurotoxicity.	[58]
***A. dracunculus* / ethanol extract**	In-vivo / Mice with xylene-induced ear edoema were used to assess the effect of the ethanolic extract on acute inflammation.	50 mg/Kg	Throughout a xylene ear edoema test, the ethanolic extract shown substantial efficacy (ED_{50} = 78.20 mg/kg).	[75]
		Anticarcinogenic activity		
***A. capillaris*/ Chloroform extract**	In-vivo / Carcinogenesis of the skin in mice caused by	-	*A.capillaris* chloroform fraction reduced tumors/mouse and incidence.	[76]

Table.I.2 continued….

	exposure to 7,12-dimethylbenz[a]anthracene (DMBA).			
		Hepatoprotectiv activity		
***A. capillaris* / 6,7-dimethylesculetin**	In-vivo / Galactosamine (D-GalN) and lipopolysaccharide (LPS) induced hepatitis in mice.	150–600 mg/kg	The hepatoprotective efficacy of 6,7-dimethylesculetin isolated from *A. capillaris* was confirmed by liver histology.	[62]
		Anti-diabetic activity		
***A. vulgaris* / Aqueous and Methanol Extract**	In-vitro/ α-Glucosidase inhibition activity - In vivo / Activity of aldose reductase inhibiting enzyme in rat lenses	-	-Capillinol's IC50 for inhibiting a-glucosidase activity was 464.53 ± 2.69 mM, indicating moderate activity. -The IC50 value for capillin's RLAR inhibiting activity was 55.34 ± 0.28 mM, indicating that it was a potent inhibitor.	[77]
***A.judaica* / water and alcoholic extracts**	In-vivo/ Single and Multiple *A. judaica* Extracts Doses on Diabetic Rat Blood Glucose	-0.25-0.5 g/kg b.wt. for the water extract. - 0.5 - 1 g/kg b.wt for the alcoholiextract	Following oral administration of aqueous extract of *A. judaica* up to 50 to 5g/kg, the obtained results demonstrated no mortalities in mice; nevertheless, the LD for the alcoholic extract was 9.17g/kg.	[78]
		Insecticidal activity		
***A.sieberi* / Essential oil**	Fumigant toxicity - *C. maculatus* - *S. oryzae* - *T. castaneum*	37-926 uL per L	S. oryzae (LCso: 4.41 uL per L) and T.castaneum (LCso: 20.31 IJ.L per L) were found to be more resistant to the fumigant toxicity of A. sieberi than C. maculatus (LCso: 1.64 IJ.L per L uL per L).	[79]

I.1.6. *Artemisia campestris* L.

Artemisia campestris L. is a chamaephyte plant classified systematically in the tribe Asteraceae and the genus *Artemisia. A. campestris* L. is most frequently known by its common names, including dgouft, beach wormwood, oum nafsa, field wormwood, field sagewort, tall wormwood, and field sagebrush. It is known as tedjok in Tamahaq, the indigenous language of the Touareg inhabitants in the South of Algeria, and as oum nafsa in Arabic [7, 80]. This species is divided into various subspecies and varieties. In Tunisia, Pottier-alapetite is classified into three different subspecies, the *A eu-campestris* subspecies includes two varieties: *Vulgaris Marss* and *odoratatissima (Desf) Bonn* and *Barr. Battandier* and Quezel have described additional varieties in Algeria, including *A. clausonis pomel, A.glutinosa (Jgay) Batt, and*

A.glutinosa (Pomel) Batt. canescens LH. Several interesting biological properties of the *Artemisia campestris* L. species have been researched [81]. They are currently the focus of attention from the phytochemical field due to the biological and chemical richness of the species as well as the production of essential oil.

- ✓ **Systematic classification** [82, 83].

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Dicotyledones
Subclass: Magnoliidae
Superorder : Asteranae
Order: Asterales
Family: Asteraceae (Compositae)
Tribe : Anthemideae Cass.
Genus: *Artemisia* L.
Species: *Artemisia campestris* L.
Vernacular names: Field sagewort
Local name: tedjok

Figure.I.6. *Artemisia campestris* L.

I.1.6.1. Botany of the *Artemisia campestris* L.

Artemisia campestris L. consists of small shrubs that are perennial, biennial, or annual, hermaphrodite, almost odorless, and grow to around 1 m in height.

Vegetative apparatus

- ✓ **Roots:** The root structure comprises a fibrous taproot or caudex and a woody taproot or caudex .
- ✓ **Stems**: Subligneous stems that stay flat at the base and rise vertically; reddish and glabrescent at the genitalia; branching trees with spreading limbs.
- ✓ **Leaves**: Alternate and green leaves, not fleshy, keeled beneath, more or less silky in adolescence, becoming glabrescent afterward, the lower petioles with 2-3 pinnatiped blades, the other sessile with less divided blades, with narrowly linear segments, mucronulated

Producing apparatus

- ✓ **Flower**: Individual flower heads (capitulum) are green or yellowish green in color, about 2-4 mm long, void and heterogeneous. Each flower head has 8–30 perfect inner disc florets and 5–20 pistillate outer disc florets. Male flowers (which are tubular, yellow, calyx-less, with five fused petals and five fused filaments, and secretion sacs on the corolla lobes) have a different structure than female flowers. The disc flowers are functionally male with decreased abortive ovaries, whereas the ray flowers are female, pistillate, and fertile (Figure.I.7) [81, 84].
- ✓ **Fuit**: The fruit is an achene in the shape of an ovoid without a pappus [85].

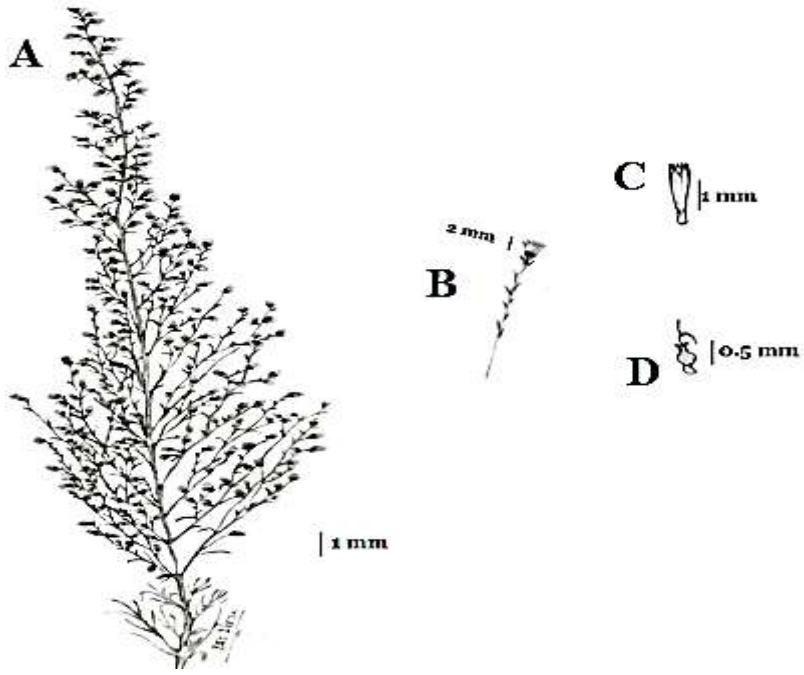


Figure.I.7. *Artemisia* campestris L. illustrations (**A**) paniculate inflorescence, (**B**) Alternate-leavesed capitula, (**C**) male flower, (**D**) female flower [86].

I.1.6.2. Geographic distribution of *Artemisia campestris* L.

Concerning the geographical distribution of *A.campestris* L., it has been indicated that this plant is widely distributed in Europe, in European Russia from the Urals to Siberia, and is considered an exotic species in North America [87]. It also occurs predominantly in the arid regions of the countries of North Africa, particularly the countries in the Mediterranean region, where it prefers a semi-arid habitat and constitutes good land cover, in addition to being a source of litter production and having the surface property of providing protection [88]. Morocco [89],

Algeria, Tunisia [90], and Libya [91] are the leading countries in which the dominance of this plant is found. A.*campestris L* grows naturally along the shoreline of Ryukyu Island in Japan [92]. However, it represents a native herbaceous species that persists at reference sites of restored dunes on North America's Great Lake [93] (Figue.I.8).

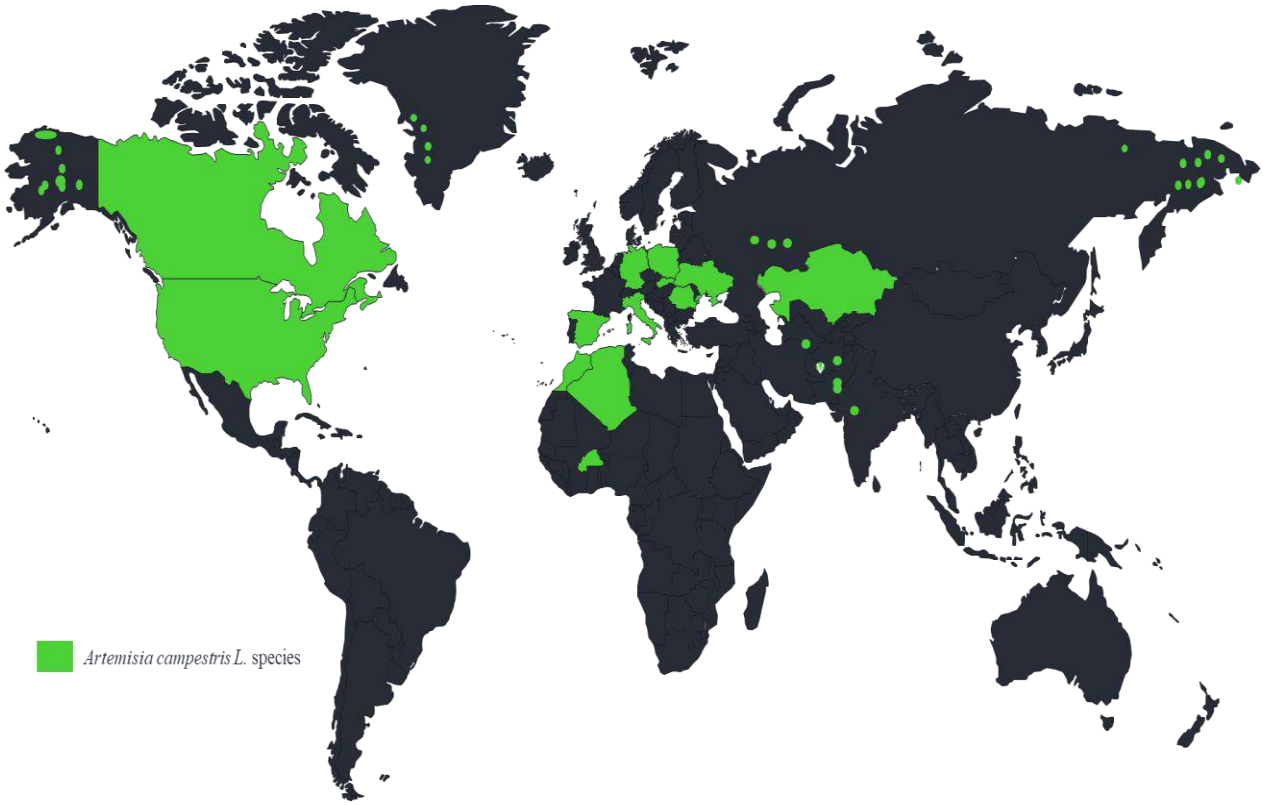


Figure.I.8. Geographic distribution of *A.campestris* L.

I.1.6.3. Folk-medicinal of *Artemisia campestris* L.

A.campestris L. is a plant that has a long history of use as a traditional medicine in a variety of countries, including Algeria, Libya, Tunisia, Morocco, Spain, Turkey, Italy, India, the United States, Canada, Iraq, and Argentina. It has been shown to be effective in treating skin problems, respiratory, and gastrointestinal [94-97]. In the Algerian region of Hoggar, the local populations of Tamanraesset use a infusion, decoction prepared of aerial parts, leaves as a treatment for gastrointestinal illnesses, fever, after childbirth, and loss of hair [98].

Additionally, it is recommended in Japan, Algeria, and Tunisia for the treatment of urinary and hepatic problems [83, 99, 100]. In Algeria, Iraq, and Tunisia, *Artemisia* campestris L. is recommended for the treatment of fever, and in Algeria [101] and India, it is used as an antivenom [102]. It is advised for the treatment of ocular disorders, and baldness, as an aid to

weight loss and hyperthyroidism [103-105]. Additional traditional applications are listed in the literature (Table.I.3).

Table.I.3. Traditional medicinal uses of *Artemisia campestris* L.

Region	Preparation method	Part used	Ethnopharmacology reports	Ref
Algeria	Infusion, decoction, poultice, powder	Leaves, fruits, aerial part, stems	gastrointestinal, analeptic, cutaneous, antidiabetic, antihypertensive, anthelmintic, diuretic, vulnerary, circulation regulator, antitussive, for post-partum, accommodation, antivenom, baldness.	[106] [107] [100] [101]
Morocco	Infusion, poultice, decoction	Flowers, leaves, aerial part, seeds	Treatments for diabetes, metabolic disease, eye disease, skin disease (melasma), digestive disease, lung disease, and allergy.	[108] [105]
Japon	Decoction	-	Renal, Liver disease, and antidiabetic.	[99] [92]
Spain	Decoction	Flowers	Baldness therapy	[104]
India	Decoction	Leaves	Antivenom	[109]
Argentin	Maceration, infusion	Stems, leaves	Treatment of bronchitis, medication for cough, and treatment for bruising	[96]
Italy	-	-	Fever lowering and ulcer treatment	[110]
Canada	Infusion, poultice	Roots, leaves	Digestive, respiratory, and Cutaneous	[97]
Serbia	-	Leaves	Antiseptic, antihelminthic, tonic	[110]
Libya	Decoction	Leaves, flowers	Anthelmintic treatment	[111]
Iraq	-	Whole plant, leaves	Fever reducer, and cough reducer	[104]
Tunisia	Decoction	Whole plant, leaves	Anti-inflammatory, Antivenom, anti-eczema, anti-rum, anthelminthic, cough suppressant, febrifuge, against digestive problems and urinary tract infections	[112]

I.1.6.4. Pharmacology of *Artemisia campestris* L.

The species *Artemisia campestris* L. has been the focus of a significant variety of scientific studies that investigate a wide range of pharmacological activities. The majority of them focused on antivenom, anti-inflammatory, antidiabetic, spasmolytic, vasorelaxant, antioxidant, and neuroprotective effects. Table.I.4 comprehensively examines the diverse pharmacological effects of *Artemisia campestris* L.

Table.I.4. Pharmacological studies of *Artemisia campestris* L.

Testd sample / Part used	Model	Dose / concentration	Results	Ref
		Antivenom activity		
Essential oil / Aerial part	-In vivo / Indirect anti-hemolytic impact of phospholyase A2 inhibition on egg yolk red blood cells	1.0–10 mg/mL	Suppression of hemolysis from both Cerastes cerastes and Macrovipera lebetina venoms (27-31%)	[113]
Decoction / Leaves	In vivo/ Hypertension induced by subcutaneous injection of Buthus occitanus tunetanus scorpion venom in pregnant and nonpregnant rats	200 mg/mL	Reduced venom-induced lung hypertrophy (1.33 g versus 1.47 g). Reduced (30%) MAP in pregnant and (10%) non-pregnant rats with extract only. Absence of a hypertensive increase in pregnant and non-pregnant rats following extract+envenomation.	[114]
		Anti-inflammatory activity		
2',4',5,7-tetrahydroxy-5',6-dimethoxyflavone, Cirsiliol/ Leaves	In vivo / Inhibition of lipoxygenase	30 Mm	Simultaneous inhibition on cirsirol (48.51%) and lypoxygenase by 2',4',5,7-tetrahydroxy-5',6- dimethoxyflavone (46.85%)	[115]
Aqueous extract / Leaves	In vivo / Mice paw inflammation following subcutaneous carrageenan injection	200 mg/Kg, b.w.	Edema volume reduction, inflammatory cell count reduction, and tissue repair following edoema .	[116]
		Antidiabetic activity		
Aqueous extract/ Aerial part, roots	In vivo/ Inhibition of α-glucosidase	-	Aqueous extracts (roots and aerial organs) inhibit microbial α-glucosidase, and a root tincture inhibits mammalian α -glucosidase	[117]
Aqueous extract/ Leaves	In vivo / Diabetes induced by alloxan in rats	200 mg/Kg	-Reduced serum glucose levels (147 mg/dl). -Elevation in serum insulin concentration by 30% -Reduction in glucose concentration by 63% after 120 minutes of the tolerance test for glucose.	[118]
		Neuroprotective activity		
Essential oil / Aerial part	In vitro / Inhibition of AChE (acetylcholinesterase)	-	Important ibition of acetylcholinesterase (IC_{50}=0.02 mg/ml)	[119]
		Antihypertensive activity		
Aqueous extract/ Aerial part	In vivo/ Blood pressure was measured using the tail-cuff technique in L-	50 mg/kg, b.w.	Protection of the rise of arterial blood pressure produced with L-NAME	[120]

Table.I.4 continued….

	NAME-induced hypertensive rats for a duration of 4 weeks.			
		Vasorelaxant activity		
Aqueous extract , Essential oil / Aerial part	in vivo / Vasorelaxant effect on normotensive rat aorta	-2 mg/ml (aqueous extract) -10-1 mg/mL (essential oil)	Both the essential oil and the aqueous extract exhibit significant vasorelaxation.	[119, 120]
		Antinociceptive activity		
Aqueous extract/ Aerial part	In vivo / Mice sense pain following formalin injection, pain mice due to the heat, and acetic acid induces stomach pain in mice after injection.	200- 400 mg/kg, b.w.	Late and early nociceptive reflexes decrease, Attenuation of the central pain sensation.	[121]
		Antileishmaniasis activity		
Essential oil / Aerial part	In vitro/ Leishmania infantum L eishmania major	10^{-3}-10^{3} µg	Growth of both Leishmania infantum and Leishmania major promastigotes was inhibited (IC_{50}=3.24 and 4.59 µg/ml, respectively).	[122]
		Antioxidant activity		
Aqueous extract/ Aerial part	In vivo / Rats exposed to oxidative stress caused by Lagocephalus puffer fish liver extract	5 mg/mL	Improvement in antioxidant enzymatic (GSH-Px in kidney SOD, and CAT, brain, and liver) and suppression of lipid peroxidation (TBARS inhibition in the kidney, brain, and liver).	[123]
Essential oil / Stems, leaves	In vivo / Deltamethrin-induced oxidative stress in rats	200 mg/kg, b.w.	Reducing conjugated dienes and MDA prevents lipid peroxidation, CAT, GSH-Px in the kidney and SOD, liver, and brain enhance	[123]
Aqueous extract/ Leaves	In vivo / Gastroesophageal reflux in rats induces oxidative stress over six hours.	100 - 400 mg/kg, b.w.	Reduced MDA inhibits lipoperoxidation, thiol enhance,reduction in hydrogen peroxide calcium and free iron, CAT, SOD, and GSH-P normalisation.	[124]
Essential oil / Aerial part	In vitro / DPPH assay	Essential oil (5 mg/ml)	IC_{50} : 94,5 mg/ml	[125]
Aqueous extract/ Leaves	In vitro / ABTS assay	0.5 mg/mL	IC_{50} = 26.01 ± 5.74 mg/ml	[126]
Essential oil / Aerial part	In vitro / Ferric Reducing Antioxidant Power assay (FRAP)	1-10 mg/mL	IC_{50} = 17.79 ± 0.08 mg/ml	[127]
Aqueous extract/ Aerial part	In vitro / β-carotène/ linoleic acid	50 mg/mL	% of inhibition of blanchiment of carotene= 64.81 %	[116]
Aqueous extract/ Aerial part	In vitro / Trapping peroxide anion	5-100 mg/mL	IC_{50}= 47,5 mg/ml	[99]
		Gastro - esophageal protection		
Aqueous extract/ Leaves	In vivo / Six hours of gastroesophageal reflux-	100- 400 mg/kg, b.w.	78% reduction in ulcer index, prevention against esophageal epithelium damage,	[124]

Table.I.4 continued….

	induced esophageal ulceration in rats.	reduced inflammatory infiltration and epithelial hyperemia

I.1.6.5. Secondary metabolites isolated from the *Artemisia* genus

I.1.6.5.1. Flavonoïds

A.campestris L. is a species that has several subspecies. Three of these subspecies, *ssp. glutinosa*, *ssp. campestris*, and *ssp. Maritima*, have been studied considerably for their flavonoid profiles. It has been reported that the plant genus *Artemisia* has a significant quantity of flavonols such as quercetin, apigenin, patuletin, acacetin, and luteolin, as well as their derivatives. The presence of flavones marked a significant abundance, including Hispidulin, Cirsiliol, and Eupatilin, among many others. *A. absinthium, A. campestris, A. glutinosa*, and *A. pygmaea* were the sources of the isolated flavanones, flavanonols, chalcones and derivatives. The Flavonoïds isolated from *Artemisia* species are reported in Table.I.5.

Table.I.5. Flavonoïds compounds isolated from *Artemisia* species.

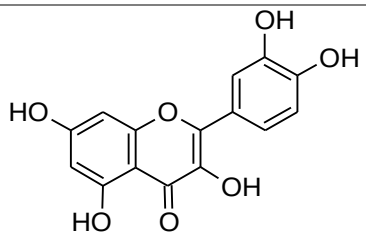
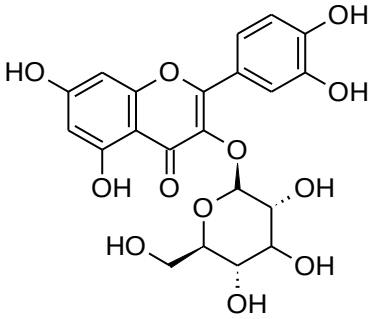
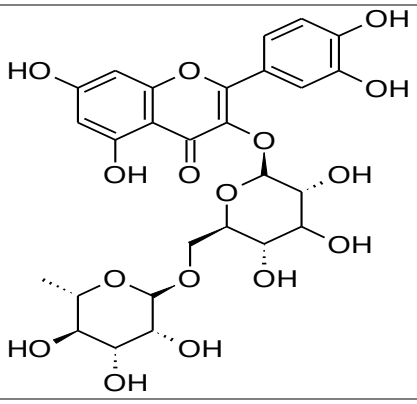
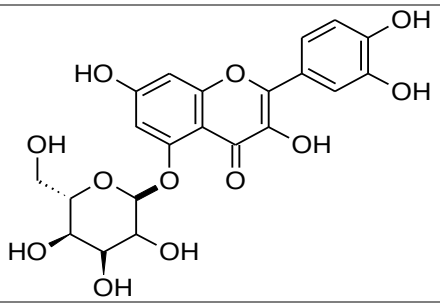
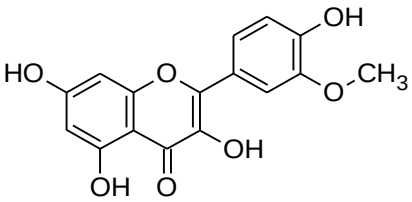
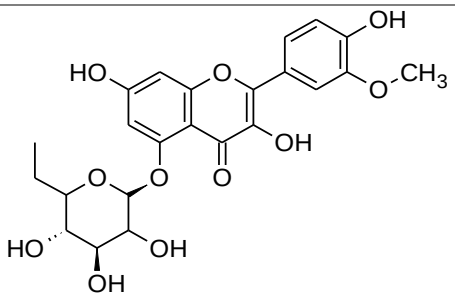
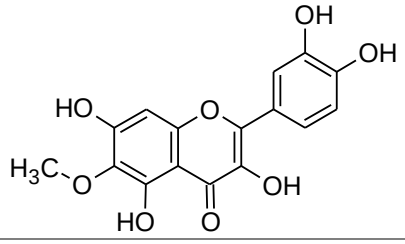
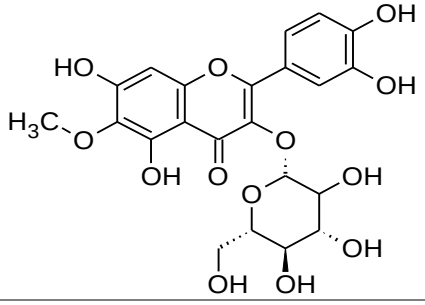
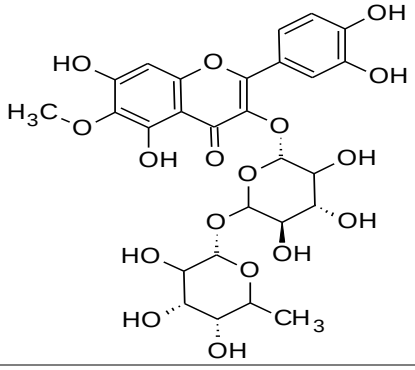
Isolated Compound	Structure	Species	Ref
	Flavonols		
Quercetin		*A. vulgaris, A. dracunculus A. absinthium*	[128]
		A. monosperma A. herba-alba	[129]
Quercctin 3-glucoside		*A. monosperma A. herba-alba*	[130]
Quercetin 3-rutinoside		*A. monosperma A. herba-alba*	[130]

Table.I.5 continued….

Quercetin 5-glucoside		*A. monosperma*	[130]
Isorhamnetin		*A. vulgaris,* *A. dracunculus* *A. absinthium*	[128]
		A. monosperma *A. herba-alba*	[129]
Isorhamnetin 5-glucoside		*A. monosperma*	[130]
Patuletin		*A.absinhium*	[128]
		A. herba-alba	[129]
Patuletin 3-glucoside		*A. monosperma*	[130]
Patuletin 3-rutlnoside		*A. monosperma* *A. herba-alba*	[130]

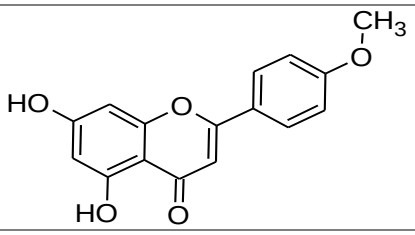
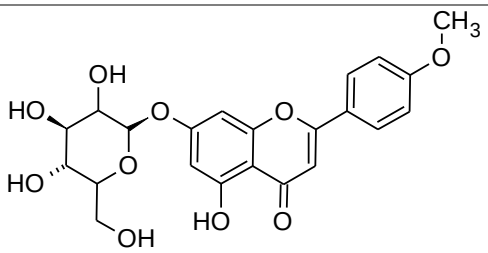
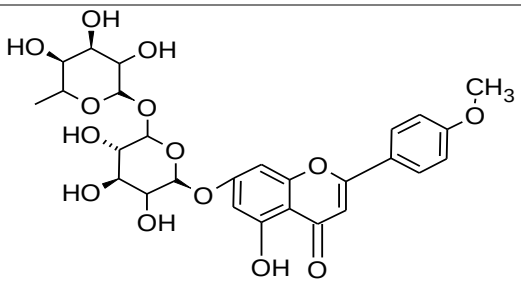
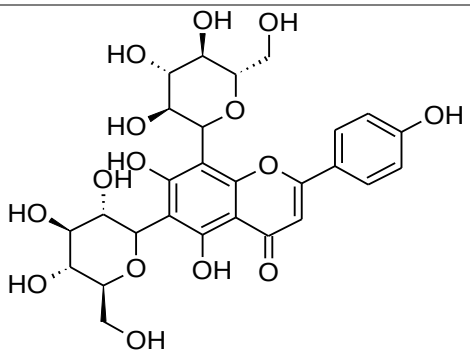
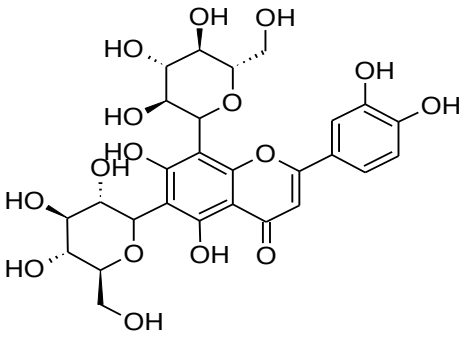
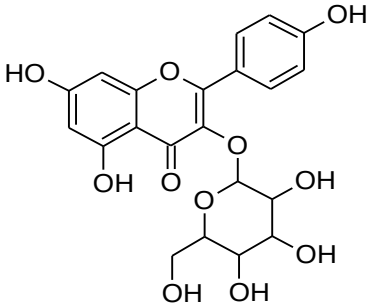
Acacetin		*A. pygmuea*	[131]
Acacetin 7-glucoside		*A. monosperma*	[129] [130]
Acacetin 7-rutinoside		*A. monosperma*	[129] [130]
Vicenin-2		*A. monosperma* *A. judaica*	[130]
		A. transiliensis	[132]
Lucenin-2		*A. monosperma*	[130]
Kaempferol 3-glucoside		*A. vulgaris,* *A. dracunculus* *A. absinthium*	[128]
		A. monosperma *A. herba-alba*	[129]

Table.I.5 continued….

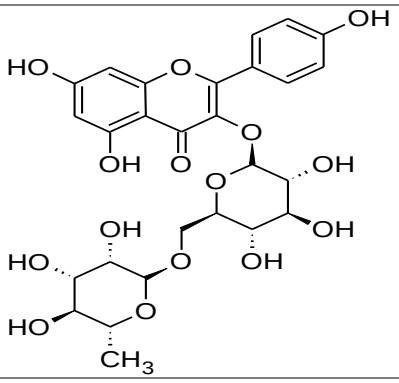
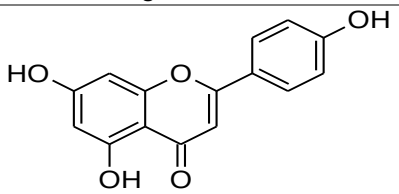
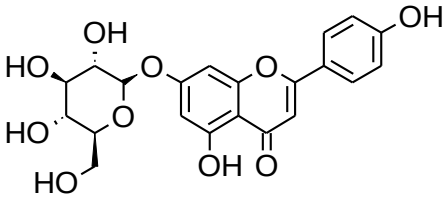
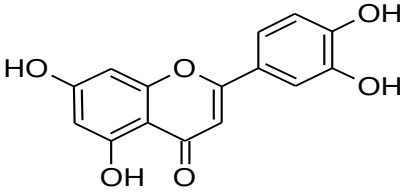
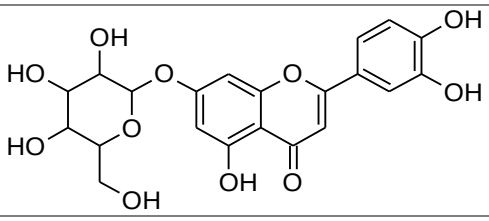
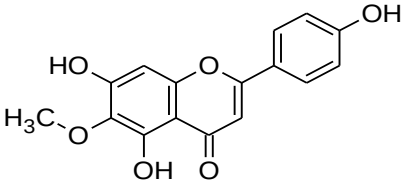
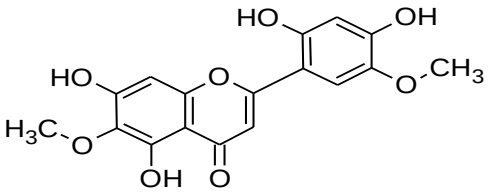
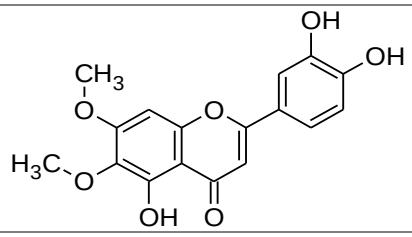
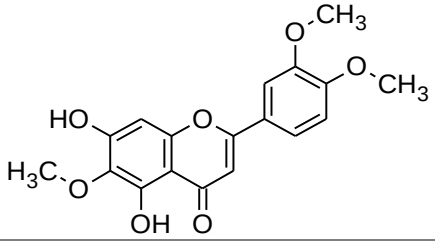
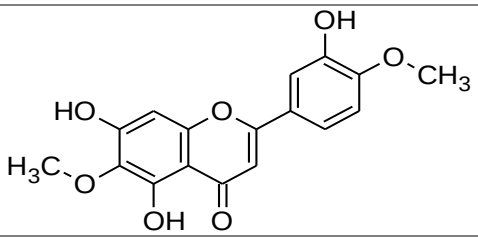
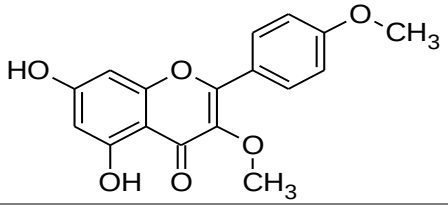
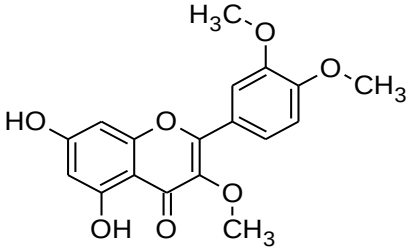
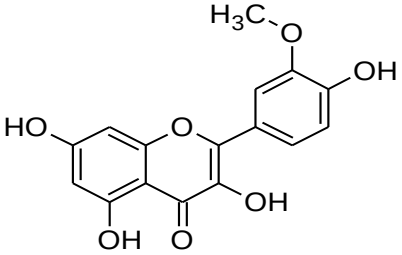
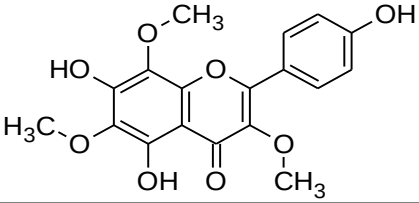
Kaempferol 3-rutinoside		*A. vulgaris, A. dracunculus A. absinthium*	[128]
		A. monosperma A. herba-alba	[129]
Apigenin		*A.ludoviciana*	[133]
		A.frigida	[134]
Apigenin 7-glucoside		*A. japonica*	[135]
Luteolin		*A.ludoviciana*	[133]
		A. tridentata	[136]
Luteolin 7-glucoside		*A. japonica*	[135]
		A. tridentata	[136]
Flavones			
Hispidulin		*A.campestris*	[137]
		A. ludoviciana	[133]
		A. frigida	[134]
5,7, 2',3'-tetrahydroxy-6, 5'-dimethoxy-flavone		*A.campestris*	[115]
Cirsiliol		*A.campestris*	[115]
		A. heba-alba	[138]

Table.I.5 continued….

		*A. mesatlantica* *A. capillaris*	[139]
Eupatilin		*A.campestris*	[115]
		A.ludoviciana	[133]
		A. frigida	[134]
Dimethoxycentaureidin		*A.campestris*	[115]
5,7-dihydroxy-3,4'-dimethoxyflavone		*A.campestris*	[100]
5,7-dihydroxy-3,4',5'-trimethoxyflavone		*A.campestris*	[100]
3,5,7,4'-tetrahydroxy-3'-methoxyflavone		*A.campestris*	[100]
5,7,4’-triihydroxy-3,6,8, trimethoxyflavone		*A.ludoviciana* *A.grayi*	[133] [140]

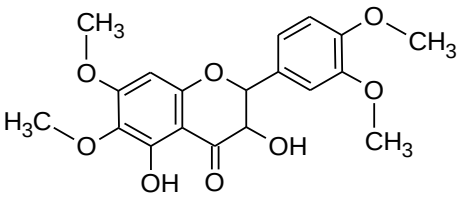
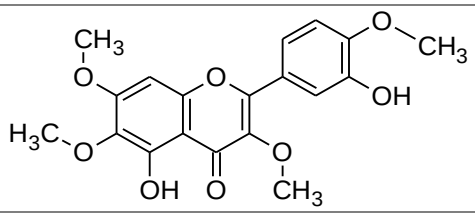
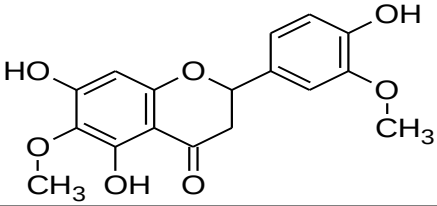
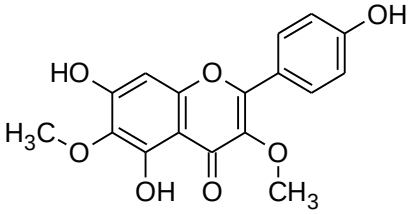
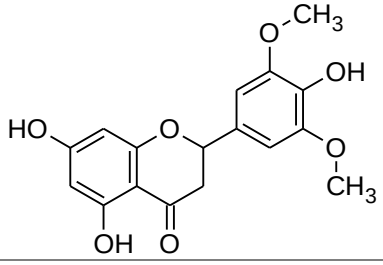
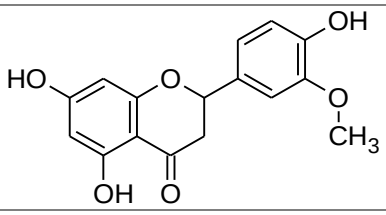
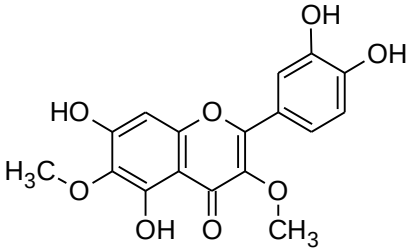
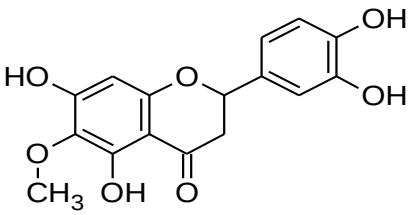
6,7,3',4'-tetramethoxy Quercetagetin		*A.ludoviciana*	[133]
Casticin		*A. annua*	[140]
Jaceosidin		*A.ludoviciana* *A.princeps* *A. frigida*	[133] [141] [142]
5,7,4'-trihydroxy-3,6-dimethoxyflavone		*A.ludoviciana*	[133]
Tricin		*A.ludoviciana*	[133]
Chrysoeriol		*A.ludoviciana*	[133]
Axillarin		*A. ludoviciana* *A. incanescens*	[133] [143]
Eupafolin		*A.ludoviciana* *A.tridentata*	[133] [136]

Table.I.5 continued….

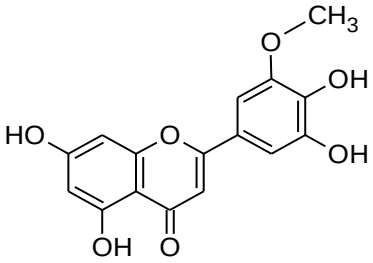
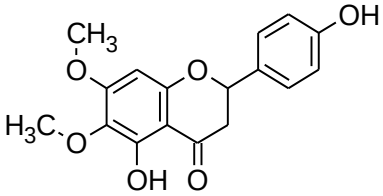
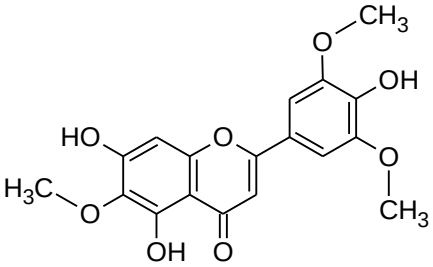
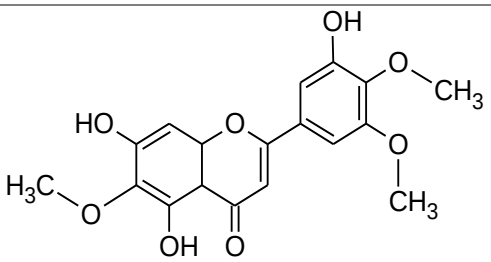
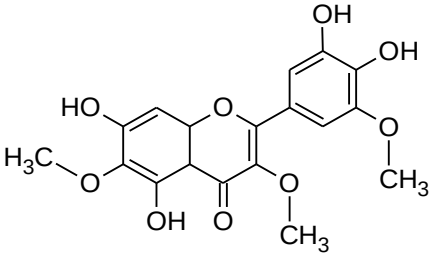
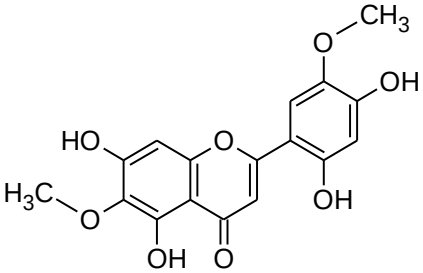
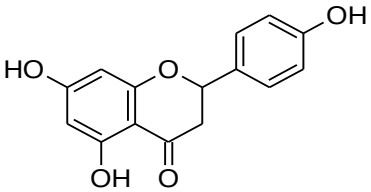
Selagin		*A.ludoviciana*	[133]
Cirsimaritin		*A. scoparia*	[144]
		A. capillaris	[139]
5,7,4'-trihydroxy-6,3',5'-trimethoxyflavone		*A.ludoviciana*	[133]
		A.frigida	[142]
5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone		*A. ludoviciana*	[133]
		A.frigida	[142]
5,7,3',4'-tetrahydroxy-3,6,5'-trimethoxyflavone		*A.ludoviciana*	[133]
		Artemisia frigida	[142]
5,7,2',4'-tetrahydroxy-6,5'- dimethoxyflavone		*A.ludoviciana*	[133]
Flavanones, Flavanonols			
Naringenin		*A.campestris*	[137] [145] [146]
		A.Glutinosa	[147]

Table.I.5 continued….

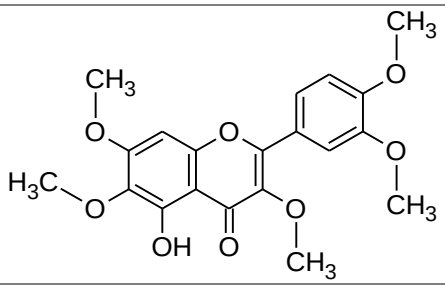
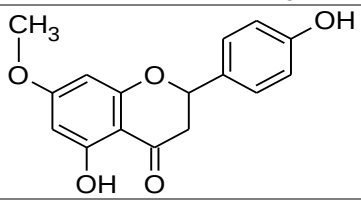
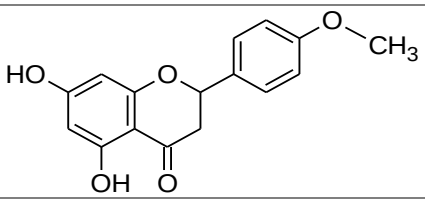
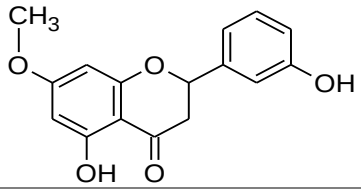
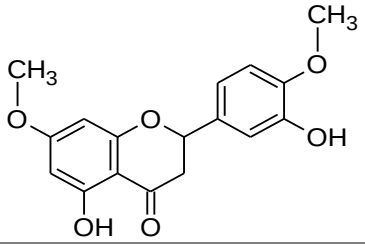
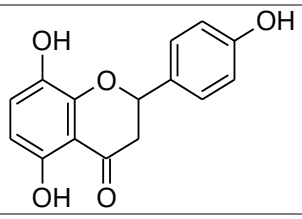
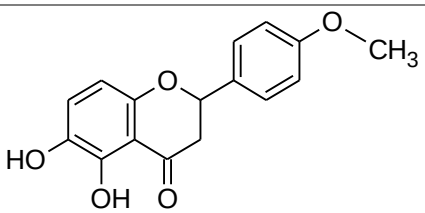
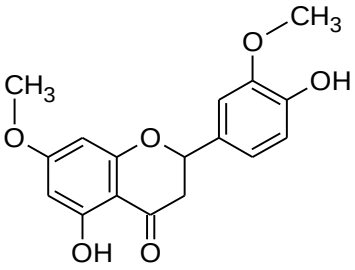
Artemisetin		*A. absinthium*	[148]
5-4'dihydroxy-7-methoxyflavanone (Sakuranetin)		*A.campestris*	[145] [146]
5.7 dihydroxy-4'-methoxyflavanone (isosakuranetin)		*A. campestris*	[145] [146]
7-methyoxy-Eriodictyol		*A. campestris*	[146]
7, 3'-dimethoxy-Eriodictyol		*A.campestris*	[146]
5,8,4'-trihydroxyflavanone		*A.campestris*	[149]
5,6-dihydroxy 4'-methoxyflavanone		*A. campestris*	[149]
5,4'-dihydroxy-7,3'-dimethoxyflavanone		*A. campestris*	[149]

Table.I.5 continued….

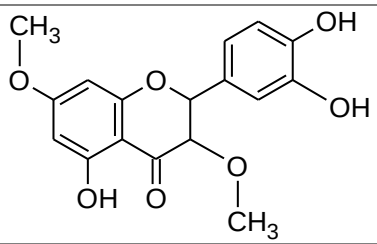
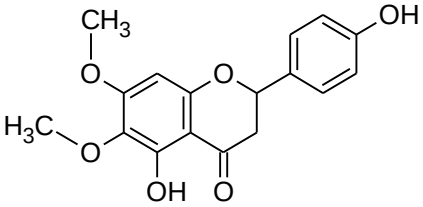
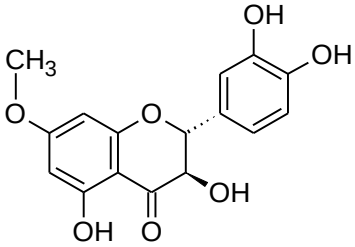
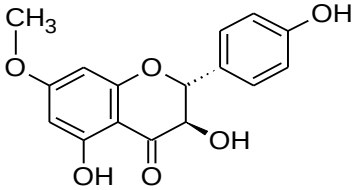
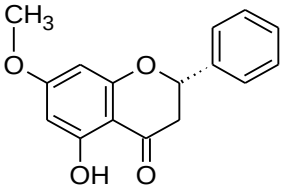
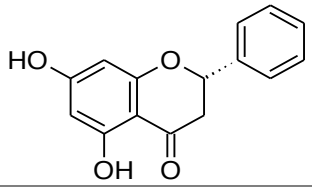
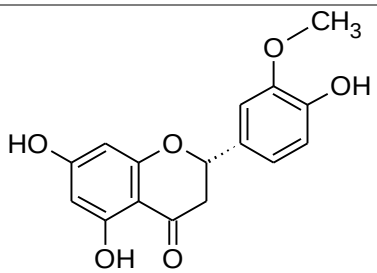
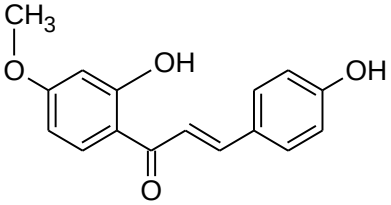
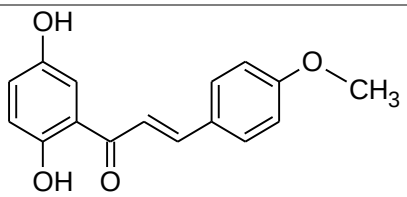
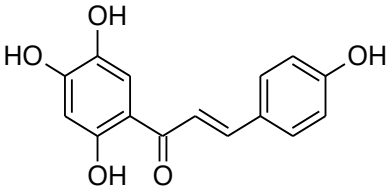
3,7-dimethoxy-,5 3',4'-trihydroxyflavanone		*A. campestris*	[149]
		A.pygmaea	[131]
5,4'-dihydroxy-6,7-dimethoxyflavone		*A.campestris*	[149]
Padmatin		*A. campestris*	[145]
		A. glutinosa	[147]
7-O-methoxy aromadendrin		*A. campestris*	[137]
Pinostrobin		*A. campestris*	[137]
Pinocembrin		*A. campestris*	[137]
Homoeriodictyol		*A. campestris*	[137]
Chalcones			
4,2'-dihydroxy-4'-methoxychalcone		*A. campestris*	[100]

Table.I.5 continued….

2',5'-dihydroxy-4-methoxychalcone		*A. campestris*	[100]
Isoliquiritigenin		*A. campestris*	[100]

I.1.6.5.2. Lignans

Various studies have been conducted on the lignans of *A. sieversiana* and *A. absinthium*, both of which contain cyclolignans. The Table.I.6 present an overview of the different structures of lignans that have been isolated.

Table.I.6. Lignans compounds isolated from *Artemisia* species.

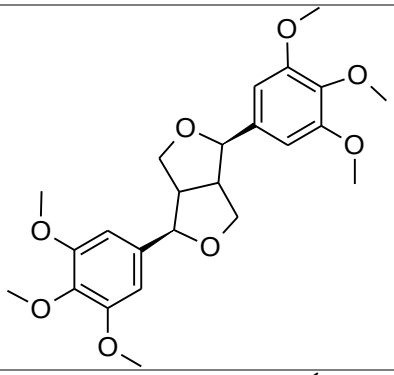
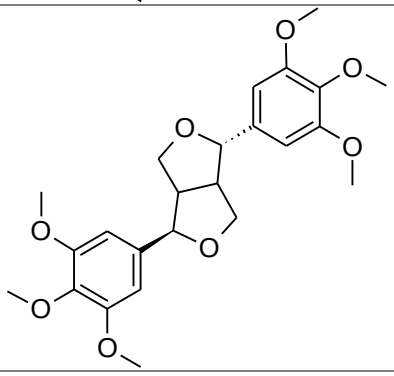
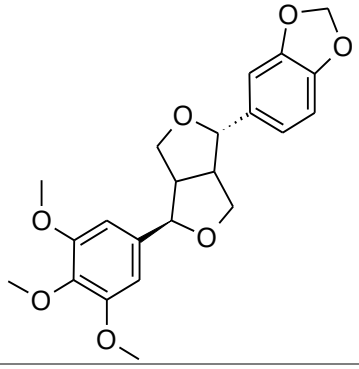
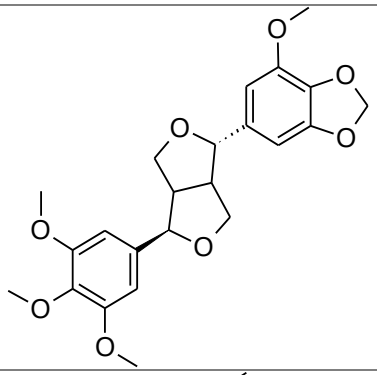
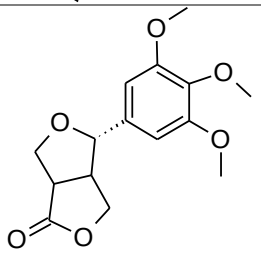
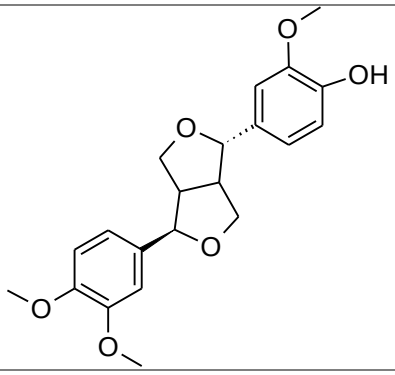
Isolated Compound	Structure	Species	Ref
Diayangambin		*A.sieversiana*	[150]
		A. absinthium	[151]
Epiyangambin		*A.sieversiana*	[150]
		A. absinthium	[148] [151]
Epiashchantin		*A.sieversiana* *A. absinthium*	[150] [151]

Table.I.6 continued….

Sesartemin		*A.sieversiana*	[150]
		A. absinthium	[148] [151]
Caruilignan A		*A.sieversiana*	[150]
		A. absinthium	[151]
(+)-Phylligenin		*A.sieversiana*	[150]

I.1.6.5.3. Coumarins

Coumarin is a natural organic aromatic heterosidic oxygenated substance with the chemical formula $C_9H_6O_2$. It is also referred to as 1,2-benzopyrone or 1, benzopyrane-2-one in the international nomenclature. There are few isolated coumarins from the genus *Artemisia*. We list 7 coumarines from 5 different species (Table.I.7).

Table.I.7. Coumarins compounds isolated from *Artemisia* species.

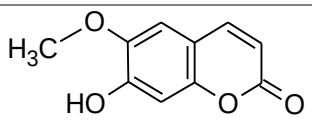
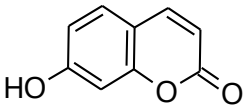
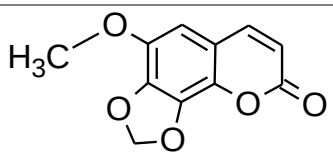
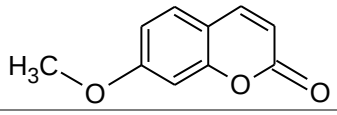
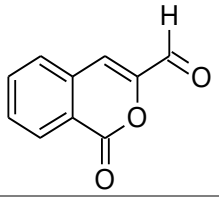
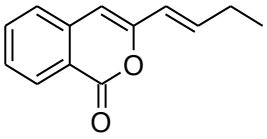
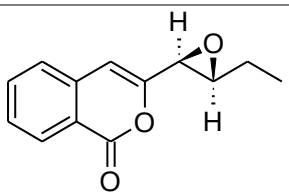
Isolated Compound	Structure	Species	Ref
Scopoletin		*A.campestris*	[146]
		A. herba alba	[152]
Umbelliferone		*A. anomola*	[153]
Dracunculin		*A. kieskiana*	[154]

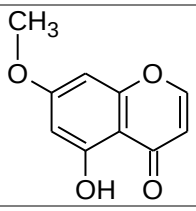
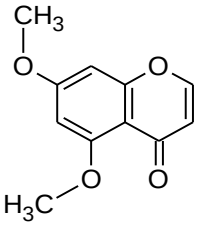
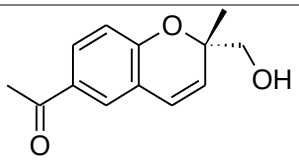
Table.I.7 continued….

Herniarin		*A. glutinosa*	[147]
Artemidinal		*A.campestris*	[100]
E-artemidin		*A.campestris*	[100]
(+) epoxyartemidin		*A.campestris*	[100]

I.1.6.5.4. Chromones

Two chromones were isolated from an acetone extract of *A.campestris* L. *ssp maritima,* and artemisenol was isolated from *A. campestris* L. *ssp. Glutinosa* (Table.I.8).

Table.I.8. Chromones compounds isolated from *Artemisia* species.

Isolated Compound	Structure	Species	Ref
5-Hydroxy-7-methoxychromone		*A.campestris*	[146]
5.7-dimethoxychromone		*A.campestris*	[146]
Artemisenol (6-acetyl-2-methyl-2-hydroxymethylchromene)		*A.campestris*	[155]

I.1.6.5.5. Acetophenones

Acetophenones have been thoroughly examined by De Pascual *et al.*, and their occurrence has been limited to the weakly acidic fraction of the hexane extract of *A.campestris L ssp. glutinosa*; the main isolated acetophenones are presented in Table.I.9.

Table.I.9. Acetophenones compounds isolated from *Artemisia* species.

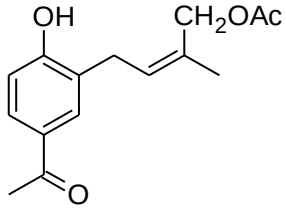
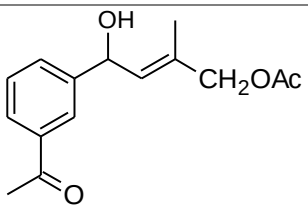
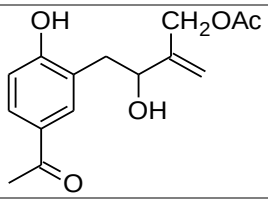
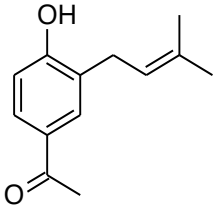
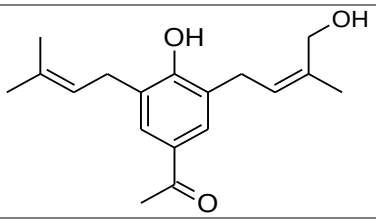
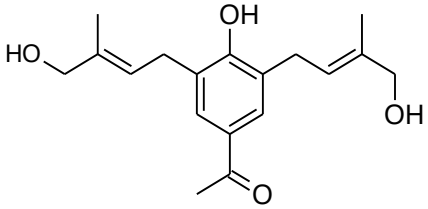
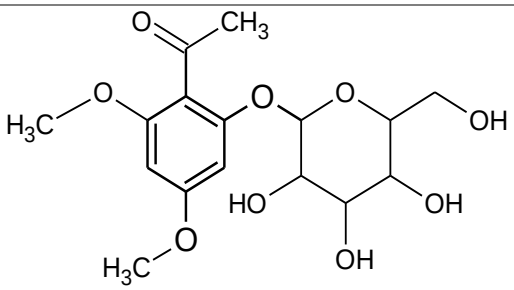
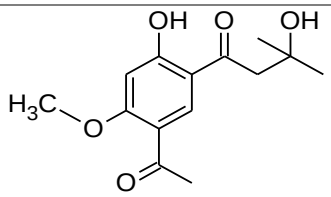
Isolated compounds	Structure	Species	Ref
3-[4-acetoxyisopent-2(Z)-enyl]-4-hydroxyacetophenone		*A.campestris*	[156]
3-[4-acetoxyisopent-2(E)-enyl]-4-hydroxyacetophenone		*A.campestris*	[156]
3-(3-acetoxymethyl-2 hydroxybut-3-enyl)-4-hydroxyacetophenone		*A.campestris*	[156]
3-(isopent-2-enyl)-4-hydroxyacetophenone		*A.campestris*	[149]
3-[4-Hydroxyisopent-2(E)-enyl]-5-(isopent-2-enyl)-4-hydroxyacetophenone		*A. campestris*	[157]
3- [4-Hydroxyisopent-2(Z)-enyl]-5- [4-hydroxyisopent-2-(E)-enyl]-4-hydroxyacetophenone		*A. campestris*	[157]
4, 6-di methoxy acetophenone-2-O-β-D-glucopyranoside		*A. Spicigera*	[158]
Glutinosol		*A. glutinosa*	[147]

Table.I.9 continued….

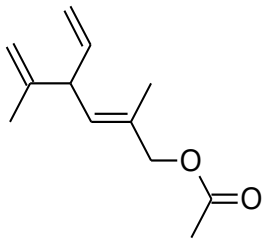
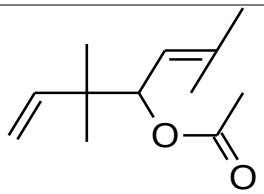
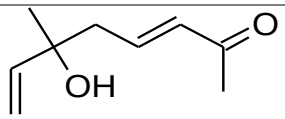
I.1.6.5.6. Terpenoids

Terpenes are compounds formed from 2-methylbutadiene branching pentacarbonyl units. Depending on the number of units, monoterpenes (2 units), sesquiterpenes, diterpenes, triterpenes, steroids, and carotenoids are formed. α-Santonin and 8α-Hydroxytaurin were both isolated from the air-dried *Artemisia* santonicum.

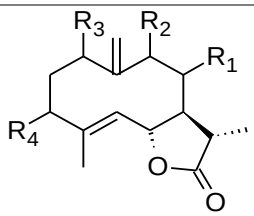
Ridentin, Ridentin-3-acetate, and Artemorin were isolated from the aerial part of *A. verlotorum* and *A. lerchiana* using chloroform and the crude extract, respectively, and Pallensin was obtained using the acetone fraction from the aerial part of *A. pallens*. Table.I.10 shows several different terpenoid compounds that belong to this genus.

Table.I.10. Terpenoids isolated from *Artemisia* species.

Monoterpenes

Isolated Compound	Structure	Species	Ref
Lyratyl acetate		*A. schimperi*	[159]
Artemisyl acetate		*A. schimperi*	[159]
1 ,5-Octadien-3-hydroxy-3-methyl-7-one		*A. schimperi*	[159]

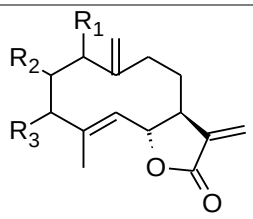
Sesquiterpenes



Germacranolides

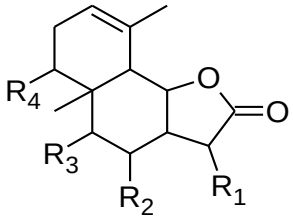
Isolated Compound	R_1	R_2	R_3	R_4	Species	Ref
Herbolide F	H	OH	H	OH	*A. herba alba*	[160]
Herbolide H	H	OH	H	H	*A. herba alba*	[160]
Shonachalin	OH	H	OH	H	*A. herba alba*	[161]
Dihydroridentin	H	H	OH	OH	*A. herba alba*	[162]
11β,13-dihydroridentin-3-acetate	H	H	OH	OAc	*A. desertii*	[162]

Table.I.10 continued….



Germacranolides

Isolated Compound	R1	R2	R3	Species	Ref
Ridentin	OH	H	OH	*A. lerchiana*	[163]
Artemorin	OH	H	H	*A. verlotorum* *A. lerchiana*	[163] [164]
Ridentin-3-acetate	OH	H	OAc	*A. verlotorum* *A. lerchiana*	[163] [164]



Eudesmanolides

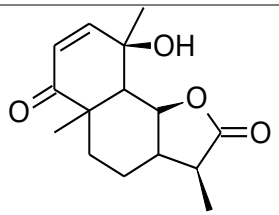
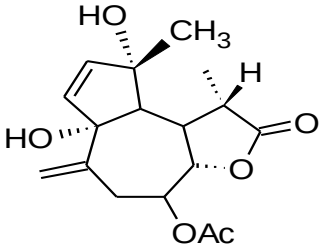
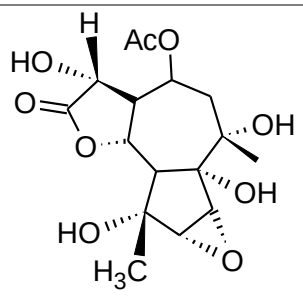
Isolated Compound	R_1	R_2	R_3	R_4	Species	Ref
Santamarin	CH_2	H	H	OH	*A. hispanica*	[165]
α-Santonin	CH_3	H	H	=O	*A.santonicum*	[166]
8α-Hydroxytaurin	CH_3	OH	H	=O	*A.santonicum*	[166]
8α-Acetoxytaurin	CH_3	OCH_3	H	=O	*A.santonicum*	[166]
Pallensin					*A. pallens*	[167]
Neoezoguaianin					*A.montana*	[168]

Table.I.10 continued….

Oyomoginin		*A.montana*	[168]

I.2. Zygophyllaceae

The Zygophyllaceae is a flowering family that includes the bean-escapade and the caltrop. It was classified as a dicot family (angiosperms)[169] . Around 27 genera and 285 species belong to the Zygophyllaceae family, further divided into five subfamilies. Therefore, approximately 3% of the Algerian desert comprises Zygophyllaceae [170].

I.2.1. Botany of the family Zygophyllaceae

Vegetative apparatus

- ✓ **Stems**: Swollen nodes are a common characteristic of stems.
- ✓ **Leaves**: The leaves can be paripinnate, trifoliolate, geminate, or simple, depending on the species. They are often opposite (less frequently spiral), stipulate and resinous, and the stipules are permanent and spiky in certain taxa [171].

Producing apparatus

- ✓ **Flowers**: The flowers are always bisexual, actinomorphic or zygomorphic, terminal or axillary, and single or clustered in racemes or cymes. The 4 or 5 sepals, valvaires or imbricate, are lanceolate, oblong, oval, or obovate, with an acute or obtuse apex and a glabrous or hairy surface. 4 or 5 valvaires or nested petals are white, creamy, yellow, pink, purple, or purple. Oblong, spatulate with 4-5 stamens up to 3 times the number of petals [172].
- ✓ **Fruits:** The fruit is typically a schizocarp or lobed capsule (each carpel is divided into 2 mericarps in certain taxa) and is rarely a berry or drupe.
- ✓ **Seeds:** the seeds might be ellipsoid, oblong, obovoid, ovoid, or flat. They can also be smooth, tuberculous, or crustaceous [171, 172].

I.2.2. Geographic distribution of the family Zygophyllaceae

The family of Zygophyllaceae is distributed in the old and new world but predominately in arid, semiarid, and saline regions in all warm areas of the world, particularly in seasonally dry deserts, in tropical and subtropical regions [7, 173].

I.2.3. Folk-medicinal and Pharmacology of the family Zygophyllaceae

Zygophyllaceae is a family of plants with many different species, many of which have remarkable therapeutic properties and are utilized in traditional medicine. The following are several examples of species that are very important from a therapeutic:

In traditional Iranian medicine, *Peganum harmala L* is known to as "Esfand," "Espand," and "Harmal" due to its wide range of medicinal uses, including as a carminative, vermifuge, aphrodisiac, galactagogue, diuretic, emménagogue, and antithrombotic [174].

In traditional Egyptian medicine, the fruits of the *Balanites aegyptiaca* Del. plant are used as an oral hypoglycemic. On the other hand, in traditional Indian medicine, the species was used to treat epilepsy and rheumatism [175]. Besides treating digestive problems such as constipation and heartburn, it can also help in treating helminthiases, jaundice, spleen illness, herpes, skin problems, malignant wounds, gonorrhea, lice, and syphilis [176].

Zygophyllum gaetulum is widely known for the anti-diabetic characteristics that it possesses [177]. In addition to those properties, it is anti-spasmodic, anti-eczema, and a helpful cure for the stomach and the *Zygophyllum album* L., which is Saharo-Mediterranean species, is used to treat diabetes and diarrhea. Its aqueous preparations are carminative, antiseptic, and stimulating [178].

In Argentina, the species *Larrea divaricata* is widely used to treat various diseases, including tumours, inflammation, arthritis, and fever [179]. In addition to the use of Zygophyllaceae in traditional pharmacopeias, a variety of studies have been carried out to validate their traditional uses and to look for novel biological potentials in these plants.

Several plants of the family Zygophyllaceae, including *Guaiacum officinale* [180], *Fagonia schweinfurthii* [181], *Peganum harmala* L. [182] and *Balanites aegyptiaca* L. [183], have been shown to exhibit antioxidant potential in recent studies.

Zygophyllaceae species are natural anti-inflammatory sources, including *Zygophyllum album* Desf. [184], *Peganum harmala* L. [182], *Balanites aegyptiaca* L. [183] and *Larrea divaricata Cav.* [185].

Zygophyllum album Desf. and *Peganum harmala L* have both been examined for their potential to replenish anticancer and antitumor compounds [182, 184].

Additional research on different species in this family has revealed a wide range of biological effects, including those that are antihyperglycemic (*Zygophyllum album* L.) and antihyperlipidemic (*Zygophyllum album* L.) [186], anticonvulsant (*Balanites aegyptiaca* L.) *De*L.) [175] , antinociceptive (*Balanites aegyptiaca* L.), cytotoxic (*Larrea tridentate* DC.) [183].

I.2.4. Chemistry of the family Zygophyllaceae

In light of their contribution to phytochemistry, Zygophyllaceae species include an abundance of secondary metabolites, notably flavonoids and alkaloids [187], triterpenoids, sterols, and saponins [173], and other compounds isolated from Zygophyllaceae owing to their potential pharmacological activity.

I.2.5. The *Fagonia* genus

The genus *Fagonia*, a member of the Zygophyllaceae family, is noteworthy since it has 35 species native to various regions of Africa, the Mediterranean Basin, the Middle East, and Asia, which is classified as eurosid I (APG II, 2003). these species are generally Shrub or herbaceous plants up to 60 cm tall. Ethnobotanically, numerous species of *Fagonia* have been utilized for the treatment of a wide variety of illnesses by traditional practitioners adhering to Ayurveda and other healing regimens [188].

I.2.5.1. Botany of the genus *Fagonia*

Vegetative apparatus

- ✓ **Stems**: Stems with glabrous, pubescent, or glandular characteristics.
- ✓ **Leaves**: Depending on their complexity, the leaves are either opposite or simple (1-, 2- or 3-foliolate., rarely up to 7 foliolate.). Folioles are typically linear to oval or obovate in form, frequently mucronate, and can have a petiole. The stipules are free and have spiny.

Producing apparatus

- ✓ **Flowers**: The five sepals have an acute-attenuated apex. The five petals are obovate, pink, or purple. There are 10 stamens and filaments without basal appendages. The ovary is (5-) locular, glandular and pubescent.

- ✓ **Fruits:** Obconical capsules assist in circumscribing *Fagonia*.
- ✓ **Seeds:** The exterior seed testa is mucilaginous without internal structures, and the aril lacks [189, 190].

I.2.5.2. Geographic distribution of the genus *Fagonia*

The disjunct distribution of *Fagonia* includes arid regions of the New and Old World. In the New World, it is found in the southwestern United States, the southwestern USA, Peru, Chile, and Mexico. In the Old World, it is found in Macaronesia, northern Africa, south of the Sahel regional transition area, southern Africa. as well as the southern regions of Europe, comprising the islands in the Mediterranean. *Fagonia* is also found in Kenya, western Asia (Turkey, Lebanon), western India, Afghanistan, and Arabian Peninsula. Several different species of *Fagonia* can be found in the Old or New World [190] (Figure.I.9). Among the 17 species of this genus found in Algeria are the *F. bruguieri, F.arabica, F.longispina, F. kahirina, and F. olivieri* [191].

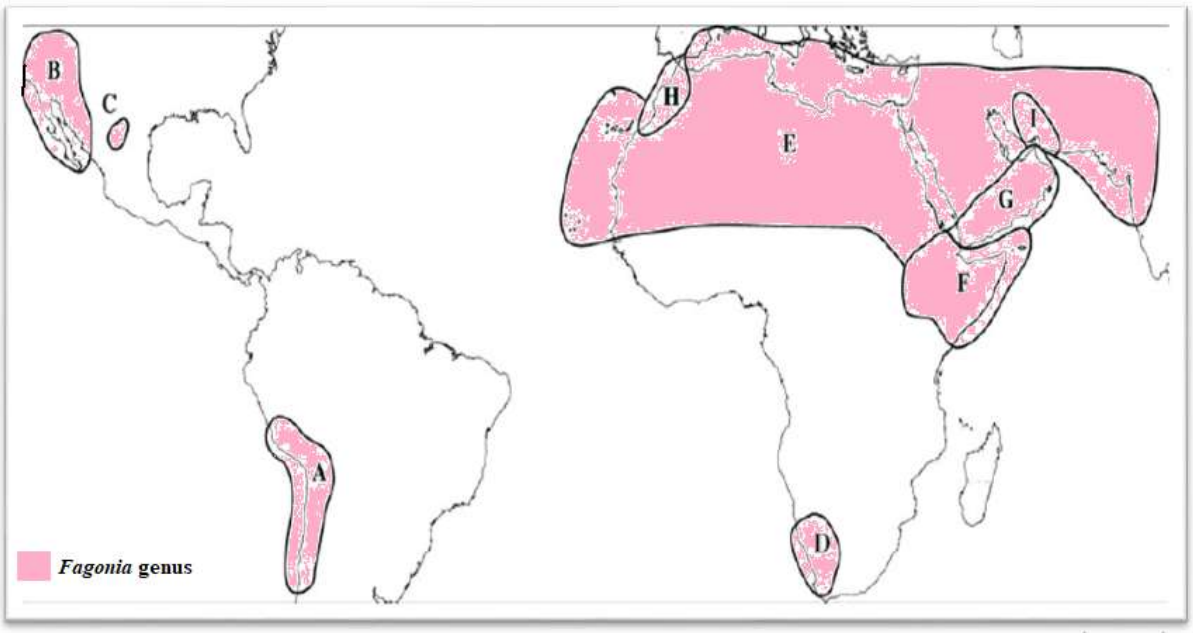


Figure.I.9. *Fagonia* distribution map with endemism areas: (**A**): South America, (**B**): North America, (**C**): north of Mexico, (**D**): south of Africa, (**E**): Sahara-Sind includes Macaronesia, (**F**): the Horn of Africa, (**G**): Oman and Yemen, (**H**): west of Morocco, and (**I**): south of Iran [190].

I.2.5.3. Folk-medicinal of the genus *Fagonia*

There are extensive and intriguing lists of illnesses that reportedly can be treated traditionally using *Fagonia* species. Depending on the type of disease being treated, the recommended therapy approach may include both an internal or exterior treatment. Due to its widespread use

in both India and Pakistan, it is widely utilized in folk medicine by the native inhabitants. Some examples are presented in the Table.I.11.

Table.I.11. Traditional medicinal uses of *Fagonia* species.

Region	Plant/ part used	Ethnopharmacology reports	Ref
Pakistan	*F.cretica*/ leaves, flowers, aerial parts	Stomach aches, urinary, vomiting, fever, asthma, liver disorders, typhoid, toothaches, and stomach aches.	[192]
	F.cretica / whole plant	Treatment for cancer	[193]
	F.arabica L / Ns	Typhoid, vomiting, leukemia, boils, dysentery, and asthma. Their infusions treat mouth pain, stomatitis, and constipation.	[194, 195]
	F.arabica L / Twigs	treat snake bites and are applied externally as a paste on tumors and swellings	[194]
	F. Olivieri / Shoot	Diabetes and cough treatment, blood purifier, and cooling agent	[196]
	F. olivieri / Ns	Vascular, early-stage cancer, and digestive diseases.	[177]
India	*F.arabica L* / Leaves, aerial parts	smallpox, skin diseases, and endothermic body reactions	[193, 195]
	F.schweinfurthii / whole plant	Poisoning, smallpox, diabetes, pain, jaundice, cough, asthma, skin rashes, liver tonic for many liver conditions	[181]
Algeria	*F. longispina* / aerial plant	digestive diseases	[189]

Ns : Not specified

I.2.5.4. Pharmacology of the genus *Fagonia*

Several investigations have been conducted to validate traditional uses of the genus *Fagonia* and investigate novel biological potentials concerning their therapeutic utilizes. The majority of these studies concern anticancer and antitumor effects, microbial resistance, antidiabetic effect, anti-inflammatory, and healing (Table.I.12).

Table.I.12. Pharmacological studies of *Fagonia* species.

Plant / Tested sample	Model	Dose / concentration	Results	Ref
		Cytotoxic, Anti-tumor activity		
***F.cretica* / methanolic extract**	-In-vivo / Brine shrimp toxicity assay -In-vivo / Potato disc assay	-0.5-10,000 ppm for Cytotoxic assay -10-1000 ppm for anti-tumor activity	- At LD50 118.89 ppm, a significant cytotoxic effect was recorded against brine shrimps - All of the tumor-inducing Agrobacterium strains tested showed significant antitumor activity (At6, At10, and At77), with At10 showing the highest tumour inhibition (77.04%).	[192]
***F. indica* / crude water extract**	In-vivo / Tumors in male and female rats were induced experimentally	-	Significantly greater tumorostatic activity in females than in males	[197]
		Thrombolytic agent activity		
***F. Arabica* / Aqueous extract**	In vivo / The clot lysis efficacy of *F. Arabica* was tested using a thrombolytic model with Streptokinase as a positive control.	10 mg/mL	*F.arabica* demonstrated a substantial % of clot lysis (75.6%) compared with Streptokinase (86.2%) (reference).	[198]
		Anti-Diabetic effect		
***F.indica* / (hexane, chloroform, methanol, and water extracts)**	-In vitro / Potential hypoglycemic effects were examined using an alpha inhibitory method. -In vivo / Wistar albino rat models were used to test the chloroform fraction's anti-hyperglycemic potential.	-20-100 ug/mL for the test in vitro -250-500 mg/kg b.w. for the test in vivo	-In vitro / The chloroform extract showed substantial activity, with an IC_{50} of 93.61±4.44 µg/mL compared to the acarbose (standard) value of 52.99±4.88 µg/mLfollowed by ethyl acetate, n-hexane, and n-butanol extracts. -In vivo / at 30 min after a meal, chloroform extract lowered postprandial blood glucose levels less than the refrence acarbose.	[199]
		Anti microbial activity		
***F.indica* / Ethanolic extract**	In vivo / Inhibition zones against gram-negative and positive bacteria: *E.coli, S. aureus, P. aeruginosa* and *B. cereus*	25- 100 mg/mL	The ethanolic extract had a strong inhibitory effect against all bacterial strains, with the best impact observed against *B.cereus.*	[200]
		Hepatoprotective activity		
***F. schweinfurthii* / ethanolic extract**	In vivo/ The hepatoprotective effect of *F. schweinfurthii* ethanolic extract was evaluated in the male wistar albino rats model	200-400mg:kg/day/ p.o.	*F.schweinfurthii* ethanolic extract effectively reduced CCl_4-induced alterations in HepG2 cells	[181]

Table.I.12 continued….

	of CCl_4-induced hepatotoxicity			
		Anti-inflammatory and wound healing activity		
***F.schweinfurthii* / Herbal gel**	In vivo/ Carrageenan induced rats paw edema and excision wound	0.5 g	Gel formulations have a gradual anti-inflammatory effect and expedite wound healing	[201]
		Clot lytic activity		
***F. Arabica* / Aqueous extract**	In vitro/ Blood co-agulation and Fibrinolysis by Microtiter plate clot lysis method	50 ug/mL	*F.arabica* lysed clots have a similar effect to streptokinases (reference =60 ug /ml) in an MPCL technique.	[198]
		Antioxidant activity		
***F. arabica* / Total extract**	In vitro/ Prevention of Oxidative Stress in PC12 Cells Due to Chemical Ischemia	1000 ug/mL	The antioxidant activity of *F.arabica* protect cells from being damaged by oxidative stress by ischemia.	[202]
***F.Longispina* / Ethanolic extract**	In vitro/ DPPH scavenging assay	0.5-0.001 mg/ml	The ethanolic extract had a scavenging efficacy (IC_{50}= 0.22±0.0075 mg/ml) comparable to that of ascorbic acid (IC_{50}= 0.24±0.0.0081 mg/ml)	[203]
***F.mollis* / (Aqueous and methanolic extracts)**	In vitro/ ABTS scavenging activity	-	IC_{50}= 72.5 ±0.6 µmol TE g^{-1} dry weight for Aqueous and 31.4± 4.5 µmol TE g^{-1} dry weight for methanolic extracts which exhibited a moderate effect compared with the reference trolox.	[204]

I.2.6. *Fagonia bruguieri* DC.

The genus *Fagonia* species possess a wide variety of active chemical constituents, which have been speculated to be accountable for the pharmacological effects of the *Fagonia* species. These studies have shown the accumulation of various secondary metabolites including flavonoids and terpenes [205]. Plants belonging to this genus have been the subject of significant attention in phytochemistry for an extended period. *Fagonia bruguieri* DC. species Saharo-arabic is most frequently known by its common name *F. echinella Boiss.* It is known as afessour in Tamahaq, the indigenous language of the Touareg inhabitants in the South of Algeria [98]. This choice was influenced mainly by the endemism of this plant in the southern Sahara, which has a distinct climate and unique environment. These factors, particularly the latter, are likely to influence the chemical composition of the vegetation.

- ✓ **Systematic classification [7, 191]**

Kingdom: Plantae
Subkingdom: Viridaeplantae
Division: Magnoliophytae
Class: Magnoliopsidae
Order: Zygophyllales
Family: Zygophyllaceae
Sub-family: Zygophylloideae
Tribe : Zygophylleae
Genus: *Fagonia* L.
Species: *Fagonia bruguieri* DC.
Vernacular names: *F. echinella Boiss*
Local name: *afessour*

Figure.I.10. *Fagonia bruguieri* DC.

I.2.6.1. Botany of the *Fagonia bruguieri* DC.

Fagonia bruguieri DC.is a small perennial (Shrub or shrublet) , pale-green plant, procumbent, reaches a maximum height of 20 cm and is thickly branched [188] (Figure.I.11).

Figure.I.11. Drawing of *Fagonia bruguieri* DC.[206].

Vegetative apparatus

- ✓ **Stems**: thickly or sparsely glandular, frequently viscid, broadly angular and with ridges of varying sizes; internodes measuring between 3 and 35 mm in length.
- ✓ **Stipules:** 1.5-25 mm long, spinescent, erect or deflexed, shorter or longer than the leaves, basal stipules longer than upper stipules, glandular.

- ✓ **Leaves**: Lower leaves 3-foliolate or sometimes 2-foliolate, upper leaves (3)-foliolate, linear to elliptic, rarely obovate, glandular, flat, with up to 0.8 mm mucro, lateral leaveslets smaller than center

Producing apparatus

- ✓ **Sepal:** persistent, ovate, apiculate, and glandular. *Fagonia bruguieri* DC.has longer-lasting sepals than most species.
- ✓ **Petal**: Petals varied from 3.5 to 5 mm, 1.7 mm claw, apiculate and soon deciduous. Violet-purple petals.
- ✓ **Fuit**: Fruit-calyx persistent, a capsule with five sides is covered in hairs, 4 mm long and 3 to 4 mm wide [188].

I.2.6.2. Geographic distribution of *Fagonia bruguieri* DC.

Fagonia bruguieri is a northern Sahara-endemic species found all over the Sahara desert. In Africa, F. bruguieri is distributed in Mauritania in the west, East Egypt, and the transition zone of the Sahel area in the south. It has a continuous distribution across Afghanistan and Pakistan to the east and reaches Yemen to the south (Figure.I.12) [188, 207].

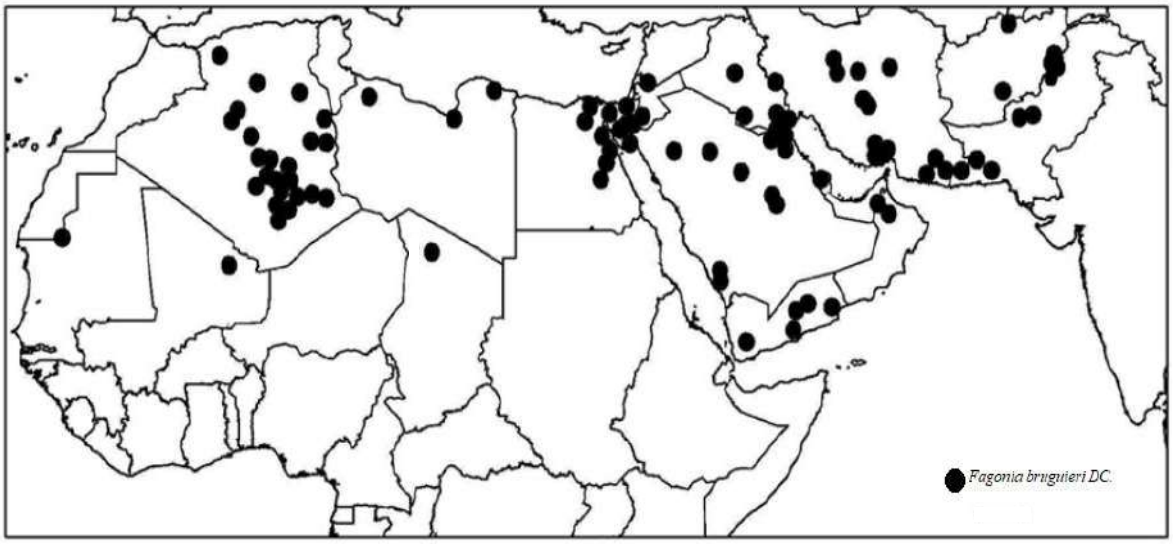


Figure.I.12. Distribution of *Fagonia bruguieri* DC.[188].

I.2.6.3. Folk-medicinal of *Fagonia bruguieri* DC.

Fagonia species are widely utilized in folk medicine as popular treatments for a wide range of skin diseases. In the Algerian region of Hoggar, the local populations of Tamanraesset use a decoction prepared of leaves as a treatment for jaundice as well as digestive problems, anemia, and kidney diseases [98]. Additional traditional applications are listed in the literature (Table.I.13).

Table.I.13. Traditional medicinal uses of *Fagonia bruguieri* DC.

Region	Preparation method	Part used	Ethnopharmacology reports	Ref
Algeria	Décoction, infusion, Powder	Leaves, aerial parts	Jaundice, Digestive diseases, anemia kidney diseases.	[98]
	Powder (internal use)	Aerial parts	Jaundice, liver insufficiency, Palpitations, anxiety	[176]
Saudi Arabia	-	Leaves	Blood and heart tonic	[178]
	boiled water	Leaves	blisters dermatitis	[208]
Pakistan	Infusion	Whole plant	Diabetes, obesity, control swatting	[209]
Iran	Decoction, infusion	Aerial parts	Appetizing, vermicide, carminative	[210]

I.2.6.4. Pharmacology of *Fagonia bruguieri* DC.

Table.I.14. Pharmacological studies of *Fagonia bruguieri* DC.

Testd sample / Part used	Model	Dose / concentration	Results	Ref
		Analgesic activity		
Methanol extrac/ whole plant	-In vivo / Acetic acid induced writhing model nd Eddy's hot plate in	250- 750 mg/kg	A 750 mg/kg dose of *F. bruguieri* methanolic extract decreased the writhing count by 62.23 %, while *F. bruguieri* improved delay in Eddy's hot plate test.	[211]
		Anti-inflammatory activity		
Methanol extrac/ whole plant	In vivo / Carrageenan-induced paw edema in adult male albino Wistar rats was used to assess anti-inflammatory effects	250- 750 mg/kg	The methanolic extract of *F. bruguieri* at 750 mg/kg showed anti-inflammatory effects in carrageenan-induced edema after 2, 3, and 4th hours.	[211]
		Anti-pyretic activity		
Methanol extrac/ whole plant	In vivo / Brewer's yeast induced pyrexia model.	250- 750 mg/kg	The yeast treatment increased rectal temperature and decreased the methanolic extract of *F.bruguieri* (98.3±0.07) at 500 and 750 mg/kg compared to diseased rats.	[211]
		Antidiabetic activity		
Crude extract / Aerial part	In vitro / amylase inhibition assay	-	Important inhibition of alpha amylase (75.5±0.5 %)	[212]
		Histopathological and biochemical hepatoprotective effect		
Ethanolic extract / Aerial part	In vitro/ Pre-treatment with ethanolic extract against CCl4-intoxicated rabbits was investigated biochemically and histologically	500 and 750 mg/kg/day PO	The ethanolic extract of *F.bruguieri* showed Hepatoprotection at 500 and 750 mg/kg, which considerably restored SGOT, SGPT, and ALP levels, whereas TB levels did not increase appreciably. Moreover, these findings were confirmed	[213]

Table.I.14 continued….

			by a histopathological study of the liver tissue.	
		Anti-allergic activity		
Boiling water, freeze-dried / whole plant	in vivo / Albino guinea pigs administered the extract orally antagonized with 20 µg/kg i.v. of histamine and 100 µg/kg i.v. of capsaicinand produced bronchoconstriction without affecting ACh and 5-HT.	200 mg/kg	Antagonisms against histamine and capsaicin were 72 % and 65 %, respectively. Treatment of guinea pigs with *F.bruguieri* for 20 minutes or orally for 2 hours prevented histamine toxicity, grasps and loss of consciousness.	[205]
		Acetylcholinesterase Activity		
Methanolic, petroleum ether, n-butanolic extracts/ Aerial part	In vivo / The haemolymph and fat body of nymphs in their last instar and adult females were tested to assess the AchE activity.	-Methanolic (7.5-3.7%) -Petroleum ether (30 -15%) -n-butanolic (30 -15%)	The petroleum ether and n-butanolic extracts showed the strongest inhibitory impact with respect to the enzyme activity in the fat body of S. gregaria nymphs, particularly in the early nymphs.	[214]
		Antioxidant activity		
Methanolic extract/ Aerial part	In vitro / DPPH radical scavenging assay	11-100 ug/mL	IC_{50} = 32.733 µM	[215]
Methanolic extract/ Aerial part	In vitro / Total antioxidant capacity(phosphomolybdenum method)	-	IC_{50} =120 µg AA Equivalent /mg methanol extract	[215]
Methanolic extract/ Aerial part	In vitro / Reducing power assay	-	IC_{50} =141 µg AA equivalent/ mg methanol extract	[215]
		Antifungal activity		
Methanolic extract/ Aerial part	In vivo / - *Aspergillus niger* - *Aspergillus Fumigatus* - *Aspergillus Flavus* - *Mucor mycosis*	100- 400 mg/kg, b.w.	The methanolic extract had antifungal effect against all of the microorganisms that were examined.	[215]

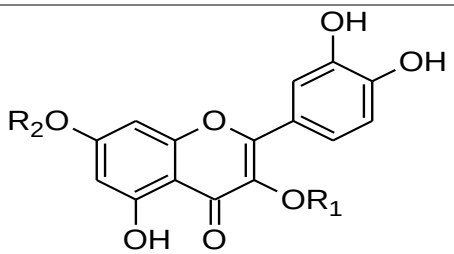
I.2.6.5. Secondary metabolites isolated from the *Fagonia* genus

I.2.6.5.1. Flavonoïds

The most abundant secondary metabolites in the genus *Fagonia* are flavonoids, predominantly O-glycosylated flavonols at the 3 and/or 7 position, including Kaempferol, Isorhamnetin, Quercetin, and Herbacetin. On the other hand, Flavones are not very common in the genus *Fagonia*. Only three derivatives of apegenin and acacetin have been isolated from the species F. taeckholmiana and F. arabica. Table.I.15→ Table.I.20 illustrate the various flavonic structures isolated from the genus *Fagonia*.

✓ **Flavonols and its derivatives**

Table.I.15. Quercetin derivatives isolated from the genus *Fagonia.*

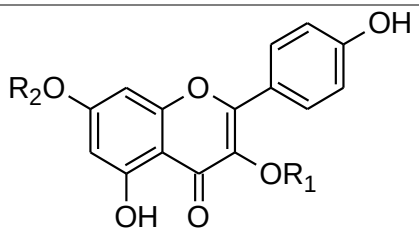


Isolated Compound	**R_1**	**R_2**	***Species***	**Ref**
Quercetin	H	H	*F.taeckholmiana*	[216]
			F. indica	[217]
Quercetin-3-O-rhamnogalactoside	Rha-Gal	H	*F. kassasii* *F.bruguieri* *F. schimperi* *F. olivieri*	[218]
			F. microphylla *F. glutinosa* *F.latifolia* *F. isotricha*	[219]
Quercetin-3-O-glucoside	Glc	H	*F. indica* *F. paulayana* *F. scabra* *F. cretica*	[220]
			F.thebaica	[221]
			F. taeckholmiana	[216]
Quercetin-3-O-galactoside	Gal	H	*F. kassasii*	[218]
			F.bruguieri *F. schimperi* *F. olivieri*	[218, 220]
			F.latifolia *F. isotricha*	[219]
Quercetin-3-O-gentiobioside	β-D-Glc^6-D-Glc	H	*F. microphylla* *F. glutinosa* *F.latifolia* *F. isotricha*	[219]
			F. kassasii *F.bruguieri* *F. schimperi* *F. olivieri*	[218]
Quercetin-3-gentiobioside-7-O-glucoside	β-D-Glc^6-D-Glc	Glu	*F. glutinosa* *F. microphylla*	[219]
Quercetin-3-O-rutinoside	Rut	H	*F. indica*	[219]
			F.boulosii	[220, 221]

Table.I.15 continued….

			F.cretica *F. tenulfolia* *F.thebaica* *F. olivieri*	[220]
			F.bruguieri *F. schimperi* *F. kassasii*	[218, 220]
Quercetin 3-O-β-D-galactopyranosyl -(1→6)- α-L-2 acetyl rhamnose-(1→3) β-D-glucopyranoside	β-D-Glc^{6}- α-L-2 acetyl Rha^{3}- D-Glc	H	*F. indica*	[217]
Quercetin-3,7-O-diglucoside	Glc	Glc	*F. glutinosa* *F. microphylla* *F. isotricha*	[219]
			F.thebaica *F.boulosii*	[220, 222]
			F. tenulfolia *F. paulayana*	[220]
Quercetin 3-O-β-D-glucopyranoside	β-D-Glc	H	*F. longispina*	[223]

Table.I.16. Kaempferol derivatives isolated from the genus *Fagonia*.



Isolated Compound	**R_1**	**R_2**	**Species**	**Ref**
kaempferol	H	H	*F. paulayana* *F. indica* *F.schweinfurthii*	[220, 224]
3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-kaempferol	Rha- Glc	H	*F. indica*	[225]
Kaempferol-3-O-rutinoside	Rut	H	*F.mollis* *F. tristis*	[226]
			F.bruguieri *F. olivieri* *F. schimperi* *F. kassasii*	[218]
			F. indica *F.schweinfurthii*	[224]
			F. isotricha *F.latifolia*	[219]
			F. indica *F.boveana*	[220]
			F. longispina	[223]
Kaempferol-3-O-glucoside	Glc	H	*F.bruguieri* *F. kassasii*	[218]
			F. glutinosa *F. microphylla*	[219]

Table.I.16 continued….

			F.schweinfurthii *F. cretica* *F. scabra* *F. longispina* *F. paulayana* *F. indica*	[220]
Kaempferol 3- O-rhamnogalactoside	Rha-Gal	H	*F.kassasii,*	[218]
			F.olivieri *F.schimperi* *F.bruguieri*	[218, 220]
			F. isotricha *F.latifolia*	[219]
kaempferol-3-gentiobioside-7- O-glucoside	Gent	Glc	*F. glutinosa* *F. microphylla*	[219]
Kaempferol-7-O-rhamnoside	H	Rha	*F.arabica*	[227]
Kaempferol-3,7-O-diglucoside	Glc	Glc	*F.isotricha*	[219]
Kaempferol-7-O-glucoside	H	Glc	*F. microphylla* *F. glutinosa*	[219]
Kaempferol-3,7-di-O-rhamnoside (kaempféritrine)	Rha	Rha	*F. taeckholmiana*	[216]
Kaempferol 3-O-β-L-arabinopyranosyl-(1→4)-α-L-rhamnopyranoside-7-O-α-L-rhamnopyranoside	Ara-Rha	Rha	*F. taeckholmiana*	[216]
Isokaempferide	CH_3	H	*F. longispina*	[223]

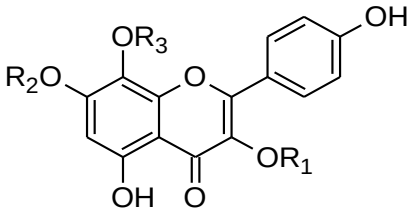
Table.I.17. Isorhamnetin derivatives isolated from the genus *Fagonia.*

Isolated Compound	**R_1**	**R_2**	**Species**	**Ref**
Isorhamnetin-3-glucoside	Glc	H	*F.thebaica*	[220, 221]
			F.scabra Forsskal *F.cretica*	[220]
			F.arabica	[220, 228]
			F. indica	[220, 224]
			F. taeckholmiana	[228]
Isorhamnetin-3,7-O-diglucoside	Glc	Glc	*F.thebaica*	[220, 222]
			F. paulayana	[220, 224]
			F.cretica *F. tenulfolia* *F.scabra Forsskal*	[220]
Isorhamnetin-3-rutinoside	Rut	H	*F. mollis*	[220, 226]
			F.schweinfurthii *F.indica*	[224]
			F. taeckholmiana *F. arabica*	[228]
			F. olivieri *F. schimperi* *F. kassasii*	[218]

Table.I.17 continued….

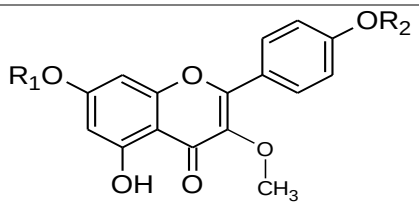
			F.boulosii *F.thebaica*	[220, 222]
			F. tristis	[226]
			F.boveana *F.bruguieri*	[220]
Isorhamnetin-3-O-rhamnoside	Rha	H	*F. indica*	[217]

Table.I.18. Herbacetin derivatives isolated from the genus *Fagonia.*



Isolated Compound	**R_1**	**R_2**	**R_3**	**Species**	**Ref**
3,8- dimethoxy herbacetin	CH_3	H	CH_3	*F. bruguieri*	[229]
				F. boveana	[230]
8-methoxy-3,glucoside herbacetin	Glc	H	CH_3	*F.thebaica*	[220]
8-methoxy-3-rutinoside-7-glucoside herbacetin	Rut	Glc	CH_3	*F.taeckholmiana*	[228]
				F. arabica	[220, 228]
Herbacetin-8-O-rutinoside	H	H	Rut	*F. Arabica*	[220, 228]
				F.thebaica	[220, 222]
				F. taeckholmiana	[228]
3,7,8-trimethoxy herbacetin	CH_3	CH_3	CH_3	*F. bruguieri*	[229]
8-methoxy-3-rutinoside herbacetin	Rut	H	CH_3	*F.boulosii* *F.thebaica*	[222]
				F. taeckholmiana	[228]
				F. Arabica	[187, 220]
				F. mollis *F.boveana*	[220]
8-methyl ether-3,7-diglucoside herbacetin	Glc	Glc	CH_3	*F. arabica*	[220, 228]
				F.taeckholmiana	[228]
8-methoxy herbacetin	H	H	CH_3	*F. tristis*	[226]

Table.I.19. Flavonols isolated from the genus *Fagonia*.



Isolated Compound	R_1	R_2	Species	Ref
Apigenin	H	H	*F. taeckholmiana*	[216]
Acacetin 7-O-rhamnoside	Rha	CH_3	*F. arabica*	[227]
Apigenin 7-O-glucoside	Glc	H	*F. taeckholmiana*	[216]

I.2.6.5.2. Terpenoids

- ✓ **Triterpenoids and saponins and its derivatives**

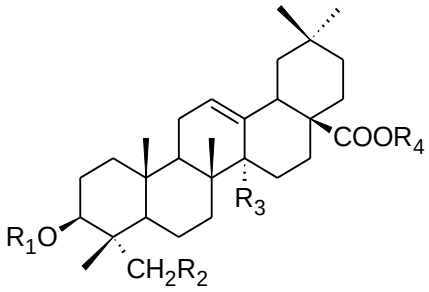
As a class, these secondary metabolites are among the most defining characteristics of the *Fagonia* genus. More than 40 new Saponin and triterpenoid compounds have been isolated from this genus. The vast majority of them are saponins that are monodesmosides or bisdesmosides, and they are distinguished by α -L- arabinopyranosyl at position 3 that is linked to structures that are of the oleanane, ursane, or taraxastane type. The research on *Fagonia* has led to the isolation and characterization of thirteen genin saponins, including oleanolic acid and ursane type 3-O-glycosides or 3,28-di-O-glycosides in the genus *Fagonia*, represented by genins: ursolic acid, 27-hydroxyursolic acid, quinovic acid. In the genus *Fagonia*, taraxastane-type saponins are rare; they are widespread among the *F. indica* species.

A novel saponin glycosylated steroid of the genus *Fagonia* 12-(4-methyl-pent-3enoyloxy)-20-(4-methyl-pent-3-enoyloxy)-3β,12β,20β-trihydroxypregnane-3-ylO-β-Dcymapyranosyl-(1→4)-3-methoxy-6-deoxy-β-D-glucopyranoside which is capable of triggering apoptosis or necrosis in cancer cells was isolated as a consequence of research into the plant anticancer activity of the plant *Fagonia indica* [231]. We classify the triterpenes and saponins into five categories (oleananes, ursanes, taraxastane, lupane, and saponin steroids), distributed across six different species (Tableau.I.21; Figure.I.13→Figure.I.16).

Table.I.20. Triterpenes and saponins isolated from the genus *Fagonia*.

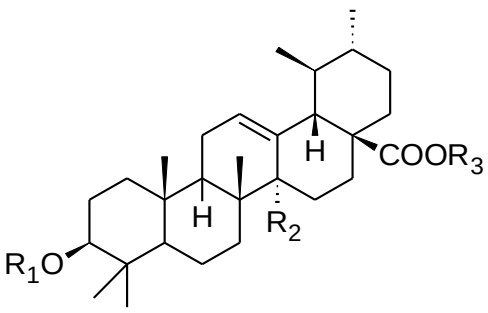
N°	Isolated Compound	Species	Ref
	Saponins of the Oleanane type		
1	3-O-β-D-xylopyranosyl (1→2) -[β-Dglucopyranosyl (1→3)]-α- L arabinopyranosyl oleanolic acid	*F. arabica*	[232]
2	3-O-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-α-L-arabinopyranosyl oleanolic acid	*F. arabica*	[232]
3	3-O-β-D-glucopyranosyl(1→3)-α-L- arabinopyranoside oleanolic acid	*F. arabica*	[227]
		F. indica	[233]
4	3-O-β-D-xylopyranosyl (1→2)-[β-D glucopyranosyl (1→3)]-α-L- arabinopyranosyl 28-O-β-D-glucopyranoside oleanolic acid	*F. arabica*	[232]
5	3-O-β-D-glucopyranosyl (1→2)-[β-D-glucopyranosyl (1→3)]-α-Larabinopyranosyl 28-O-β-D-glucopyranoside oleanolic acid	*F. arabica*	[232]
6	3-O-α-L-rhamnopyranosyl-(1→3)-6'-*O*-methyl-*β*-D-glucuronopyranoside oleanolic acid	*F.mollis*	[234]
7	3-*O*-*α*-L-rhamnopyranosyl (1→3)-6'-*O*-methyl-*β*-D-glucurono pyranosyl 28-*O*-*β*-D-glucopyranoside oleanolic acid	*F.mollis*	[234]
8	3-O-sulfonyl- acide 27-hydroxyoleanolic-28-O-β-D-glucopyranoside	*F.arabica*	[235]
9	3-O-[β-D-glucopyranosyl (1→2)] [β-D-glucopyranosyl (1→3)]-α-L-arabinopyranosyl- acid 27-hydroxy oleanolic-28-O-β-D-glucopyranosyl ester	*F.glutinosa*	[236]
10	3 β-O-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl] acide olean-12-èn-27-al-28-oic 28-O-[β-D-glucopyranosyl(1→6)-β-D-glycopyranosyl] ester	*F. cretica*	[237]
11	Hederagenin 3-O-β-D-glucopyranosyl	*F. cretica*	[238]
12	Hederagenin 3-O-β-Dglucopyranosyl-(1 →5)-α-L-arabinofuranosyl-(1 →4)-α-L-rhamnopyranosyl	*F. cretica*	[238]
13	hederagenin 3-O-β-D glucopyranosyl-(1 →4)-α-L-rhamnopyranosyl	*F. cretica*	[238]
Table.I.21 continued….	**Saponins of the ursane type**		
14	3-O-{[β-D-glucopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-α-L-arabinopyranosyl} ursolic-28-O-[β-Dglucopyranosyl] ester	*F. arabica*	[233]
15	3-O-[β-Dglucopyranosyl(1→2)][β-D-glucopyranosyl(1→3)]-α-L arbinopyranosyl ursolic-28-O-β-Dglucopyranosyl ester	*F. glutinosa*	[236]
16	ursolic 3-*O*-[*β*-D-glucopyranosyl(1→3)]-*α*-L-arabinopyranosyl	*F. glutinosa*	[236]
17	3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl ursolic-28-O-β-D-glucopyranosyl ester	*F. indica*	[236]
18	3-O-β-Dxylopyranosyl(1→2)-[β-D-glucopyranosyl (1→3)-α-L arabinopyranosyl -27-hydroxy ursolic28-O-β-D-glucopyranoside	*F.arabica*	[232]
19	3-O-[2-O-sulfo-α-L-arabinopyranosyl] 27-hydroxyursolic 28-O-β-D-glucopyranoside	*F.arabica*	[235]

20	3-O-[2-O-sulfo-α-L-arabinopyranosyl] -27-hydroxyursolique	*F.arabica*	[235]
21	3-O-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl quinovic 28-O-β-D-glucopyranoside	*F. arabica*	[227]
22	3-O-β-D-glucopyranosyl quinovic 28-O-β-D-glucopyranoside	*F.cretica*	[239]
23	3-O-β-D-2-O-sulfonyl glucopyranosyl quinovic	*F. indica*	[240]
	Triterpenes and saponins of the taraxastane type		
24	3β,O-β-D-glucopyranosyl-23-hydroxytaraxer-20-en-28-oic	*F. indica*	[177]
25	23,28-di-O-β-D-glucopyranosyl taraxer-20-en-28-oic	*F. indica*	[177]
26	3β, 28-di-O-β-D-glucopyranosyl-23- hydroxytaraxer-20-en-28-oic	*F.mollis*	[241]
27	21,22α-epoxy-23-O-β-D-glucopyranosyl-nahagenin	*F. indica*	[177]
28	3β,23-disulfonyl-nahagenin	*F. indica*	[235]
29	fagonicin	*F. indica*	[242]
30	3β- hydroxy-23-O-β-Dglucopyranosyl-taraxer-20-en-28-carboxy-O-β-D-glucopyranosyl	*F. indica*	[242]
31	3β- hydroxy-23,28-di-O-β-D-glucopyranosyl-acide taraxer-20-en-28-oic	*F. indica*	[242]
	Lupane-type triterpenes		
32	3β, 19, 21-trihydroxy-20(29)-lupéne	*F. indica*	[243]
33	Lupeol	*F. indica*	[243]
	Saponin Steroid		
34	12-(4-methyl-pent-3enoyloxy)-20-(4-methyl-pent-3-enoyloxy)-3β,12β,20β-trihydroxypregnane-3-ylO-β-Dcymapyranosyl-(1→4)-3-methoxy-6-deoxy-β-D-glucopyranoside	*F. indica*	[231]



	R_1	R_2	R_3	R_4
1	β-D-xyl^2-β-D-Glc^3-α-L-Ara	H	CH_3	H
2	β-D-Glc^2-β-D-Glc^3-α-L-Ara	H	CH_3	H
3	β-D-Glc^3-α-L-Ara	H	CH_3	H
4	β-D-xyl^2-β-D-Glc^3-α-L-Ara	H	CH_3	β-D-Glc
5	β-D-Glc^2-β-D-Glc^3-α-L-Ara	H	CH_3	β-D-Glc
6	α-L-Rha^3-6'-O-Me-β-D- GlcA	H	CH_3	H
7	α-L-Rha^3-6'-O-Me-β-D- GlcA	H	CH_3	β-D-Glc
8	SO_3H	H	CH_2OH	β-D-Glc
9	β-D-Glc^2-β-D-Glc^3-α-L-Ara	H	CH_2OH	β-D-Glc
10	β-D-Glc2-α-L-Ara	H	CHO	β-D-Glc^6-β-D-Glc
11	β-D-Glc	OH	CH_3	H
12	β-D-Glc^4-α-L-Ara^4*f*-α-L-Rha	OH	CH_3	H
13	β-D-Glc^4-α-L-Rha	OH	CH_3	H

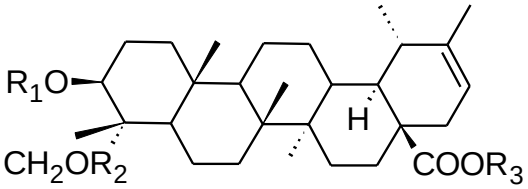
Figure.I.13. Oleanane saponins isolated from the genus *Fagonia*.



	R_1	R_2	R_3
14	β-D-Glc^2-α-L-Ara^3-α-L-Ara	CH_3	β-D-Glc
15	β-D-Glc^2-β-D-Glc^3-α-L-Ara	CH_3	β-D-Glc
16	β-D-Glc3-α-L-Ara	CH_3	H
17	β-D-Glc^3-α-L-Ara	CH_3	β-D-Glc
18	β-D-xyl^2-β-D-Glc^3-α-L-Ara	CH_2OH	β-D-Glc
19	2-O-Sulfonyl-α-L-Ara	CH_2OH	β-D-Glc
20	2-O-Sulfonyl-α-L-Ara	CH_2OH	H
21	β-D-Glc^3-α-L-Ara	COOH	β-D-Glc

22	β-D-Glc	COOH	β-D-Glc
23	2-O-Sulfonyl-β-D-Glc	COOH	H

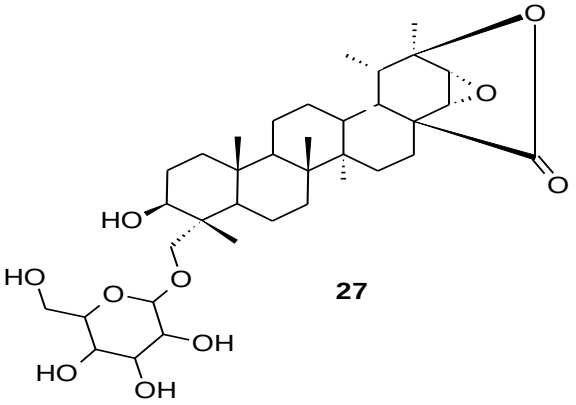
Figure.I.14. Ursan saponins isolated from the genus *Fagonia*.



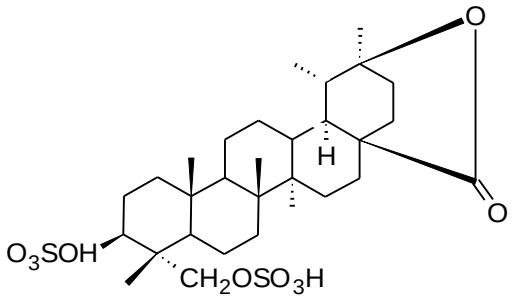
24 : R1= β-D-Glc, R2=H R3=H

25 : R1= H, R2= β-D-Glc R3= β-D-Glc

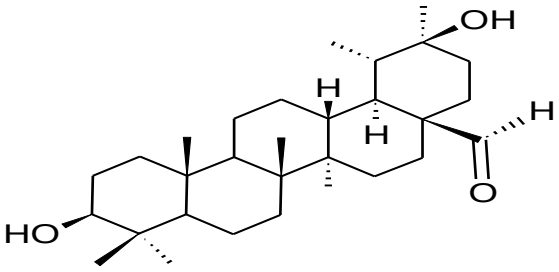
26 : R1= β-D-Glc R2= H R3= β-D-Glc



27



28



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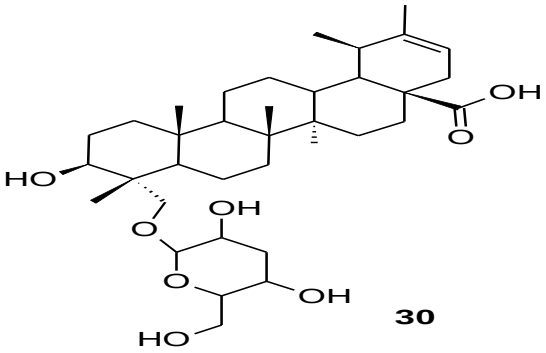
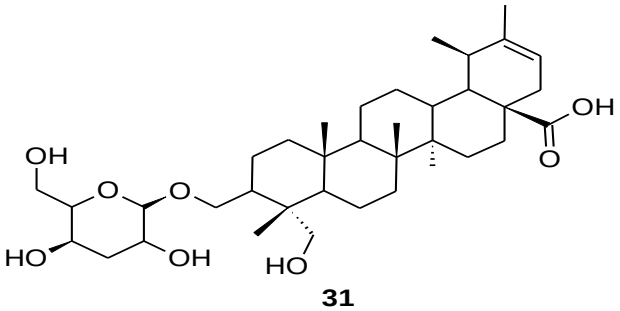
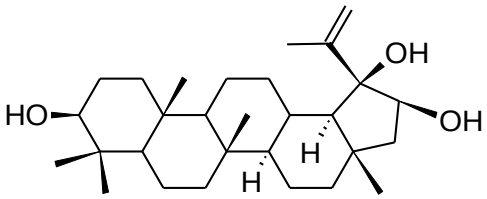


30



31

Figure.I.15. Triterpenes and saponins of the taraxastane type isolated from the genus *Fagonia*



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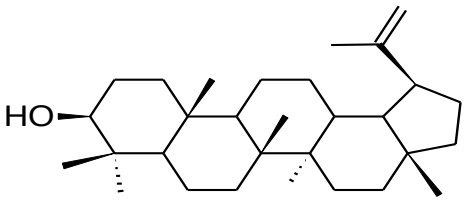
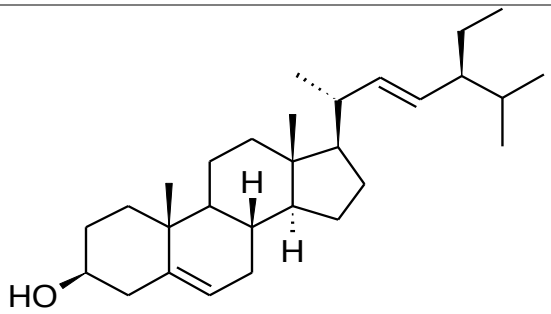
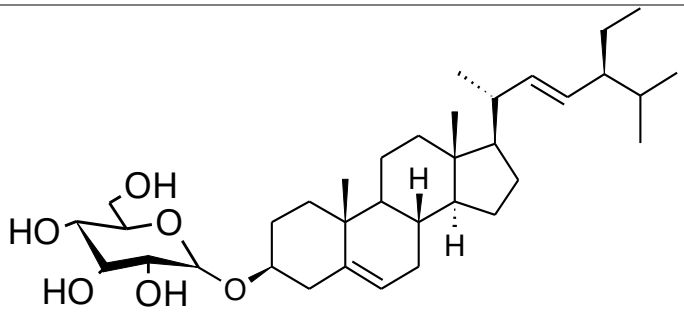


33

Figure.I.16. Lupan-type triterpenes isolated from *Fagonia.*

- **Steroids and its derivatives**

Table.I.21. Steroids isolated from *Fagonia* genus.

Isolated Compound	Structure	Species	Ref
Stigmasterol		*F.indica*	[217]
β-sitosterol-3-O-β-D-glucoside		*F.indica* *F.cretica*	[239] [244]

- **Diterpene and its derivatives**

Table.I.22. Diterpenes isolated from *Fagonia* genus.

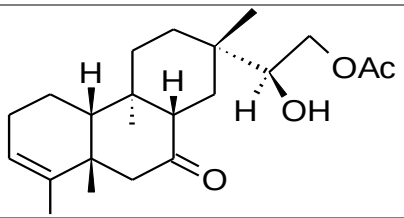
Isolated Compound	Structure	Species	Ref
7-β- hydroxyfagonene		*F.bruguieri*	[229]

Table.I.23 continued….

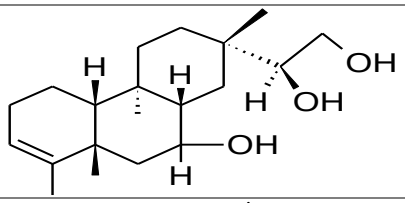
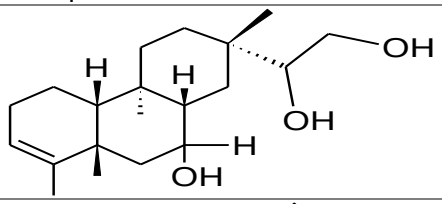
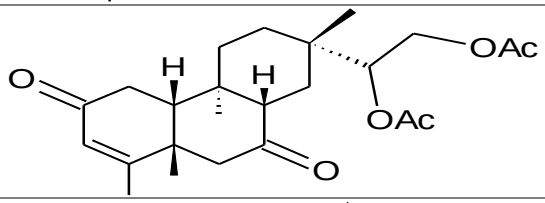
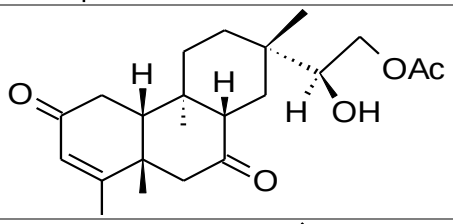
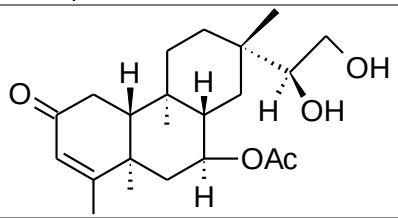
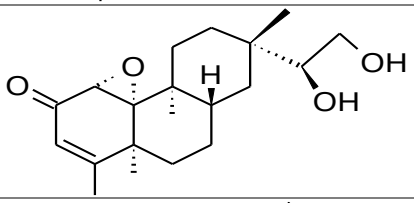
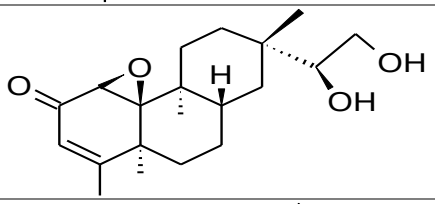
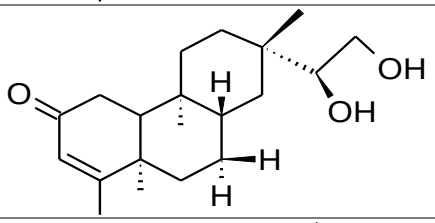
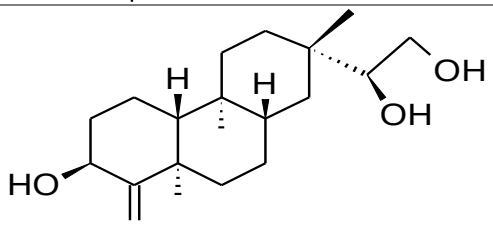
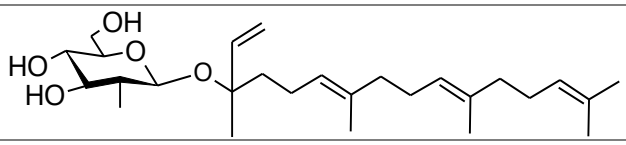
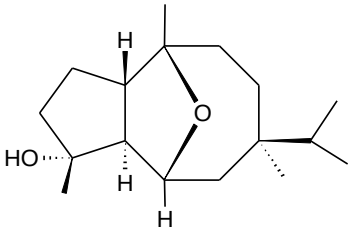
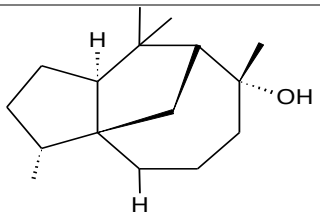
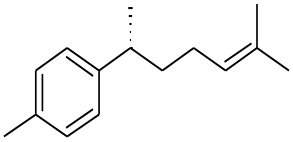
15,16-dihydroxy-7-oxo-cis-ent-erythrox-3-ene (fagonone)		*F.bruguieri*	[245]
7-α-hydroxyfagonene		*F. glutinosa*	[245]
2,7-Dioxofagonene		*F. glutinosa*	[245]
16-O-acetyl-2,7-dioxofagonene		*F. glutinosa*	[245]
7-*O*-acetyl-2-oxo-5-epi-fagonene		*F. glutinosa*	[245]
1α,10α-epoxy-2-oxofagonene		*F. glutinosa*	[246]
1β,10β-epoxy-2-oxofagonene		*F. glutinosa*	[246]
2-oxofagonene		*F. glutinosa*	[246]
3*β*,15,16-trihydroxy-erythrox-4(18)-ene		*F. boveana*	[230]

Table.I.23 continued….

7β-Hydroxy-5-epifagonene		*F.mollis*	[240]

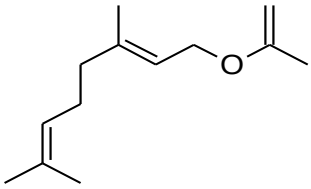
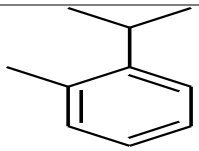
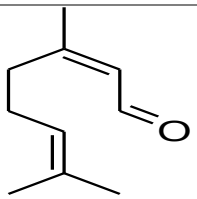
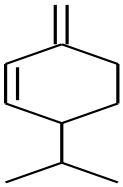
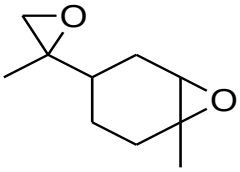
- **Sesquiterpene and its derivatives**

Table.I.23. Sesquiterpene isolated from *Fagonia* genus.

Isolated Compound	Structure	Species	Ref
6,10-epoxy-4α-hydroxy guaiane		*F. boveana*	[230]
α-cedrol		*F.cretica*	[247]
α-curcumen		*F.cretica*	[247]

- **Monoterpene and its derivatives**

Table.I.24. Monoterpene isolated from *Fagonia* genus.

Isolated Compound	Structure	Species	Ref
Geraniol acetate		*F.cretica*	[247]
o-Cymene		*F.cretica*	[247]
α-citral		*F.cretica*	[247]
β-phellandrene		*F.cretica*	[230]
α-limonene diepoxide		*F.cretica*	[230]

Chapter II: Valorisation of Bioactive Compounds

II.1. Introduction

Each plant species has a multitude of substances, which proceed from primary or secondary metabolism. Primary metabolism is responsible for the production of carbohydrates, proteins, lipids, minerals, and nucleic acids [248], all of which are directly engaged in the significant pathways of basal cell metabolism [249]. Secondary metabolites are a category of compounds distinguished by their complexity, a large number of metabolites (at least 30,000 have been characterized), and subsequent categorization into distinct chemical classes [250]. These compounds include phenolic compounds, flavonoids, tannins, saponosides, essential oils, and alkaloids, all of which have many applications in the food and pharmaceutical industries.

II.2. Phenolic compounds

Phenolic compounds are secondary metabolites that are distinctive to the plant kingdom. They constitute an extensive set of more than 8,000 molecules with an extremely wide range of chemical characteristics [251] from the basic phenolic molecules, which have a low molecular weight, such phenolic acids, through the most highly polymerized compounds, such as tannins [252]. The distinguishing structural feature of all these substances is the existence of at least one benzene nucleus directly linked to at least one hydroxyl group, whether free or performing some other function: ether, ester, or heteroside [253]. The basic structures of phenolic compounds allow for the categorization of these compounds into a variety of different classes, the most important of which are tannins, flavonoids, and phenolic acids [254].

II.2.1. Phenolic acids

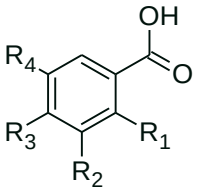
Acid-phenols are a class of organic compounds characterized by the presence of both a carboxylic function and a phenolic hydroxyl [255]. In the field of phytochemistry, the use of this word is restricted to the derivatives of benzoic acid or cinnamic acid, which together make up the two subclasses:

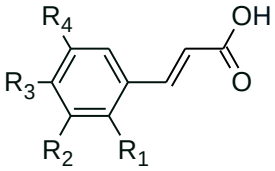
- **Hydroxybenzoic acid**

Hydroxybenzoic acids are typically found in the form of esters or glycosides [256]. Hydroxybenzoic acid, protocatechuic acid, vanillic acid, gallic acid, and salicylic acid are plants' most common hydroxybenzoic acids (Figure II.1) [257].

- **Hydroxycinnamic acids**

Cinnamic acid derivatives (hydroxycinnamic acids) have a basic C_6-C_3 structure for example: caffeic acid, ferulic acid, p-coumaric, and sinapic acid (Figure.II.1). They are members of the phenylpropanoids family. The most common type of simple phenolic compound is a hydroxycinnamic acid. Free forms of these acids are extremely uncommon outside of frozen, sterilized, or fermented processed foods. Quinic acid, shikimic acid, and tartaric acid have glycosylated derivatives or esters that constitute the bound forms [258, 259].





Benzoic acids	**R_1**	**R_2**	**R_3**	**R_4**	**Hydroxycinnamic acids**
p-hydroxybenzoic acid	H	H	OH	H	p-coumaric acid
Protocatechuic acid	H	OH	OH	H	Caffeic acid
Vanillic acid	H	OCH_3	OH	H	Ferulic acid
Gallic acid	H	OH	OH	OH	
Syringic acid	H	OCH_3	OH	OCH_3	Sinapic acid
Salicylic acid	OH	H	H	H	
Gentisic acid	OH	H	H	OH	

Figure.II.1. Structure of principal phenolic acids [258].

II.2.1.1. Biosynthesis

The production of hydroxybenzoic acids begins with the hydroxylation of benzoic acid, which has a fundamental structure of the type C_6-C_1. Following that, these phenolic hydroxyls OH can be methylated (Figure.II.2) [260]

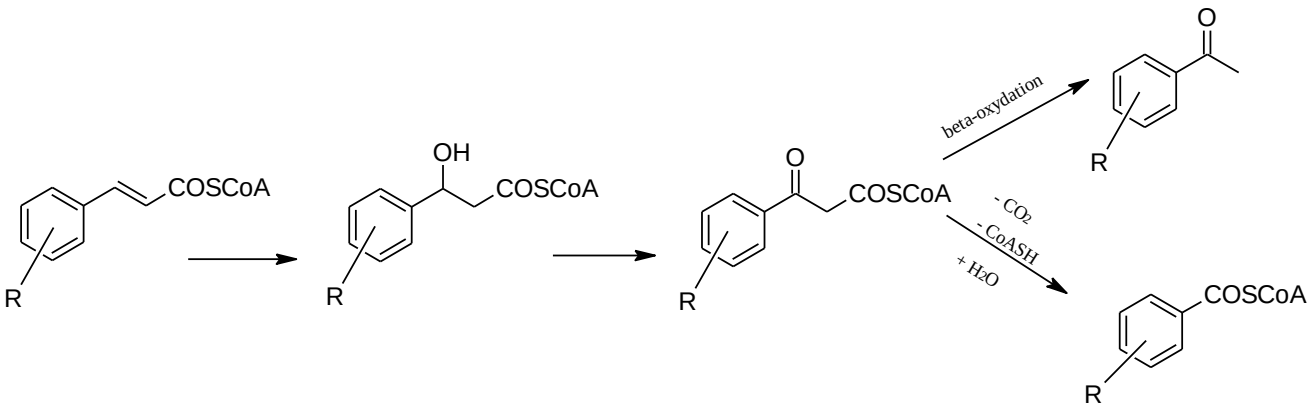


Figure.II.2. Biosynthesis of benzoic acid derivatives [255].

The reaction of cinnamic acid derivatives consists of the condensation of phosphoenolpyruvate (PEP) with erythrosis-4-phosphate to create 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP), which leads to aromatic amino acids (phenylalanine, tyrosine) and, via deamination of the latter, to cinnamic acids and their derivatives (benzoic acid, acetophenone, lignans, coumarin, etc.) (Figure.II.3).

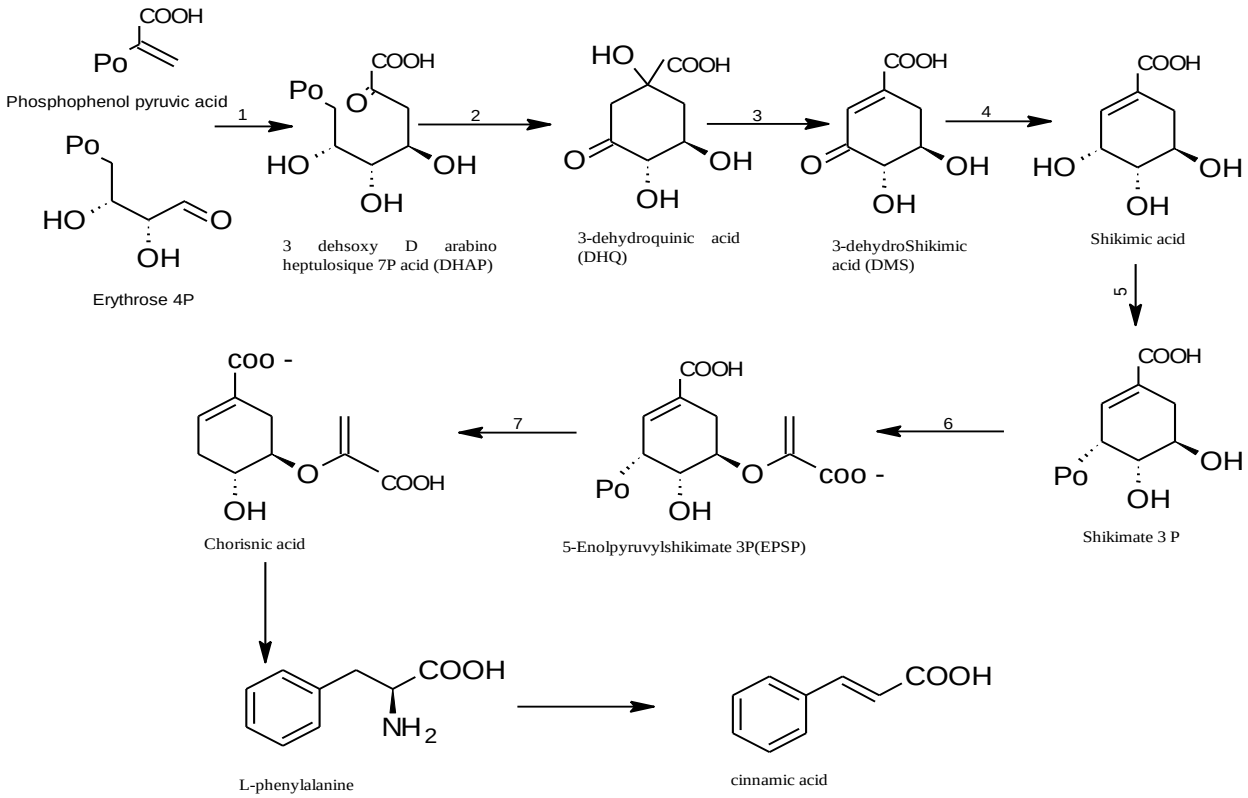


Figure.II.3. Biosynthesis of cinnamic acid derivatives [255].

II.2.2. Flavonoids

The term "flavonoid" is used to designate a broadly diverse group of naturally occurring compounds members of the polyphenols class. They are regarded as practically all plant peppers due to their prevalence [261]. These substances have a low atomic weight, and all have the same fundamental structure, which consists of 15 carbon atoms grouped in three cycles of C_6-C_3-C_6: A, B, and C, each of which has one or more hydroxyl groups as a substituent (Figure.II.4) [258, 262].

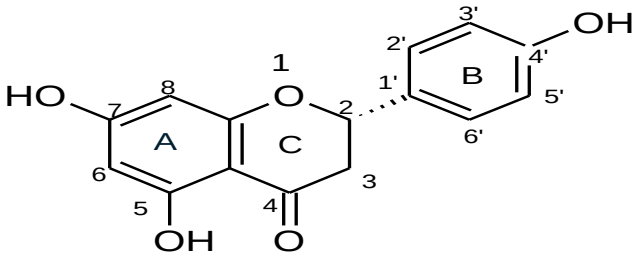


Figure.II.4. Structure of a flavonoid

Flavonoids are commonly found in their glycosylated form in nature. In the presence of sugar, the molecule's characteristics are altered compared to its glycosylated analog. In particular, the molecule's solubility and hydrophobicity are affected. The formation of the bond between aglycone and ose is typically accomplished by one of the phenolic hydroxyls, particularly those in position 3 (for flavonols) or position 7 (for flavones) and sometimes in positions 6 or 8 if these positions are hydroxylated. D-glucose, L-rhamnose, D-glucuronic acid, D-galacturonic acid, L-arabinose, D-xylose, and rarely D-allose, and D-mannose are the predominant monosaccharides in their constitution. D-glucose and L-rhamnose are the most predominant sugars in flavonoids [258, 263].

Different types of flavonoids can be distinguished based on the oxidation degree of the central heterocycle C, the degree of unsaturation, and the positions of the hydroxyl groups on the nuclei A and B. The most extensively reported and well-characterized of these groups are the following six: flavonoids, flavanones, flavonols, isoflavones, flavonols, flavones, and anthocyanidins [258, 264].

II.2.2.1. Biosynthesis

This fundamental structure is the final product of the two various pathways for the synthesis of phenolic compounds. The phenylpropanoid unit, which includes the B nucleus and the carbon bridge, is produced by the shikimic acid pathway from phenylalanine, whereas the A nucleus

is formed via the condensation of 3 acetate patterns via the malonic acid pathway. The combination of these two components takes place through the condensation of a phenylpropanoid, 4-coumaryl, with three malonyl CoA molecules, which results in the formation of tetrahydroxychalcone. This, in turn, will result in the formation of all flavonoids (Figure.II.5) [258, 263].

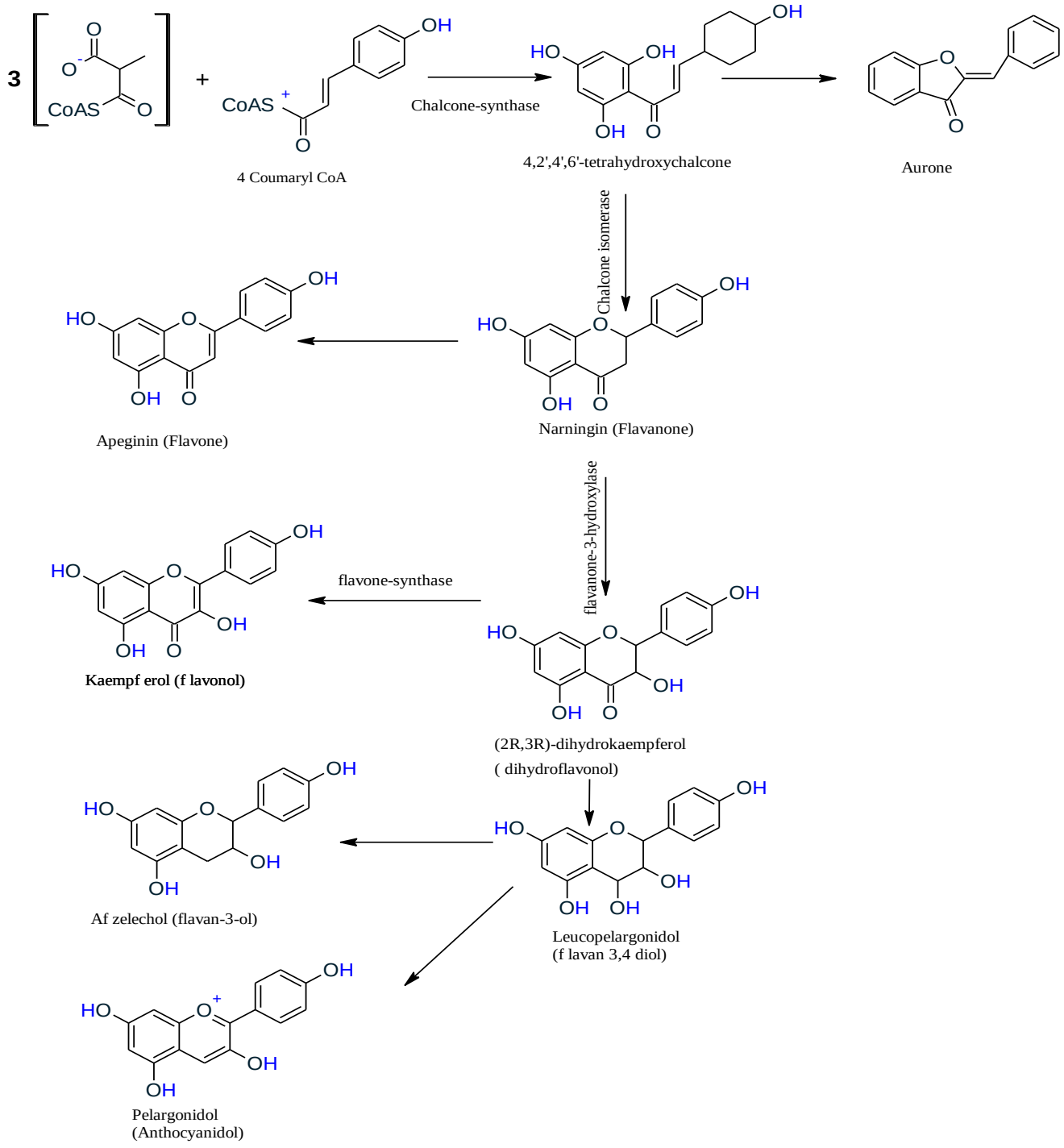


Figure.II.5. Flavonoid biosynthesis [255].

II.2.3. Tannins

Tannins are a class of polyphenols that possess a high molecular weight and may be found in nearly every part of a plant, including the bark, the wood, the leaves, and the roots [265]. These substances are highly hydroxylated molecules that, when coupled with carbohydrates, proteins, and digestive enzymes, can form insoluble complexes that decrease digestibility [266].

Hydrolyzable tannins and condensed tannins are the two categories into which natural tannins can be divided according to their chemical structure [267].

Condensed tannins or proanthocyanidins are dimers or polymers of flavan-3-ols, mainly catechin and catechols, including profisetinidin, prorobinetinidin, procyanidin, prodelphinidine, and other polysaccharides [268]. In type A proanthocyanidins, these units are bound by a single C-C bond (interflavanic) C_4-C_8 or C_4-C_6 (Figure II.6). In type B, they are linked by a double interflavanic bond (C_4-C_8 or C_4-C_6) and (C_2-O-C_7) [268, 269].

Hydrolyzable tannins are sugar-phenolic acid oligos or polyesters (Figure.II.6). Sugar is commonly glucose. Gallic acid or hydroxydiphenic acid (HHDP) and its derivatives make up phenolic acid for gallic tannins and elylagic tannins, respectively [270].

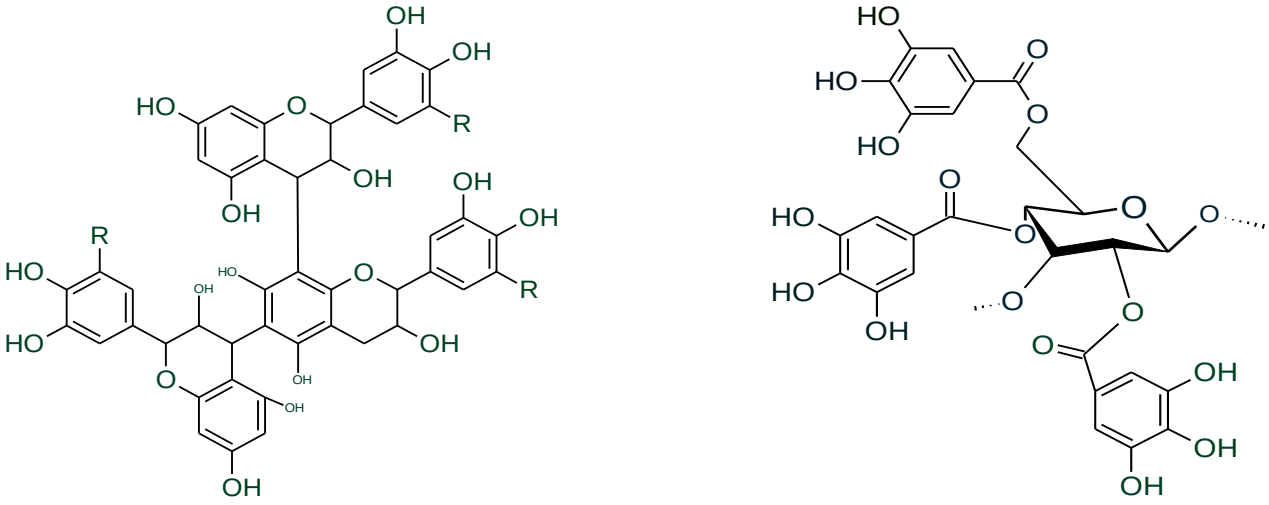


Figure.II.6. Basic structure of tanins. **A** : Condensed tannins ; **B** : Hydrolysable tannins [271].

II.3. Biological properties of phenolic compounds

The bioactive compounds produced by the various parts of the plant are involved in a variety of occurrences that are protective not only for the plant itself but also for the human organism. In light of the fact that the body's baseline production of free radicals is crucial to many functions and should not be eliminated, it makes sense to investigate the therapeutic potential

of naturally occurring antioxidant compounds that can act in the prevention of degenerative diseases so lengthy as they are taken in controlled doses.

A/ . Phenolic acids play a crucial function in protecting against the oxidative stress that leads to degenerative illnesses such as cancer, cardiovascular disease, and Alzheimer's...[272, 273].

B/ . Flavonoids are among the most representative of the compounds synthesized by plants through their secondary metabolisms; these aromatic, medicinal, and cosmetic compounds give plants evolutionary advantages. It contains numerous valuable ingredients that benefit both the plant and humans.

C/ . Tannins exert their biological and pharmacological effects mainly through their capacity to precipitate proteins, such as salivary proteins, resulting in an astringent flavour [254]. Tannins are able to act as antioxidants due to the presence of phenol nuclei and hydroxyl groups in their structure. Numerous investigations have demonstrated that these bioactive compounds possess an antibacterial impact on a wide variety of pathogens (bacteria, viruses) [274]. Moreover, tannins are natural chelators that act in the digestive tract to prevent iron absorption [275].

D/ . Terpenic compounds also have antifungal and antiparasitic capabilities and well-known anticancer effects [276]. Several studies have demonstrated limonene, myrcene, and sabinene to have stimulant and anticancer properties [277].

E/ . Saponins have a function known as adjuvant activity, which affects the immune system. Additionally, saponins have the capacity to enhance the absorption of a very wide variety of different compounds [278].

II.3.1. Antioxidant properties

In recent years, natural antioxidant's medicinal properties have drawn a great deal of attention. It is the capability of a molecule to inhibit or prevent the oxidation of biological substances triggered by free radicals or other molecules that are considered pro-oxidants [279].

II.3.1.1. Free radicals

A free radical or reactive oxygen species is defined as any molecule, atom, or independent ion that contains one or more single electron(s) occupying the outer orbital space. Free radicals are extremely unstable and rapidly react with other components to acquire the electron required for

stability (Figure.II.7), producing new stable radicals through a cascade of chemical reactions [280].

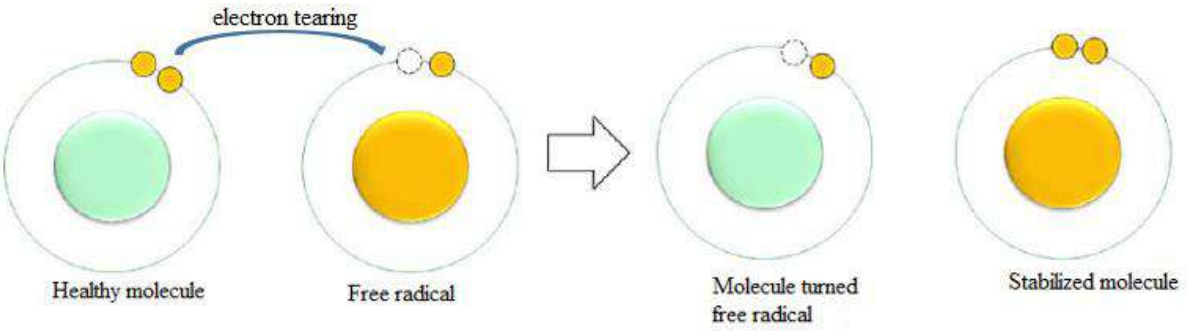


Figure.II.7. Oxidizing stress mechanism.

II.3.1.2. The different reactive oxygen species

Reactive oxygen species (ROS) are free radicals derived from oxygen oxidation, such as: superoxide radical ($O_2^{\bullet}$), radical hydroxyl ($OH^{\bullet}$), OxydeNitric ($NO^{\bullet}$), and peroxynitrite ($ONOO^{-}$), and reactive nitrogen species (RNS) are a subcategory of free radicals derived from the oxidation of oxygen and nitrogen. There are additional reactive species but not free radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3), and these species can act as precursors to radicals [281].

II.3.1.3. Oxidative stress

Oxidizing stress is generally defined as an imbalance between an organism, organelle, or cell's oxidizing systems and antioxidant capacities [282]. Physiologically, free radicals, such as tissue mediators or energy or defense response leftovers, are constantly created in minute amounts, although defense mechanisms carefully regulate these amounts under normal conditions [283]. The balance of antioxidants to pro-oxidants is stable under physiological conditions [284].

II.3.1.4. Phenolic compounds as antioxidants

An antioxidant is any substance that delays or prevents the oxidation of biological substances; these compounds react with free radicals, rendering them harmless. The antioxidant activity of phenolic compounds is affected by several factors, including the number and position of hydroxyl groups on the aromatic ring, the binding position of the hydroxyl groups, the type of

substituent, and the glycosylation of flavonoid molecules [285]. The glycosylation of flavonoids reduces their activity compared to the corresponding aglycones, while certain hydroxyl groups on the nucleus increase antioxidant activity. Substituents in cycles A and B, as well as double binding 2,3 (instauration), and the 4-oxo group in the C nucleus, also affect flavonoid's antioxidant activity [286, 287].

Several research studies have demonstrated that phenolic compounds have the ability to act as antioxidants through a variety of modes of action, including the chelation of transition metals, the hardening of singlet oxygen, the trapping of free radicals, and the inhibition of oxidative enzymes (Figure.II.8) [288].

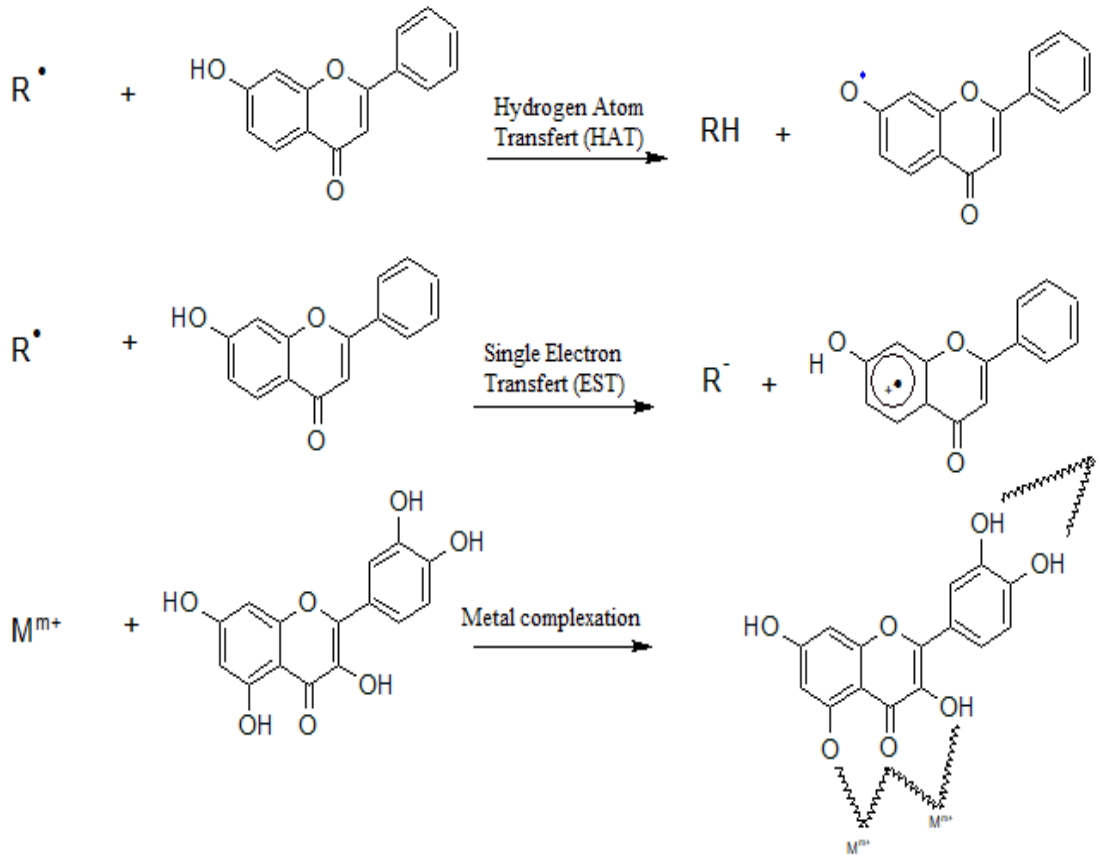


Figure.II.8. Simplified presentation of antioxidant mechanisms of phenolic compounds [289].

The delocalization of the free radical can be stabilized by donating a hydrogen atom, which allows the free radical to acquire an electron by first removing it from another molecule. The deactivation of the molecule's excited state might occur either through a chemical or a physical mechanism, which results in the hardness of the singular oxygen. The generation of free radicals

is inhibited or reduced as a direct result of all these reactions. They prevent free radical chain reactions from continuing, delay their initiation, or slow them ultimately [285].

In large part, free oxygen radicals are produced in living organisms due to the presence of transition metals. There are two distinct oxidation states of iron (ferrous and ferric ions).

Although ferric iron (Fe^{3+}) has no biological activity, depending on the conditions, particularly pH and oxidation via the Fenton reaction, with the production of hydroxyl radicals, Fe^{+3} can be reduced to an active form (Fe^{2+}). The formation of free radicals can result in the peroxidation of lipids, the alteration of proteins, and even damage to DNA. Phenolic compounds have the ability to inactivate metal ions and could potentially inhibit metal-dependent activities. The capacity of polyphenols to chelate metals is correlated with the existence of polyphenols ortho-dihydroxylates, which are characterized as molecules that include either a catechol or galloyl group...etc [285].

Flavonoids have the ability to block a wide variety of $O_2^{\bullet-}$ and other ROS enzymes. Some of these enzymes include xanthine oxidase, protein kinase C, cyclooxygenase, lipooxygenase, monooxygenase microsomal, and glutathione S-Transferase. Furthermore, flavonoids containing half catechol on cycle B have been shown to inhibit mitochondrial succinoxidase and NADH oxidase [290].

II.3.2. Anti-inflammatory activity

In vitro studies have shown that various flavonoids have the ability to alter the metabolism of platelet arachidonic acid. Therefore, at relatively high concentrations, myricetin and quercetin are able to inhibit the activity of cyclooxygenase as well as lipoxygenase. At low concentrations, lipoxygenase is preferentially inhibited. The results of certain studies suggest that they have potent anti-inflammatory effects without inducing ulcers [291].

In vivo, Subcutaneous administration of hesperidin had considerable anti-inflammatory effects in rats with edema caused using a model of carrageenan and dextran [261].

II.3.3. Anti-diabetic activities

Numerous research studies have shown that active biomolecules in plant extracts have anti-diabetic effects. They carry out their functions according to various mechanisms of action to regulate blood glucose, and these processes can vary depending on the category [292]. Herbal therapy is highly regarded and widely utilized to treat metabolic disorders like diabetes, obesity, and dyslipidemia in Africa [293]. The anti-diabetic effects of polyphenols and polysaccharides

have been the subject of substantial research, which has revealed that these compounds exert their effects via the following mechanisms:

II.3.3.1. Reduction of the adsorption of sugar

Several compounds prevent the transformation of starch into simple ose by preventing the activity of the digestive enzymes alpha-glucosidase and alpha-amylase. This mechanism of action has been seen in polyphenols, including alkaloids [292], tannins such as geraniin [294], flavonoids notably quercetin [295], and certain phenolic acids (curcumin, caffeic acid, p-coumaric acid), and diterpenes.

Studies have demonstrated that polysaccharides (mucilages, pectins, and proteoglycans) significantly inhibit the intestinal absorption of sugars. This is accomplished in part by increasing the viscosity, which in turn causes a delay in the reabsorption of sugars [296].

II.3.3.2. Inhibition of enzymes involved in the occurrence and complications of diabetes related to obesity

Inhibition of enzymes involved in the occurrence and complications of diabetes related to obesity including aldose reductase (AR), protein kinase C (PKC), end products of glycation (AGEs), and protein phosphatase 1B (PTP-1B). This mechanism of action has been proven by flavonoids, tannins, phenolic acids, and oligosaccharides [297].

II.3.3.3. Increase in Glucose Transport

Increase in Glucose Transport in muscle and fat cells, attributable to alkaloids, phenolic acids, polysaccharides, saponosides, and flavonoids [292].

II.3.3.4. Increase in insulin secretion

Tannins, alkaloids, and saponosides were found to have antidiabetic effects in both the pancreas and the liver. This activity was demonstrated by increased insulin secretion by the pancreas, which was associated with the regeneration of beta cells. Additionally, Flavonoids and polysaccharides also show insulin-mimetic activity [292].

II.3.4. Anti-cholinesterase activity

In humans, two cholinesterases are distinct in terms of their synthetic medium, structure, mechanism of action, physiological function, and activity indicator.

Acetylcholinesterase, also known as globular cholinesterase or real cholinesterase, itis an enzyme produced in nerve tissue and red blood cells. Specifically, it is found in the synapses of the brain, lungs, and spleen, as well as the Nervous and neuromuscular junction. Its

physiological function ensures the proper functioning of acetylcholinergic synapses by preventing neurotransmitter buildup. Additionally, it is abnormally present in amniotic fluid cases of neural tube closure failure [298].

Butyryl-cholinesterase (also known as serum cholinesterase or pseudo-cholinesterase) has a significantly broader affinity. It can hydrolyze a wide range of synthetic and natural esters, including acetylcholine and succinylcholine. The liver is the primary source of its synthesis, but it is also present in the plasma/serum, pancreas, intestine, and other organs [298].

The activity of each of these enzymes can be ascertained through the application of a colorimetric method, which involves the monitoring of the hydrolysis kinetics with the assistance of a specific substrate: Butyrylthiocholine, which is exceptionally stable at room temperature, is used for serum cholinesterase, while acetylthiocholine is used for globular cholinesterase [298].

The treatment of Alzheimer's disease has included the use of acetylcholinesterase inhibitors, often known as AChE. Anti-cholinesterase compounds primarily target the cholinergic system and are the only cure for AD [299]. AD is a neurodegenerative disorder predominantly affecting the elderly, with memory loss being the most prominent symptom [298, 300].

Several synthetic medications are available to treat cognitive dysfunction and memory loss caused by AD, such as tacrin, donepezil, and rivastigmine-based natural products, as well as galantamine. The secondary effects of these approved drugs, such as the development of gastrointestinal diseases and bioavailability issues, limit their use [301-303].

In Far Eastern countries, plants have been utilized to enhance cognition for centuries. Diverse pharmacological investigations have revealed promising leads in the search for novel anticholinesterase in these plants. Cognitive improvements were attributed to Panax ginseng, while the compound Huperzine A obtained from Huperzia serrata was reported beneficial in treating Alzheimer's disease [304, 305].

The Ellman colorimetry method was used to examine cholinesterase inhibitory effects of methanol extracts of seven herbs (Acorus calamus, Acorus gramineus, Bupleurm facaltum, Dioscorea batatas, Epimedium E. koreanum., Poria cocos et Zizyphi jujuba). They were used in traditional Korean medicine to promote memory and cognition in the elderly. Extracts of both A. calamus and E. koreanum were found to inhibit the enzyme significantly. In light of

recent research on these herbs' chemical and biological activity and others that have been investigated, we discuss the possible origins of their well-known properties [299].

II.4. Mineral elements

In addition to supplying the human body with protein, energy, and vitamins, plants also provide the body with minerals. Studies have shown that about half of the elements in Mendeleyev's table are biologically necessary for man, making mineral elements essential, notably micro- and macronutrients, for different metabolisms. Simultaneously, both major and trace elements play an essential part in forming and repairing the physical body in both health and illness [306]. The mineral profile of plants is determined on residue using various methods (ICP-OES, INAA, RNAA, AAS…).

Besides the organic components that make up more than 90% of the dry residue by weight (C: 40-50%, O: 42-45%, H: 6-7%), the mineral elements are classified into two [307]:

- Macro-elements (Ca, Mg, P, Na, K…) with a concentration greater than 10 mmol kg^{-1}
- Micro-elements or trace elements (Mn, Zn, Cu, Mo...) Less than 10 mmol kg^{-1}

On the other hand, the chemical elements are divided in terms of importance or not into three groups [308]:

- Essential chemical elements
- Non-essential chemical elements
- The toxic elements

According to some investigations, antioxidants are thought to include minerals and trace elements, including magnesium, zinc, and selenium. Quantitative assessments of various element concentrations are important for determining the plant's effectiveness and association with secondary metabolites and treating various diseases and perceptions of their pharmacological activity [309].

PART 2:

EXPERIMENTAL

PART

Chapter III: Extraction, Chemical Composition, Separation and Purification

III.1. Plant Material

Artemisia campestris. L and *Fagonia bruguieri* DC.plants were harvested during the flowering season in March 2018 from the Oued Tifouguine and Oued Amsel regions, respectively Tamanrasset –Algeria (22°47'13" N, 5°31'38" E), in the Sahara Desert. Figure.III.1 illustrates the locations of the harvest. Taxonomically, the plants were identified by Dr. Reggani Adelmalek from the University of Tamanrasset and Dr. Halis Yousef from the Center for Scientific and Technical Research on Arid Regions on the basis identification described by Quezel and Santa [310]. Under the codes "Ac. TMT: 03/18" and "Fb. TMT: 03/18," standard samples were kept in the Herbarium of the department's Saharan Resources Valorization and Promotion Laboratory. Dry aerial parts were pounded with an electric blender and stored in paper wrappings until they were required. The weight of the dry aerial parts was 500 g for the *A.campestris. L* species and 300 g for the *F.bruguieri* DC. species, respectively.

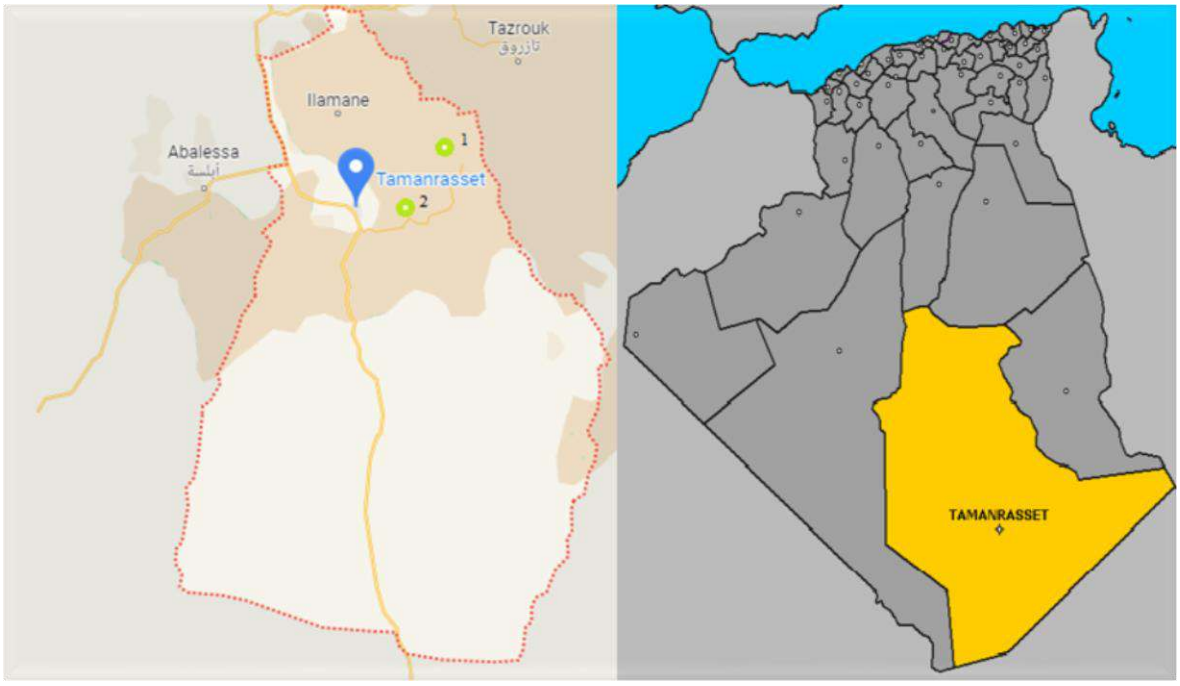


Figure.III.1. The geographical position of the collects, Tamanrasset (Hoggar) - Algeria.

III.2. Preparation of Extracts

Aerial parts of *A.campestris.* L. and *F.bruguieri* DC. were prepared by maceration in hydro-methanolic solution (methanol / Water) (80: 20) (v / v) for 24h at room temperature. The method of extraction was conducted three times with the renovation of the solvent. Through

Whatman filter paper N°1, the mixture was separated then the filtrate was evaporated in a rotary evaporator (BUCHI, R-100, flawil, Switzerland) at 40 °C. After filtration and concentration under vacuum, the hydromethanolic extract is diluted with distilled water at the rate of 50 ml per 100 g of a dried powdered plant at room temperature for 24 hours and then filtered. The resultant solution has carried out a series of liquid-liquid extractions utilizing solvents of progressively higher polarity, commencing with the Petroleum ether, Dichloromethane, Ethyl Acetate, and finally with n-Butanol. Each extraction is repeated three times to obtain dryness to extracts. The Figure.III.2 summarizes the extraction procedure. The two investigated plants both perform the same extraction procedure.

The obtained organic phases (non-polar: Petroleum Ether, weakly polar: Dichloromethane, medium-polar: Ethyl Acetate, polar: n-Butanol), in addition to the phase Residual aqueous and hydroethanolic extracts, are all concentrated dry under reduced pressure, weighed, and then recovered with methanol. Yields of different fractions are presented in Table.III.1.

Table.III.1. Yield of different fractions of *Artemisa campestris* L. *and Fagonia bruguieri* DC.

Extracts		Yield (%)
AcME	*Artemisa campestris* Methanol Extract	9.23 %
AcPEE	*Artemisa campestris* Petroleum Ether Extract	0.05 %
AcDE	*Artemisa campestris* Dichloromethane Extract	0.35 %
AcEAE	*Artemisa campestris* Ethyl Acetate Extract	0.79 %
AcBE	*Artemisa campestris* Butanol Extract	1.45 %
AcAE	*Artemisa campestris* Aqueous Extract	7.95 %
FbME	*Fagonia bruguieri* Methanol Extract	8.30 %
FbPEE	*Fagonia bruguieri* Petroleum Ether Extract	1.94 %
FbDE	*Fagonia bruguieri* Dichloromethane Extract	0.17 %
FbEAE	*Fagonia bruguieri* Ethyl Acetate Extract	0.11 %
FbBE	*Fagonia bruguieri* Butanol Extract	4.23 %
FbAE	*Fagonia bruguieri* Aqueous Extract	4.89 %

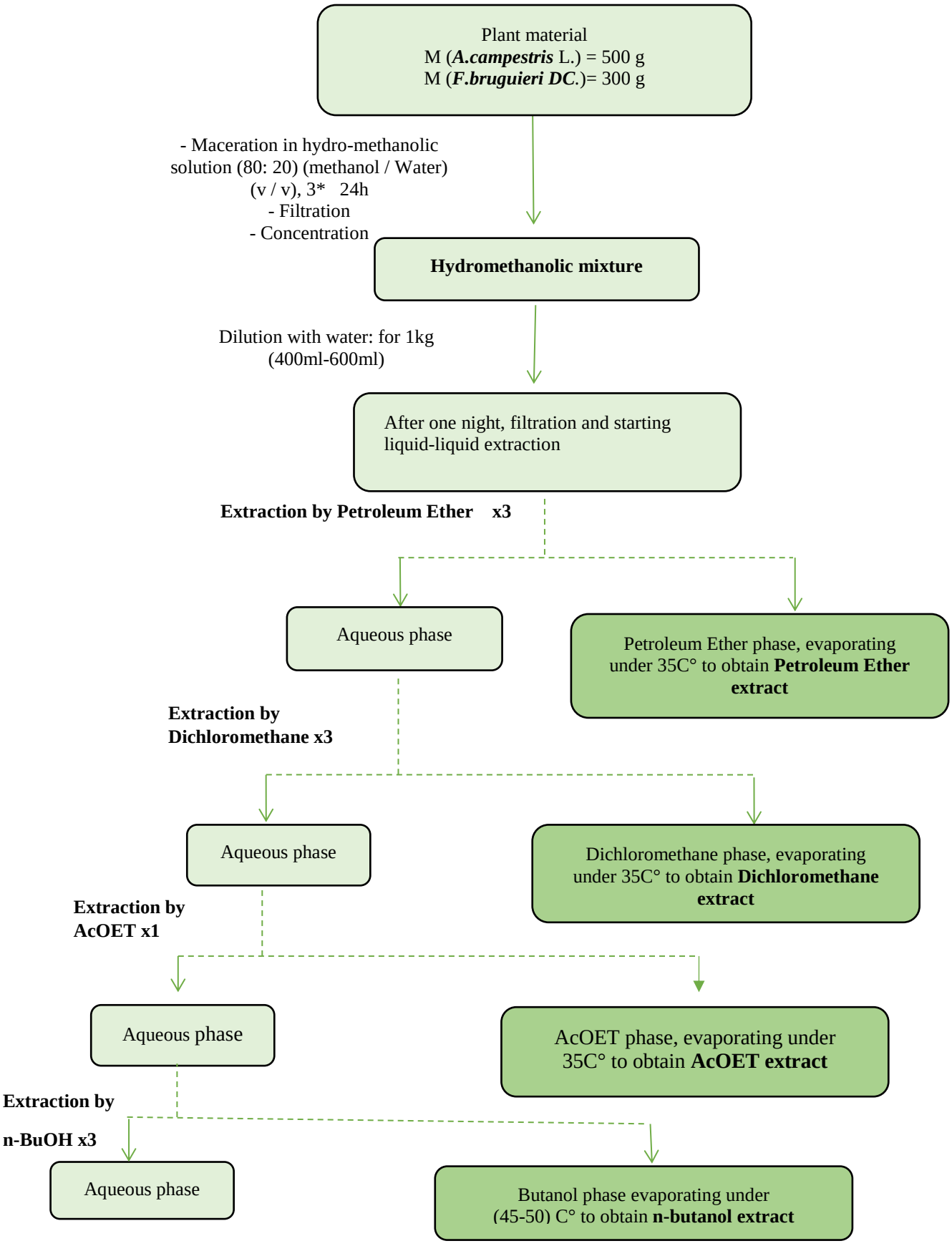


Figure.III.2. The protocol of extraction for different fractions of *A.campestris* L., and *F.bruguieri* DC.

III.3. Qualitative Phytochemical Screening

The process of phytochemical screening involves performing a series of straightforward tests on natural compounds derived from plants. This screening has the ability to detect the presence of products that belong to many classes of bio-phytochemically active substances that are collectively known as secondary metabolites.

These are the preliminary colorimetric, solubility, and precipitation experiments that are being performed to demonstrate the presence of broad groups of secondary metabolites. The screening for phytochemicals in this study was carried out in accordance with Harborne's methods [311, 312].

III.3.1. Flavonoids test

A few turns of magnesium were added to 5 ml of different fractions of *A.campestris* L., and *F.bruguieri* DC. (while stirring), then 5 drops of concentrated hydrochloric acid were carefully added. The appearance of red color indicates the presence of flavonoid

III.3.2. Alkaloids Test

It is accomplished by mixing 5 ml of each extract with 1 ml of diluted H_2SO_4 (1/2). Two tubes are prepared by using two different reagents, one is the Mayer reagent, and the other is the Wagner reagent. After 15 minutes, the formation of turbidity or precipitate is a clear indication that alkaloids are present.

III.3.3. Tannins Test

To demonstrate the presence of tannins, we mixed 1 ml of each extract with 1 ml of water and 1 to 2 drops of $Fecl_3$ (1%).

- The appearance of a dark green or green blue color indicates the presence of tannins.
- The appearance of a dark green color indicates the presence of catechic tannins.
- The appearance of a blue-green color indicates the presence of gallic tannins.

III.3.4. Terpenoids Test

Salkowski test: targeted for identification of terpenes, each of the extracts had five (5) ml evaporated until they were dry. After thoroughly combining the residue with 2 ml of chloroform, we continued to add 0.5 ml of concentrated sulphuric acid in order to form a

layer. The presence of terpenoids was indicated by the interface's coloring, which was a reddish brownish red.

III.3.5. Coumarins Test

The various extracts were divided between two test tubes after being diluted with distilled water by a ratio of 1/2. The first tube was employed as a control, and 0.5 ml of a solution that had been titrated to 10% NH_4OH was added to the second tube. The presence of coumarin can be detected by observing a blue or green fluorescence when exposed to an ultraviolet lamp at 365 nm.

III.3.6. Reducing compounds Test

Their confirmation is done by placing 2 ml of the different extracts in a test tube, followed by adding 2 ml of the Fehling liqueur within the same tube; the mixture is then put in a bath of boiling water for 8 minutes. Obtaining a red brick precipitate indicates the presence of reducing compounds.

III.3.7. Saponins Test

Foam test: The different extracts were brought up to their final volume of 20 ml by diluting them with distilled water. A graduated cylinder was used to shake the suspension for 15 minutes. A layer of foam measuring two centimeters high revealed the presence of saponins.

III.4. Mineral Analysis using the INAA and ICP-OES techniques

The Analysis of mineral elements was carried out at the Nuclear Research Centre of Birine (CRNB) Ain Oussera, Algeria and also at the Nuclear Research Centre of Draria (CRND).

INAA and ICP-OES were utilized in order to determine the majority of the trace elements that were detected.

III.4.1. Instrumental Neutron Activation Analysis (INAA)

The samples selected for this analysis were finely powdered and weighed on an analytical balance of 110-120 mg. We have prepared three copies for each type, which will then be packaged in high-purity aluminum envelopes. In this section, we have provided standards with the same matrix as the analyzed samples. These standards were NIST-SRM 1570a, WEPAL-IPE 172 and WEPAL-IPE 189. During each step of the analysis procedure, the certified standards are required to be with the samples that will be analyzed. The following

step, which will be the validation and quality control of the results that were achieved, will use the application of standards. Each sample and standard was arranged inside the irradiation capsule (high purity aluminum cylinder) to ensure that each target is subjected to the same amount of neutron flux [313, 314].

III.4.1.1. Irradiation and Measurement

The samples and the standards were put inside a clean capsule made of cylindrical aluminum. At the irradiation site used by the CRNB and CRND research reactors, this thermal neutron flux of 1013 $n.cm^{-2}.s^{-1}$ is applied to the irradiation capsule. The irradiation capsule is exposed to the neutron flux for a total of 6 hours during the experiment. After a decrease of 2 days, the powder was placed into new polyethylene capsules, and a gamma spectrometer was used to measure all of the samples as well as the standards. At the start, the Ge(Hp) detector was calibrated in terms of energy and efficiency (resolution of FWHM is 1.8 keV at 1332.5 keV of γ-peak of ^{60}Co and relative efficiency equal (35 %)), coupled with a digital gamma spectrometry system, after two different decay times (medium, and longtime). In order to properly adjust the peaks in the spectrum in accordance with the channels, it is essential that this step of the analysis process be performed. The first measure analyzes elements having medium half-life radionuclide's like Na, Ba, and Br, was determined after a decay time of three days, the counting time of each sample was about 5000s and the source-detector distance was 15cm. On the other hand, the activities of long life radionuclide's like Co, Eu, Fe and Zn were determined after decay time of 20 days, and different counting time comparing to the previous half-life radionuclide's which was about 7200s for each sample. After the initial measurement, the gamma spectra of the samples and standards are gathered after 20 days of degradation to determine the elements over long periods for a collecting time of 14400 seconds. At the end, we determined the concentrations of the elements (major and trace elements) using the equation of INAA given by the Equation (III.1) [315]:

$$\rho(mg/kg) = \left(\left[\frac{N_p/t_c}{DC}\right]_a / \left[\frac{N_p/t_c}{DC}\right]_s \right) * \frac{(\rho W)_s}{W_a} \qquad \text{(III.1)}$$

where the subscripts a and s refer to the sample and the standard, respectively, N_p is the net photo-peak counts, W is the sample mass, D = [exp (-λ t_d)] is the decay factor, and C = [1-exp (-λ t_m)]/ (-λ t_m) is the counting factor, λ decay constant, t_c, t_d and t_m counting, decay and measurement times, respectively.

The gamma-ray spectrometry of the irradiated samples in this study, was carried out by using the nuclear parameters listed presents in (Table.III.2) [316].

Table.III.2. Optimum experimental conditions and nuclear data employed in this study.

Elements	Radio-nuclide	Half life	γ - peaks (keV)	Cooling time
Barium	^{131}Ba	11.50 d	496.3	20 d
Bromine	^{82}Br	35.30 h	554.3, 698.08, 776.5	2-5 d
Cobalt	^{60}Co	5.2714 y	1173.2, 1332.5	20 d
Chromium	^{51}Cr	27.7025 d	320.1	20 d
Cesium	^{134}Cs	2.0648 y	604.7, 795.8	20 d
Europium	^{152}Eu	13.537 y	124.78,344.28,1408.01	20 d
Iron	^{59}Fe	44.5 d	1099.3, 1291.6	20 d
Potassium	^{42}K	12.360 h	1524.7	2-5 d
Molybdenum	^{98}Mo	65.94 d	140.51	20 d
Sodium	^{24}Na	14.9590 h	1368.6	2-5 d
Rubidium	^{86}Rb	18.631 d	1077	20 d
Antimony	^{124}Sb	60.20 d	169, 602.7	20 d
Scandium	^{46}Sc	83.83 d	889.3, 1120.5	20 d
Strontium	^{153}Sr	64.84 d	514.01	20 d
Thorium	^{233}Th	26.967 d	300.1, 311.9	20 d
Uranium	^{215}U	2.3565 d	106.13,228.18,277.60	2-5 d
Zinc	^{65}Zn	244.26 d	1115.5	20 d

III.4.1.2. Quality Control and Quality Assurance (QC/QA)

Quality control validation of analyzes is an important step of the quality of results, in this investigation, for the relative approach of the INAA, were used WEPAL-IPE 172 and WEPAL-IPE 189 were used as the standard, while NIST- SRM 1570a were used as the control samples.

To assess the quality of the obtained results, the parameters test of Z-score and U-score were determined, they were calculated according to the following equations (III.2 and III.3), the results are found to be acceptable if Z-score ≤3 and satisfactory if U-score ≤ 1.

$$Z_{score} = \frac{\left|X_{Lab} - X_{Ref}\right|}{\mu_{Lab}} \quad \text{.....(III.2)}$$

$$U_{score} = \frac{\left|X_{Lab} - X_{Ref}\right|}{\sqrt{\mu_{Lab}^2 + \sigma_{Ref}^2}} \quad \text{.....(III.3)}$$

With:

X_{Lab}, μ_{Lab}, X_{Ref} and σ_{Ref} : are the laboratory results, standard deviation, the recommended and standards uncertainties, respectively [317].

III.4.2. Inductively Coupled Plasma ICP-OES Analysis

The analysis of the mineral contents was performed using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) [318, 319]. Following the procedure depicted in Figure.III.3, samples are dissolved in preparation for analysis. A sample of *A. campestris* L. and *F. bruguieri* DC. is powdered and weighs 500 mg. Afterward, each powder is put into a distillation flask with a capacity of 200 mL and is equipped with an effective reflux condenser system (Figure I.3). After that, the powder was mixed with 3 mL of HNO_3 with a concentration of 65% (Sigma Aldrich-Germany), as well as 3 mL of hydrochloric acid with a concentration of 37% (Sigma Aldrich-Germany), and 1 mL of H_2O_2 with a concentration of 30%. To ensure that all of the powder was transformed into a liquid, the mixture was heated to 105°C and then agitated for 90 minutes. A 50 mL volumetric flask was used to properly filter the mixture through a membrane filter with a pore width of 0.45 m (MF-Millipore® Membrane Filter, Germany). In order to reduce microparticles made during the analysis process, they don't get in the way of taking in liquid samples or stop the nebulization of the solution by the plasma used in ICP-OES procedures. The filtred sample was then diluted with 50 mL distilled water and transferred to a new tube, where it was allowed to settle and homogenize. Finally, the supernatant was subjected to ICP-OES analysis to determine the minerals in the sample; each sample was examined in triplicate .

The concentration of chemical elements in the samples is calculated using the following equation [318, 319]:

$$C(sample)(mg/kg) = \frac{\mathrm{C}(Sample\ \mathrm{per\ ICP(mg/L)} * \mathrm{V(mineralisation)(L)}}{\mathrm{test\ sample\ mass(kg)}} \quad \text{(III.4)}$$

Where; C: concentration.

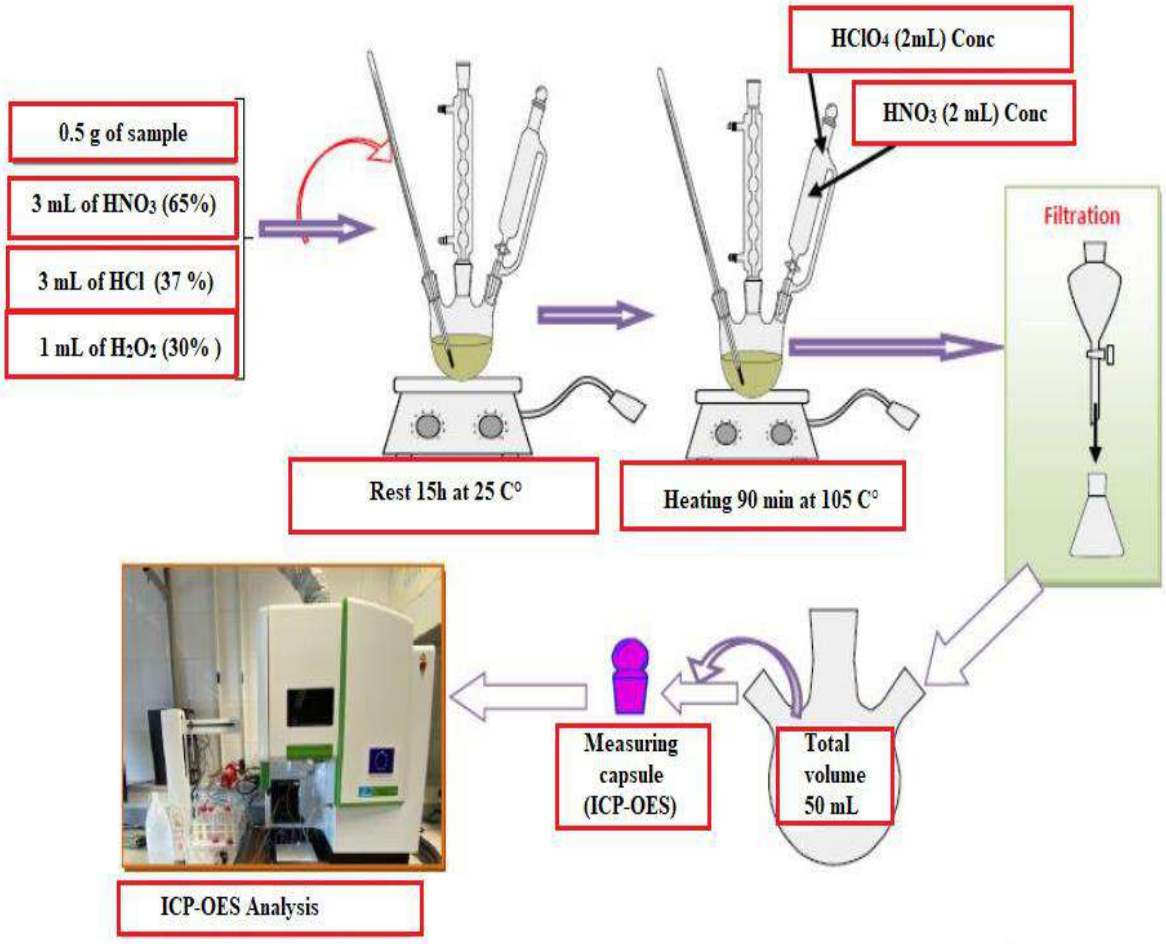


Figure.III.3. Protocol for mineral element analysis using ICP-OES.

III.5. Analytical Chromatography Techniques

The Analysis of HPLC and LC-MS-MS was carried out at the Laboratory of Valorization and Technology of Saharan Resources (VTRS) University Echahid Hamma Lakhdar El-Oued, Algeria, and Scientific & Technical Research Center in Physico-Chemical Analysis (CRAPC), Ouargla Algeria.

III.5.1. Quantitative analysis of phenolic compounds by High-Performance Liquid Chromatography-Ultraviolet (HPLC-UV)

III.5.1.1. Preparation of extracts for HPLC-UV analysis

The various extracts of *A. campestris* L. and *F. bruguieri* DC. (10 mg) were diluted in methanol (HPLC grade). Before injecting the sample solutions into the HPLC, solutions were filtered through a Millipore nylon filter disk with a Millipore 0.45 m.

III.5.1.2. Preparation of polyphenol standard

In order to prepare a stock solution of polyphenol, 10 mg of pure polyphenol standard was first dissolved in methanol (HPLC grade). It was sonicated for about 10 min and then carried to volume with the mobile phase. Daily working standard solutions of polyphenols were prepared by proper dilution of each one with the mobile phase. After injecting each solution into the column three times with a volume of 20 µl, the peak areas and retention times were recorded.

III.5.1.3. Chromatographic instruments and HPLC analysis conditions

High-performance liquid chromatography analysis of the extracts and standards was performed using a shimadzu model prominence liquid chromatography. At room temperature, a Pura disc 25 mm and a Terumo syringe with a volumetric capacity of 5 cc/ml were used to inject 20 µl of analyte solution into an HPLC valve. On a Thermo scientific (HPLC-RP 18), column CTO-20AC (250mm x 4,6mm) packed with C18 stationary phase with a particle size of 5µm , phenolic compounds were separated at a flow rate 1mL/min. A binary gradient linear system consisting of acetonitrile (A) and 0.2% acetic acid in water (B) was used. The elution gradient was as follows: (0-0.1 min) 90% B; (0.01-6 min) 86% B, (6-16 min) 83% B, (16-23 min) 81% B, to 77% B in 28 min, held at 77% B in 28-35 min, to 60% B in 38 min, to 90% B in 50 min, detected by UV-SPD-20 Å detector (operating at 268 nm). In order to identify the chromatographic peaks, we compared the retention times of our analyses with those of reference chemicals (Figure.III.4). The phenolic composition were quantified by plotting a standard curve with the respective standards (Table.III.3).This procedure is required for the purpose of performing a qualitative and quantitative analysis of a different extract of *A.campestris.* L. and *F.bruguieri* DC.collected in the Saharan region of Algeria. The results of each examination were recorded in triplicate.

III.5.1.3. LOD and LOQ

The detection limit is the minimum concentration in a sample that can be estimated but not commonly quantified under the experimental requirements established. The quantitation limit is the lowest analyte concentration in a sample, which can be evaluated with adequate accuracy and precision. The values for LOD and LOQ are given in Table.III.3.

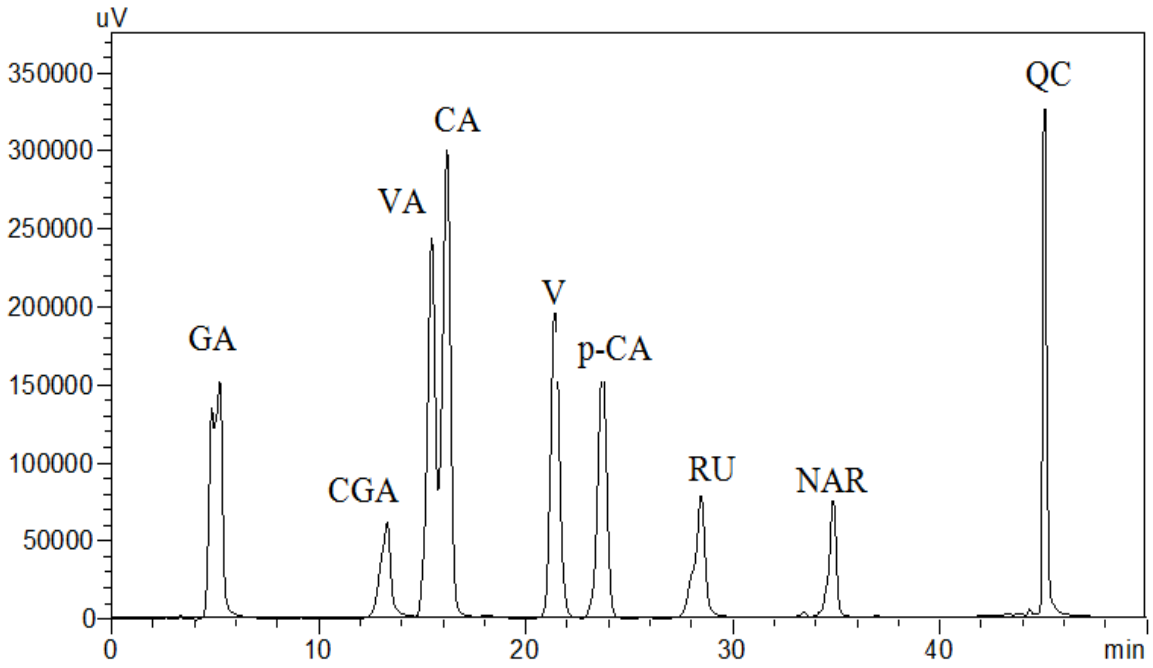


Figure.III.4. High-performance liquid chromatography (HPLC) chromatogram of phenolic compounds. Detected compounds are: GA: Gallic Acid; CGA: Chlorogenic Acid; VA: Vanillic acid; CA: Caffeic acid; V: Vanillin; *p*-CA—p-Coumaric Acid; RU: Rutin; NAR: Naringin and QC: Quercetin.

Table.III.3. Retention times (Rt), calibration curves, regression coefficients, Detection and quantification limits for phenolic compounds.

Compound	Rt (min)	Calibration curve	R^2	LOQ (µg/mL)	LOD (µg/mL)
Gallic acid	5.29	Y=23616x-7232	0.9986	0.36939	0.12189
Chlorogenic acid	13.392	Y=39775x-1881	0.9963	0.41308	0.13631
Vanilic Acid	15.531	Y=65077x+33	0.9885	0.09577	0.03192
Caffeic acid	16.277	Y=72328x-52	0.9992	0.44863	0.14804
Vanilin	21.46	Y=58930x+64	0.9933	0.11350	0.37833
p-Coumaric acid	23.817	Y=157538+122	0.9996	0.09385	0.03097
Rutin	28.37	Y=28144x-24	0.9854	0.39912	0.13304
Naringin	34.788	Y=19379x-212	0.9922	0.52711	0.17570
Quercetin	45.047	Y=45378x+177	0.9988	0.13475	0.04491

III.5.2. Quanlitative analysis of phenolic compounds by high-performance liquid chromatography coupled to tandem mass spectrophotometry (LC-MS/MS)

It is a methodology of physicochemical analysis that depends on mass measurement to detect, identify, and quantify molecules of interest. The basic concept behind this technique is the separation of charged molecules (ions) in the gaseous phase according to their mass-to-charge ratios (m/z). In addition, mass spectrometry can be used to characterize the molecular chemical structure by fragmenting the molecules [320].

III.5.2.1. Preparation of extracts for LC-MS/MS analysis

Before performing an LC-MS/MS analysis, the diffrents extracts were diluted in methanol (LC grade) at a concentration of 1mg/1mL and then filtered through a 0.2 µm syringe filter.

III.5.2.2. Chromatographic instruments and LC-MS/MS analysis conditions

A qualitative analysis of constituents present in different fractions of *A. campestris.* L. and *F. bruguieri* DC. as performed using UPLC-ESI-MS-MS Shimadzu 8040 Ultra-High sensitivity with UFMS technology equipped with binary bump Nexera XR LC-20AD. Separation was achieved with an Ultra-force C18 column (I.D. 2.5 mm ×100 mm, 1.8 µm particle size; Restek) at 25 °C oven temperature. The chromatographic separation was carried out using a mixture of 30% (water, 0.1% formic acid) as mobile phase A and 70% methanol as mobile phase B. The duration of gradient elution was applied as: 0 min 100% B; 0.10 min 5% B; 10 min 15% B; 30 min 95% B; 45 min 15% B; 50 min 5% B; 60 min 100% B. The flow rate was established at 0.3 mL/min, while the injection volume was 6 µl, passed through a Millex-LCR (PTFE) filter with 0.22 µm pore sizes. The separation was performed at room temperature, while the run lasted for 60 min.

The ESI conditions employed in the MS/MS are as follows:

- 230 KPs CID gas; −6.00 Kv conversion dynode.
- 350 °C interface temperature; 250 °C DL temperature.
- 3.00 L/min nebulizing gas flow, 400 °C heat block.
- 15.00 L/min drying gas flow.

The ion trap mass spectrometer was used in both negative and positive ions with MRM mode (multiple reaction monitoring). Accurate identification was made according to their typical fragments by comparing the mass spectra with published literature.

III.6. Separation and purification

All the extracts of *A.campestris* L. and *F.bruguieri* DC. were treated by using the thin-layer chromatography (TLC) analytical to select the rich extract and good systems for separation, elution, or elution system, and stationary phases, that would give the best results, we have compared each of the extracts of *A.campestris* L. *and F.bruguieri* DC.by using silica gel and polyamide TLC.

The comparison and the results of our analytical work led us to choose, separate, and isolate the constituents of the butanol extracts of *A.campestris* L. *and F.bruguieri* DC., for its richness.

III.6.1. Isolation of the constituents of the Butanol fraction of *Artemisia campestris* L. aerial part AcBE

The AcBE fraction (6 g) was slurried with a ½ amount of celite, dried, powdered and subjected to silica gel (150 g) [4.3 (ID) × 50 (L) cm] flash column, which was eluted with the gradient elution with $CHCl_3$-MeOH (98:02;V/V), (95:05; V/V), (93:07; V/V), (90:10; V/V), (85:15; V/V), (80:20; V/V), (77:23; V/V), (75:25; V/V), (70:30;V/V), (65:35; V/V), (60:40; V/V), (55:45; V/V), (50:50; V/V), (40:60; V/V), (30:70;V/V), (20:80; V/V) and finally with 100 % MeOH.

Each fraction was monitored by TLC on silica gel using diffrents systems: $CHCl_3$- EtOAc-MeOH ; $CHCl_3$-AA-MeOH (12:0.5:1; 5:0.2:1; 3:0.2:1 V/V/V) and $CHCl_3$-MeOH (11:1; 9:1; 4:1 V/V) as developers. Similar fractions were combined and concentrated under reduced pressure to give six main fractions from A - F. All TLC plates were sprayed with the acidic solution. The scheme of separation and isolation of the butanol fraction of aerial part *A.campestris* is presented in Figure.III.5 and Figure.III.6.

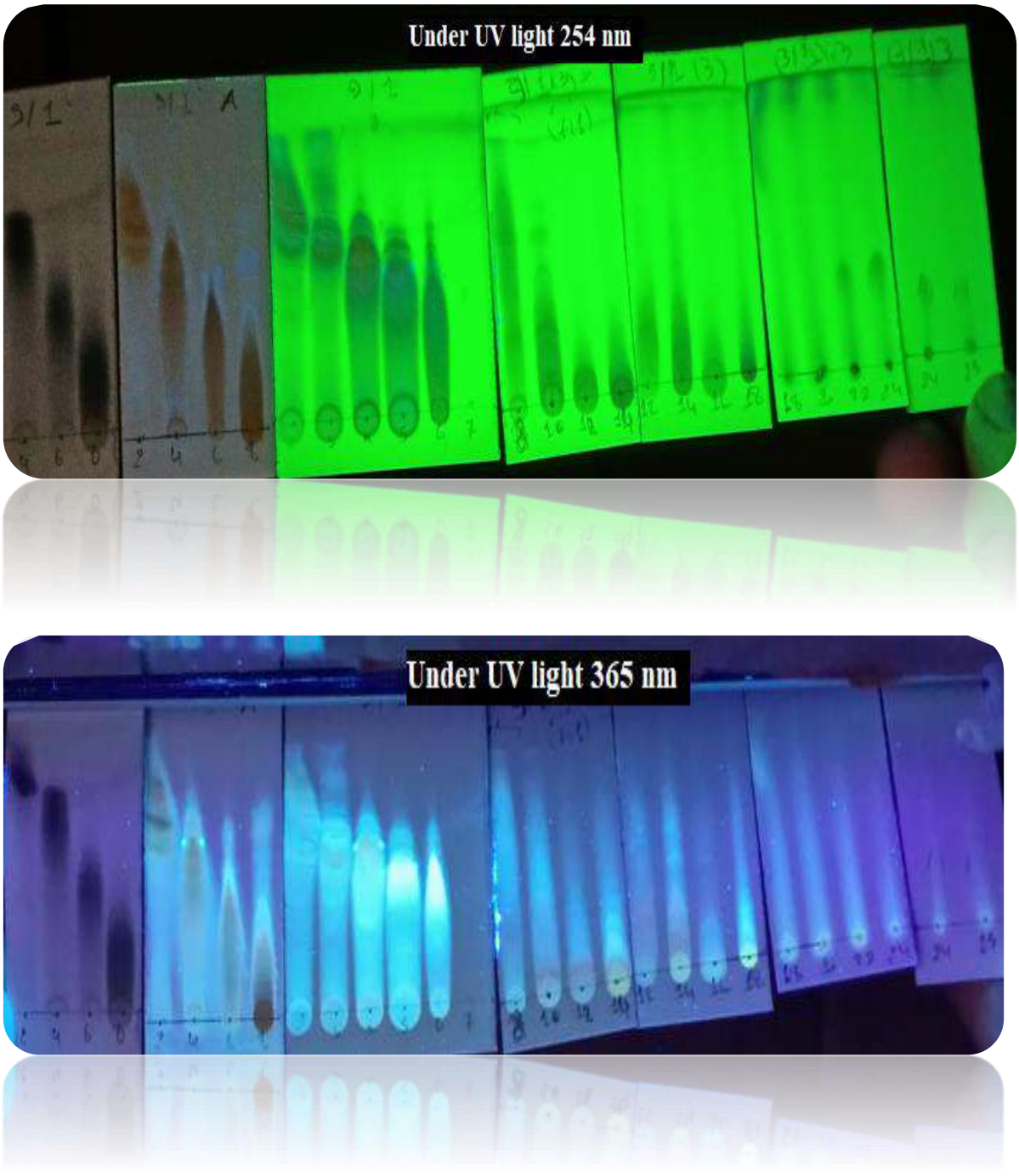


Figure.III.5. Follow-up the TLC fractions silica gel column of AcBE after revelation with acidic solution.

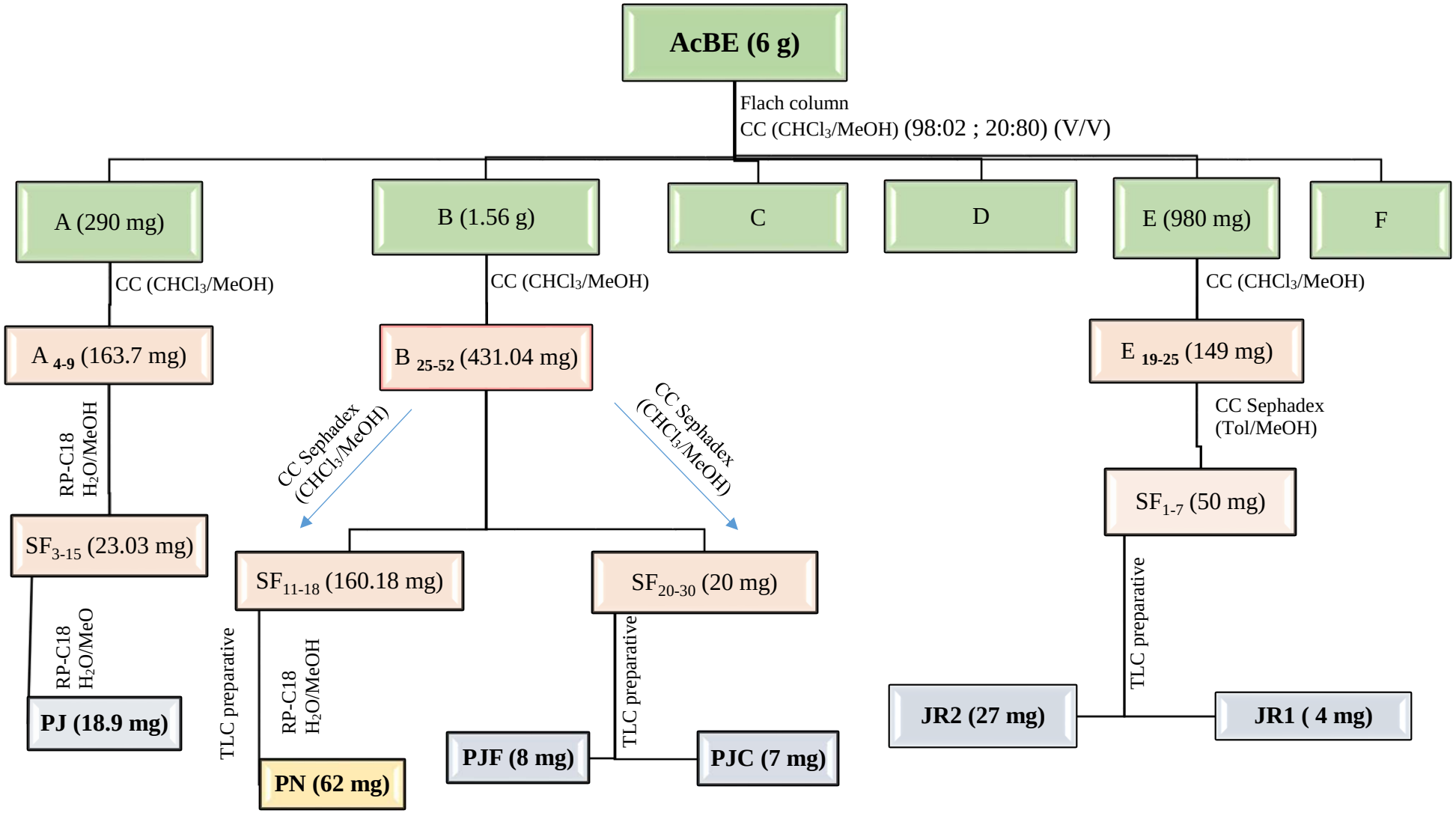
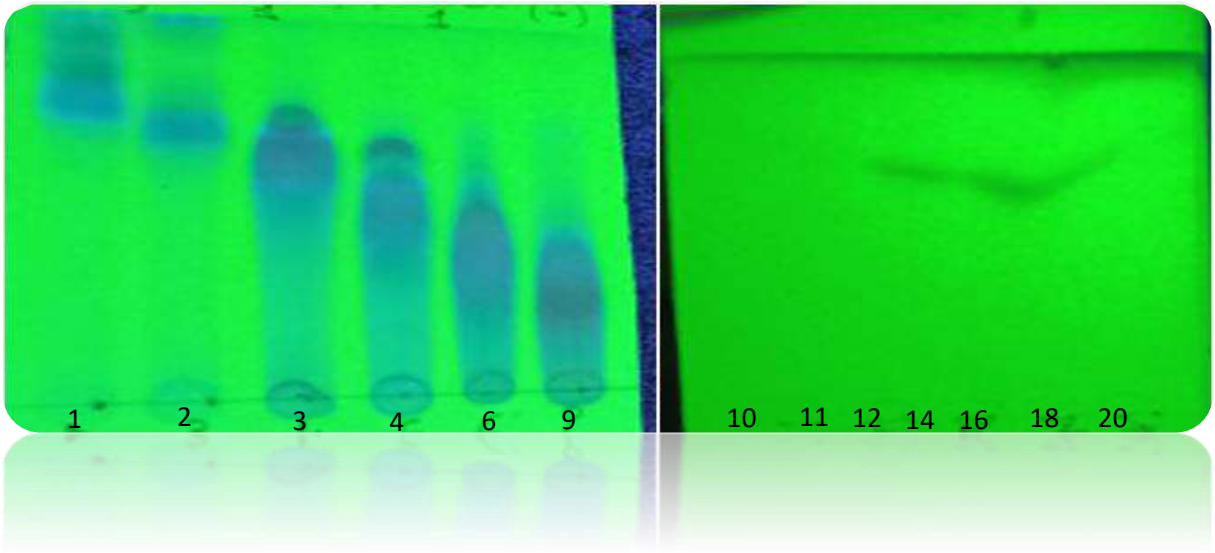


Figure.III.6. Scheme of the fractionation and isolation of pures compounds from AcBE aerial parts.

Fraction A of AcBE column (290 mg) was subjected to silica gel column using $CHCl_3$/MeOH as eluent, we obtained 20 sub-fractions.

Sub-fractions were collected and tested on a TLC plate (Figure.III.7).

Under UV light 254 nm



Under UV light 365 nm

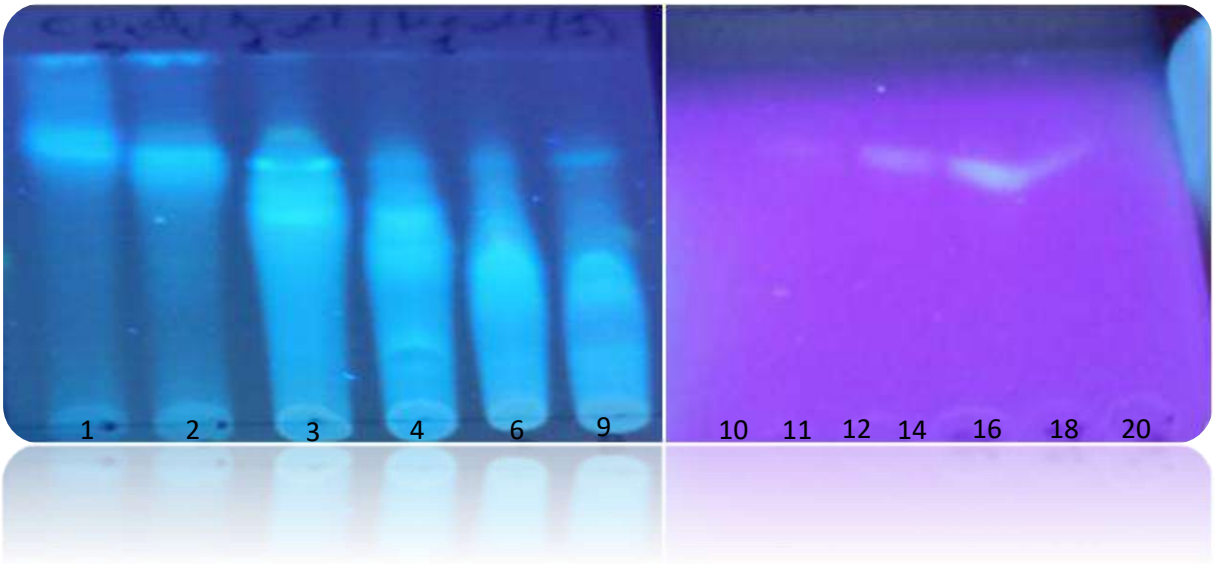


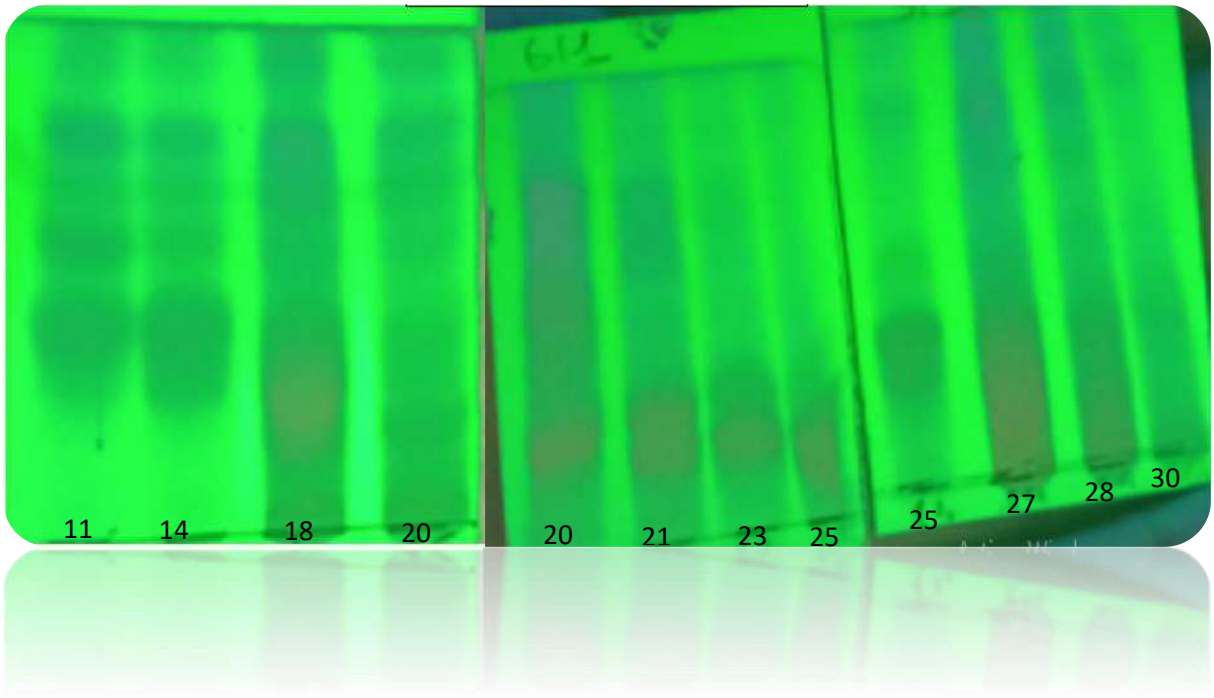
Figure.III.7. Follow-up the separation of fraction A.

- **Sub-fraction $A_{4\text{-}9}$**: 163.7 mg was chromatographed over by the RP-C18 column eluted by the H_2O % to MeOH % gradient system to yield non-pure fraction that have a mixture of three compounds. This mixture presented in **$SF_{3\text{-}15}$**, which is purified again many times by the RP-C18 column (H_2O % to MeOH %) to obtain pure yellow compound **PJ (18.9 mg).**

Fraction B (1.56 mg) was subjected to silica gel comlumn using $CHCl_3$/MeOH as an eluent (50 g) [2.7 (ID) × 50 (L) cm] CC, we obtained 16 sub-fractions.

Some of those sub-fractions were collected and tested on a TLC plate (Figure.III.8).

Under UV light 254 nm



Under UV light 365 nm

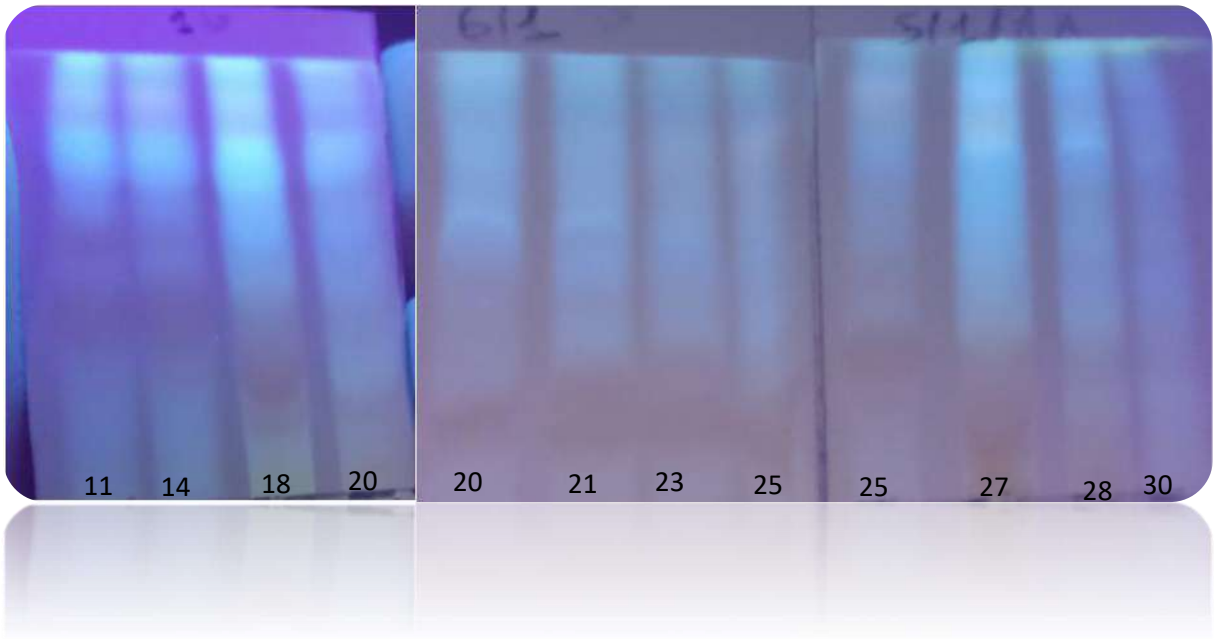


Figure.III.8. Follow-up the separation of fraction B.

- **Sub-fraction $B_{25\text{-}52}$** (431.04 mg) underwent CC of LH Sephadex using gradient system $CHCl_3$/H_2O. The **$SF_{11\text{-}18}$** was separated on reversed phase RP-C18 with the system

H_2O/MeOH then, it was purified on TLC preparative with $CHCl_3$/AA/MeOH (5:0.5:1 ; V/V/V) ×3 to give PN (62 mg). The **SF_{20-30}** was subjected to TLC preparative using the system $CHCl_3$/MeOH (5:1; V/V) ×5 to afford two pure compounds **PJF** (8 mg), and **PJC** (7 mg) respectively.

Fraction E (980 mg) was subjected to silica gel using gradient elution $CHCl_3$-MeOH (9:1 V/V), we obtained 5 sub-fractions.

- **Sub-fraction E_{19-25}** (149 mg) was separated on LH Sephadex CC using $CHCl_3$-MeOH (4:1 V/V). This separation produced more than 20 fractions. The fractions **SF_{1-7}** were collected and put throught one more purification step on TLC preparative using the system $CHCl_3$/AA/MeOH (5:0.2:1; V/V/V) ×3 to afford two pure compounds **JR1** (4 mg), and **JR2** (27 mg) respectively Figure.III.9.

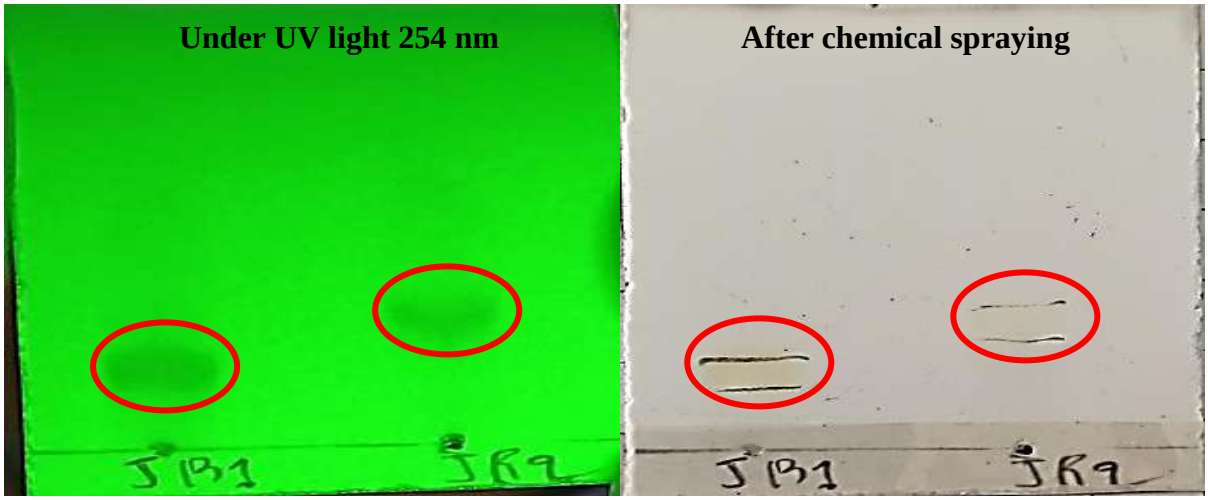


Figure.III.9. TLC purification of JR1 and JR2 compounds.

III.6.2. Isolation of the constituents of the Butanol fraction of *Fagonia bruguieri* DC. aerial part (FbBE)

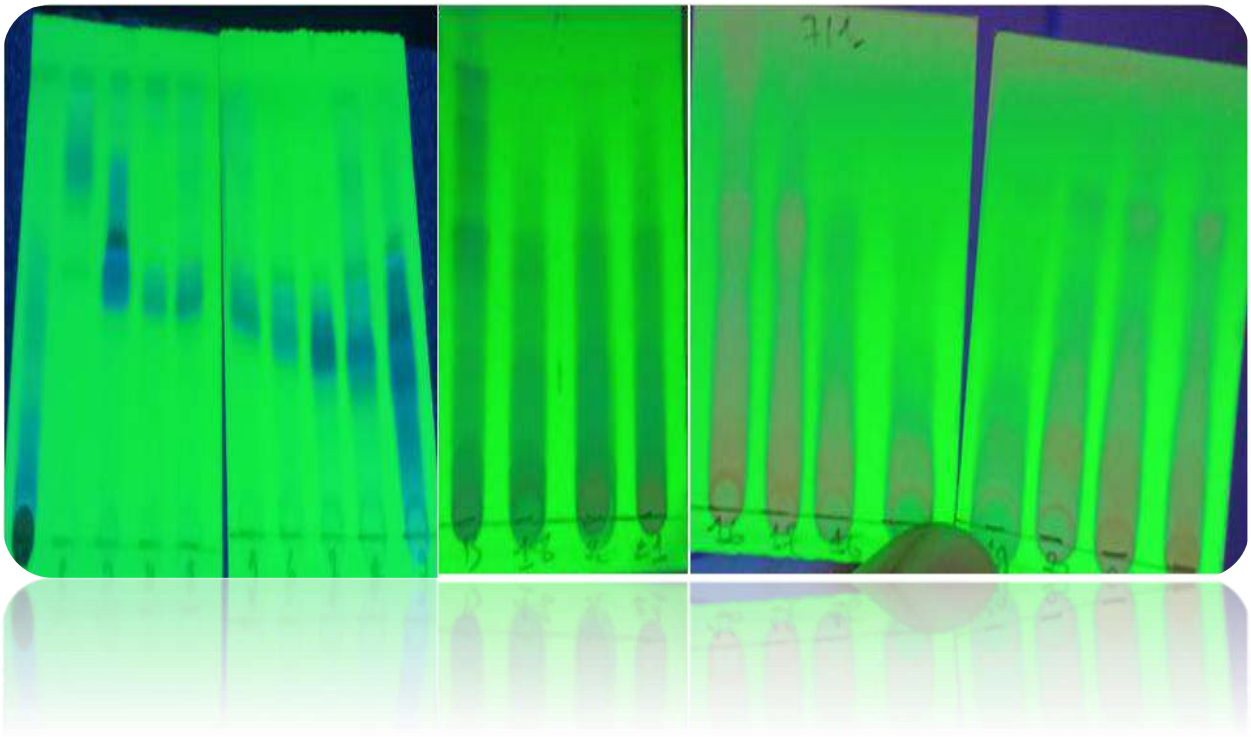
The Butanolic fraction (5 g) was subjected to silica gel CC which was eluted with $CHCl_3$-MeOH systems (95:05; V/V), (90:10; V/V), (86:14; V/V), (80:20; V/V), (77:23; V/V), (75:25; V/V), (70:30; V/V), (65:35; V/V), (60:40; V/V), (55:45; V/V), (50:50; V/V), (30:70; V/V) (20:80; V/V), and 100 % MeOH.

The eluate was collected in fractions (50 ml each) to give five fractions (A - E).

The fractions were monitored by TLC on silica gel using the system: $CHCl_3$-MeOH (15:1 V/V), $CHCl_3$-MeOH (9:1 V/V), $CHCl_3$-MeOH (7:1 V/V) and $CHCl_3$-EtOAc-MeOH (10:0.5:1; 7:1:1; V/V/V).

The scheme of separation and isolation of the FbBE of leaves aerial part is presented in Figure.III.10 and Figure.III.11.

Under UV light 254 nm



Under UV light 365 nm

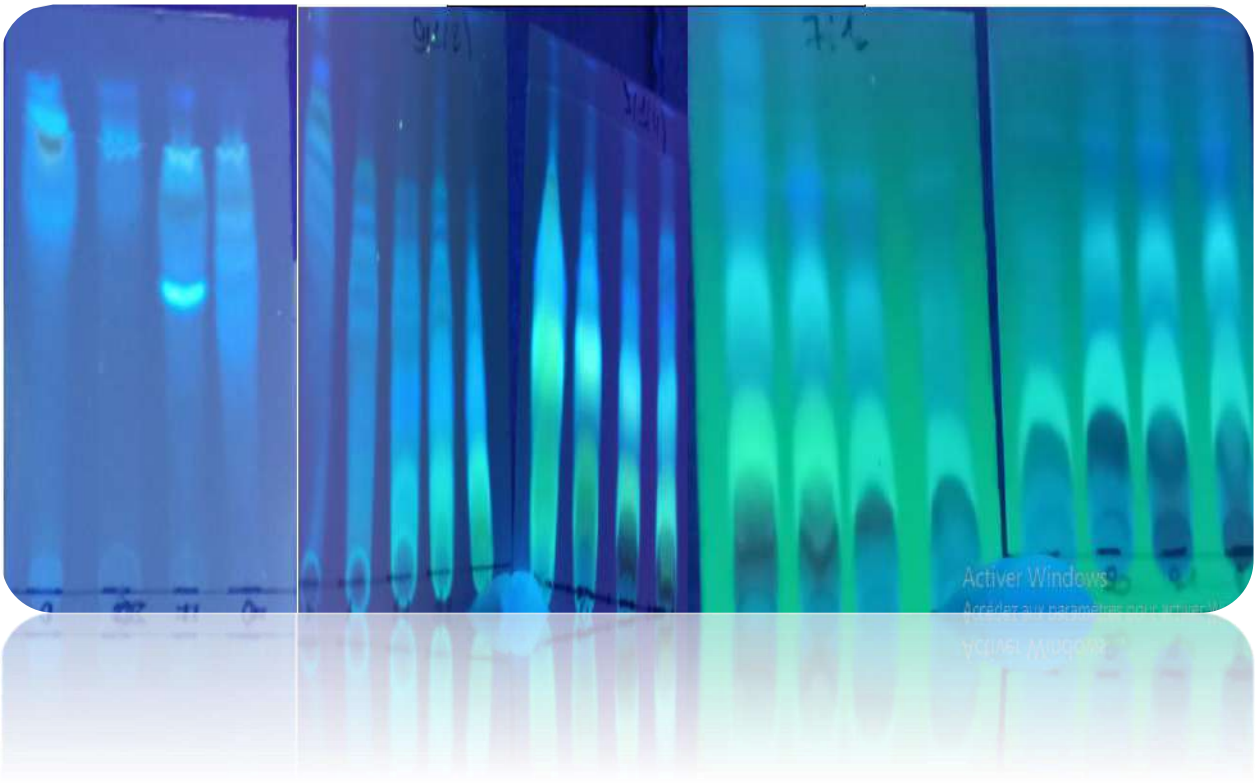


Figure.III.10. Follow-up the TLC fractions silica gel column of FbBE.

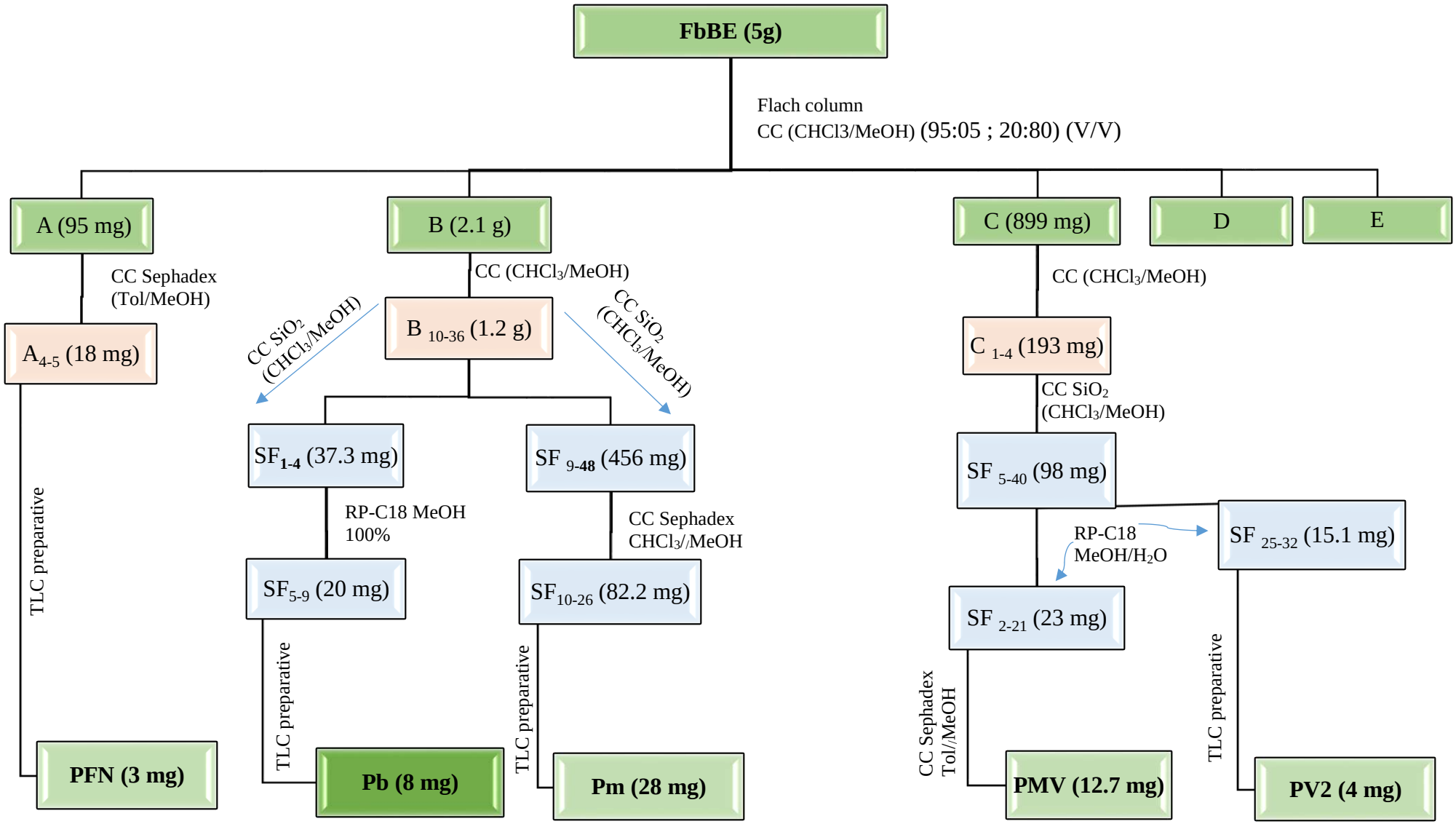


Figure.III.11. Scheme the fractionation and isolation of pures compounds from FbBE aerial parts.

Fraction A (95 mg) was subjected to Sephadex LH-20 column using gradient elution $CHCl_3$-MeOH (7:3). We obtained a sub-fractions which is collected and tested on a TLC plate presented as $A_{4\text{-}5}$ (Figure.III.12).

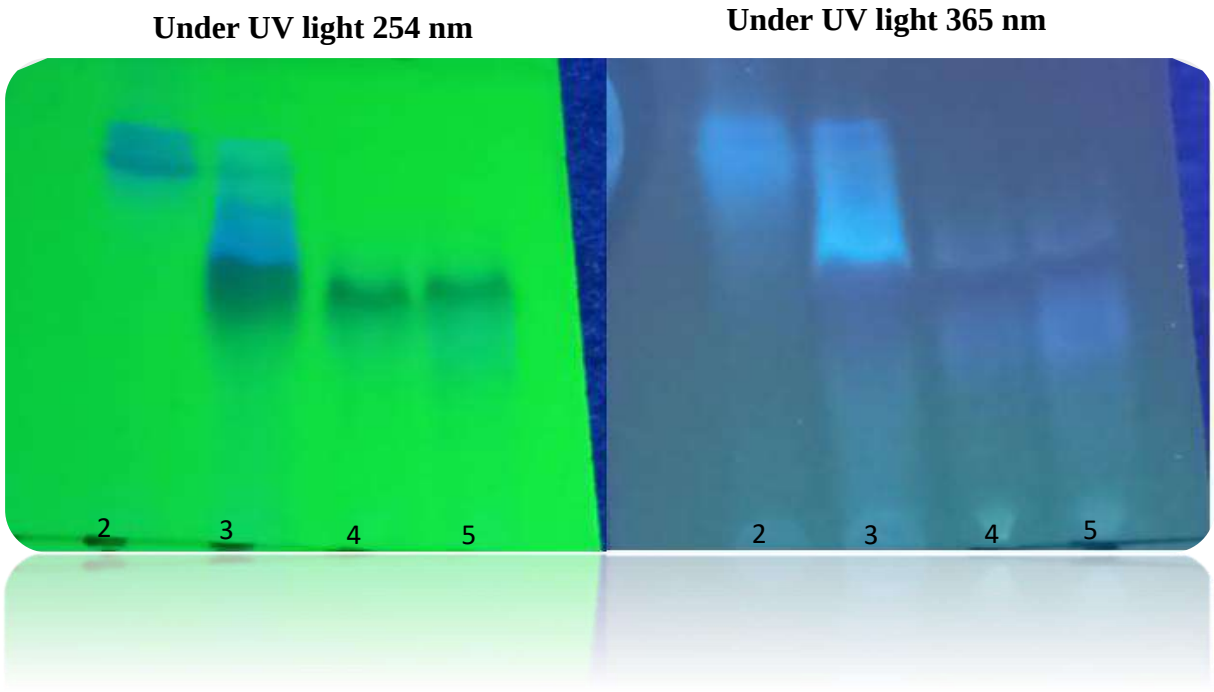
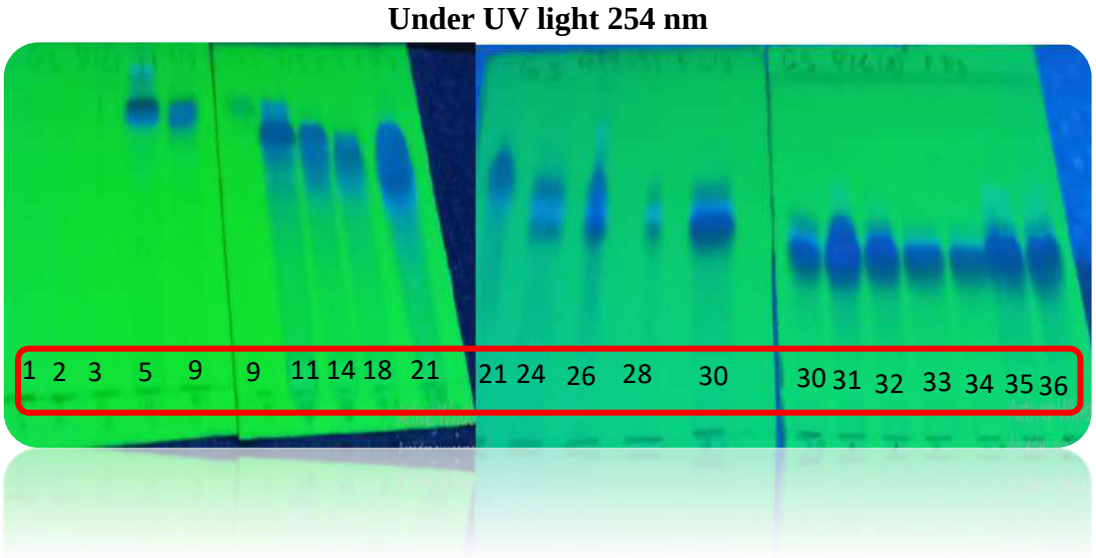


Figure.III.12. Follow-up the separation of fraction A.

- **Sub-fraction $A_{4\text{-}5}$** (18 mg) was subjected to TLC preparative using the system $CHCl_3$/AA/MeOH (5:0.5:1 ; V/V/V) ×4 to afford a crystals yellowish pure compound **PFN (3 mg).**

Fraction B (2100 mg) was subjected to CC silica gel using $CHCl_3$-MeOH as an eluent (100 g) [2.7 (ID) × 50 (L) cm] CC, we obtained 29 sub-fractions.

Sub-fractions were collected and tested on a TLC plate (Figure.III.13).



After chemical spraying

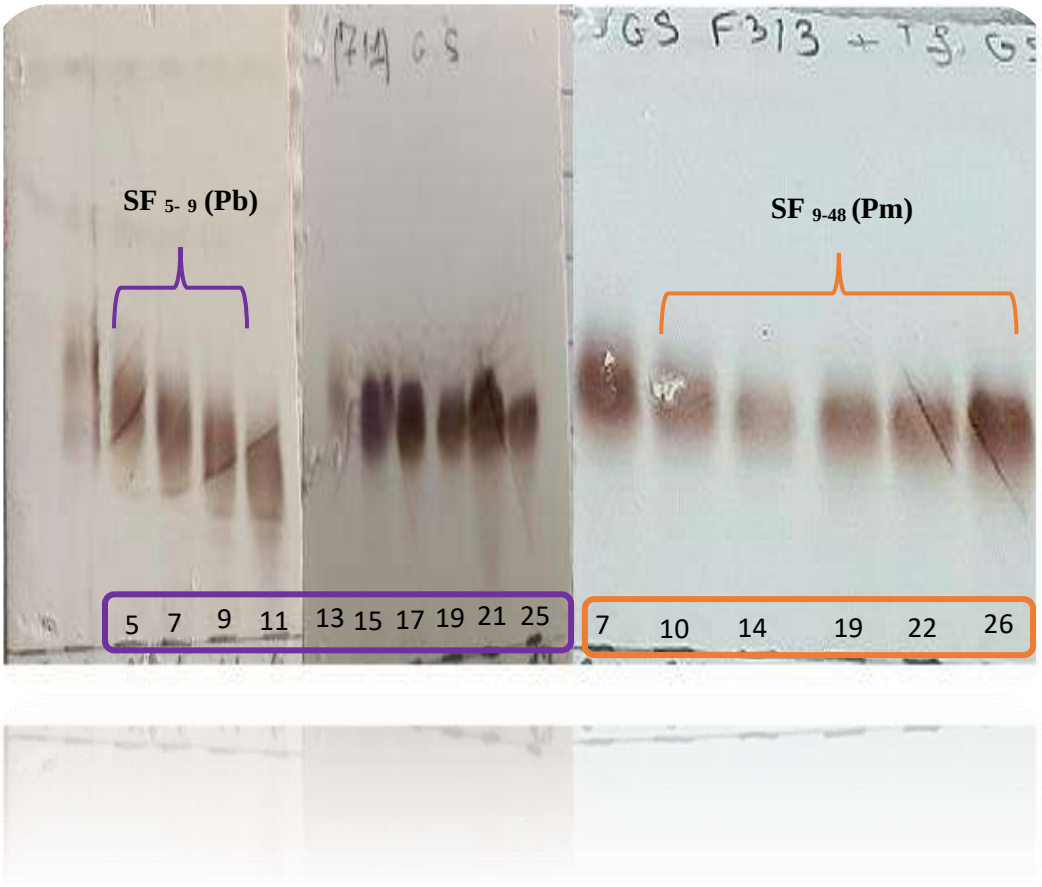


Figure.III.13. Follow-up the separation of fraction B.

- **Sub-fraction SF$_{1-4}$** (37.3 mg) underwent RP-C18 column eluted by the 100% of H_2O. All the fractions tested on TLC where similar ones were collected to get B$_{5-9}$.

SF$_{5-9}$ was purified on TLC preparative using the system $CHCl_3$/AA/MeOH (7:0.2:1 ; V/V/V) ×3 to afford a white crystals pure compound **Pb** (8 mg). On the other hand, the **SF$_{9-48}$** subjected to CC of Sephadex LH-20 with $CHCl_3$-MeOH (70 %: 30 %) and purified with TLC preparative using the system $CHCl_3$/MeOH (6:1 ; V/V) ×3 to give brown powder presented a pure compound **Pm** (28 mg).

Fraction C (899 mg) was subjected to CC silica gel using gradient elution $CHCl_3$-MeOH, we obtained 37 sub-fractions.

- **Sub-fraction C$_{1-4}$** (193 mg) was chromatographed by using column silica gel eluted with the $CHCl_3$-MeOH gradient system to yield:

SF$_{5-40}$ (98 mg) was separated on RP-C18 column using H_2O-MeOH (7:1; V/V) to obtain a subfractions **SF$_{2-21}$** contain a mixture of two compounds.

SF$_{2-21}$ was purified by CC of Sephadex LH-20 with Tol-MeOH to afford **PMV** (12.7 mg).

SF_{25-32} was recollected from **C_{1-4}** and put throught one more purification with TLC preparative using the system $CHCl_3$/AA/MeOH (7:0.5:1 ; V/V/V) ×5 to give pure white powder compound **PV2** (4 mg).

Chapter IV: Biological Activities

IV. Biological Activities

The Biological activities were examined at Biotechnology Research Center (CRBT) Constantine, Algeria and the Laboratory of Valorization and Technology of Saharan Resources (VTRS) University Echahid Hamma Lakhdar El-Oued, Algeria.

The investigation of the biological properties and total bioactive content was performed on the various extracts of *A. campestris* L. and *F.bruguieri* DC.aerial parts:

AcME: *Artemisa campestris* L. Methanol Extract

AcPEE: *Artemisa campestris* L. Petroleum Ether Extract

AcDE: *Artemisa campestris* L. Dichloromethane Extract

AcEAE: *Artemisa campestris* L. Ethyl Acetate Extract

AcBE: *Artemisa campestris* L. Butanol Extract

AcAE: *Artemisa campestris* L. Aqueous Extract

FbPEE: *Fagonia bruguieri* DC. Petroleum Ether Extract

FbDE: *Fagonia bruguieri* DC. Dichloromethane Extract

FbEAE: *Fagonia bruguieri* DC. Ethyl Acetate Extract

FbBE: *Fagonia bruguieri* DC. Butanol Extract

FbAE: *Fagonia bruguieri* DC. Aqueous Extract

IV.1. Total bioactive components assessment

IV.1.1. Total phenolic content (TPC)

The total polyphenols content of the different extracts is determined by using the Folin-Ciocalteu reagent method. In the presence of polyphenols, the middle Alkaline is reduced to tungsten oxide and molybdenum, producing a blue color. This reagent, known as the FCR reagent, is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), which is reduced as a consequence of the oxidation of phenols to a mixture of tungsten oxides (W_8O_{23}) and molybdenum oxides (Mo_8O_{23}). The resulting blue coloration is proportional to the total phenol content and exhibits maximum absorption at 750 -765 nm [321].

Protocol

Total phenolic in the extracts of *A.campestris* L. and *F.bruguieri* DC. were carried out as stated by Singleton & Rossi. (1965) using the Folin-Ciocalteu reagent with slight modifications [322]. Briefly, 20 µL of the extract was mixed with 75 µL of sodium carbonate 7.5 % and 100 µL of (1:10) Folin-Ciocalteau reagen to adapt a microplate protocol as reported by Müller *et al*. (2010) [323]. The mixture was incubated at room temperature in the dark for 2h. The absorbance was recited at 765 nm. The calibration curve was achieved using gallic acid at increasing concentrations (0-200 µg /ml) (Figure.VI.1). The phenolic amounts were defined as (µg GAE/mg dried extract).

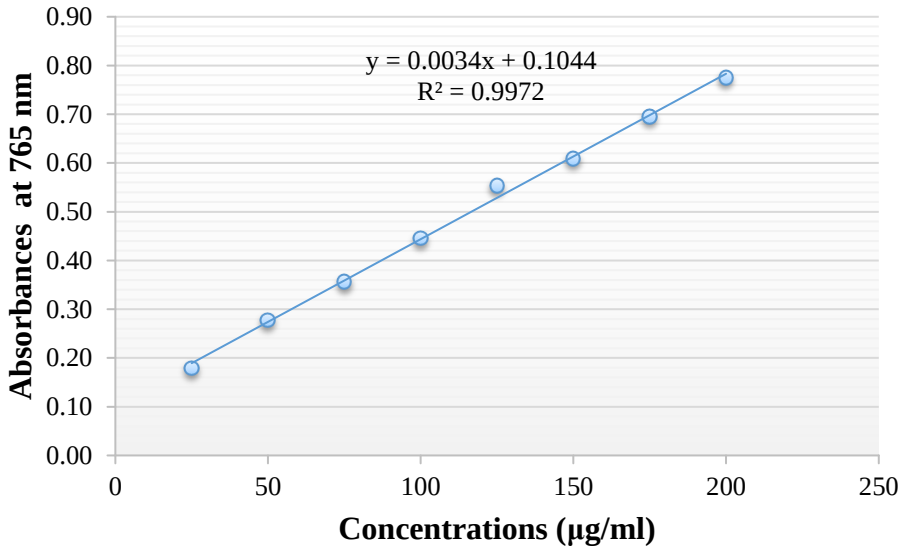


Figure.IV.1. Gallic acid calibration curve for TPC quantification.

IV.1.2. Total flavonoids content (TFC)

Flavonoids are quantified by the aluminum trichloride ($AlCl_3$) colorimetric method. The dosage of flavonoid extracts depends on forming a complex between Al^{+3} and flavonoids. The complex forms a yellow compound that absorb in the visible at 415 nm.

Protocol

Total flavonoids in the extracts of *A.campestris* L. and *F.bruguieri* DC. were evaluated by using the aluminium coluorimetric method Topçu *et al.* (2007) with some modifications [324]. Shortly, 130 µL (MeOH) was added to 50 µL of extract solution (1mg/mL), then 10 µL aluminium nitrate (10%) $Al(NO_3)_2$, $9H_2O$) and 10 µL potassium acetate (1 M) (CH_3COOK) were added. The mix was allowed to attain at room temperature for 40 min. The absorbance was measured by a microplate reader (Perkin Elmer, Enspire) at 415 nm, and the standard curve

was plotted with quercetin (0-200 µg / ml) (Figure.IV.2); the total flavonoid amounts were expressed in µg quercetin equivalent per mg of dried extract (µg QE/mg).

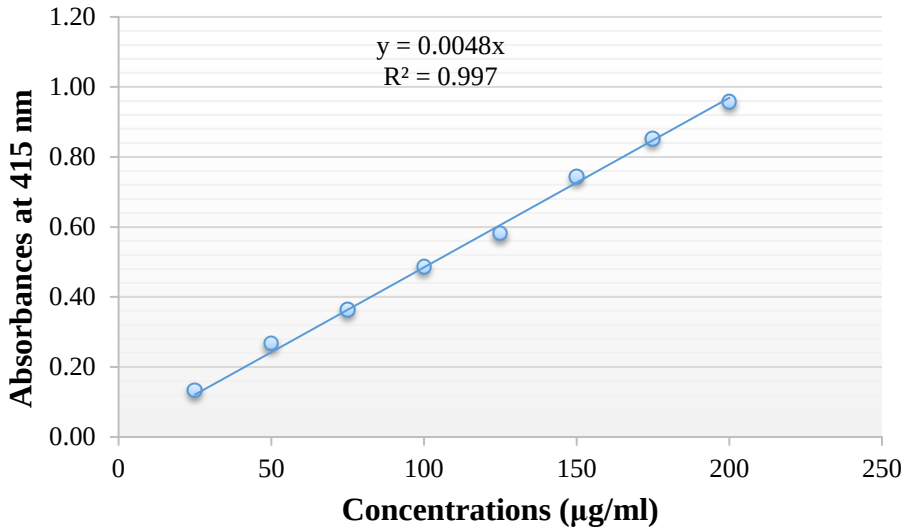


Figure.IV.2. Quercetin calibration curve for TFC quantification.

IV.2. Antioxidant Activity

Seven methods were used to investigate the antioxidant activity of the various extracts of *A.campestris* L. and *F.bruguieri* DC: DPPH• scavenging, ABTS scavenging, β-carotene-linoleic acid bleaching, Reducing Power (RP), Cupric Reducing Antioxidant Capacity (CUPRAC), O-Phenanthroline Chelating and Galvinoxyl scavenging (GOR).

Preparation of dilutions

In order to prepare a series of dilutions of different samples, the various extracts and standards were dissolved in methanol, 500 µl of this stock solution (known concentration) were mixed with 500 µl of methanol to arrive at a 1/2 dilution, and continuing in this manner. This procedure is repeated for further dilutions until 1/64 (Figure.IV.3).

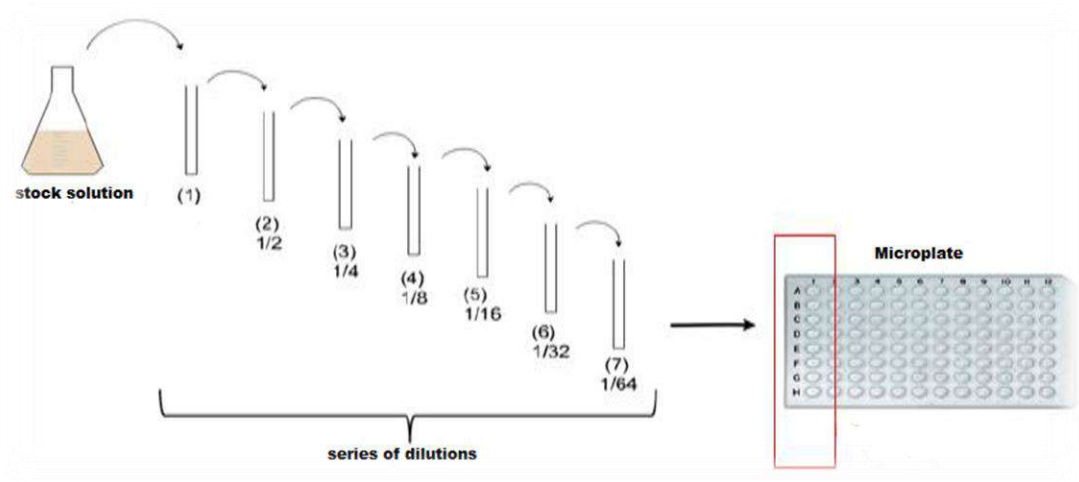


Figure.IV.3. Preparation of dilutions of different extracts.

IV.2.1. DPPH• Scavenging Activity

Due to its stability as free radicals and simplicity of analysis, DPPH (2,2 diphenyl-1-picrylhydrasyl) is the most widely utilized substrate for rapid and direct assessment of antioxidant activity. It absorbs in the visible region between 515 and 520 nm in wavelength. The fundamental concept behind this approach is the transformation of violet DPPH (2,2-diphenyl-1-picrylhydrazyl) into yellow 2,2-diphenyl-1-picrylhydrazine (Figure.IV.4). However, when an antioxidant reduces it, this wavelength becomes less efficient. The intensity of the colour is inversely related to the medium's antioxidants' ability to produce H^+ protons [325].

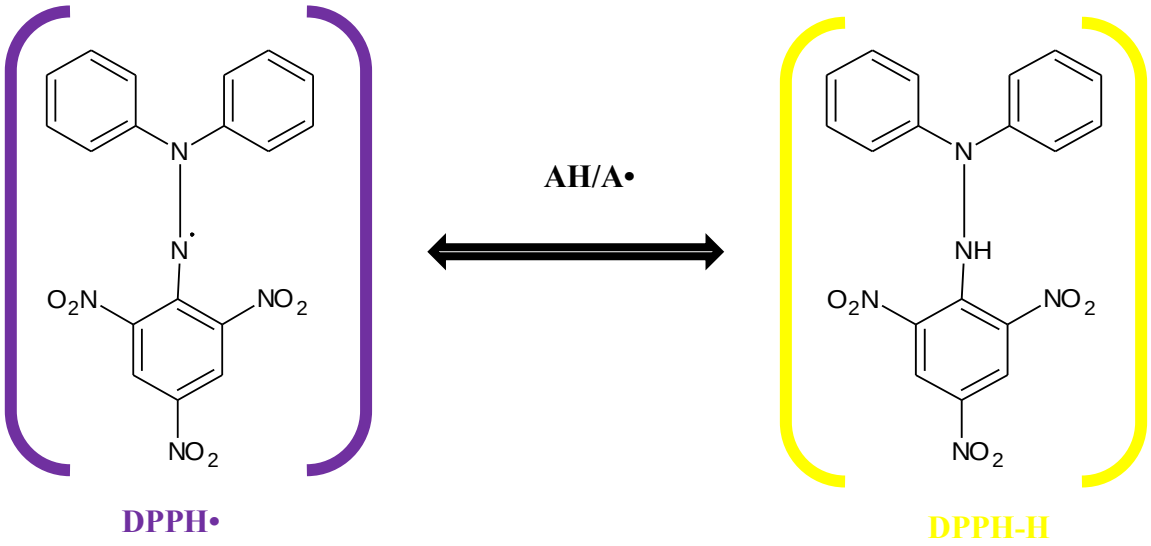


Figure.IV.4. The mechanism for reducing the DPPH• radical by an antioxidant [326].

Protocol

The capacity of *A.campestris* L. and *F.bruguieri* DC. to inhibit the 2,2-diphenyl-1-picrylhydrazyl free radical was performed according to the procedure reported by Blois (1958) [327]. In a 96-wells microplate, 160 µL of a DPPH solution (0,4 mM) were placed in the presence of 40 µL of different concentrations. For 30 min, the microplate was kept at room temperature in the darkness. Using a microplate reader (Perkin Elmer Enspire, Singapore), the absorbance was recited at 517 nm, and BHT, α-Tocopherol, and BHA were used as standards. Free radical trapping activity increased when the reaction mixture had a lower absorbance. The capacity to scavenge DPPH in percentage (%) was calculated using the following equation (IV.1):

$$\textbf{\% Inhibition} = [(Abs_{CN} - Abs_{Ext})/Abs_{CN}] \times 100 \quad (IV.1)$$

Where; Abs_{CN}: is the absorbance of the control reaction.

Abs_{Ext}: is the absorbance of the extract.

IV.2.2. $ABTS^{\bullet+}$ Scavenging Activity

2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) is a radical cation that is readily formed by oxidation in the presence of potassium persulfate, resulting in a blue-green-coloured solution. Absorbance measurements at the appropriate wavelength allowed for determining this radical's concentration (Figure.IV.5). The absorbance at 734 nm decreases when an antioxidant is added to a solution containing these radical cations. This decrease frequently depends on time, concentration, and the examined samples' antioxidant activity [328].

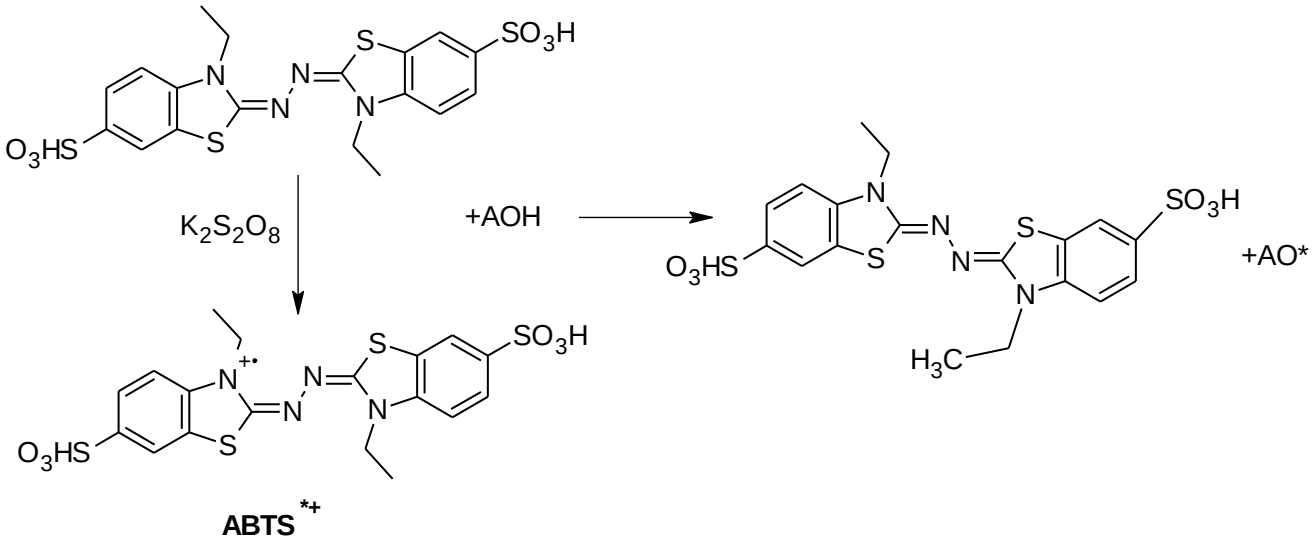


Figure.IV.5. Formation and trapping of the radical $ABTS^{+}$ by an antioxidant donor of $H^{\bullet}$ [329].

Protocol

According to the procedure described by Re *et al*. [328], the extracts of *A. campestris* L. and *F.bruguieri* DC. were conducted to determine their ability to scavenge free radicals in the presence of ABTS (2,2'-azinobis (3-ethylbenzothiazoline)-6-sulfonic). After reacting an aqueous solution of ABTS at 7 mM with 2.45 mM of potassium persulfate ($K_2S_2O_8$) for 16 hours, the cation $ABTS^{\bullet+}$ was generated during storage of the mixture in the dark at room temperature. Following dilution, the absorbance of the $ABTS^{\bullet+}$ solution was 0.700 ± 0.020. In a 96-well microplate reader, PerkinElmer Multi-mode Plate Reader EnSpire, USA, 40 µl of each sample prepared in methanol at various concentrations was combined with 160 µl of the $ABTS^{\bullet+}$ mixture and kept in the darkness for 10 min. A wavelength of 734 nm was used to measure the absorbance. The antioxidant standards were BHT and BHA, respectively. The following equation was utilized to calculate the inhibition percentage of $ABTS^{\bullet+}$:

$$\textbf{\% inhibition} = [Abs_{734\ plank} - Abs_{734\ sample} / Abs_{734\ plank}] \times 100 \qquad (IV.2)$$

Abs_{734} blank: absorbance of control reaction. Abs_{734} sample: absorbance of test sample.

IV.2.3. β-Carotene-Linoleic Acid Bleaching Activity

This system is founded on the idea; when yellow beta-carotene's color is destroyed by its reactivity with the radicals generated during the oxidation of linoleic acid in an emulsion. Antioxidants slow the bleaching of β-carotene, which is determined by spectrophotometric monitoring at 470 nm. Therefore, the absorbance value is obtained by reading it at time 0 and then again during two hours. This approach is sensitive because of the high absorption of beta-carotene, despite being slower than DPPH. The use of 96-well microplates has recently allowed for improvements in the methodology. This technique is frequently used for testing the antioxidant potential of a wide variety of samples, including pure chemicals, plant extracts, seeds, fruits, and vegetables [330].

Protocol

Using a model system (β-carotene/linoleic acid), we evaluated the ability of *A. campestris* L. and *F.bruguieri* DC. extracts to inhibit β-carotene [331]. β-carotene/linoleic acid emulsion was obtained by dissolving 0.5 mg of β-carotene in 1 ml of chloroform. This mixture was transferred to a flask containing 20 µL linoleic acid and 200 µL tween 40. A vacuum evaporator was utilized to remove the chloroform, and [50-70 ml] of hydrogen peroxide H_2O_2 was added for *A. campestris* L.; *F.bruguieri* DC.; respectively, followed by vigorous agitation. At 470 nm,

the absorbance of the combination was adapted to between 0.8 and 0.9. then, 160 µL of the emulsion was mixed with 40 µL of the plant extracts or synthetic antioxidants (BHA and BHA) at diverse concentrations. This mixture sets in each well of the 96-well microplate. After incubating the microplate at 50 °C, The absorbance was determined using a wavelength of 470 nm and a range of times beginning at t=0 min and finishing at t=120 min, each having 30 min intervals. The percentage of inhibition was expressed using the following equation:

$$I\ \% = 1- [(A_{S0} - A_{St}) / (A_{C0} - A_{Ct})] \times 100 \qquad (IV.3)$$

I (%): percentage of inhibition

A_{S0}: absorbance value of β-carotene content in the presence of the extract measured at t = 0.

A_{C0}: absorbance value of β-carotene content in the presence of negative control measured at t=0.

A_{St}: absorbance value of β-carotene content in the presence of the extract measured at t = 120 min.

A_{Ct}: absorbance value of β-carotene content in the presence of negative control measured at t = 120 min.

IV.2.4. Cupric Reducing Antioxidant Capacity (CUPRAC) Activity

The assay is a follow-up on the decreased enhanced absorbance of the copper-neocuproin complex [Nc_2 - Cu^{2+}] due to the reduced copper-neocuproin once an antioxidant is present (Figure II.6). At a wavelength of 450 nm, this reaction is determined by measuring the absorbance [332]. The principle of this test is based on the transformation of phenolic hydroxides into quinones, which takes place as a result of the reduction of the Cu^{+2}-Nc complex, resulting in the formation of a chromogenic complex of Cu^{+2}-Nc that absorbs light at 450 nm (Figure.IV.5)

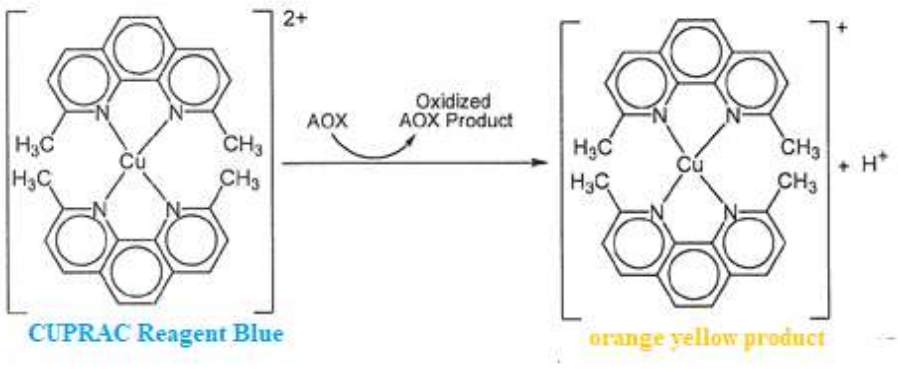


Figure.IV.6. Reduction of the Cu^{+2}-Nc chromogenic complex [332].

Protocol

In the CUPRAC method, the measurement is based on reducing copper ions (Cu^{2+}) [332]. A solution was assembled by mixing the following volumes: 50 µl of Cu (II) (10 mM), 50 µl of neocuprin (7.5 mM) , and 60 µl of CH_3COONH_4 buffer solution (1 M, pH = 7.0). Then 40 µL of samples at different concentrations were added to the initial mixture. The resulting mixture was put in darkness at room temperature for 1 hour. The absorbance was measured at 450 nm using the microplate reader (Perkin Elmer Enspire, Singapore). The antioxydant standards were BHA and BHT. The concentration given an absorbance of 0.5 ($A_{0.5}$) was calculated from the absorbance curve at different concentrations.

IV.2.5. Reducing Power Assay

The presence of reducers in plant extracts causes the reduction of ferric iron (Fe^{3+}) in the ferricyanide complex to the ferrous iron form (Fe^{2+}); this is the iron form considered more stable (Figure.IV.7). It is a colorimetric reaction characterized by a color change. It can be determined by spectrophotometric detection as having a high absorbance (green colour) at 593 nm [333].

Thus, the formation of this complex will demonstrate the reducing power that determines the antioxidant capacity of a compound [334].

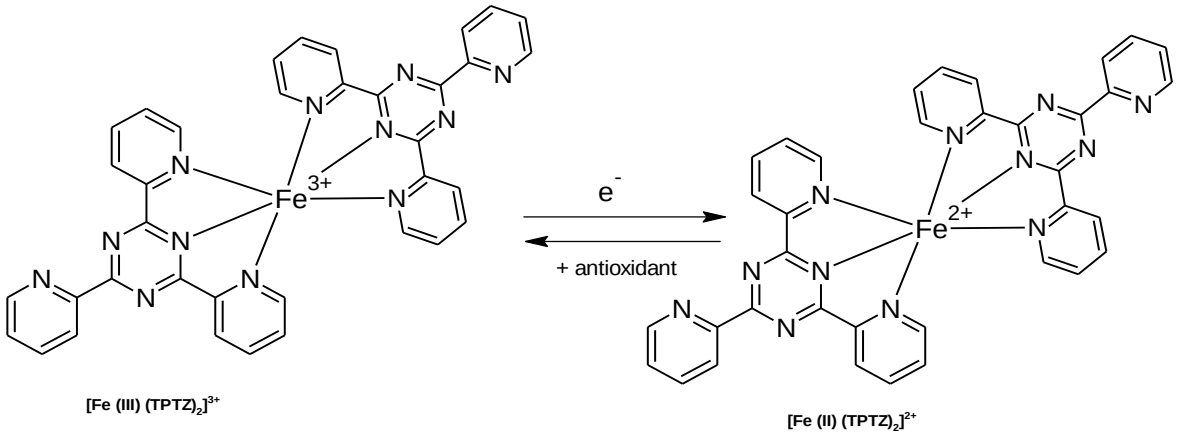


Figure.IV.7. Reduction of Fe^{+3} by an antioxidant in the reducing power test [333].

Protocol

The capacity of the samples to reduce Fe^{3+} contained in the complex $K_3Fe(CN)_6$ to Fe^{2+} was assessed by reducing iron ions. The Oyaizu method (1986) was used to perform the study that established the reducing power [335]. In brief, 10 µl of each extract or standard at various concentrations was mixed with 40 µl of phosphate buffer solution (0.2 M, pH 6.6) and 50 µl 1% potassium ferricyanide. The mixture was then kept at 50°C for 20 minutes. Afterwards, 10

µL of a 0.1% ferric chloride solution, 50 µL of 10% trichloroacetic acid, and 40 µL of distilled water were added. The absorbance measurement at 700 nm was recorded with the assistance of a microplate reader (Perkin Elmer, EnSpire, Singapore). In the experiment, the reference points utilized were BHA and ascorbic acid.

IV.2.6. Galvinoxyl (GOR) Scavenging Activity

Galvinoxyl is a relatively stable free radical commonly used in antioxidant tests (Figure.IV.8). The decrease of this radical can be easily detected using a spectrophotometer since its characteristic maximum absorption in the visible range (λmax = 432 nm) disappears when trapped. Galvinoxyl has a higher reactivity towards phenolic compounds than DPPH [336].

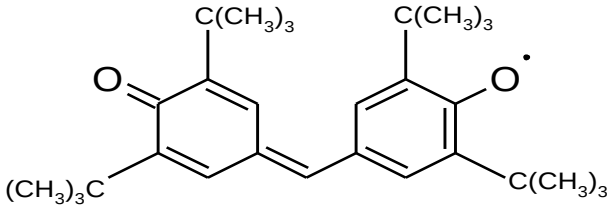


Figure II.8. Chemical structure of galvinoxyl [337].

Free radical traps such as hydrogen donors are responsible for the reduction of galvinoxyl, as shown in the following reaction :

$$\mathbf{G^{\bullet} + IH \rightarrow GH + I^{\bullet}} \quad \text{(IV.4)}$$

Where; **G$^{\bullet}$**: Galvinoxyl; **GH**: Reduced galvinoxyl; **IH**: free radical donor of hydrogen; and **I$^{\bullet}$** Corresponding IH radical [336].

Protocol

Shi *et al.* (2001) method was used to determine Galvinoxyl radical (GOR) antioxidant assay [336]. For this investigation, 40 µl of various extract concentrations in methanol were combined with 160 µl of a galvinoxyl solution in methanol at a concentration of 0.1 mM. After 120 minutes of incubation at room temperature in darkness, the absorbance of the resultant solution was determined using spectrophotometry at 428 nm. The standards were BHT and BHA and the methanolic solution of galvinoxyl was used as a control. The inhibition percentage I (%) was expressed as formula (IV.1)

IV.2.7. O-Phenanthroline Activity

It is a colorimetric antioxidant activity of iron reduction that is utilized for the purpose of determining the antioxidant capacity. It is based on the formation of the ferrous complex-phenanthroline in the presence of polyphenols (Equation .IV.5), which has a color of red [338].

Fe (III)+ 1,10-Phenantroline ⟶ Fe(II)-1.10 phenantroline + oxidizing antioxidant (IV.5)

Protocol

According to Szydowska-Czerniak *et al.* approach, the O-phenanthroline chelating activities of the extracts were determined [338]. A mixture of 30 µL O- phenanthroline (0.5 % in methanol), 110 µL methanol, 50 µL $FeCl_3$ (0.2 %), and 10 µL of samples or standards at varying concentrations was prepared (BHA; BHT), then the mixture was incubated at room temperature for 20 minutes before the examination. A 96-well microplate reader was used to measure the absorbance of an orange-red solution at 510 nm.

IV.2.8. Antioxidant Activity Evaluation via Electrochemical Method

IV.2.8.1. $O_2^{-\cdot}$ free radical scavenging assay

Cyclic voltammetry (VC) is one of the most important experimental techniques for studying redox systems intended to be employed as rechargeable energy sources. This technique is differentiated by its benefits of simplicity, sensitivity, and rapidity in collecting data on redox processes, the kinetics of charge transfer reactions, or the reversibility of the electrochemical system that has been investigated. The antioxidant capacity of plants may be estimated with the technique (VC).

Cyclic voltammetry (VC) is an electrochemical inversion technique controlled by the potential. It is usually used for the purpose of studying reactions and mechanisms. The procedure involves measuring the current intensity produced by applying a linear ramp in potential at a positive or negative scan rate. The potentials are swept in a cycle at a constant speed; after sweeping towards the anode potentials and performing oxidation. The cathodic potential is exploited by switching the direction of potential variation. Voltammograms therefore record the anodic and cathodic polarization curves, also referred to as cyclic voltammograms (Figure.IV.9).

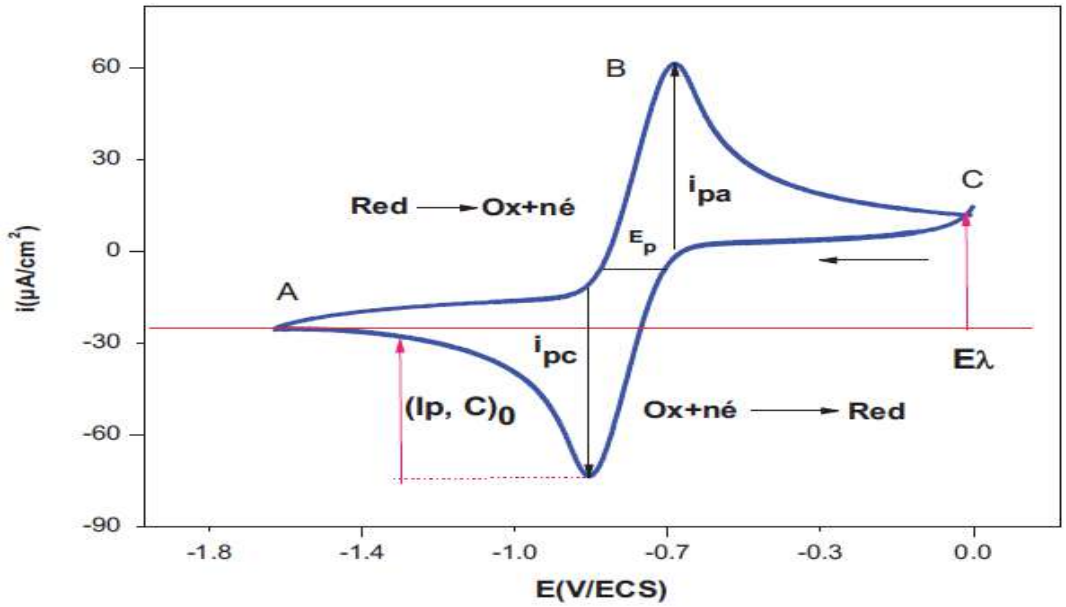


Figure.IV.9. Cyclic voltammogram for a reversible system.

In a reversible process, where the recorded current is plotted against the potential, the analyte exhibits oxidation (Epa) and reduction (Epc) peaks in the forward and reverse scans, respectively. The current against potential plot indicates that the anodic peak current (Ipa) occurs at the anodic peak potential during the anodic scan. In contrast, the cathodic peak current (Ipc) occurs at the cathodic peak potential during the cathodic scan (Figure II.9).

Protocol

The cyclic voltammetry methodology is used to generate the superoxide anion radical $O_2^{-\cdot}$ in the electrode's diffusion layer by reducing oxygen to an electron in the DMF solution. The electrochemical examination was carried out with a PGZ301 voltammeter equipped with VoltaMaster 4 V 7.08 software (Radiometer Analytical SAS, France). The measurements are performed using an electrochemical cell with a capacity of 15 ml and a three-electrode system; that includes the following components:

- A working electrode made of glassy carbon (GC) with a geometric area of 0.013 cm^2, the electrode was manually polished using 4000 silicon carbide sandpaper to provide a homogeneous and smooth surface. For each cyclic voltammetry testing, we did make sure the polishing was adequate for the test.
- An auxiliary electrode is made of a platinum wire as a counter.
- Reference electrode made of a Hg/Hg_2Cl_2 paste-covered wire (3,0 M KCl).

The electrochemical cell was saturated with high-purity commercial oxygen for 15 minutes before each experiment to study the interaction of superoxide anion free radicals. Each assay, extract or standard volume was added to 15 ml of 0.1 M DMF/nTBuNBF4 solution. Afterwards, the voltammograms were recorded in the absence and presence of progressively increasing concentrations of the different extracts that were investigated. Voltammograms were obtained in cyclic voltammetry extracts of A.*campestris* L. and *F.bruguieri* DC. with a sweep speed of 0.1 $V.s^{-1}$; region d'électro-activity (0, -1,6 V). The Free $\boldsymbol{O_2^{-\cdot}}$ radical scavenging data of *A. campestris* L. *and F.bruguieri* DC. extracts were used to determine IC_{50} values. The IC_{50} value was defined as the sample concentration (mg/mL) that inhibits $O^{\bullet-}$ radical formation at 50%.

$$I(\%) = \frac{I_{pa0} - I_{pas}}{I_{pa0}} \times 100 \qquad \text{(IV.6)}$$

Where; I_{pa0} and I_{pas} are the anodic peak current densities of the superoxide anion radical in the absence and presence of *A. campestris* L. and *F.bruguieri* DC. extracts respectively.

IV.3. Enzymes Inhibitory Activity

IV.3.1. Evaluation of anti-Alzheimer activity

IV.3.1.1. Cholinesterase Inhibitory Activity

Cholinesterases are enzymes that degrade acetylcholine, a cholinergic neurotransmitter involved in cognitive activities. Inhibition of these enzymes will reduce acetylcholine and consequently increase cholinergic effects [339]. The inhibitory effect of the aerial parts of *A.campestris* L. *and F.bruguieri* DC. on butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) was evaluated by employing the method of Ellman *et al*., [340] (Figure.IV.10).

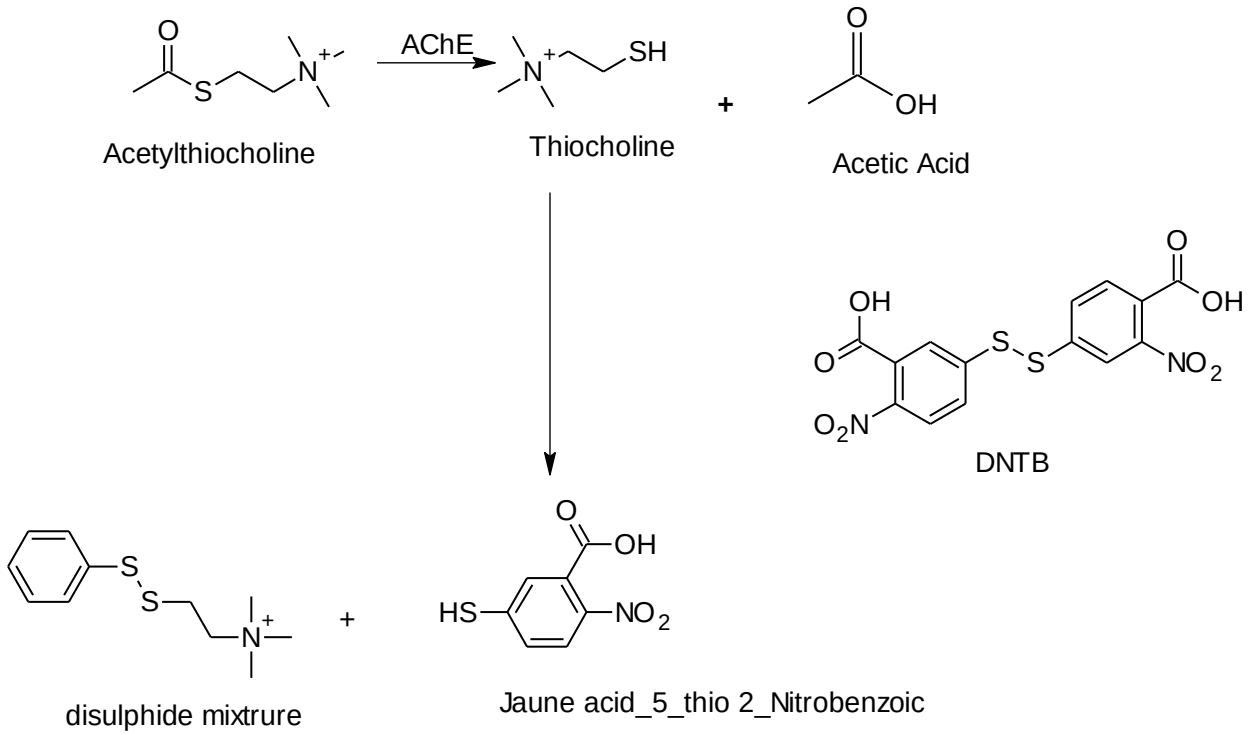


Figure.IV.10. Chemical mechanism of the Ellman *et al*., method [340].

Protocol

Both acetylcholinesterase (AChE) from electric eels and butyrylcholinesterase (BChE) from horse serum were utilized. As substrates, iodide acetylthiocholin and butyrylthiocholin chloride were used. DTNB [5,5-dithio-bis (2-nitrobenzoïc)] was employed in the measurement of anti-cholinesterase activity. Methanol was the selected solvent for dissolving both the test samples and the controls. Briefly, a mixture composed of 150 µL sodium phosphate buffer (100 mM, pH:8.0), 10 µL of sample test or galantamine (reference molecule), and 20 µL BChE (6.85×10-3 U) AChE or (5.32×10-3 U) solution were added. Incubation at 25 C° for 15 min was realized; then 10 µL of 0.5mM DTNB was added along with 10 µL of acetylthiocholine iodide or butyrylthiocholine iodide. Using a 96-well microplate reader, Perkin Elmer Multimode Plate Reader EnSpire (National Center of Biotechnology Research; Constantine, Algeria), the absorbance was recited at 412 nm for 15 min. The hydrolysis of these substrates was monitored by spectrophotometry via the formation of a yellow 5-thio-2-nitrobenzoate anion, the product of DTNB's interaction with thiocholine. This anion was released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, and the reaction was measured at a wavelength of 412 nm.

The inhibitory activity was measured by comparing the reaction rates of samples relative to the blank samples using the formula:

$$\textbf{Inhibition \%} = [(E - S) / E] \times 100 \qquad (IV.7)$$

E: activity of the enzyme without sample, S: activity of the enzyme with the sample. Galantamine was used as a reference compound (positive control).

IV.3.2. Evaluation of anti-diabetic activity

The antidiabetic activity of the various extracts of *A. campestris* L. *and F.bruguieri DC.*, aerial part, was evaluated using the alpha-amylase inhibition method.

IV.3.2.1. Alpha -amylase Inhibitory activity

Alpha-amylase is a digestive enzyme generated by the glands in the salivary glands and pancreas. It is one of the most important endo-amylases. Its function is to hydrolyze the (1-4) glycosidic bonds inside the starch chains to generate maltose molecules, also known as α-glucose disaccharides. This enzyme is important for absorbing and breaking down the carbohydrates in food. This enzyme can be a target for treating type 2 diabetes [341].

Acarbose is a well-known medication employed in the drug industry as an inhibitor of the enzyme alpha-amylase. It delays carbohydrate digestion and reduces postprandial plasma glucose levels [342]. On the other hand, it might produce undesirable side effects such as diarrhea, gastrointestinal obstructions, and ulcers [343]. Plants' naturally occurring α-amylase inhibitors, such as flavonoids and phenolic compounds, are recommended as a safe and effective approach for treating and preventing type 2 diabetes [344].

Protocol

The α -amylase inhibitory activity was investigated according to Zengin *et al*., 2014 [345], using the iodine/potassium iodide method, with slight modifications. The reaction mixture was prepared in a 96-well microplate by adding 25 µL of the sample at various concentrations with amylase solution in 1U of sodium phosphate buffer (pH = 6.9 with 6 Mm NaCl). After incubating the resultant solution at 37 C° for 10 min, the reaction was initiated by adding 50 µL of 1% starch solution. A control was simultaneously prepared without the enzyme solution. Re-incubation for 20 min at 37 °C was done, followed by the addition of 25 µL 1M HCl and 100 µL of iodine-potassium iodide solution to stop the reaction.

The absorbance was measured at 630 nm, and the % inhibition of α -amylase was estimated as follows:

$$\mathbf{I\ \%} = 1 - [(Abs_a - Abs_b) - (Abs_c - Abs_d) / (Abs_a - Abs_b)] \quad (IV.8)$$

- Abs_c = Absorbance (Extract, Starch, Enzyme, IKI, HCl).
- Abs_d =Absorbance (Extract, sodium phosphate buffer, IKI).
- Abs_b = Absorbance (solvent vol Extract, Enzyme, Starch, HCl, IKI).
- Abs_a = Absorbance (solvent vol Extract, sodium phosphate buffer, Starch, HCl, IKI).

IV.3.3. Evaluation of anti-obesity activity

IV.3.3.1. Pancreatic Lipase Inhibitory Activity

Protocol

For determining lipase inhibitory activity against pancreatic lipase, the method of Souza *et al.*, (2011) was performed to determine the inhibitory capacity with slight change [346]. In this experiment, the studies extracts were diluted in dimethyl sulfoxide (DMSO) at the concentration of 4 mM. An aliquot containing 100 µL of pancreatic Lipase solution in Tris-HCl buffer (pH = 8) was mixed with 50 µL of each sample at various concentrations. After incubation of 20 min at 37 C°. Subsequently, the addition of 50 µL of p-NPP (p-Nitrophenol Palmitate) drove the reaction to initiated after incubation at 37 ◦C for 120 min. Orlistat was used as a positive control. A blank with DMSO as a substitute for enzyme solution was prepared. The absorbance of lipase products (p-nitrophenol) was read at 410 nm at t=0 min and t=120 min using a 96-well microplate reader (Perkin Elmer, Enspire). All samples were analyzed in triplicate. Percent pancreatic lipase inhibition was determined using the following formula [35]:

$$\mathbf{I\ (\%)} = [(AbsA - Absa) - (AbsB - Absb)/ (AbsA - Absa)] \times 100 \quad (IV.9)$$

Where:

AbsA: The activity in the absence of an inhibitor.

Absa : the negative control in the absence of an inhibitor.

AbsB: the activity in the presence of an inhibitor.

Absb: the negative control in the presence of an inhibitor.

IV.3.4. Anti-inflammatory Activity (BSA denaturation inhibition)

The BSA denaturation inhibition method was used to establish the anti-inflammatory activity of the different extracts of *A. campestris* L. and *F.bruguieri* DC. aerial parts.

The fundamental concept of BSA denaturation inhibition is to prevent BSA from being denaturized by heating at (72°C).

The heat-induced denaturation of albumin causes a reaction that mimics the production of antigens associated with Type III hypersensitivity. This reaction is involved in the pathogenesis of disorders such as serum diseases, glomerulonephritis...etc [347].

Protocol

The in vitro anti-inflammatory efficacy of the *A. campestris* L. and *F.bruguieri* DC. extracts was determined using the method described by Kandikattu *et al*., (2013), with slight modifications [348]. After adding 0.5 ml of 0.2 % BSA solution in Tris Buffer (pH 6.8) to 0.5 ml of each extract concentration or standard (Diclofenac), the mixture was then placed in an incubator at 37 ° C for 15 min. It was then placed in a water bath at 72 °C for 5 min. After cooling the tubes, the turbidity (protein precipitation) was read at 660 nm using a spectrophotometer. In order to calculate the percentage of the amount to which the proteins were prevented from denaturation, the following equation was applied:

$$\textbf{\% Inhibition} = [[Abs_{control} - (Abs_{sample}-Abs_{white})] / Abs_{control} * 100] \qquad (IV.10)$$

Abs_{sample}: Sample (0.5 ml extract + 0.5 ml BSA), Abs_{white}: White (0.5 ml extract + 0.5 ml Tris-phosphate (pH: 6.8)).

IV.3.5. Photoprotective Activity

IV.3.5.1. *Sun protection factor activity (SPF)*

The skin is the biggest organ in the body, covering 1.5-2 m^2. It protects the body from UV radiation; the latter is found in three subclasses: UVA, UVB, and UVC. UVB is the most dangerous radiation; it can trigger skin diseases like melanoma [349]. Natural compounds such as polyphenols and vitamins have an impact that is beneficial and preventive against the ROS which is produced by these rays [350].

The sun protection factor, abbreviated SPF, is a quantitative measure of the effectiveness of a sunscreen formulation. Sunscreen representatives are identified using an in vitro methodology

based on spectrophotometric measurement of diluted solutions as described by Mansur *et al.*, (1986) [351].

Protocol

The SPF of *A. campestris* L. and *F.bruguieri* DC. extracts was determined in vitro to investigate its ability to protect against UV damage (SPF) [351] . First, the samples were diluted in absolute methanol to a concentration of 2 mg/mL (2000 ppm). As shown in Table 1, the absorbances were measured at different wavelengths, starting with 290 nm to 320 nm, each 5 nm using a multimode microplate reader (Perkin Elmer Enspire, Singapore). Each measurement was performed in triplicate, and the SPF value was calculated by applying the below mathematic equation:

$$\text{SPF spectrophotometric} = CF * \sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda) \qquad \text{(IV.11)}$$

CF: correction factor (= 10); EE: erythemal effect spectrum; I: solar intensity spectrum; Abs: absorbance of sunscreen product, EE* I: is a constant calculated by Sayre *et al*. and is displayed in Table.IV.1 [352].

Table.IV.1. The Normalized product function used in the calculation of sun protection factor (SPF).

Longueur d'onde λ (nm)	EE (λ)x I(λ) (Normes)
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0837
320	0,0180
Total	1

According to the recommendations of the European Commission in 2006 [353], Table.IV.2 illustrates the different protection categories displayed on the products, as well as the sun protection factor values which correspond to all those protection categories.

Table.IV.2. Protection categories displayed on solar products according to the measured protection factors, as recommended by the European Commission, 2006.

Indicated category	Protection factor indicated	Sun protective factor measured	Recommended minimum UVA protection factor	Recommended minimum critical wavelength
«Minimum protection »	6	6 - 9,9	1/3 of the sun protection factor indicated on the label	370 nm
	10	10 - 14,9		
«Medium Protection »	15	15 - 19,9		
	20	20 - 24,9		
	25	25 - 29,9		
High » « protection »	30	30 - 49,9		
	50	50 - 59,9		
«Very high protection»	50+	60 ≤		

Statistical Data Analysis

All tests were carried out in triplicate. Data were recorded as mean±standard variation (SD) of three measurements. The obtained results were statistically analyzed using SPSS descriptive statistics (IBM SPSS Statistics, version 21 PL) and one-way ANOVA (GraphPad Prism 5 program), followed by Turkey's post hoctest for multiple comparisons and differences with *p* Values are considered significantly different at $p<0.05$.

PART 3:

RESULTS & DISCUSSION

Chapter V: Quantitative analysis and Structural Elucidation

V.1. Qualitative phytochemical screening

Artemisia campestris L.

Based on the information shown in the Table.V.1 below, it can be stated that *A. campestris* L. is an extremely rich source of flavonoids, particularly in the AcEAE and AcDE extracts. Similarly, both the AcAE and the AcBE showed a significant content of reducing chemicals and saponins. Coumarin has a moderately positive effect on AcEAE, but it has a substantial positive content in AcME and AcDE. Terpenoids have a moderately positive appearance in AcME, AcDE and AcPEE but a negatively appearance in AcAE. An abundance of saponins can be detected in various *A. campestris* L. extracts. Conversely, tannins are present but in low concentrations. All *A. campestris* L. extracts were negative for the presence of alkaloids in a precipitation test using the Dragendorff reagent.

Table.V.1. Phytochemical screening results of *Acampestris* L.

Extracts	**secondary metabolites**						
	Flavonoids	**Alkaloids**	**Tannins**	**Terpenoids**	**Coumarins**	**Reducing compounds**	**Saponins**
AcME	++	-	+	++	+++	+++	+++
AcPEE	+	-	+	++	+	+	++
AcDE	++	-	+	++	+++	++	+++
AcEAE	+++	-	+	+	++	+++	+++
AcBE	++	-	+	+	+	+++	+++
AcAE	+	-	-	-	-	+	+

(+++): Strongly positive; **(++)**: Moderately positive; **(+)**: Weakly positive; **(-)**: Negative.

Fagonia bruguieri DC.

The data presented in the Table.V.2 below demonstrate that the aerial part of *F.bruguieri* DC.is abundant in flavonoids, except FbPEE and FbAE, which showed a low flavonoid content. Salkowski's reaction confirms the presence of Terpenoids in FbME, FbPEE, FbDE, and FbEAE and negative in FbBE and FbAE. Catechins tannins show a significant positive reaction in the FbME, FbPEE, FbDE, and FbEAE tests; however, the FbBE and FbAE showed a weak positive reaction. For the coumarin test, there is a strong presence in FbME and FbDE; there is also a low presence of Reducing compounds in the various extracts. The same is for saponins; all of the different extracts of the *F.bruguieri* DC. plant have shown positive test outcomes. The presence of alkaloids in FbME and FbPEE was confirmed by precipitation processes using the Dragendorff reagent.

Table.V.2. Phytochemical screening results of *F.bruguieri* DC.

Extracts	secondary metabolites						
	Flavonoids	**Alkaloids**	**Catechins tannins**	**Terpenoids**	**Coumarins**	**Reducing compounds**	**Saponins**
FbME	+++	+	++	+++	+++	+	+++
FbPEE	+	+	++	+++	+	+	+++
FbDE	+++	+	++	+++	+++	+	+++
FbEAE	+++	-	++	+++	+	+	++
FbBE	+++	-	+	-	-	+	++
FbAE	+	-	+	-	-	+	++

(+++): Strongly positive; **(++)**: Moderately positive; **(+)**: Weakly positive; **(-):** Negative

The fact that the classes of secondary metabolites found in the chosen species have the potential to have a correlation with the therapeutic properties of those species justifies the widespread use of those species in traditional medicine practiced by the inhabitants of the Tamanrasset region.

Several investigations were carried out on the phytochemical investigation of *Artemisia campestris* L. plant; according to the findings of Douaouya and al., (2022), the plant Artemisia *campestris* L. from the Aures-Algerie region contains flavonoids, saponins, tannins, alkaloids, reducing compounds, and coumarin [354]; however, terpenes were not found in the sample. These findings are almost identical to our own. Similarly, the study by Naili *et al*. (2010) revealed that the methanolic crude extractof this species contains saponosides, Alkaloids, tannins, terpenes, and flavonoids [355].

Few phytochemical studies have been conducted on the species *Fagonia bruguieri* DC. Irshad *et al.* (2022) demonstrated different classes of secondary metabolites, such as alkaloids, flavonoids, terpenoids, and saponin, were found in the methanol extract of Fburguieri throughout a chemical screening [211]. In contrast, several phytochemical studies on the genus *Fagonia* have demonstrated its richness in secondary metabolites, with a distinct predominance of flavonoids [218, 220], and diterpenes [245].

V.2. Mineral Analysis

The ICP-OES and INAA analytical methods were used to identify the major, minor, trace, and ultra-trace element concentrations in the samples selected for further investigation.

V.2.1. Calculation Of Elementary (Mineral) Concentrations

The element concentrations in the plants studied with NAA and ICP-OES were determined using Equations (III.1) and (III.4) , respectively in Part 2: Chapter III.

Table.V.3 shows the main concentrations of mineral elements found in *A. campestris* L. and *F.bruguieri* DC.using two techniques (INAA and ICP-OES). The results obtained were arranged in values as mean and standard error (±SD), based on assessment measured on the basis of triplicate analyses. twenty one elements were found in our samples as reported.

Table.V.3. Concentrations in mg/kg of the chemical elements in the plant *A. campestris* L. and *F.bruguieri DC.*

Elements	***A. campestris* L. Mean ± SD***		***F.bruguieri DC.* Mean ± SD***	
	ICP-OES	**INAA**	**ICP-OES**	**INAA**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Br	Nd	16.1409±14.4970	Nd	85.4020±119.2283
Ca	10538.1 ± 69.9	Nd	Nd	Nd
Cd	0.0988±0.0098	Nd	0.0999±0.0199	Nd
Co	1.28±0.148	1.4625 ± 0.2267	0.899±0.099	0.8208±0.0545
Cr	1.083±0.551	1.1982 ± 0.3272	Nd	1.1392±0.2418
Cs	Nd	0.1566± 0.0414	Nd	0.2211±0.0557
Cu	70.22±0.94	Nd	37.81±0.39	Nd
Eu	Nd	0.0393 ±0.0181	Nd	0.0202±0.0095
Fe	1181.8±82.37	1264.2366± 56.3983	490.7±10.51	512.7699± 16.3994
Li	46.6±4.45	Nd	25.5±1.10	Nd
Mg	990.7±3.14.5	Nd	933.8±253.5	Nd
Mn	41.06±6.51	Nd	9.25±3.68	Nd
Mo	0.29±0.068	Nd	0.498±0.059	Nd
Na	298.41±1.319	311.7137± 19.0049	762.55±13.33	752.5544±11.1111
Ni	2.75±0.43	Nd	1.690±0.248	Nd
Pb	0.68±0.305	Nd	0.59±0.099	Nd
Sc	Nd	0.0220 ± 0.0024	Nd	0.0059±0.0007
Se	Nd	0.1680 ± 0.0925	Nd	0.3244±0.1746
Sr	23.93±0.768	25.3373± 6.9346	168.55±14.03	157.6644±16.1226
Ti	20.38±1.61	Nd	6.26±0.66	Nd
Zn	47.07±0.797	43.7270 ± 3.3415	38.12±0.358	36.3133±1.2809

*__SD__: standard deviation n=3 (All values expressed on dry weight basis). **Nd**: Not detected

Based on this research, it can be deduced that our samples contain three different groups of chemical components, some of which are major, minor, and trace. The components found in the studied plants can be grouped into three categories based on their biological properties:

1) Essential elements and nutrients: Ca; Fe; Na ; Zn ; Cr ; Mg ; Mn ; Co; Cr.

2) Potentially toxic elements in the top-down content model such as Pb; Cd.

3) The remaining chemical elements are non-essential.

V.2.2. Evaluation of Results and Quality – Quality Assurance / Control (QC / QA)

The results of the mineral compounds in the plants analyzed were validated using the following standards: NIST-SRM 1570a, WEPAL-IPE 172 and WEPAL-IPE 189 irradiated simultaneously with the samples studied. This methodology enables us to evaluate the effectiveness of the analyses and the relevance of the results obtained on the other hand and to validate the entire analytical procedure employed in this study, from sample preparation through irradiation and measurement. Table.V.4 to Table.V.6 provides an overview of these latter items. Note that the statistical parameters for evaluating the results acquired by U-score and Z-score were computed for the QC/QA concept by equations III.2 and III.3 respectively (Prat 2: Chapter III). Their assessments indicate that the majority of concentration values are located in the allowable ranges: $\leq |1|$, and $\leq |3|$ U-score and Z-score parameters, respectively.

Table.V.4. Assessment of the quality of the analytical results based on the comparison between the calculated and certified values in standard reference of NIST-SRM 1570a; values express means ± standard error (n = 3).

Elements mg/kg	NIST-SRM 1570a			
	measured values	certified values	U-score	Z-Score
Ba	-	-	-	-
Br	-	-	-	-
Ca	15128±0.2	15260±66	0.07	1.99
Co	0.394±0.078	0.393±0.03	0.01	0.03
Cr	-	-	-	-
Cs	-	-	-	-
Fe	-	-	-	-
K	28511±500	29000±260	0.87	1.88
Na	17600±0.12	18210±230	0.5	2.65
Rb	12.45±0.985	12.7±1.6	0.13	0.15
Sb	-	-	-	-
Sr	54.54±4.66	55.54±0.5	0.21	1.99
Zn	82.65±5.68	82.3±3.9	0.05	0.09

(-): not reported

Table.V.5. Assessment of the quality of the analytical results based on the comparison between the calculated and certified values in standard reference of WEPAL-IPE 172 ; values express means ± standard error (n = 3).

Elements mg/kg	WEPAL-IPE 172			
	measured values	certified values	U-Score	Z-Score
Ba	17.62±2.37	16.4±0.99	0.48	1.24
Br	1.763±0.361	2.1±1.265	0.26	0.27
Ca	-	-	-	-
Co	17.62±2.37	16.4±0.99	0.48	1.24
Cr	1.763±0.361	2.1±1.265	0.26	0.27
Cs	17.62±2.37	16.4±0.99	0.48	1.24
Fe	67.84±6.51	64.4±6.55	0.37	0.53
K	-	-	-	-
Na	-	-	-	-
Rb	8.763±1.167	9.07±0.536	0.23	0.56
Sb	0.0372±0.0105	0.0482±0.005	0.92	2.03
Sr	43.77±3.14	42.2±2.11	0.42	0.75
Zn	33.21±2.31	31.6±2.19	0.51	0.74

(-): not reported

Table.V.6. Assessment of the quality of the analytical results based on the comparison between the calculated and certified values in standard reference of WEPAL-IPE 189; values express means ± standard error (n = 3).

Elements mg/kg	WEPAL-IPE 189			
	measured values	certified values	U-Score	Z-score
Ba	74.52±9.518	80.1±5.96	0.5	0.94
Br	48.34±29.64	40.6±6.9	0.25	1.12
Ca	6346.5±30.2	6730±402	0.95	0.95
Co	0.0775±0.0159	0.0789±0.0132	0.06	0.1
Cr	0.919±0.267	1.18±0.228	0.74	1.14
Cs	0.0903±0.0236	0.089±0.0157	0.04	0.07
Fe	104.4±12.8	110±7.4	0.38	0.76
K	36500.4±4213.1	37100±2160	0.13	0.28
Na	-	-	-	-
Rb	7.91±0.59	7.5±0.501	0.52	0.82
Sb	0.013±0.007	0.0095±0.0019	0.5	1.88
Sr	-	-	-	-
Zn	19.12±1.39	20.1±1.32	0.51	0.74

(-): not reported

V.2.3. Assessment of dietary intake of trace elements according to the RDA

The Recommended dietary allowances (RDA) are determined using scientific evidence to establish the minimum and maximum amounts of nutrients a human needs to maintain optimal health [356]. Macronutrients and micronutrients, often known as trace elements, compose the majority of the mineral elements required in the body's metabolism. These can be divided into three groups based on their nutritional value:

- Essential trace elements, also called micronutrients, are parts of hormones, vitamins, and catalysts for the enzymatic systems of the metabolic process inside cells.
- The mainly essential elements.
- Toxic and non-toxic elements that have no metabolic function in a living organism make up the non-essential trace elements [357].

From a nutritional perspective, there is a growing interest in determining the levels of macro-elements and oligo-elements in foods like calcium, iron, potassium, sodium, selenium, and zinc, all of which play a significant role in human health. Furthermore, one of the most important sources of toxic elements for the human body is its consumption of unhealthy foods and drinkable water . In light of this, it must be careful monitoring of their intake.

Plants used for medicinal purposes can be an excellent source of several essential elements. On the other hand, the amount of consumption must be carefully controlled and monitored . In the case of the investigated plants, the values for daily intake and average intake per person for essential and toxic elements must be controlled.

In this study, the amounts of some essential or toxic elements were determined by calculating the assumption that a person consumes 10 g (dry weight) per personne. The recommended nutrient intake values are listed in Table.V.7, along with the recommended daily requirement/tolerance limits and the estimated values.

Table.V.7. Intake values (mg/day, person) of certain essential elements and tolerable daily intake for adults (70 kg) (Tolerable Daily Intake) of certain toxic elements according to the food and agricultural organization (FAO/WHO; 2011).

Plants studied; Person-age	**RDA and AI of essential chemical elements**									**TDI (70 kg adult (BW)) of potentially toxic chemicals**		
	Ca[*] (mg/ day)	**Na** [a] (mg/ day)	**Fe**[*] (mg/ day)	**Mg**[*] (mg/ day)	**Se**[*] (µg/ day)	**Mn** [a] (mg/ day)	**Mo**[*] (µg/ day)	**Zn**[*] (mg/ day)	**Cr**[*] (µg/ day)	**Pb** (mg/day/ person)	**Cd** (µg/day/ person)	**Br** (mg/da/ person)
***A.campestris* L.**	105.38	2.98	11.81	9.90	1.68	0.41	2.9	0.47	10.83	0.06	0.988	0.16
F.bruguieri DC.	Nd	7.62	4.90	9.33	3.24	0.09	4.9	0.38	Nd	0.05	0.999	0.85
Children 1–3 years	600	1000	9	50	20	1.2	17	5	11	Nd	Nd	Nd
Men 19-50 years	600	1500	17	340	55	2.3	45	12	35	0.3	60	70
Women 19-50 years	600	1500	21	310	55	1.6	45	10	25	0.3	60	70
Pregnant women 19-50 years	1200	1500	35	310	60	2.0	50	12	30	0.3	60	70
Lactation 19-50 years	1200	1500	21	310	70	2.6	50	12	45	0.3	60	70

[*]**RDA**: Recommended daily allowance, expressed in mg/day per person for adult men. All values are expressed on the dry weight. **Nd**: Not determined.

[a]**AI**: Adequate intake, expressed in mg/day, per person for adult men and women).

As a preliminary observation, the assessment of the probable intake values for consumption of these samples reveals that the levels of toxic elements are lower than the reference toxicological values (Figure.V.1 and Figure.V.2). This was determined by comparing the levels of toxic elements to the reference toxicological values [358]. They were discovered to conform with

dietary recommendations, with the exception of iron, when they consumed 10 grams per day of the plant *A.campestris* L. According to the findings, the average daily consumption of iron is higher than the amount advised for Children 1–3 years (9 mg/day) [359].

Regarding the remaining essential components, each one is well below the permissible limits recommended by the WHO/FAO commission. It is important to note that the amount of toxic elements present is significantly lower than the reference toxicological values and agrees with the nutritional recommendations[358] (Table.V.7).

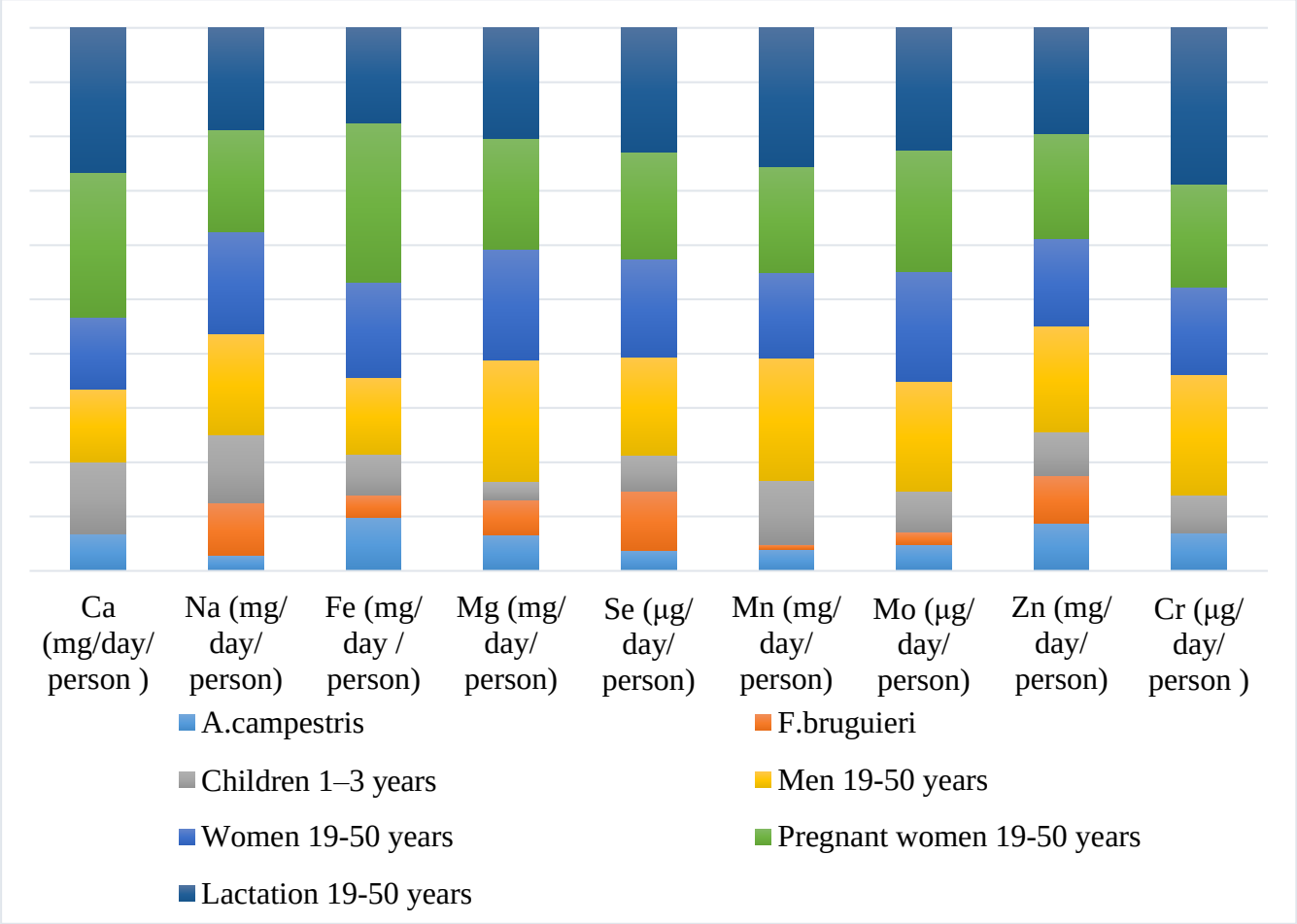


Figure.V.1. Comparison between measured values and the limit tolerance of essential elements.

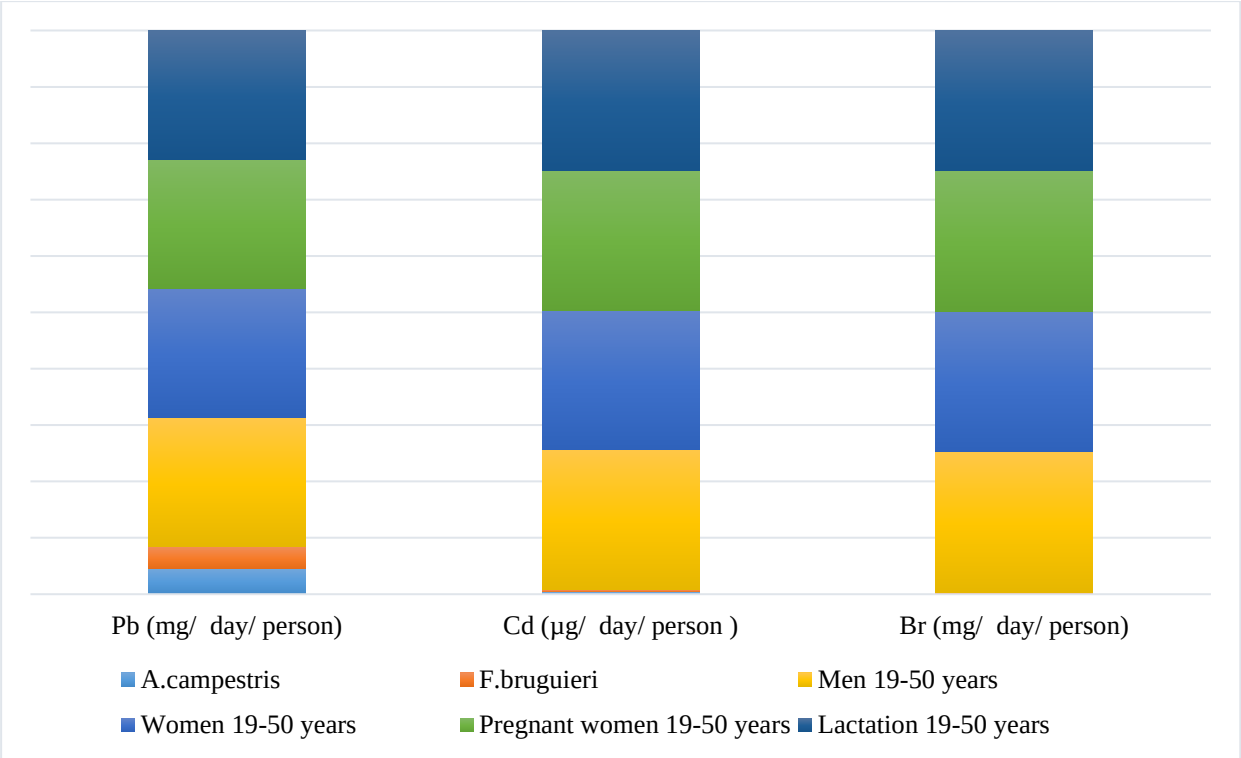


Figure.V.2. Comparison between measured values and the Tolerable Daily Intake of toxic elements.

According to the World Health Organization, traditional herbal treatments are natural substances used to cure and alleviate a wide range of diseases [360]. Monitoring the mineral content of medicinal plants is considered one of the most critical aspects of safety before evaluating pharmaceutical use. This report provides the content of mineral concentrations found in the aerial part of the two plants studied and assesses the potential risks that these concentrations represent to human health.

There is currently no research available at this time concerning the multi-element content (mineral compounds) of the plants *A.campestris* L. and *F.bruguieri* DC.This research tries to fill this gap by utilizing the ICP-OES and Instrumental Neutron Activation Analysis (INAA) techniques to provide scientific evidence on the mineral composition of *A.campestris* L. and *F.bruguieri* DC., as well as their elemental concentrations.

Description and inputs of essential trace elements for the human body:

- **Calcium**: Ca is the only mineral most abundant in the human body, with approximately 1 to 1.2 kilograms in adults. The main function of this element is building and renewing the skeleton. Indeed, 99% of calcium contributes to the formation and strength of bones and teeth [361]. In this investigation, the amounts of calcium were found to have a level

of 10538.1 mg/kg in *A. campestris* L., on the other hand, *F.bruguieri* DC. shows no evidence of the presence of Ca. In contrast, the *A. campestris* L. plant is calcium-rich. A daily dose of 10 grams of this plant provides 105.38 mg of calcium/per person, recommended by the World Health Organization WHO [359].

- **Chromium:** Cr is an oligo-element that plays a significant role in the body's operation. Its principal function is to control insulin secretion by the pancreas, which allows for stable levels of blood sugar to be maintained (blood sugar) [362]. The concentration of Cr in the plant *A. campestris* L. measures 1.083 mg/kg in content. It is necessary to mention that the plant *F.bruguieri* DC. does not detect even a trace of Cr; hence it is regarded as Cr poor. When consuming the plant *A. campestris* L., the WHO/FAO recommended RDA value must be considered [359].
- **Iron:** Fe is one of the essential oligo-elements for blood; therefore, it is important to consume iron daily. It is involved in many physiological functions. As a constituent of hemoglobin and the red pigment found in blood cells, it plays a role in various tasks crucial to the body's physiology. One of these functions is the transportation and storage of oxygen in the blood [363]. The iron concentrations in the plants examined ranged from 490.7 to 1181.8 mg/kg; according to the results of the analyses carried out on the plants, *A. campestris* L. has the highest concentration of iron, whereas the plant *F.bruguieri* DC. has the lowest concentration of iron. The Joint Committee on Food and Agriculture (FAW) and the World Health Organization (WHO) recommend that an individual's iron consumption should not exceed safe limits, even though iron is essential to human health . The recommended daily dose of *A. campestris* L. is less than 9 mg to reduce or eliminate the risk of iron toxicity [359].
- **Zinc:** Zn is an essential trace element for human health. It is involved in many enzyme reactions and plays a critical role in immune function [364]. The zinc (Zn) contents in the investigated plants ranged from 38.12 to 47.07 mg/kg. The plant *A. campestris* L. has the highest concentration, while the species *F.bruguieri* DC. has the lowest concentration. This nutritional intake is much less critical than that recommended by the DRA (5-12 mg/ day) [359].
- **Molybdenum**: Mo is also an essential oligo-element in the body. It is The key organs responsible for its storage are the liver as well as the kidneys and it found in minimal amounts in the body, Insufficiency of Mo in the body leads to dysfunction in the liver, in addition to dental problems and inflammation of a tooth [365]. The results of this analysis demonstrated that the concentrations of molybdenum (Mo) ranged from 0.29

to 0.498 mg/kg. The *A. campestris* L. plant contained the lowest concentrations of the chemical under inquiry.

- **Manganese:** Mn is one of the essential oligo-elements, non-synthetic by the body. It contributes to the process through which our body metabolizes lipids and carbohydrates. In addition to these benefits, it helps regulate blood sugar levels and fights free radicals [366]. In contrast, When present in excessive concentrations, it becomes toxic. The human body has approximately 20 mg of manganese, primarily distributed in the bones, liver, and kidneys [367]. This study's manganese concentrations range from 9.25 to 41.06 mg/kg. The lowest and highest concentrations are for plant *A. campestris* L. and plant *F.bruguieri* DC., respectively.
- **Magnesium**: Mg is an essential mineral for many human body functions. However, it is best known for preserving nervous and muscular balance. Mg is a cofactor in more than three hundred enzyme activities necessary for physiological function [368]. According to our research findings, magnesium levels vary from 933.8 to 990.7 mg/kg. The magnesium concentrations of both *A.campestris* L. and *F.bruguieri* DC. are nearly the same, and both are excellent sources of magnesium.
- **Sodium**: The crust of the Earth contains a total of 2.83% sodium in its many forms, which makes sodium the sixth most prevalent element on the planet [369]. Seawater, salt lakes, alkali lakes, and mineral spring waters all contain trace amounts of sodium salts. The levels of Na that were recorded throughout this research ranged from 299.41 to 762.55 mg/kg. The *A.campestris* L. plant has the least amount of concentration. The largest concentration of sodium is found in the *F.bruguieri* DC. plant.
- **Cobalt**: Co is a necessary element for human health. It owes its crucial feature as a central binding atom to vitamin B12 (also known as cobalamin), which is required for folate and fatty acid metabolism. Vitamin B12, which contains cobalt, is a potent tool against anemia and iron deficiency in the blood. However, high levels of Co can have very adverse health effects [370, 371]. Co values ranged from 0.89 to 1.28 mg/kg in our study. *A. campestris* L. has the most cobalt and *F.bruguieri* DC. The least concentration (Table.V.7). The recommended dietary allowance for cobalt has not been established because vitamin B12 is the only form in which it can be found. Adults in the United Kingdom were recommended to consume 2.4 µg per day of vitamin B12, pregnant women were recommended to consume 2.6 µg per day, and lactating women were recommended to have 2.8 µg per day [359].

According to the results of the experiments, the following is the order in which the other mineral concentrations were found in *A. campestris* L.: Cu (70.22 mg/Kg) > Li (46.6 mg/Kg)> Sr (23.93 mg/Kg)> Ti (20.38 mg/Kg)> Ni (2.75 mg/Kg) and for *F.bruguieri* DC. The concentration of the other mineral decreased in the order: Sr (168.55 mg/Kg)> Cu (37.81 mg/Kg) > Li (25.5 mg/Kg)> Ti (6.26 mg/Kg)> Ni (1.690 mg/Kg).

A.campestris L. and *F.bruguieri* DC. showed Cu content (70.22; 37.81 mg/Kg, respectively) outside the WHO permissible limits. The maximum allowable level of copper in fruit samples, according to FAO/WHO, is 4.5 mg/kg [372]. Although the plant is typically taken in lower quantities, there is still a potential for overexposure to Cu. Therefore it is important for people that consume *A. campestris* L. or *F.bruguieri* DC. to take that into consideration.

V.2.5. Potentially toxic elements and tolerable daily intake (TDI)

Considerable research has focused on toxicological concerns and the adverse effect that trace metals have on human health. Adopting maximum acceptable limits for their daily consumption can have negative health effects. Among these toxic chemicals harmful to human health are lead (Pb) , (Br) brom, and cadmium (Cd). Table.V.7 shows that these potentially toxic elements (Pb, Br and Cd) are present at trace levels, as are the remaining non-essential chemical elements [358, 359].

- To validate the outcomes of our attempts, we analyzed the following reference

standards : NIST-SRM 1570a, WEPAL-IPE 172, and WEPAL-IPE 189.

For this type of analysis, the judicious choice of these standards and conditions of irradiation, decay, and measurement would unquestionably increase the quality of the results. U-score and Z-score are two statistical parameters commonly used in this context, along with comparisons to certified, calculated findings from these standards.

The U-score evaluation considers both the measurement uncertainties and the uncertainty of the value that was found. Nevertheless, in the case of the Z-score, the measurement's uncertainty is not considered in the performance evaluation. In contrast, the approach's performance is significantly improved when the U-score is used.

In these contexts, we observe that the calculated values match the verified values for the majority of the analyzed elements. According to the statistical evaluation parameters, the results obtained can be interpreted as satisfactory from an analysis perspective. The values of the Z-

score and U-score statistical parameters reveal that CRM validation is the most recommended approach in ISO17025:2005. Most elements have close calculated and certified values.

V.3. Quantitative analysis of phenolic compounds by High-Performance Liquid Chromatography-Ultraviolet (HPLC-UV)

The chemical profiles of *A. campestris* L. and *F.bruguieri* DC. extracts are examined by HPLC-UV/Vis analysis shown from Figure.V.3 to Figures.V.12, respectively. Table.V.8 and Table.V.9 illustrate the quantitative concentrations of different phenolic compounds present in *A. campestris* L. and *F.bruguieri* DC., respectively. Identified compounds in *A. campestris* L. extracts were mainly flavonoids and phenolic acids: Gallic Acid, Chlorogenic Acid, and Vanillic Acid. Caffeic Acid, Vanillin, p-Coumaric Acid, Rutin, Naringin, and Quercetin, respectively. While for *F.bruguieri* DC. The predominantly phenolic compounds present were: Chlorogenic Acid, Vanillin and Caffeic Acid.

***Artemisia .campestris* L.**

Table.V.8. Identification and quantification of phenolic compounds in *A. campestris* L. extracts by HPLC analysis.

Compounds	AcME	AcPEE	AcDE	AcEAE	AcBE	AcAE
	µg/g[a]	µg/g[a]	µg/g [a]	µg/g [a]	µg/g [a]	µg/g [a]
Gallic Acid	1334.055	Nd	278.442	802.691	3154.304	1334.055
Chlorogenic Acid	Nd	117.585	39204.708	235792.291	13226.078	16142.949
Vanilic Acid	545.453	Nd	2344.484	21345.290	1626.680	545.453
Caffiec Acid	899.929	Nd	11863.529	57950.598	782.706	647.164
Vanilin	1033.090	20.151	53735.058	29910.325	29.976	92.957
p-Coumaric Acid	772.620	22.229	40586.604	23532.508	5514.425	772.620
Rutin	9563.946	167.193	45582.77	722742.147	2681.402	1597.944
Naringin	66.334	Nd	28051.266	40755.792	11334.485	66.334
Quercetin	Nd	Nd	84370.322	Nd	Nd	38023.359

[a] **Concentration**: µg /g, **Nd**: Not Detected.

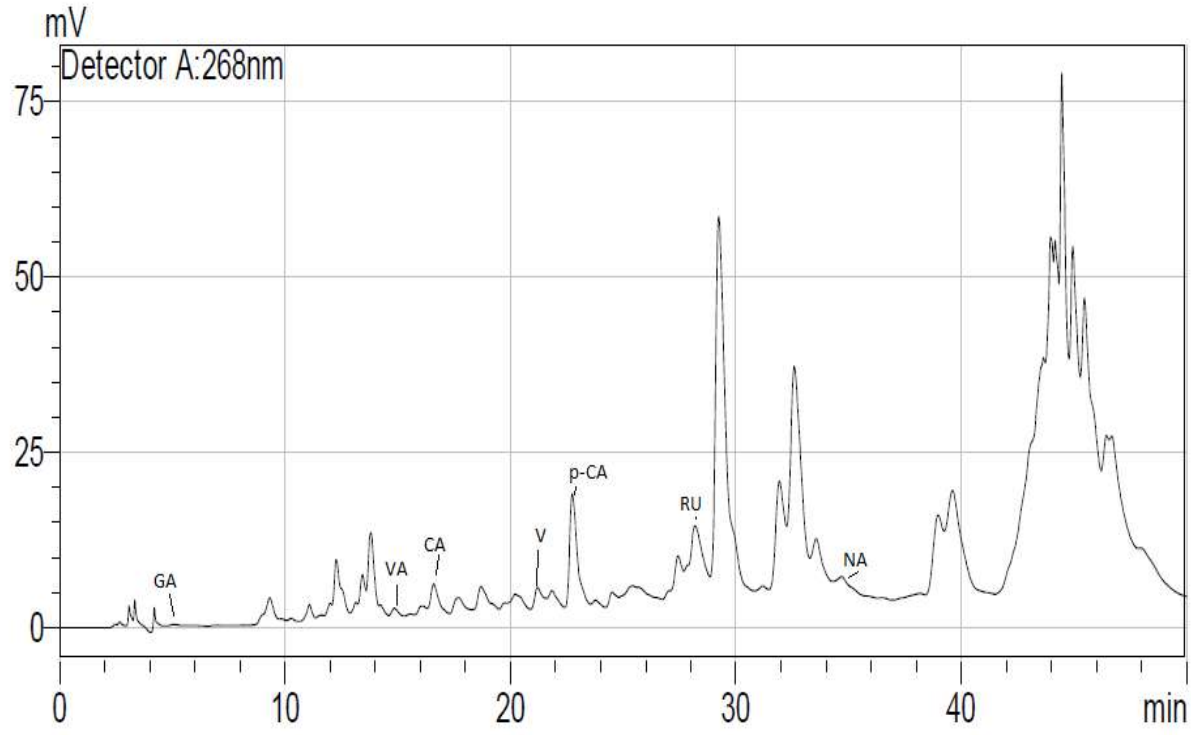


Figure.V.3. Phenolic compounds content identified by HPLC chromatogram in AcME. Detected Constituents are: GA: Gallic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid; RU: Rutin and NAR: Naringin.

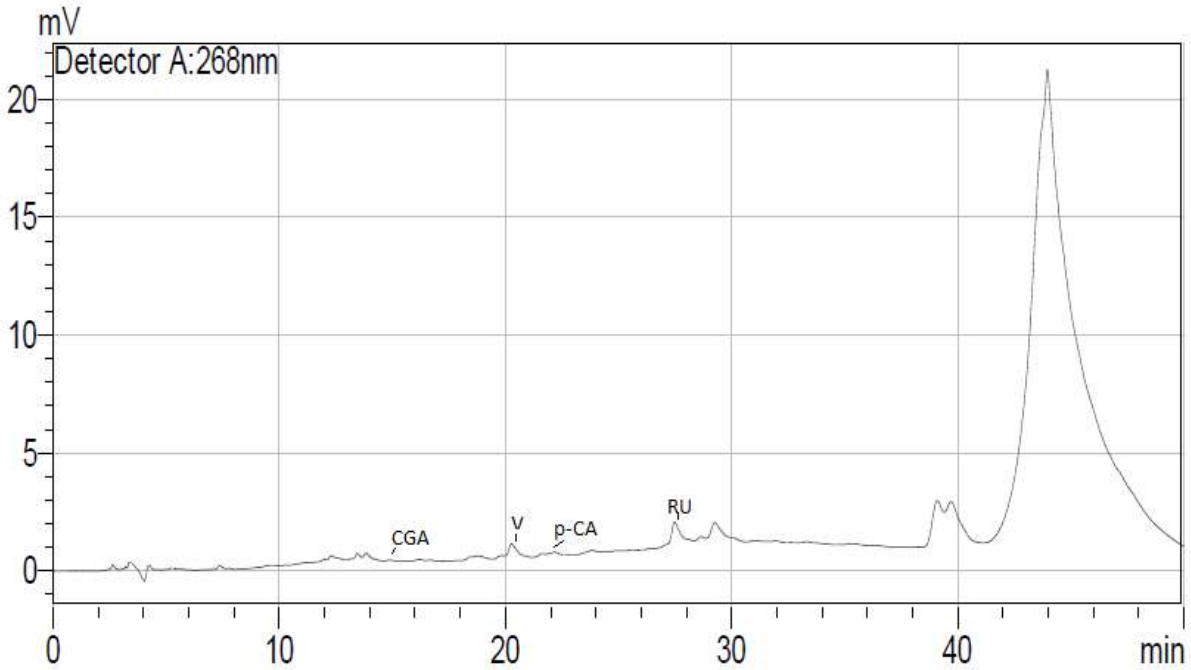


Figure.V.4. Phenolic compounds content identified HPLC chromatogram in AcPEE. Detcted compounds are: CGA: Chlorogenic Acid; V: Vanillin; p-CA: p-Coumaric Acid and RU: Rutin.

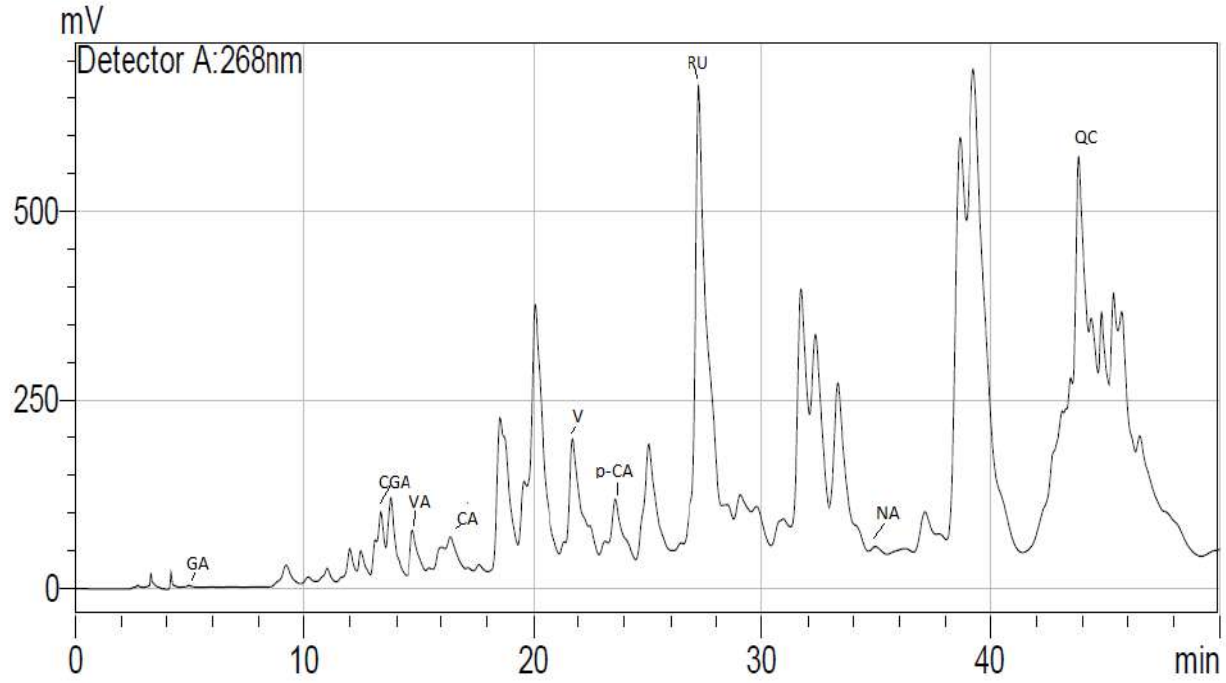


Figure.V.5. Phenolic compounds content identified by HPLC chromatogram in AcDE. Detected compounds are: GA: Gallic Acid; CGA: Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid; RU: Rutin; NAR: Naringin and QC: Quercetin.

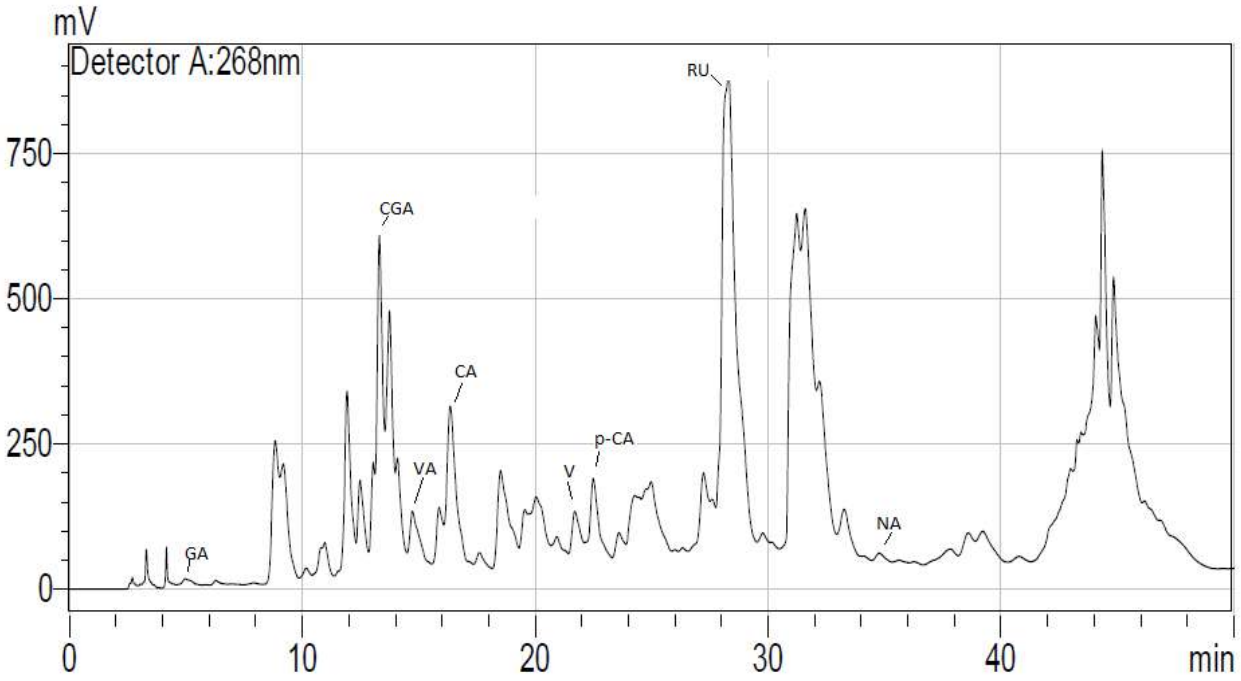


Figure.V.6. Phenolic compounds content identified by HPLC chromatogram in AcEAE. Detected compounds are: GA: Gallic Acid; CGA: Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid; RU: Rutin and NAR: Naringin.

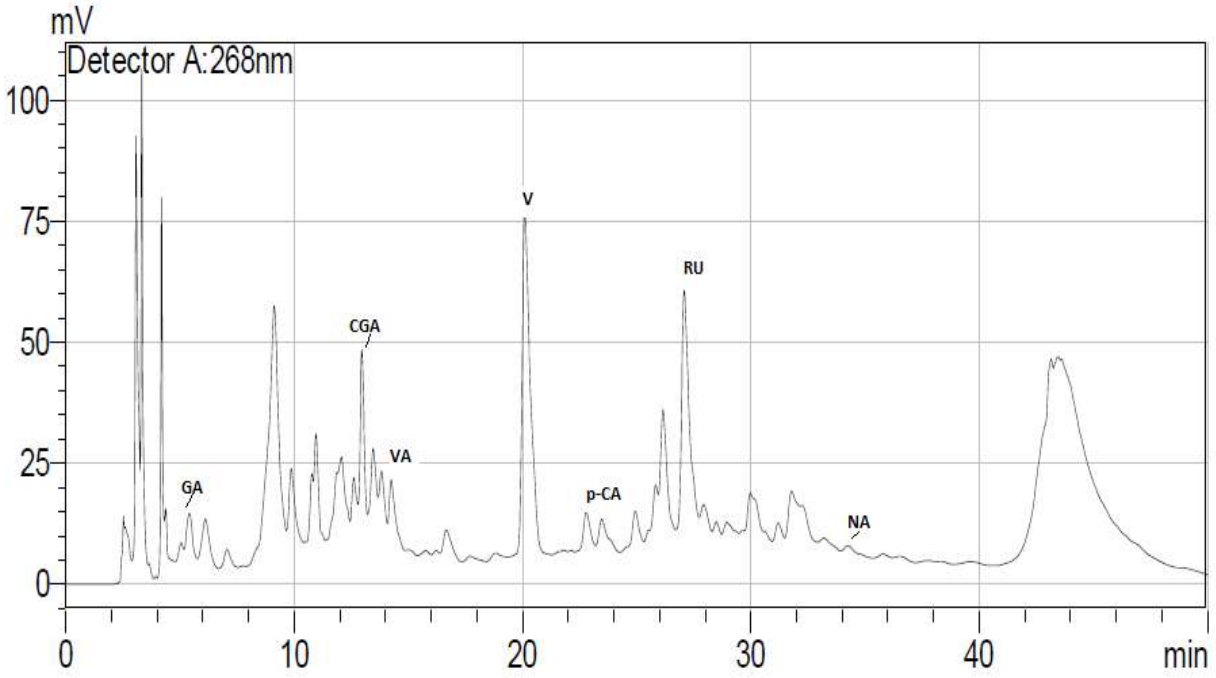


Figure.V.7. Phenolic compounds content identified by HPLC chromatogram in AcBE. Detected compounds are: GA: Gallic Acid; CGA: Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid; RU: Rutin and NAR: Naringin.

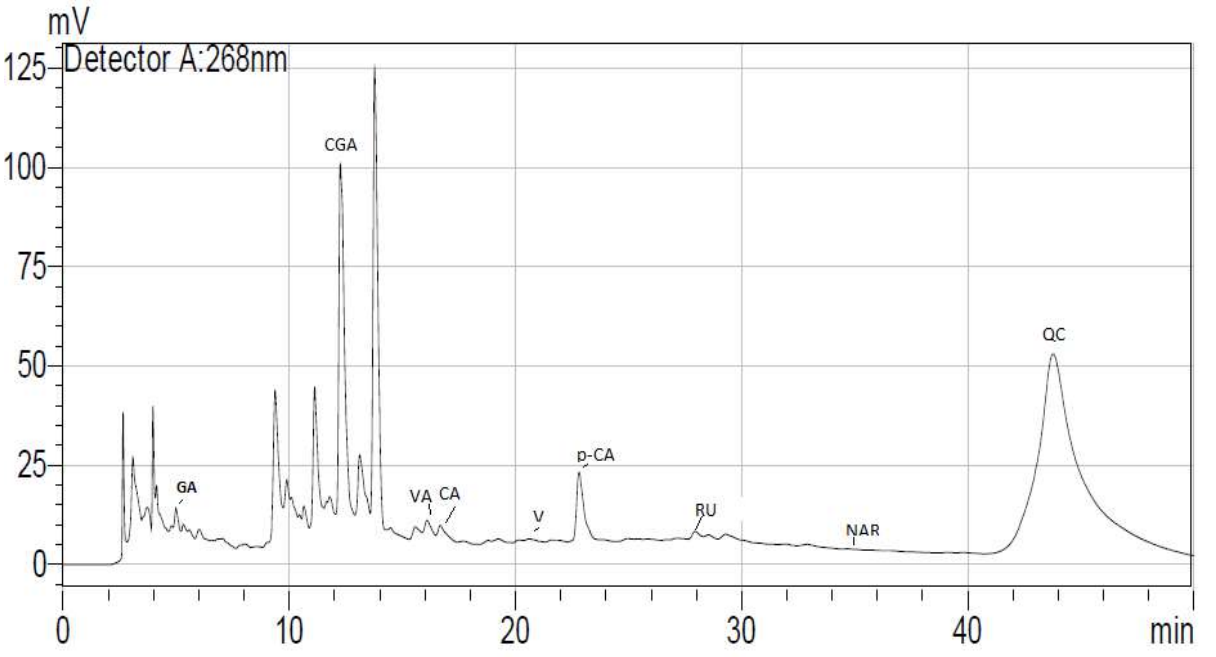


Figure.V.8. Phenolic compounds content identified by HPLC chromatogram in AcAE. Detected compounds are: GA: Gallic Acid; CGA: Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid; RU: Rutin; NAR: Naringin and QC—Quercetin.

***Fagonia bruguieri* DC.**

Table.V.9. Identification and quantification of phenolic compounds in *F.bruguieri* DC. extracts by HPLC analysis.

Compounds	FbDE	FbEAE	FbBE	FbAE
	µg/g [a]	µg/g [a]	µg/g [a]	µg/g [a]
Gallic Acid	36.319	6069.119	443.929	Nd
Chlorogenic Acid	39204.708	16744.195	11546.326	506.254
Vanilic Acid	314.296	2120.357	3843.992	Nd
Caffiec Acid	2364.374	4427.20	12138.486	941.260
Vanilin	2884.439	4793.101	13234.030	880.688
p-Coumaric Acid	Nd	4469.027	88.332	Nd
Rutin	7486.977	19288.402	Nd	3016.557
Naringin	8906.54	10997.703	24190.309	Nd
Quercetin	Nd	2533.959	38023.359	Nd

[a] **Concentration**: µg /g, **Nd**: Not Detected.

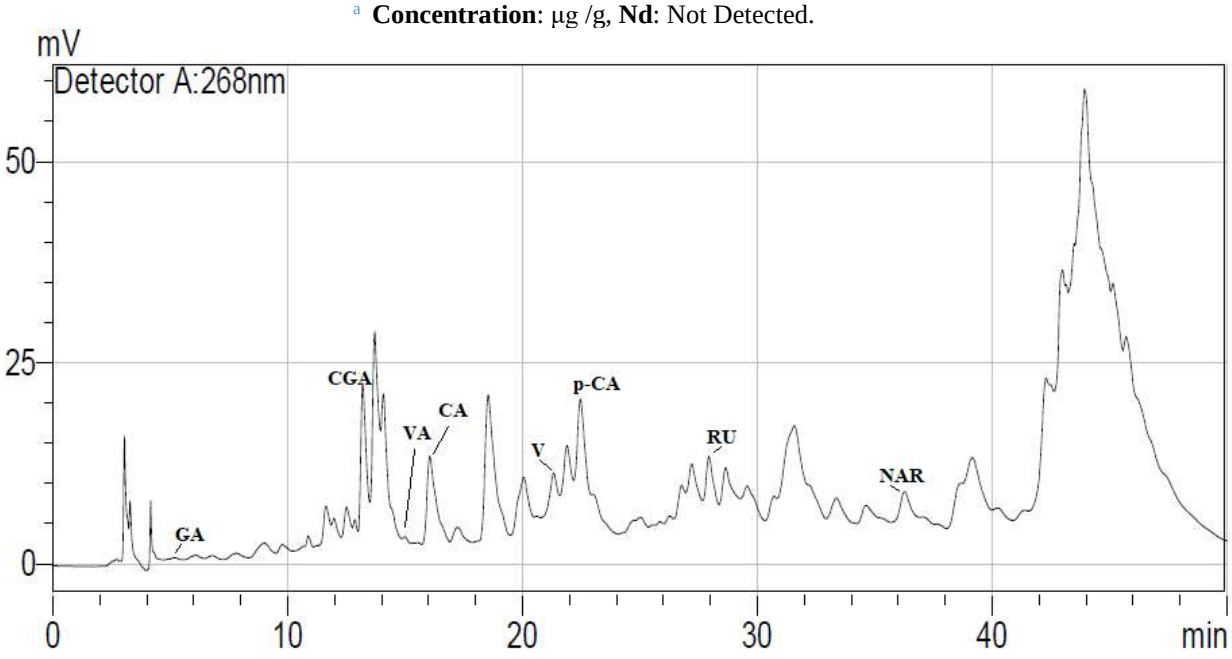


Figure.V.9. Phenolic compounds content identified by HPLC chromatogram in FbDE. Detected compounds are: GA: Gallic Acid; CGA : Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid. and NAR: Naringin.

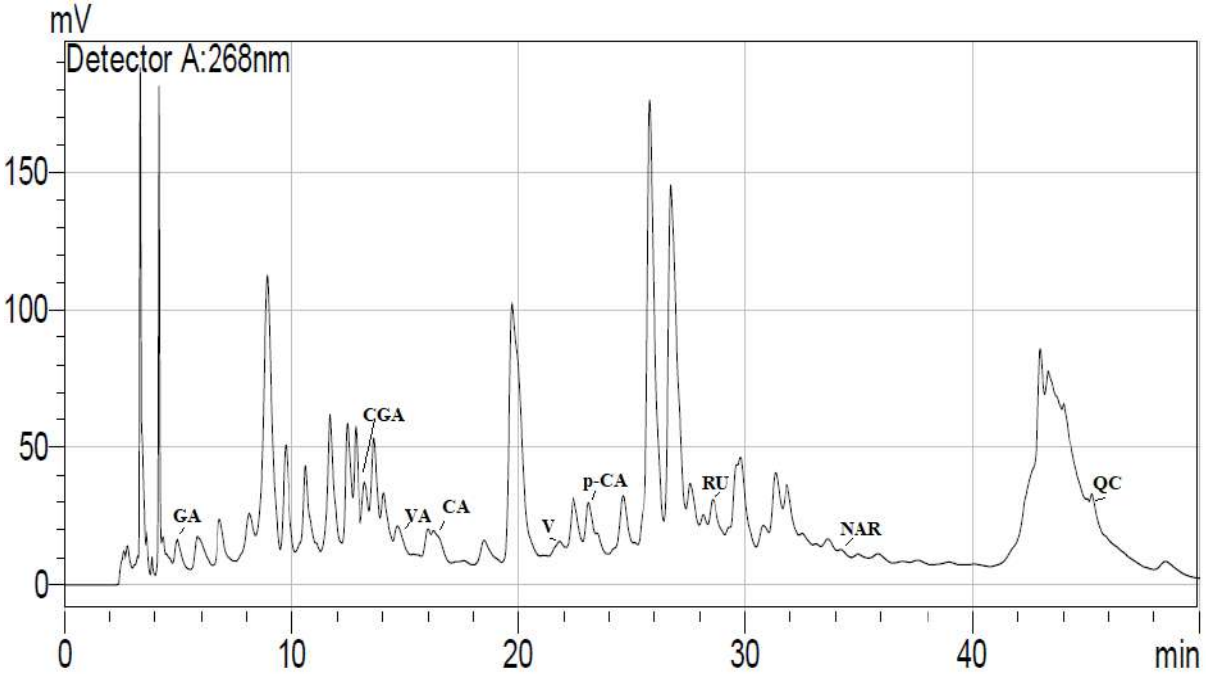


Figure.V.10. Phenolic compounds content identified by HPLC chromatogram in FbEAE. Detected compounds are: GA: Gallic acid; CGA : Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid; NAR: Naringin and QC: Quercetin.

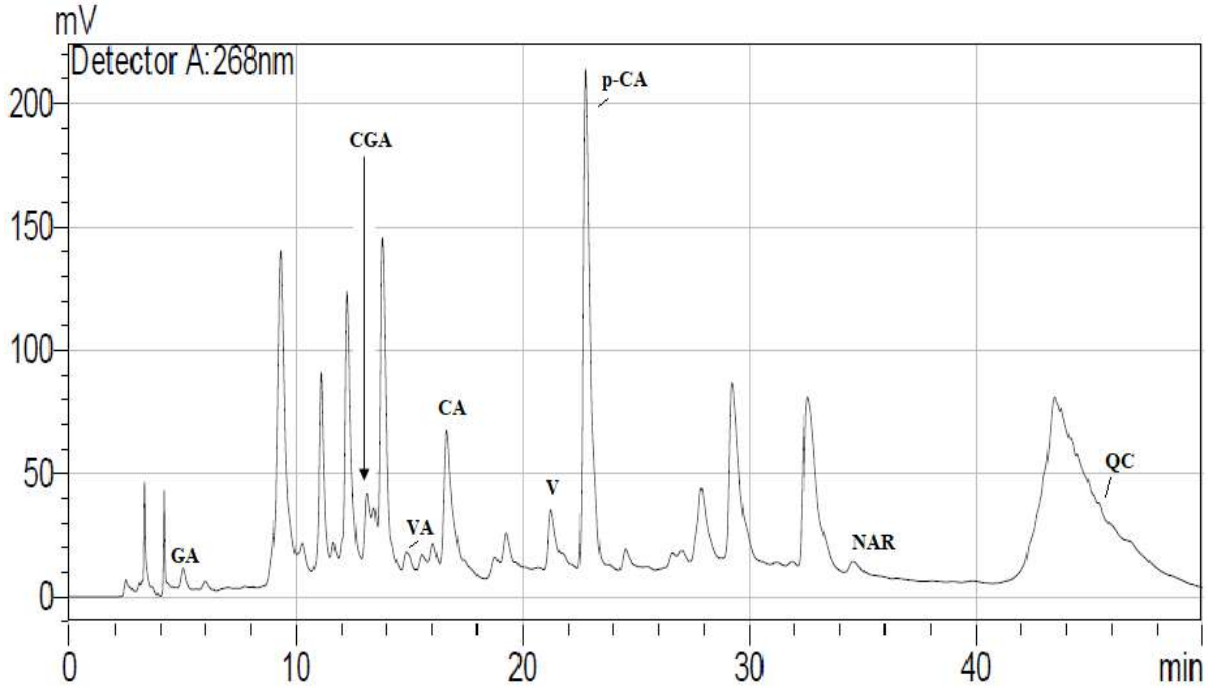


Figure.V.11. Phenolic compounds content identified by HPLC chromatogram in FbBE. Detected compounds are: GA: Gallic Acid; CGA : Chlorogenic Acid; VA: Vanillic Acid; CA: Caffeic Acid; V: Vanillin; p-CA: p-Coumaric Acid and NAR: Naringin.

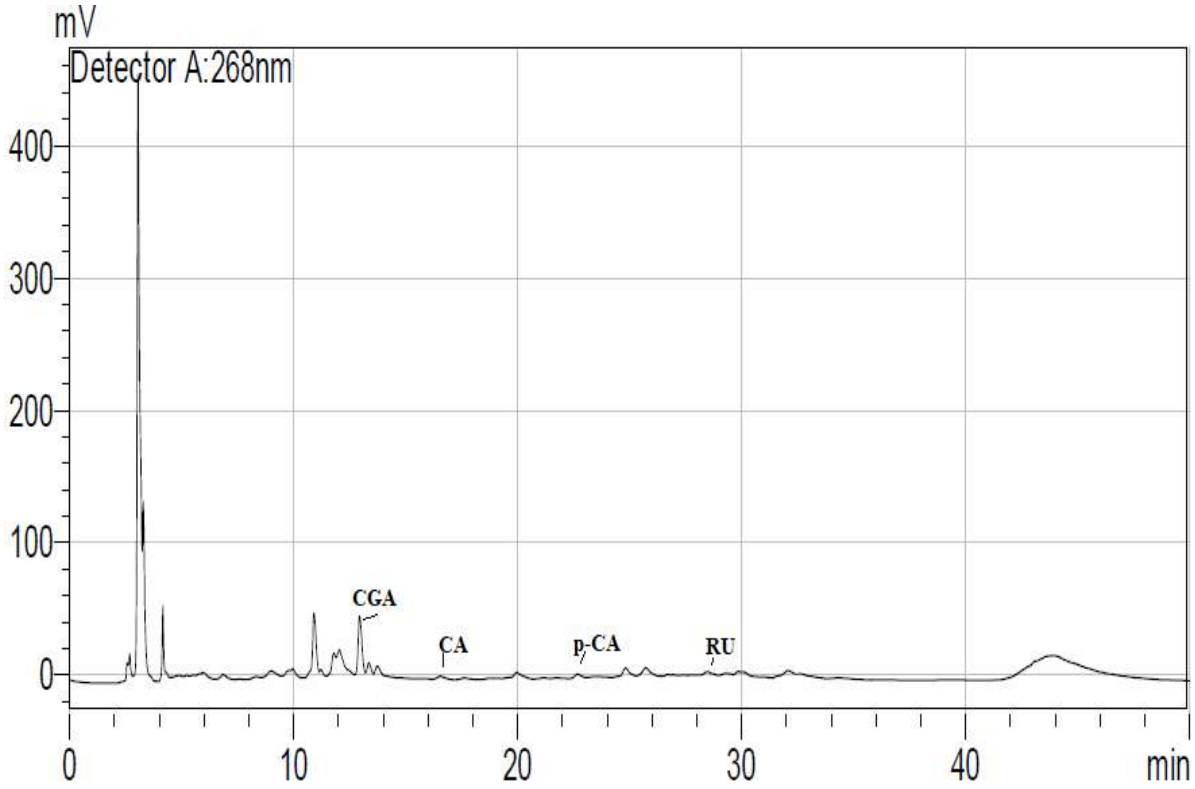


Figure.V.12. Phenolic compounds content identified HPLC chromatogram in FbAE. Detected compounds are: CGA : Chlorogenic Acid; CA: Caffeic Acid; p-CA: p-Coumaric Acid and RU: Rutin.

In order to characterize the chemical components that medicinal plants contain, emergence analysis techniques were specifically designed to perform chemical analysis.

According to the HPLC- UV/Vis profile of *A. campestris* L. extracts, the most abundant compounds in terms of flavonoids and phenolic acids were AcEAE and AcDE (Table.V.8). In contrast to the above, the AcEAE contained a high amount of Chlorogenic Acid, Vanillic Acid, Caffeic Acid, Rutin, and Naringin (235792.291, 21345.290, 57950.598, 722742.147, 40755.792 µg/g, respectively). In terms of flavonoids, AcDE exhibited an interesting amount of Vanillin, p-Coumaric Acid, and Quercetin (53735.058, 40586.604, and 84370.322 µg/g, respectively) were the flavonoids identified in the AcDE in the greatest abundance.

Interestingly. Gallic Acid was prominent in the AcBE (3154.304 µg/g). On the other hand, the flavonoids and phenolic acids identified in the AcBE, AcME, and AcAE were present in a concentration that was considered to be medium. The AcPEE was poor in flavonoids and phenolic acid constituents, and it contained only a minimum of Chlorogenic Acid, Vanillin, p-Coumaric Acid, and Rutin (117.585; 20.151; 22.229 and 167.193 µg/g, respectively).

Based on the HPLC-UV/Vis profiling of *F.bruguieri* DC. extracts, the compounds with the highest concentration of flavonoids and phenolic acids are classified as FbBE and FbEAE, respectively (Table.V.9). Compared to those above, the FbBE has a significant amount of Vanillic Acid, Caffeic Acid, Vanillin, Naringin, and Quercetin (3843.992, 12138.486, 13234.030, 24190.309, 38023.359 µg/g, respectively). Gallic Acid (6069.119 µg/g) and p-Coumaric Acid (4469.027 µg/g) were abundant in FbEAE with regards to phenolic acid concentration, where the phenolic acid identified in the FbEAE in the greatest abundance. While the flavonoids Rutin identified in the FbEAE (19288.402 µg/g) in the greatest abundance. Chlorogenic Acid (39204.708 µg/g) was remarkably abundant in the FbDE. On the contrary, the concentration of flavonoids and phenolic acids detected in FbDE was regarded as moderate. Minimal phenolic acids and flavonoids were found in the FbAE; Chlorogenic Acid, Caffiec Acid, Vanillin, and Rutin (506.254, 941.260, 880.688, 3016.557 µg/g, respectively).

V.4. Quanlitative analysis of phenolic compounds by high-performance liquid chromatography coupled to tandem mass spectrophotometry (LC-MS/MS)

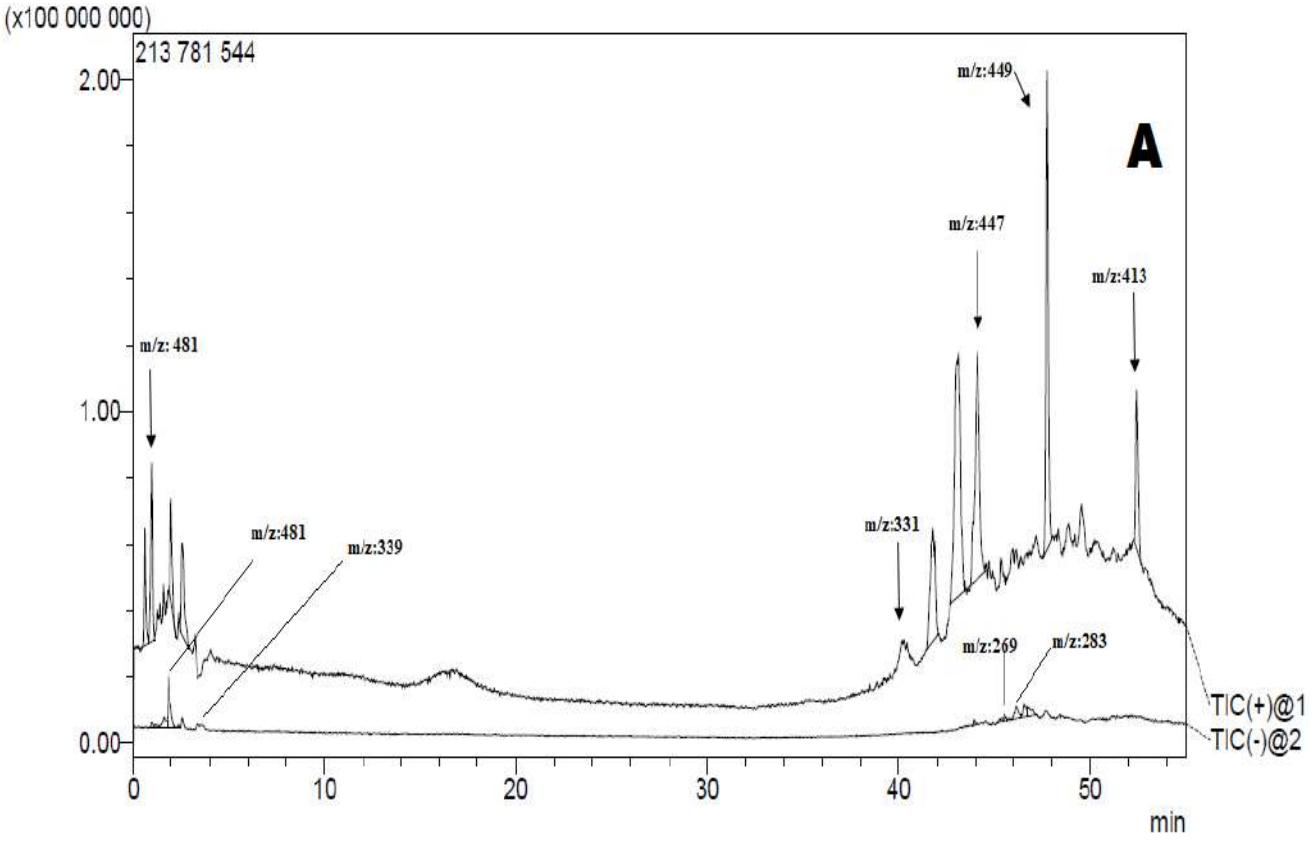
Following optimization of the UPLC-ESI-MS-MS conditions, analysis of AcDE; AcEAE; AcBE; FbDE; FbEAE and FbBE were performed in full scan with negative and positive ions mode. The UPLC-ESI-MS-MS and mass spectra of the compounds identified from the various extracts of *A. campestris* L. and *F. bruguieri* DC. are shown in Figure.V.13 and Figure.V.15, respectively. Table.V.10 and Table.V.10 provides the retention times (Rt), mass to charge ratios (m/z), and chemical formulas of substances that have been proposed based on data that has been previously identified in the genus *Artemisia* and *Fagonia*, respectively.

***Artemisia campestris* L.**

Table.V.10. Phenolic profile determined by LC-MS-MS in fractions from *A.campetris* compared with literature (retention time (Rt), not identifie (NI)).

Extract	t_R (min)	Ionisation mode (m/z)	m/z	Tentatively identified compound	Molecular formula	Ref
AcDE	0.645					
AcEAE	0.627	$[M+H]^+$	172	NI	NI	-
AcBE	0.646					
AcDE	0.983	$[M+H]^+$	481	15-O-β-D-glucopyranosyl-11β,13-dihydro urospermal A	$C_{21}H_{30}O_{10}$	[373]
AcEAE	0.986					
AcBE	0.987	$[M+H]^+$	437	NI	NI	-
AcEAE	1.629	$[M+H]^+$	365	NI	NI	-
AcDE	1.998					
AcEAE	1.764	$[M+H]^-$	353	5-O-caffeoylquinic acid	$C_{16}H_{18}O_9$	[374]
AcBE	1.995					
AcDE	2.564					
AcEAE	2.144	$[M+H]^+$	381	NI	NI	-
AcBE	2.568					
AcDE	41.787					
AcEAE	41.831	$[M+H]^+$	331	Jaceosidin	$C_{17}H_{14}O_7$	[375]
AcBE	41.805					
AcDE	43.128					
AcEAE	43.130	$[M+H]^+$	367	NI	NI	-
AcBE	43.083					
AcDE	44.107					
AcEAE	44.133	$[M+H]^+$	447	Pelargonidin-3-O-glucuronide	$C_{21}H_{19}O_{11}$	[376]
AcBE	44.115					
AcDE	47.738	$[M+H]^+$	449	Cyanidin-3-O-glucoside	$C_{21}H_{20}O_{11}$	[376]
AcEAE	47.771					

AcDE	52.406					
AcEAE	52.432	$[M+H]^+$	413	Arteminorin B	$C_{21}H_{16}O_9$	[377]
AcBE	52.427					
AcBE	47.747	$[M+H]^+$	493	Malvidin 3-O-glucoside	$C_{23}H_{25}ClO_{12}$	[376]
AcDE	0.978					
AcEAE	0.974	$[M-H]^-$	239	NI	NI	-
AcBE	0.970					
AcDE	1.600	$[M-H]^-$	369	NI	NI	-
AcBE	1.597					
AcDE	1.854					
AcEAE	1.646	$[M-H]^-$	339	Esculetin-6-O-glucoside	$C_{15}H_{16}O_9$	[378]
AcBE	1.871					
AcDE	2.387					
AcEAE	2.134	$[M-H]^-$	403	NI	NI	-
AcBE	2.565					
AcEAE	0.218	$[M-H]^-$	453	3-hydroxyphloretin 6'-O-hexoside	$C_{21}H_{24}O_{11}$	[375]
AcDE	45.530	$[M-H]^-$	269	Apigenin	$C_{15}H_{10}O_5$	[379]
AcBE	45.522					
AcDE	46.137					
AcEAE	46.135	$[M-H]^-$	283	Acacetin	$C_{16}H_{12}O_5$	[380]
AcBE	46.112					
AcEAE	3.552	$[M-H]^-$	198	NI	NI	-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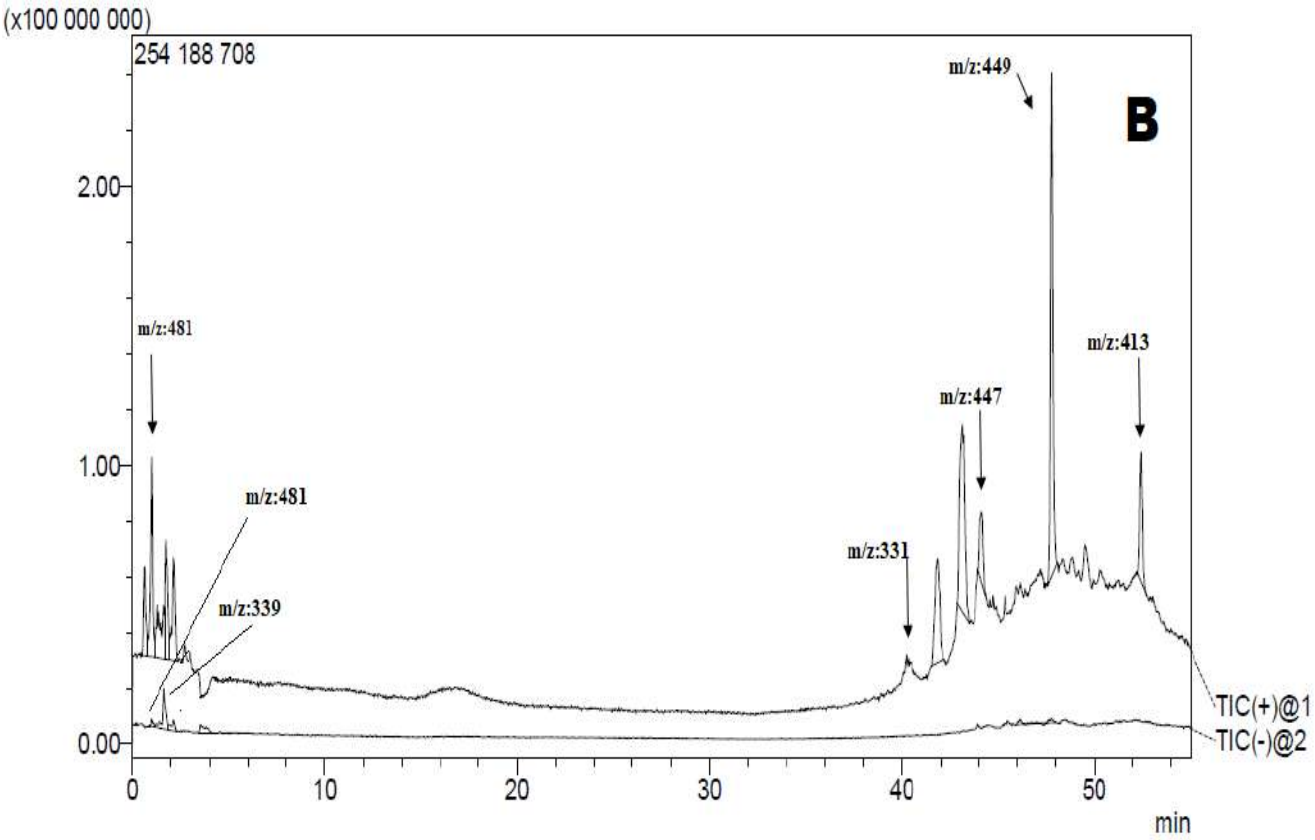
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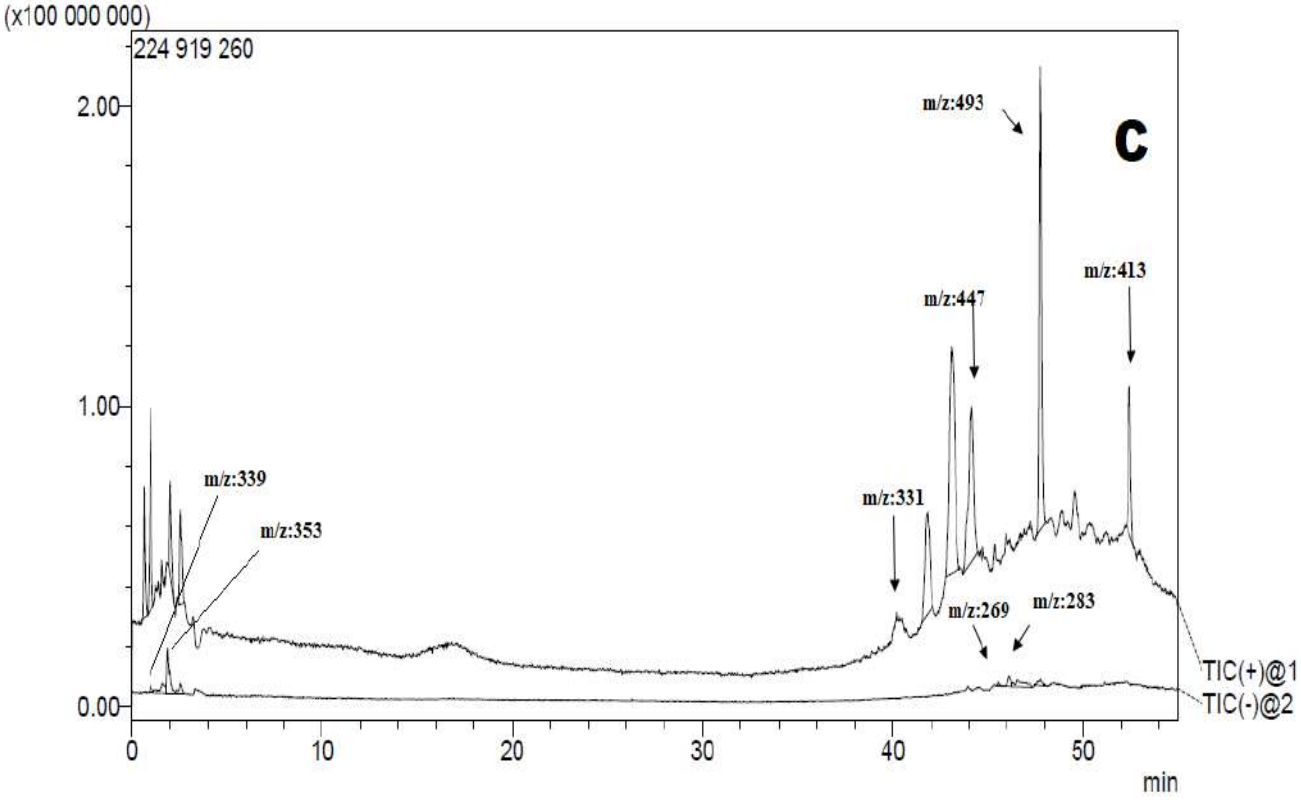
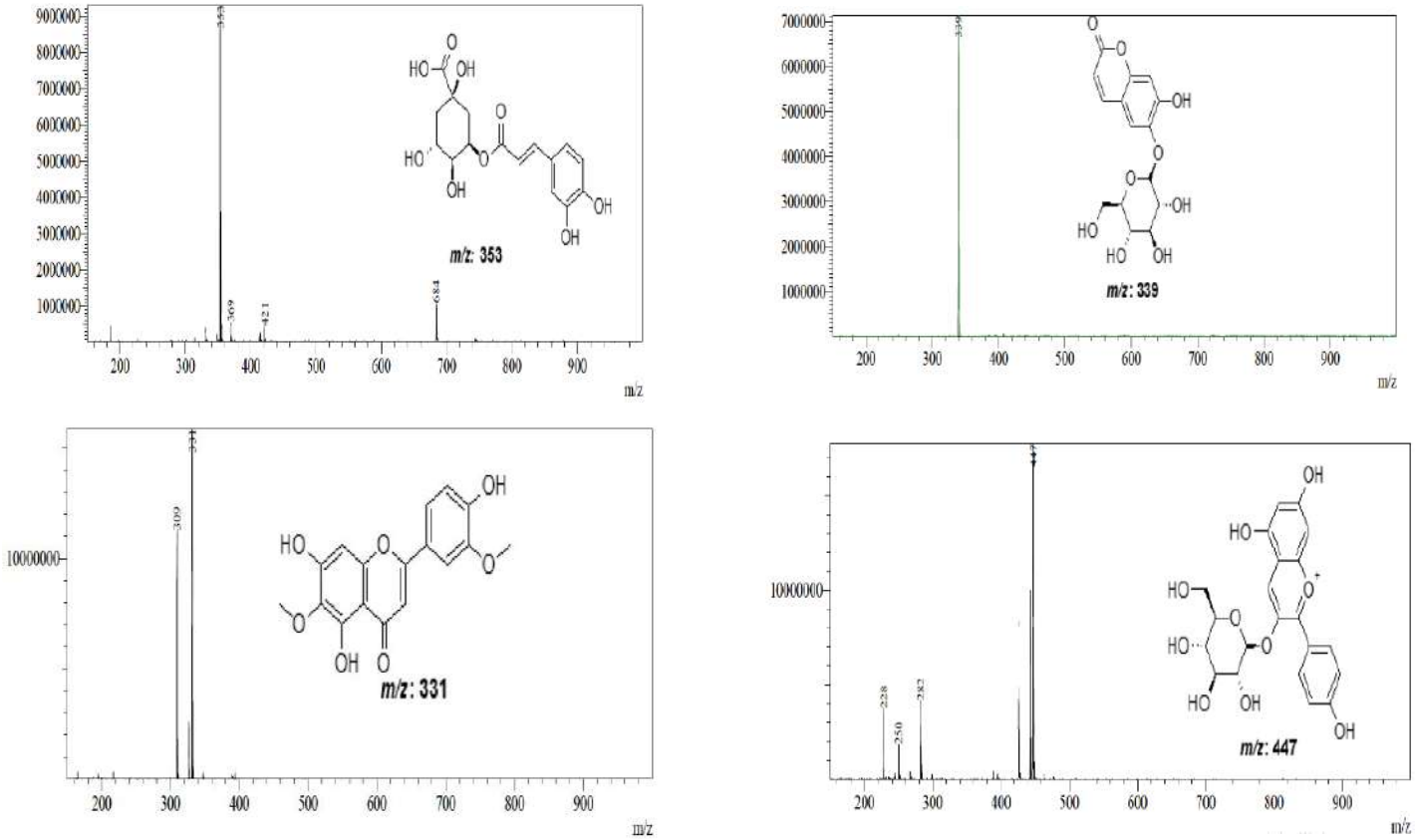


Figure.V.13. Total ion current (TIC) profile of AcDE (A) AcEAE (B) and AcBE (C).



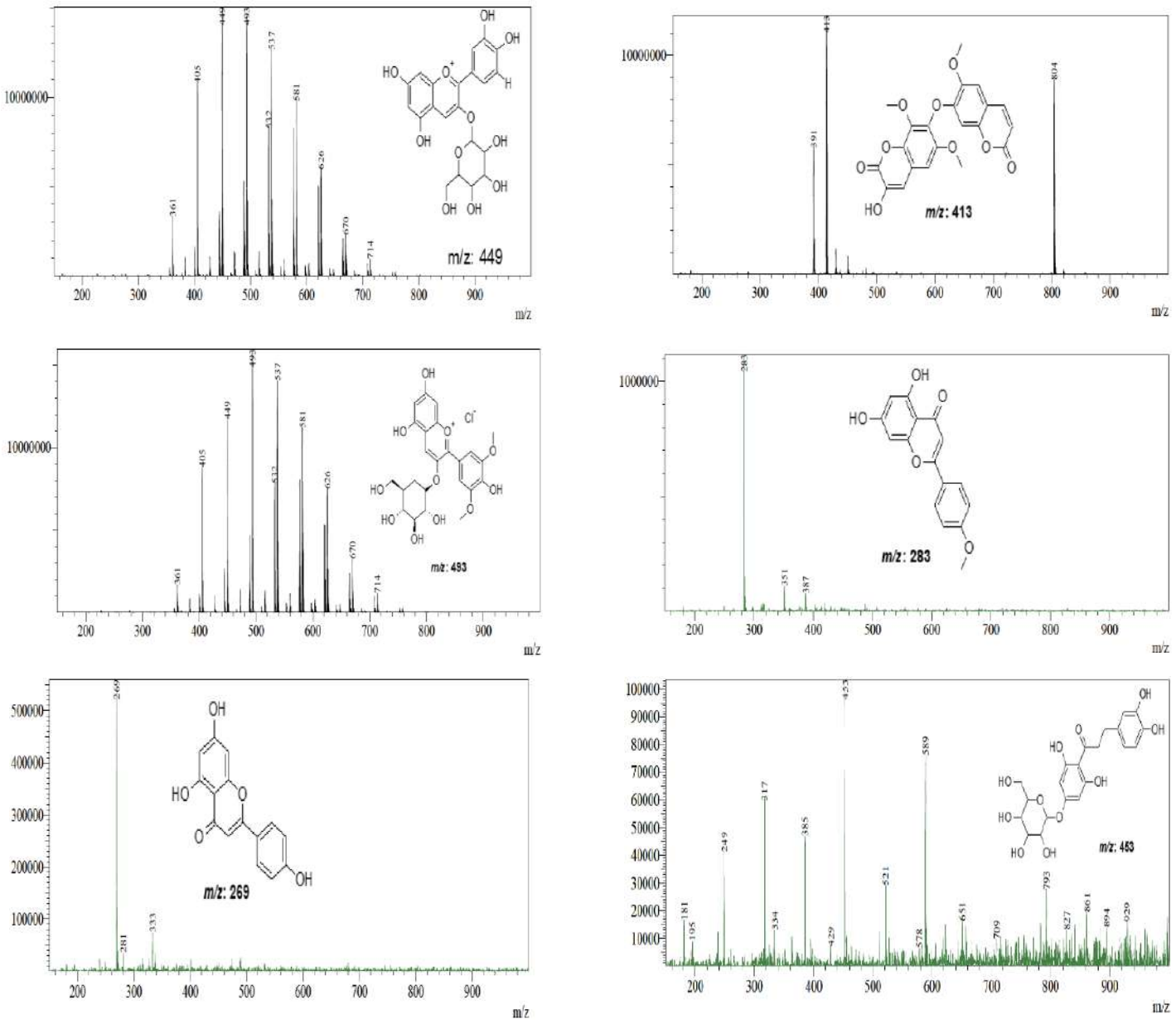


Figure.V.14. Mass spectra of detected compounds from *A. campestris* L. extracts.

The outcomes of LC-MS-MS analysis based phytochemical investigation on the aerial parts of AcDE, AcEAE, and AcBE (Table.V.10) conducted to the tentative identification of 11 compounds Figure.V.14. Flavonoids make up the vast majority of these molecules, which can be found in the various extracts of *A.campestris* L. Flavonoids were present as flavones, including Apigenin [379], Acacetin [380], and Jaceosidin (trihydroxy-3, 6-dimethoxyflavone) [375]. Recently, Carazzone *et al.*, reported Cyanidin 3-O-galactoside, Pelargonidin-3-O-glucuronideand Malvidin 3-O-glucoside in Cichorium intybus, a species from the family Asteraceae[376], which were determined based on their m/z values (Table.V.10). These three anthocyanidins have *m/z* values of 449 (AcDE, AcBE), 447 (AcDE, AcEAE, AcBE) and 493

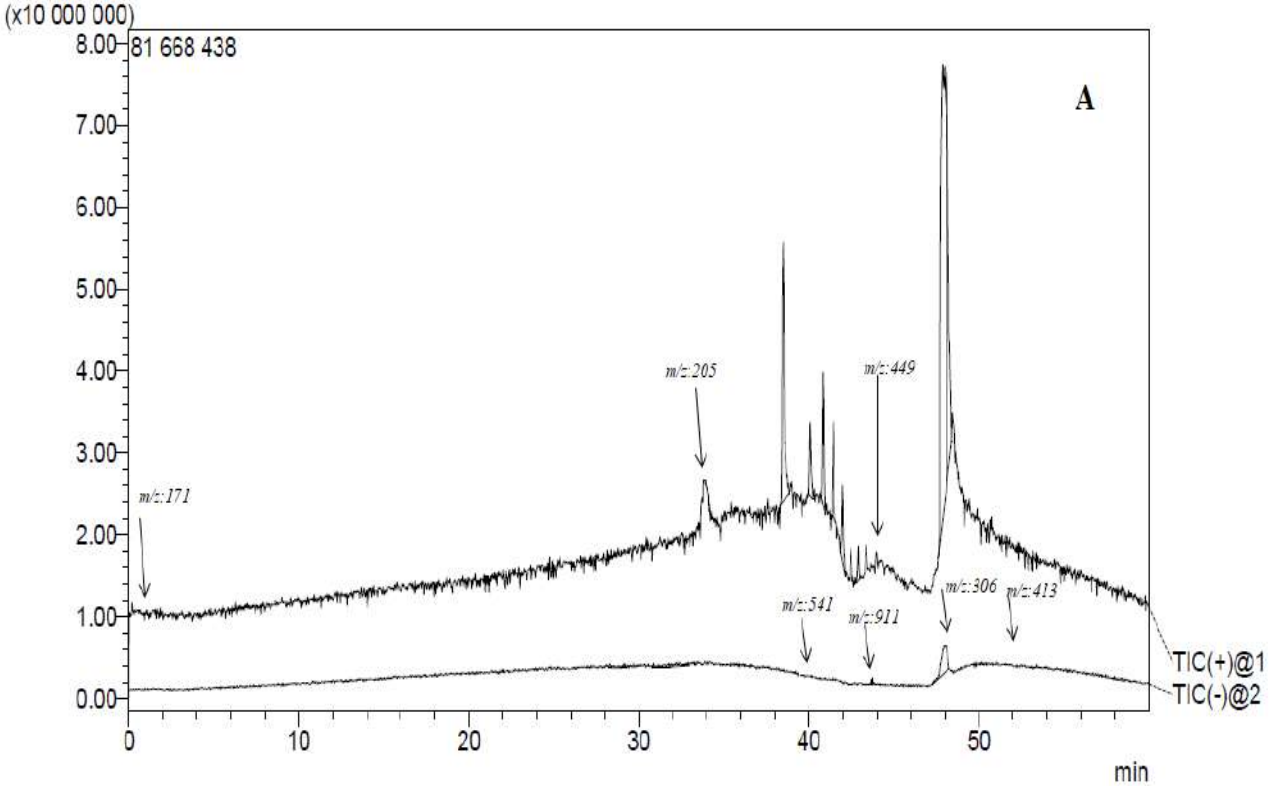
(AcBE), respectively. Full scan and MS spectra of AcDE, AcEAE, and AcBE confirmed the presence of derivative coumarins in all three fractions: 3-hydroxy- 6'-dimethoxy-7-(6'-methoxy-7'-coumarinyloxy) coumarin (Arteminorin B); produced protonated molecular ions at 413 *m/z*, This compound was found in Artemisia minor and its identity was verified to be bicoumarin by He *et al.*, [377]. In addition to a $[M-H]^-$ ion at 339 *m/z*, which was assigned as Esculetin-6-O-glucoside [378]. Moreover, AcDE, AcEAE, and AcBE chromatograms revealed consistency at m/z 353 in the negative ion mode, which led to the tentative identification of quinic acid derivative as 5-O-caffeoylquinic acid [374]. The dihydrochalcone derivative that was identified in AcEAE gave a positive ionisation at m/z 453; therefore, 3-hydroxyphloretin 6'-O-hexoside was proposed to be the identity of the compound [375].

Fagonia bruguieri DC.

Table.V.11. Phenolic profile determined by LC-MS-MS in fractions from *F. bruguieri* DC. compared with literature (retention time (Rt), not identifie (NI)).

Extract	t_R (min)	Ionisation mode (m/z)	m/z	Tentatively identified compound	Molecular formula	Ref
FbBE	1.019	$[M+H]^+$	351	Andrographolide	$C_{20}H_{30}O_5$	[381]
FbDE	47.769	$[M+H]^+$	449	Kaempferol-7-O-glucoside	$C_{21}H_{20}O_{11}$	[382] [383]
FbBE	52.183	$[M+H]^+$	317	Isorhamnetin	$C_{16}H_{12}O_7$	[382] [383] [384]
FbBE	1.397	$[M+H]^+$	353	3-caffeoylquinic acid	$C_{16}H_{17}O_9$	[384]
FbDE 0.633 **FbEAE** 0.477 **FbBE** 0.549		$[M+H]^+$	171	NI	NI	-
FbBE	1.690	$[M+H]^+$	382	Atricarpan D	$C_{23}H_{26}O_5$	[385]

FbDE	28.306	$[M+H]^+$	205	NI	NI	-
FbEAE	2.176	$[M-H]^-$	393	Xanthoangelol	$C_{25}H_{28}O_4$	[386]
FbEAE	43.007	$[M-H]^-$	443	5-Methoxy- podophyllotoxin	$C_{23}H_{24}O_9$	[381]
FbDE **FbEAE** **FbBE**	52.450 51.076 1.943	$[M-H]^-$	413	Podophyllotoxin	$C_{22}H_{22}O_8$	[381]
FbEAE	51.461	$[M-H]^-$	610	Herbacetin 8-rutinoside	$C_{27}H_{30}O_{16}$	[382] [383]
FbEAE	46.504	$[M-H]^-$	577	Isovitexin 2″- *O*-rhamnoside	$C_{27}H_{30}O_{14}$	[387]
FbDE **FbEAE** **FbBE**	48.088 48.120 48.117	$[M-H]^-$	306	Epigallocatechin	$C_{15}H_{14}O_7$	[388]
FbDE **FbEAE** **FbBE**	42.901 42.866 42.869	$[M-H]^-$	911	Oleanolic acid-3-O-β-D-glucopyranosyl-(1→3)-[(β-D-glucopyranosyl- (1→2)]-α-L-arabinopyranoside	$C_{47}H_{76}O_{17}$	[389] [232]
FbDE **FbEAE**	40.064 40.048	$[M-H]^-$	541	Feruloyl N-tryptophan hexoside	-	[390] [391]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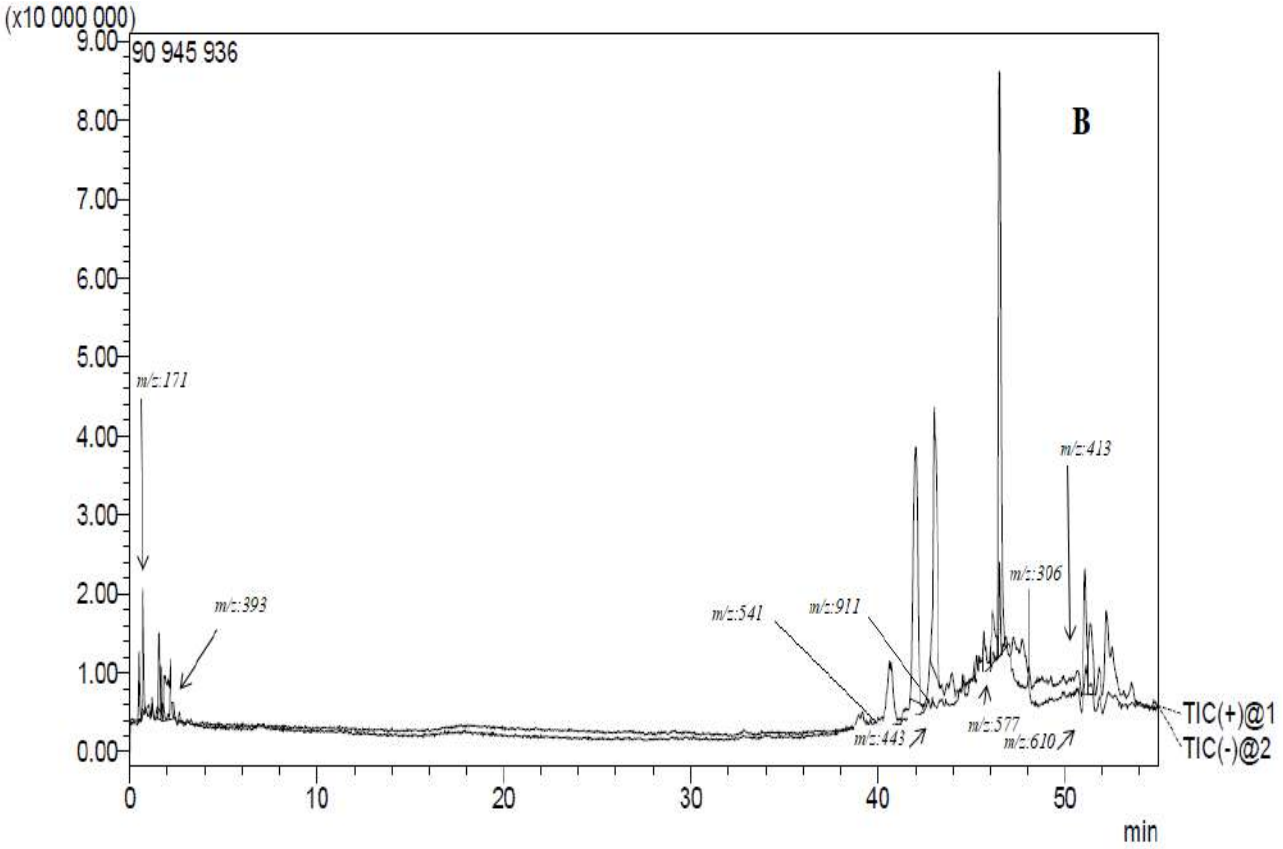
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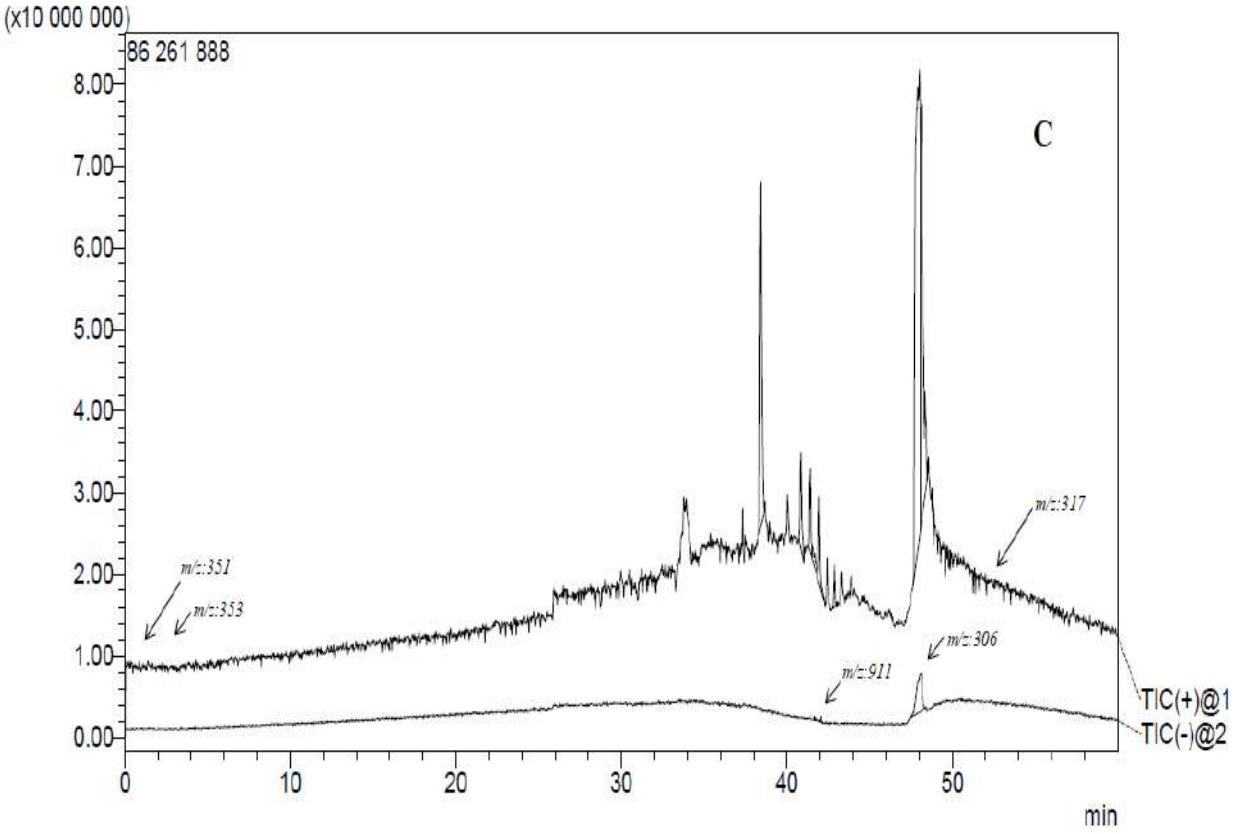
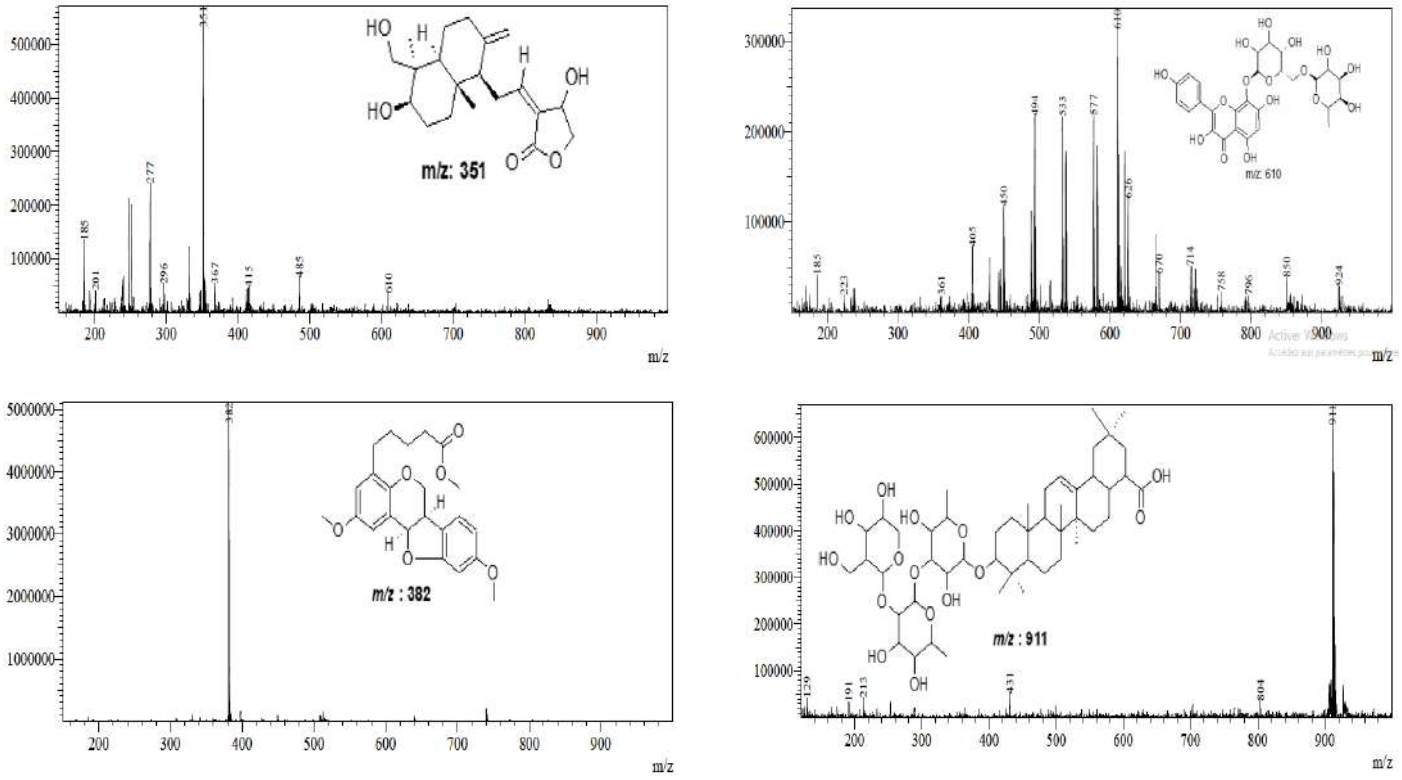


Figure.V.15. Total ion current (TIC) profile of FbDE (A) FbEAE (B) and FbBE (C).



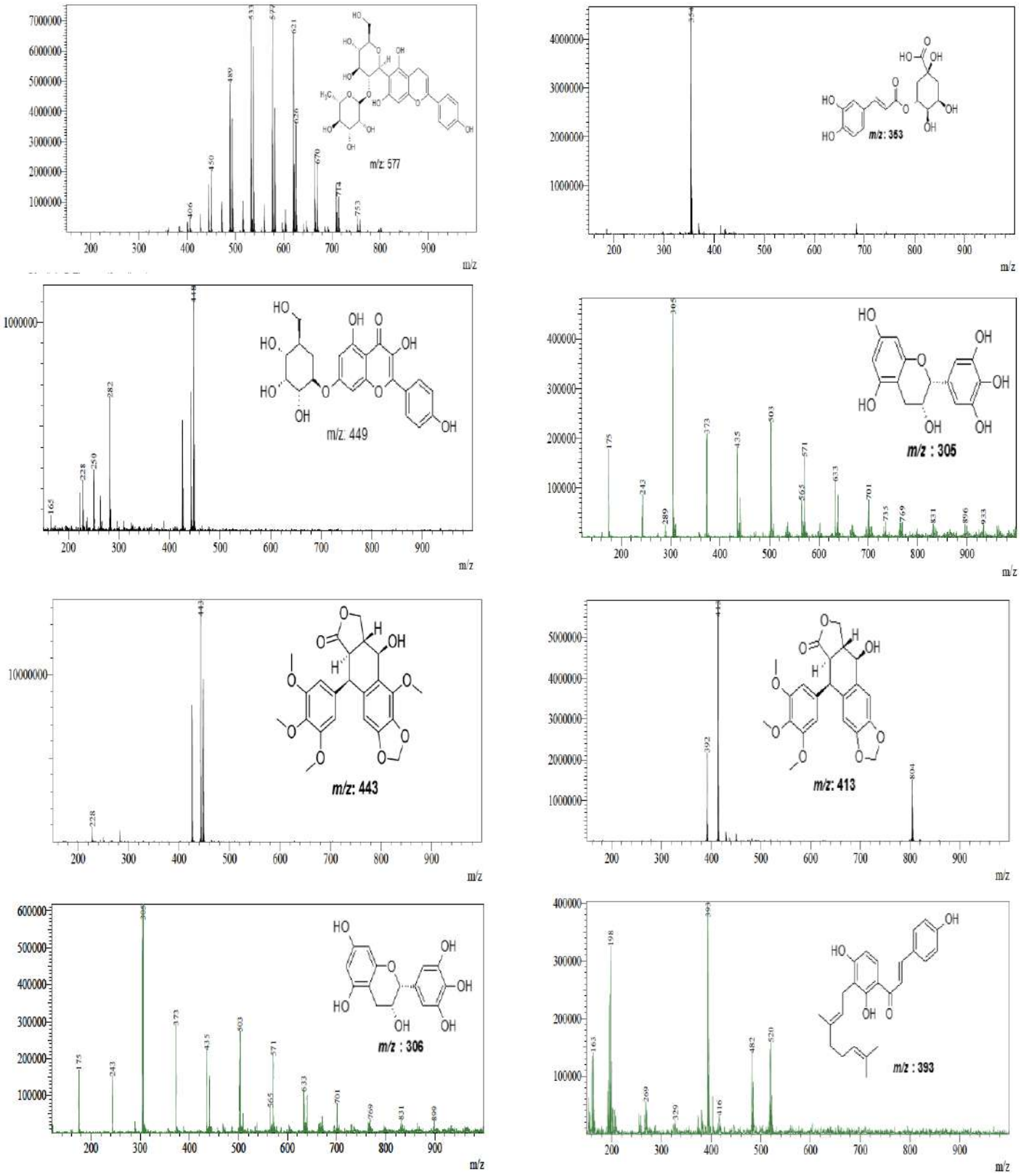


Figure.V.16. Mass spectra of detected compounds from *F. bruguieri* DC. extracts.

The findings of a phytochemical investigation based on LC-MS-MS analysis that was conducted on the aerial parts of FbDE, FbEAE, and FbBE (Table 3), led to the tentative identification of 13 constituents Figure.V.16.

To our knowledge, no previous information regarding the phenolic composition of *Fagonia bruguieri* DC. has been published. Although the phenolic composition of *Fagonia cretica* and *Fagonia indica*, particularly in plants from Pakistan origins, has only been described in a limited number of studies.

Chromatographic profiles of the plants' aerial parts are shown in Figure.V.15 and a summary of the retention times and MSn spectral data of the discovered compounds are provided in Table.V.11, together with a description of where in the various *Fagonia bruguieri* DC. extracts they can be found.

Wide varieties of metabolites were found in the different organic extracts, which may be associated with the various functions they served. Andrographolide, Atricarpan D, and 3-caffeoylquinic acid, in addition to Isorhamnetin, were exclusively identified in FbBE, whereas 5-Methoxy- podophyllotoxin was only found in FbEAE.

The preponderance of these compounds, known as flavonoids, are present in the different extracts of *F. bruguieri* DC. plant. The extracts of *F. bruguieri* DC. included four flavones, three of which were C-glycosylated flavones and one of which was a monomethoxy flavone.

An intense $[M+H]^+$ (449 m/z) ion could be seen in FbDE's full mass spectrum when measured at RT=47.7 min. Kaempferol-7-O-glucoside was determined to be the identity of this Compound through a comparison of its mass spectrometric data with published research; in fact, it has been identified in *F. cretica* by Miranda *et al.,* (2022) and Yadano Kumbi et *al.,* [383] (2019) [382]. Kaempferol-7-O-glucoside has been previously isolated in F. microphylla and *F. glutinosa* by Saleh al-Wakeel et *al.,* (1992) [219].

Peaks with $[M-H]^-$ ions at 577 m/z and $[M-H]^-$ ions at 610 m/z in the FbEAE chromatogram, respectively, suggested the chemical formulae $C_{27}H_{30}O_{14}$ and $C_{27}H_{30}O_{16}$, respectively. These findings led us to conclude that the compounds in the discussion were isovitexin 2'-O-rhamnoside (577 m/z) [387] and herbacetin 8-rutinoside (610 m/z) [382], [383], based on their chromatographic profiles and the molecular ions referenced in the

literature . Therefore, based on the comparison of its UV spectrum and the reported accurate masses with those obtained from another species of *Fagonia*, these peaks have been provisionally classified as O or C-glycosylated flavones.

The protonated ion $[M+H]^+$ at m/z 317 was obtained from the eluting peak in FbBE at 52.18 min. The preliminary analysis led to the identification of this chemical as Isorhamnetin. As far as we can tell, this is the first tentative identification of this monomethoxyflavone in *F.bruguieri DC.*, while it has been reported in other Fagonia species [382-384], notably *F.indica* and *F.cretica* .

Epigallocatechin; produced deprotonated molecular ions at 306 m/z; this molecule was identified in *Fagonia cretica*, and its identity was confirmed by Tabassum *et al*., (2022) [388]. Full scan and MS spectra of FbDE, FbEAE, and FbBE revealed the presence of flavan 3-ol in all three fractions.

The current study was able to identify one quinic acid derivative. The protonated molecular ion $[M+H]^+$ at m/z 353, which was observed in the eluting peak's mass spectra with a retention time of 1.39 min, enabled the formula $C_{16}H_{17}O_9$ to be determined. Therefore, the identity of this compound attributed to 3-caffeoylquinic acid. This substance was previously identified in a Zygophyllaceae family species, *cuneifolia* L., and potentially identified in F.*bruguieri* DC. for the first time [4].

According to their MS spectra, two lignans were discovered in the aerial part of F.*bruguieri* DC. extracts. The first compounds with the attribute eluted in FbDE at 52.45 min, FbEAE at 51.07 min, and FbBE at 1.94 min had the same characteristic. In addition, the mass spectra of these compounds showed similarities in the existence of $[M-H]^-$ ions at m/z 413. On the basis of this, it has been provisionally identified as podophyllotoxin. Despite this, they are being reported for the first time in *F.bruguieri* DC.

The second lignan was detected in the FbEAE chromatogram at 43.01 min, producing a $[M-H]^-$ ion at 443 m/z. This peak's UV and full MS spectra enabled the estimation of the chemical formula, which was found to be $C_{22}H_{24}O_8$. These results are in agreement with data that had been previously reported for 5-Methoxy-podophyllotoxin [1].

The existence of feruloyl derivatives (feruloyl N-tryptophan hexoside) was confirmed by the mass spectra of peaks that eluted at 40.06 and 40.04 min in FbDE and FbEAE respectively.

These mass spectra exhibited $[M-H]^-$ ions at m/z 541. The MS spectra are in agreement with the data that was already reported for feruloyl N-tryptophan hexoside [390] [391]. According to our best knowledge, this is the first time the Fagonia genus has been mentioned.

In addition, the FbBE chromatogram showed consistency at m/z 393 in the negative ion mode, which led to the tentative designation of prenylated chalcones as xanthoangelol [6].

FbBE chromatogram recorded at RT = 1.01 min produced $[M+H]^+$ ions at m/z 351, which were used to confirm a tentative identity of Andrographolide by comparing the relevant published data [381]. It is the first time that this labdane diterpenoid has been tentatively determined to be present in this species.

At RT 1.69 min, the chromatogram of FbBE yielded $[M+H]^+$ ions at m/z 382 and was tentatively identified as Atricarpan D ($C_{23}H_{26}O_5$) based on data from Viqar Uddin Ahmad *et al*., (2006), who isolated this pterocarpan from a BuOH extract of the whole plant of Zygophyllum eurypterum (Zygophyllaceae) [5].

An intense $[M-H]^-$ ion was detected in the full mass spectrum of FbDE, FbEAE, and FbBE at RT= 42.9, 42.86, and 42.86, respectively. This compound was identified as Oleanolic acid-3-O-β-D-glucopyranosyl-(1→3)-[(β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranoside (triterpenoid saponins) by comparison of its MS data with literature [389] [232]; in fact, it was previously isolated and identified in *Fagonia arabica* by Toshio Miyase *et al.*, (1995) [232].

V.5. Structural Elucidation

This part concerns the structural's elucidation of 3 compounds among the 11 isolated as part of our work. The structures of our compounds were elucidated by using the spectrophotometer RMN, mono-dimensional (^1H, ^{13}C, DEPT), and bi-dimensional (HSQC, COSY, and HMBC).

V.5.1. Identification of compounds isolated from of *Artemisia campestris* L. aerial parts (AcBE)

V.5.1.1. Compound I: (PN)

The compound was obtained as a brown amorphous powder soluble in the methanol. Using an acid solution and heating makes a black spot. This product is not visible under UV light.

An examination of the ^{1}H NMR spectrum (Figure.V.17, Figure.V.18) of this compound reveals the presence of signals only in the osidic region from δ_H 3.26 ppm to 5.32 ppm with intensities indicating that this compound PN is a polysaccharide, which distributed as follows:

- A doublets at δ_H = 5.30 ppm (d, *J*= 3.8 *Hz*) integrated 1H attributable to H-1 proton corresponds to the anomeric proton, with a small coupling constant indicating the presence of sugar in the α configuration.
- Peaks at δ_H = 3.33 ppm (dd, *J*= 9.8; 3.8 *Hz*) integrated 1H attributable to H-2 proton.
- Peaks at δ_H = 3.60 ppm (dd, *J*= 9.3; 4.3 *Hz*) integrated 1H attributable to H-3 proton.
- A doublets at δ_H = 3.26 ppm (d, *J*= 9.6 *Hz*) integrated 1H attributable to H-4 proton.
- Peaks at δ_H = 3.82 ppm (d, *J*= 2.4 *Hz*) integrated 1H attributable to H-5 proton.
- A doublet of doublet integration at δ_H = 3.60 ppm (dd, *J*= 9.3; 4.3 *Hz*) attributable to H_a-6 proton.
- A doublet of doublet signal at δ_H = 3.69 ppm (dd, *J*= 9.3, 3.1 *Hz*) attributable to H_b -6 proton.
- A doublet of doublet signal at δ_H = 3.45 ppm (dd, *J*= 7.0, 2.6 *Hz*) attributable to H_a -1‘ proton.
- A doublet integration at δ_H = 3.54 ppm (d, *J*= 8.6 *Hz*) attributable to H_b -1‘ proton.
- Peaks at δ_H = 3.99 ppm (d, *J*= 8.2 *Hz*) integrated 2H attributable to H-3‘ proton.
- A doublet of doublet integration at δ_H= 3.93 ppm (dd, *J*= 9; 6.7 *Hz*) attributable to H-4‘ proton.
- A doublet of doublet signal at δ_H = 3.74 ppm (dd, *J*= 6.9; 2.0 *Hz*) was attributed to H-5‘.
- A doublet integration signal at δ_H = 3.66 ppm; *J*=3.9 *Hz* was attributed to H-6‘.

The presence of only one anomeric proton in this polysaccharide indicates another sugar that does not contain an anomeric proton regarding the number of signals in the osidic region. Regarding its chemical shifts, we proposed that this polysaccharide contains fructose because this latter does not have an anomeric proton.

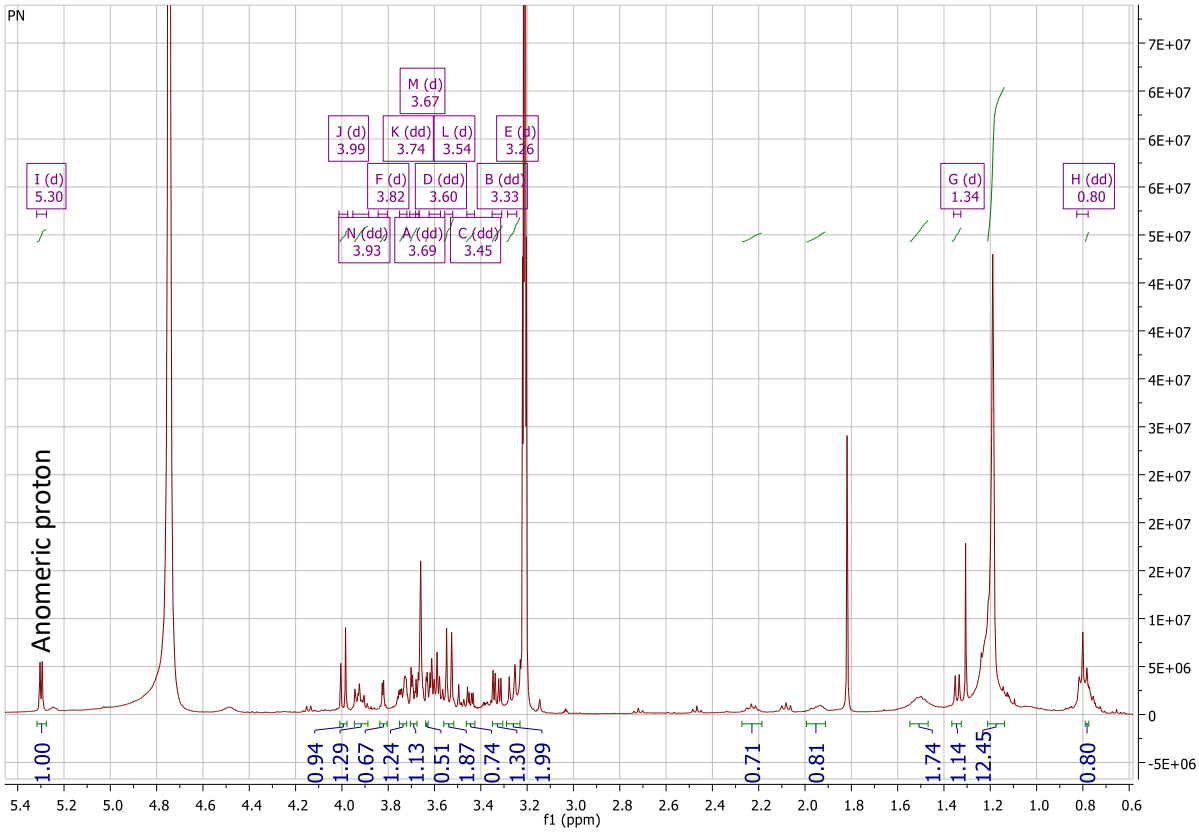


Figure.V.17. ^{1}H NMR spectrum of compound PN (400 MHz, Methanol-*d4*).

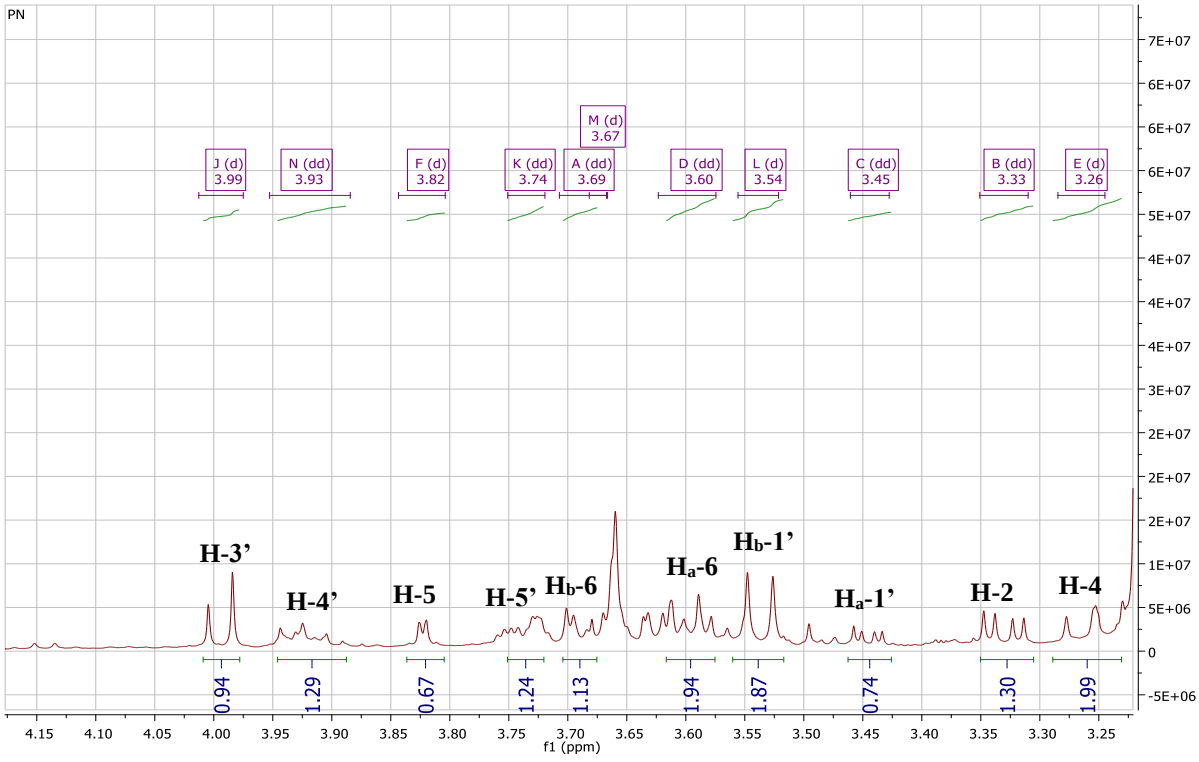


Figure.V.18. ^{1}H NMR spectrum spread out (3.25-4.15 ppm) of compound PN (400 MHz, Methanol-*d4*).

The ^{13}C NMR (Figure.V.19) and DEPT 135° spectrum (Figure.V.20) indicated the presence of twelve carbons, including [392]:

- Two anomeric carbons.
- Three carbons in group (CH_2) are linked to alcohol function.
- Eight carbons in group (CH) are linked to alcohol function.

The combined analysis of spectra ^{13}C RMN, and DEPT 135° reveals the presence of two sugar residues in the form of two hexoses located in the osidic region. The first hexose contains an anomeric proton at δ_H = 5.30 ppm (J = 3.8 *Hz*), and the small coupling constant indicates the presence of a sugar of configuration α; as a proposition, we suggest that this first hexose is the type of α-glucose.

The second sugar residue has shown the characteristic signal of quaternary carbon. We supposed that this residue could be fructose from their chemical shifts and $J_{1,2}$ (^{13}C RMN, and DEPT135°).

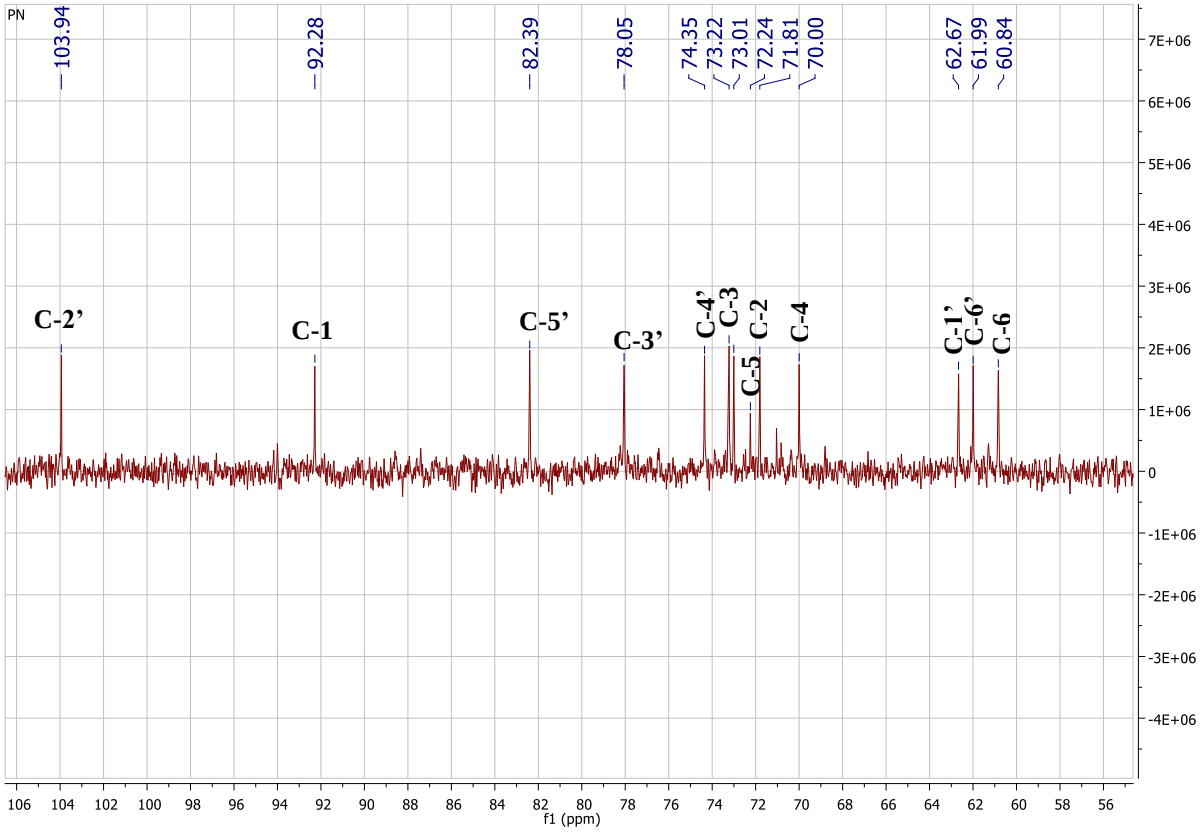


Figure.V.19. ^{13}C NMR Spectrum of compound PN (Methanol-*d4*, 100 MHz).

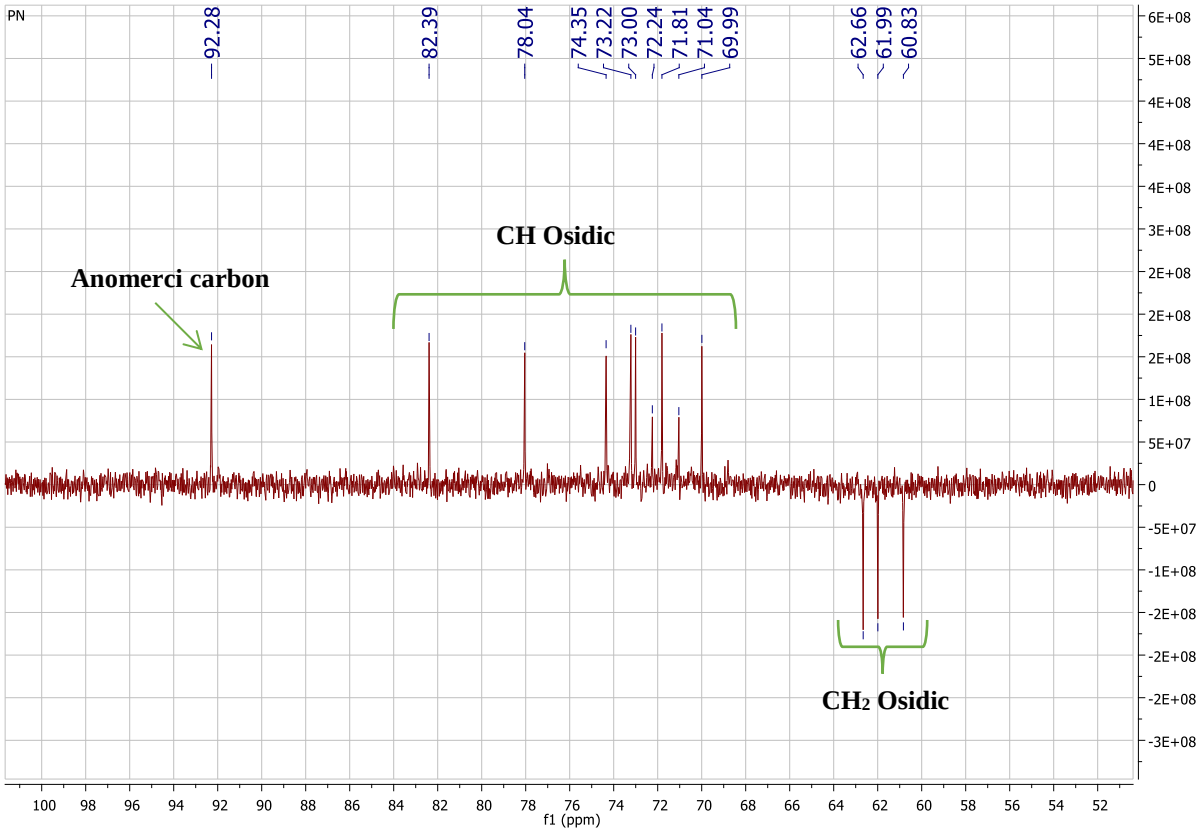


Figure.V.20. DEPT 135 ° Spectrum of compound PN (Methanol-*d4*, 100 MHz).

In the HMBC experiment (Figure.V.21), the connectivity was observed from correlation between H-1_a (δ_H = 5.30 ppm) with a coupling constant (3.8 *Hz*) and C-2 carbon (δ_C 71.81 ppm) as well as another correlation with C-3 (δ_C 73.22 ppm), respectively. The identified C-3 also shows a correlation spot with proton resonant at 3.26 ppm (d, *J*= 9.1 *Hz*), corresponding to H-4. This last show a correlation with carbon resonant at 72.24 ppm attributable to C-5.

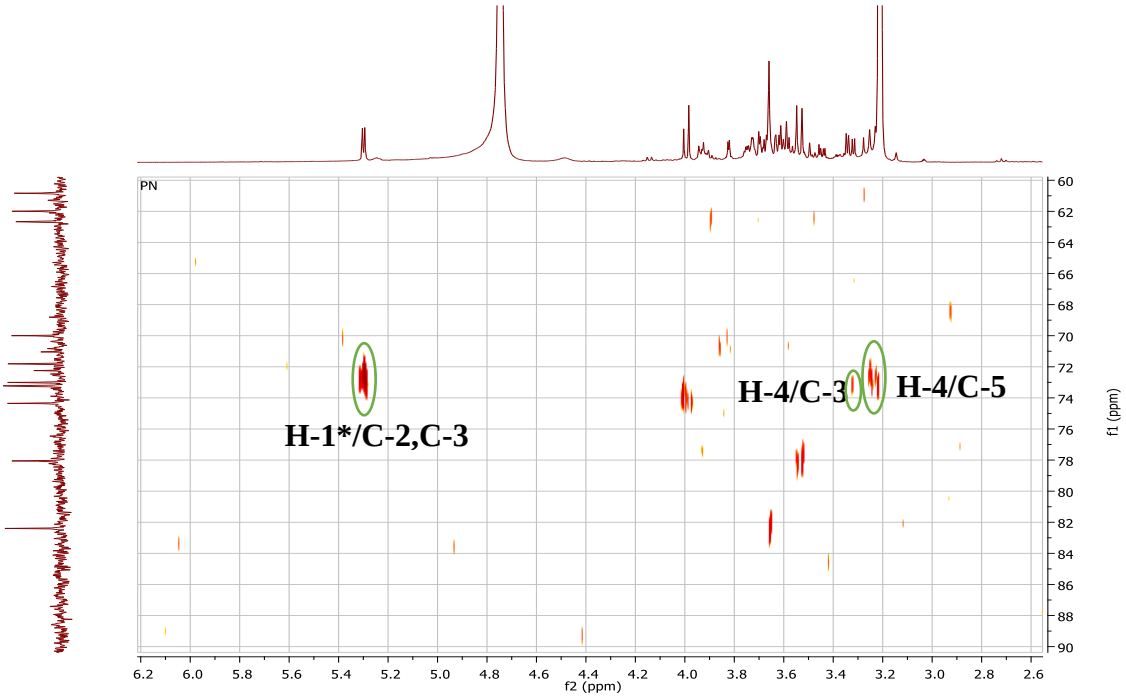


Figure.V.21. HMBC spectrum spread out of the first hexose.

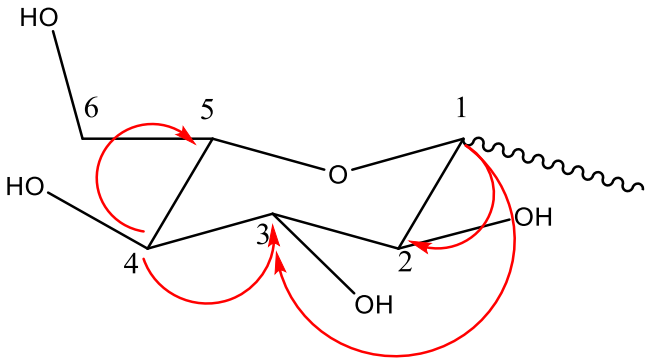


Figure.V.22. The HMBC correlations of the first hexose.

By examining the complete analyses HSQC spectrum (Figure.V.23) of this hexose, it is feasible to identify the corresponding protons and carbons:

- C-1 to δ_C 92.28 ppm due to its correlation with H-1 (d, *J*= 3.8 *Hz*).
- C-2 to δ_C 71.81 ppm due to its correlation with H-2 (dd, *J*= 9.8; 3.8 *Hz*).
- C-3 to δ_C 73.22 ppm due to its correlation with H-3 (dd, *J*= 9.3; 4.3 *Hz*).
- C-4 to δ_C 70.00 ppm due to its correlation with H-4 (d, *J*= 9.6 *Hz*).
- C-5 to δ_C 72.24 ppm due to its correlation with H-5 (d, *J*= 2.4 *Hz*) .
- C-6 to δ_C 60.84 ppm due to its correlation with H-6_a (dd, *J*= 9.3; 4.3 *Hz*) and H-6_b (dd, *J*= 9.3; 3.1 *Hz*).

The full chemical shift assignment of all the protons and carbons in this hexose and the coupling constants measured are in full agreement with those reported in the literature. Thus deduced to be α-glucose.

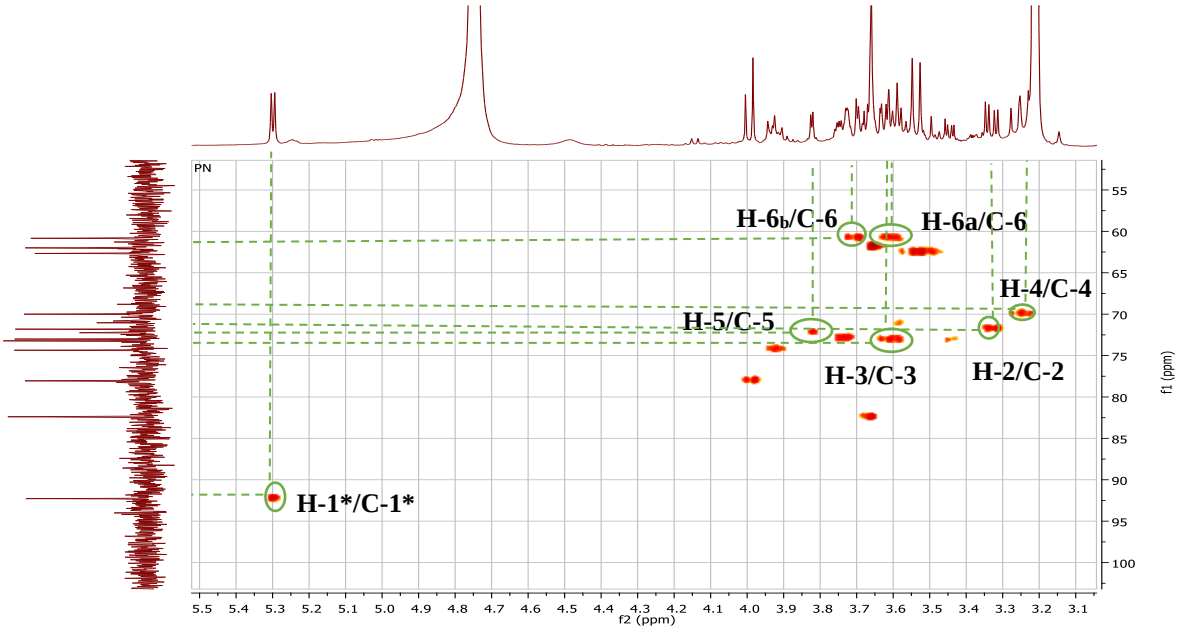


Figure.V.23. HSQC spectrum spread out of first hexose.

The HMBC spectrum has allowed to assign the position of quaternary carbon C-2' of the second hexose, the spectrum shows correlations between the protons H_b -1' δ_H = 3.54 ppm (d, *J*= 8.6 *Hz*), and the quaternary carbon resonating at δ_C = 103.94 ppm, thus attributable to the C-2' position. In the same spot of this H_b -1' which also shows correlations with H-3' protons, to allocate the carbons C-1' and C-3' respectively, resonant at δ_C 62.67 and 78.05 ppm respectively. The proton H-6', resonant at δ_H= 3.66 ppm in form doublet integration therefore attributable to the C-6' (δ_C= 61.99) position, which shows correlations with C-5' resonant at δ_C 82.39 ppm in HMBC spectrum. Moreover, the same spectrum shows a correlation spot between proton H-3' and C-4' at δ_C= 74.26 ppm (Figure.V.24).

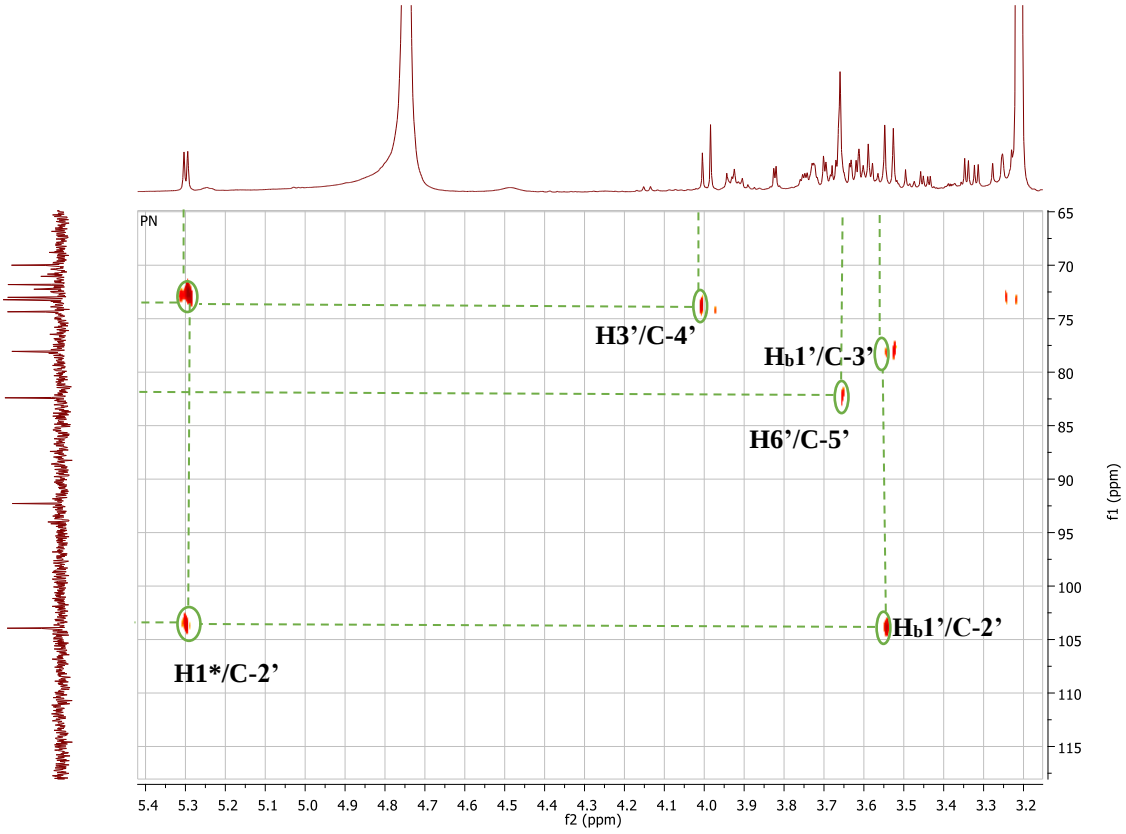


Figure.V.24. HMBC spectrum spread out of the second hexose.

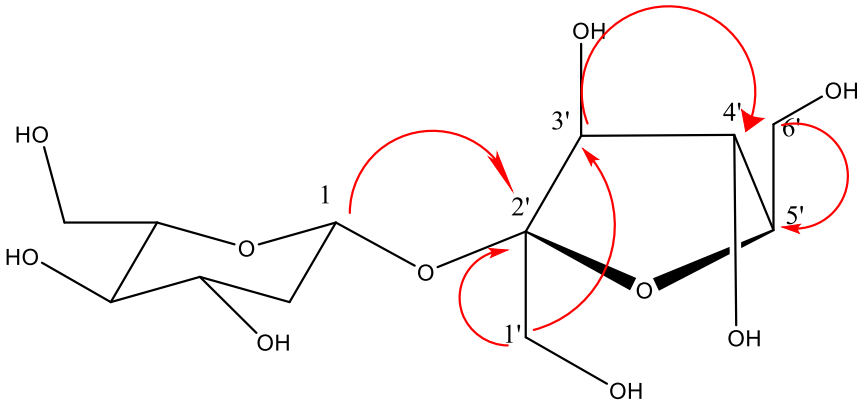


Figure.V.25. The HMBC correlations of the second hexose.

The HSQC spectrum gives the following additional clues (Figure.V.26):

- C-1‘ to δ_C 62.67 ppm due to its correlation with H_a -1‘ and H_b -1‘.
- C-3‘ to δ_C 78.05 ppm due to its correlation with H-3‘.
- C-4‘ to δ_C 74.35 ppm due to its correlation with H-4‘.
- C-5‘ to δ_C 82.39 ppm due to its correlation with H-5‘.
- C-6‘ to δ_C 61.99 ppm due to its correlation with H-6‘.

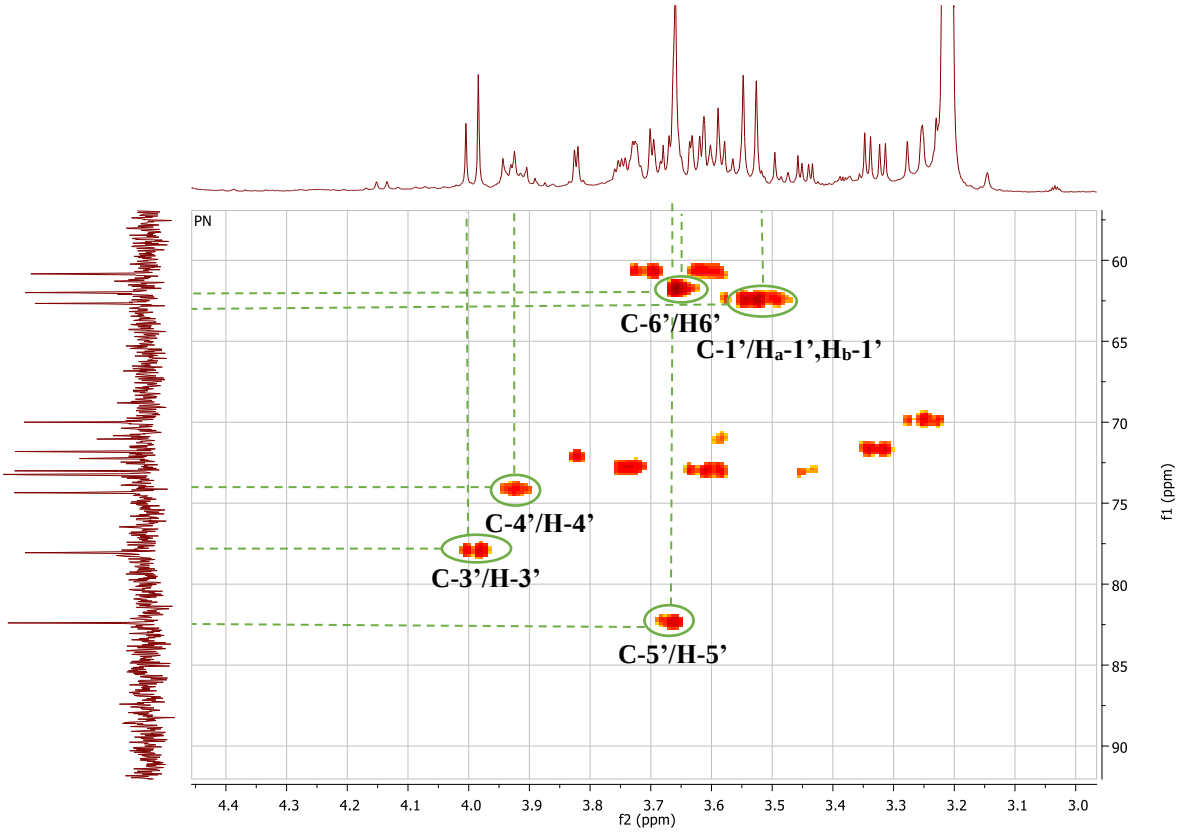


Figure.V.26. HSQC spectrum spread out of second hexose.

By comparing with the literature [393], the set of these NMR spectroscopic data confirms the suggested structure of the second hexose, which is β-fructose.

Linkage of α- glucose to β-fructose was established by an HMBC experiment. A cross-peaks were observed between the anomeric proton resonant at δ_H 5.30 ppm with a coupling constant *J*= 3.80 *Hz* attributable to H- 1* with carbon resonant at δc_q 103.94 corresponding to C-2ˋ. The HMBC experiment enabled the determination of the connections between sugars. Hence, alpha-glucose is linked to the O-2 of beta-fructose. The completeness of the spectroscopic data, confirmed by comparison with those in the literature and collected in the Table.V.12, led to the structure of disaccharide, it is α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (saccharose), as shown in Figure.V.27.

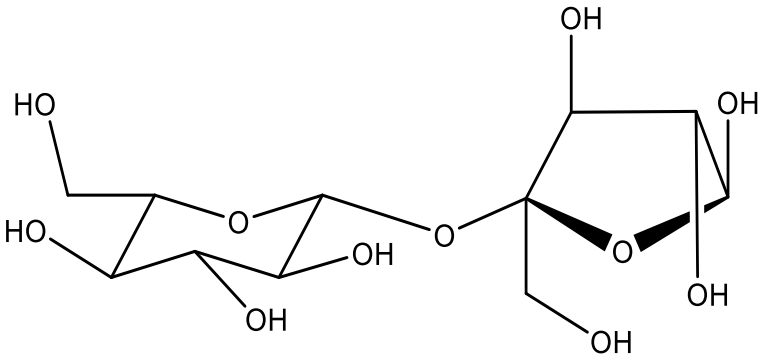


Figure.V.27. Structure of α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside .

Table.V.12. ^{13}C NMR and ^{1}H NMR spectral data of compound PN (400 M*Hz*, 125 M*Hz*, Methanol-d4).

Position	***δC* (ppm)**	***δH* (ppm)**	***multiplicity***	***J(Hz)***
1	92.28	5.30	d	3.8
2	71.81	3.33	dd	9.8; 3.8
3	73.22	3.60	dd	9.3; 4.3
4	70.00	3.26	d	9.6
5	72.24	3.82	d	2.4
6'	60.84	H_a 3.60	dd	9.3; 4.3
		H_b 3.69	dd	9.3; 3.1
1'	62.67	H_a 3.45	dd	7.0; 2.6
		H_b 3.54	d	8.6
2'	103.94	-	-	-
3'	78.05	3.99	d	8.2
4'	74.35	3.93	dd	9; 6.7
5'	82.39	3.74	dd	6.9; 2.0
6'	61.99	3.66	d	3.9

V.5.2. Identification of compounds isolated from of *Fagonia bruguieri* DC. aerial parts (FbBE)

V.5.2.1. Compound II: Pb

The compound was obtained as a white amorphous powder soluble in the methanol. Using an acid solution and heating makes a bleu spot. This product is not visible under UV light.

The ^{13}C NMR spectrum of the compound Pb was recorded at 400 M*Hz* in MeOH-*d4*, as shown in Figure.V.28. This spectrum reveals multiple signals, including the following:

- δc=154.63 ppm ; δc=104.62 ppm ; δc= 134.28 and δc=140.62 ppm shows the presence of an aromatic ring.
- δc=130.22 ppm et δc=128.58 ppm which indicates the presence of two ethylenes (CH=CH).
- Signals: δc=104.15 ppm; δc= 81.43 ppm; δc= 76.43 ppm; δc= 70.03 ppm; δc= 76.96 ppm and are present of the carbon atoms of a sugar.
- The presence of two groups of CH_2OH methylenes resonant with δc= 61.10 ppm; δc=62.07 ppm and a methoxyl (CHO) at δc=55.07 ppm.

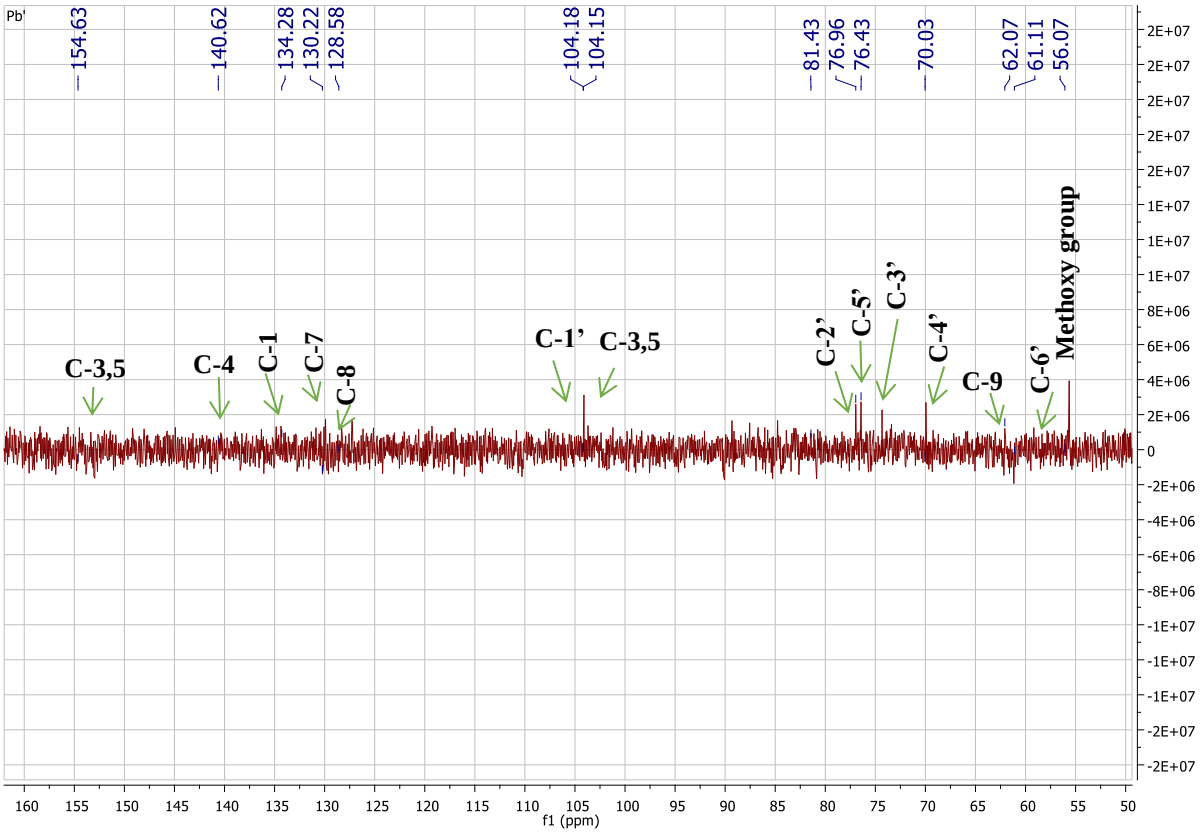


Figure.V.28. ^{13}C NMR Spectrum of compound Pb (100 M*Hz*, MeOH-*d4*).

Examination of the ^{1}H NMR spectrum (Figure.V.29→ Figure.V.31) of the Pb compound confirms these data by the presence of signals:

- A singulet signal with δ_H =6.75 ppm of 2H integration due to two equivalent protons of the aromatic nucleus (δ_C =104.15) points towards a tetra-substitution of this aromatic cycle.
- A doublet of doublet signal with integration of 1H at δ_H = 6.56 ppm (J = 14.05; 9.6 *Hz*) and 1H integration triplet doublet at δ_H=6.33 ppm (J =15.8; 5.6 *Hz*). The multiplicity of this signal and the values of the chemical shift and coupling constants confirm the presence of a trans-configuration double ethylene bond in this molecule and place a CH_2 group in a vicinal position relative to this ethylene CH.
- A doublet of doublets at δ_H =4.22 ppm (J = 5.56; 1.47 *Hz*). The values of the coupling constants indicate that this grouping is close to the previous ethylene CH. Chemical shift value indicates that this CH_2 group is oxygenated (δ_C = 62.07).
- One singulet δ_H=3.86 ppm 6H integration (δ_C =56.07 ppm) due to two equivalent methoxyl groups, therefore, two aromatic carbon cores (δ_C = 154.63 ppm).
- A doublet at δ_H=4.87 ppm (J = 7.44) is attributable to an anomeric proton of sugar configuration β.

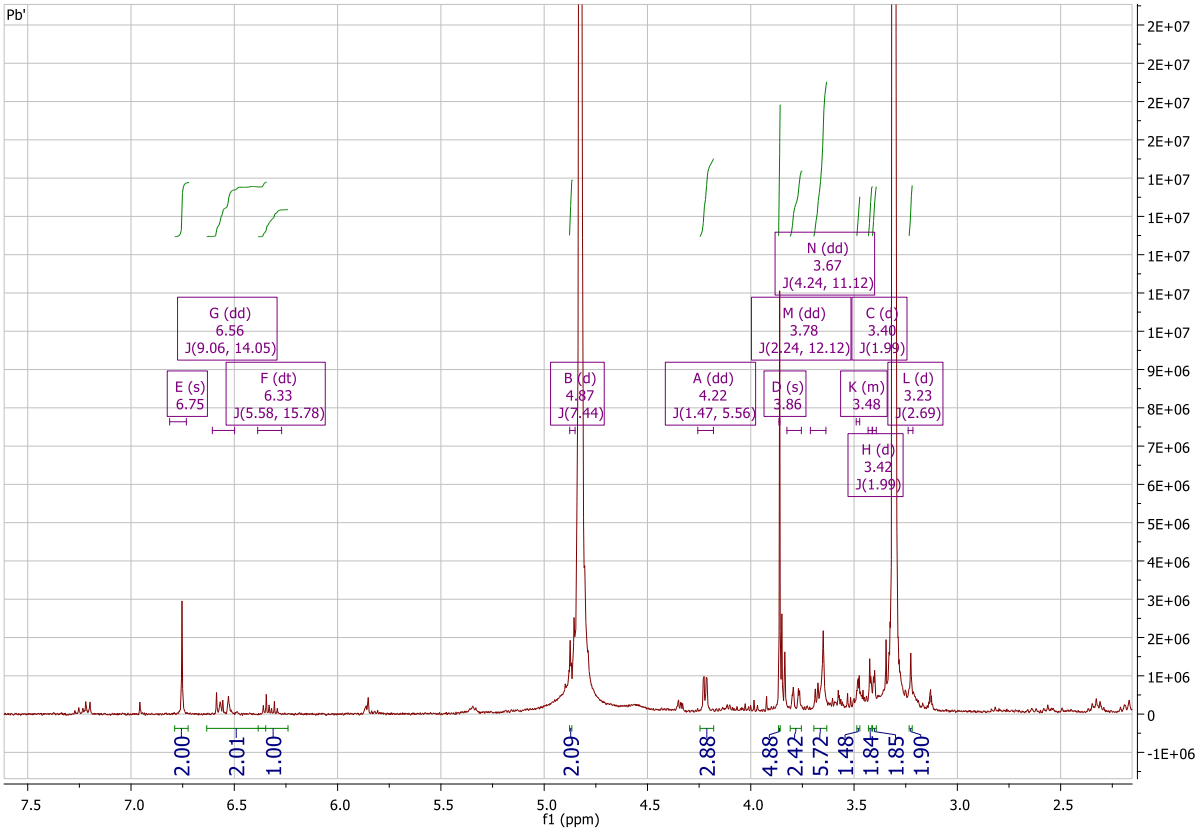


Figure.V.29. ^{1}H NMR spectrum of compound Pb (400 MHz, Methanol-*d4*).

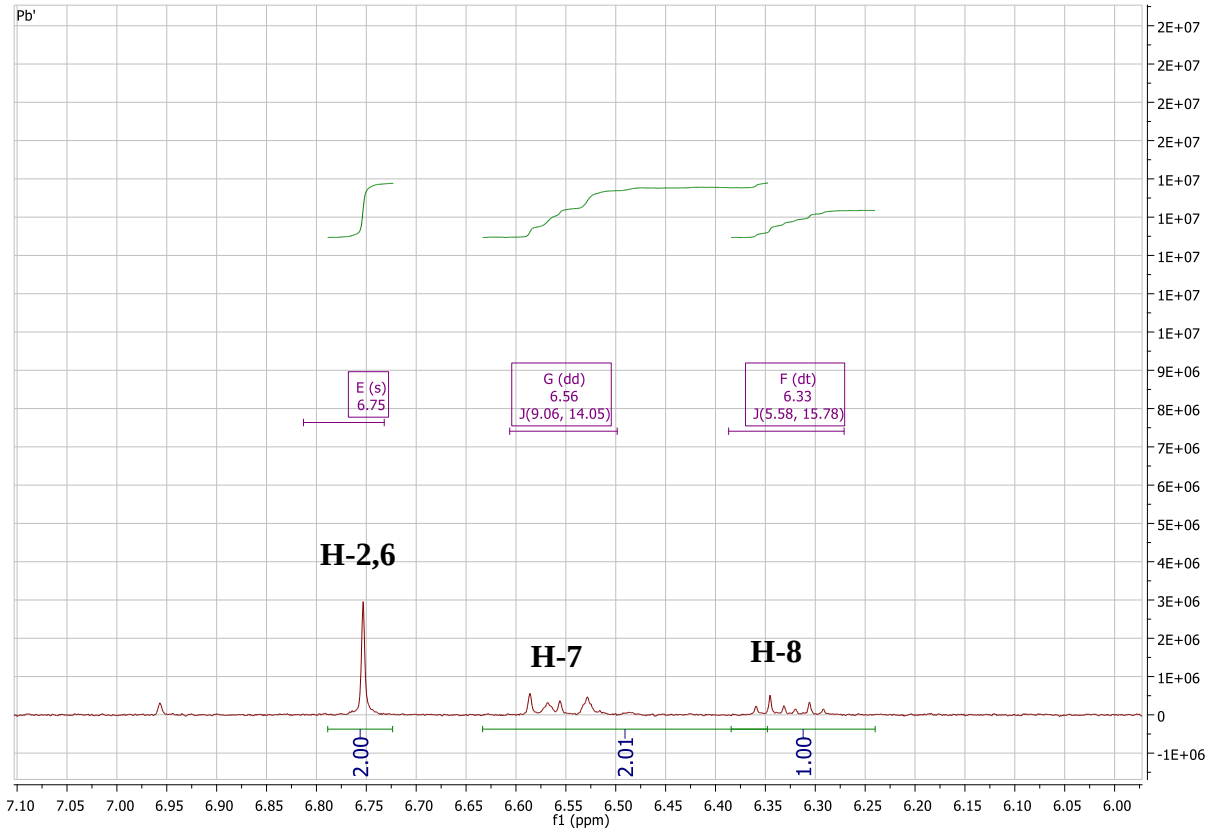


Figure.V.30. [1]H NMR spectrum spread out (6.00-7.10 ppm) of compound Pb (400 *MHz*, Methanol-*d4*).

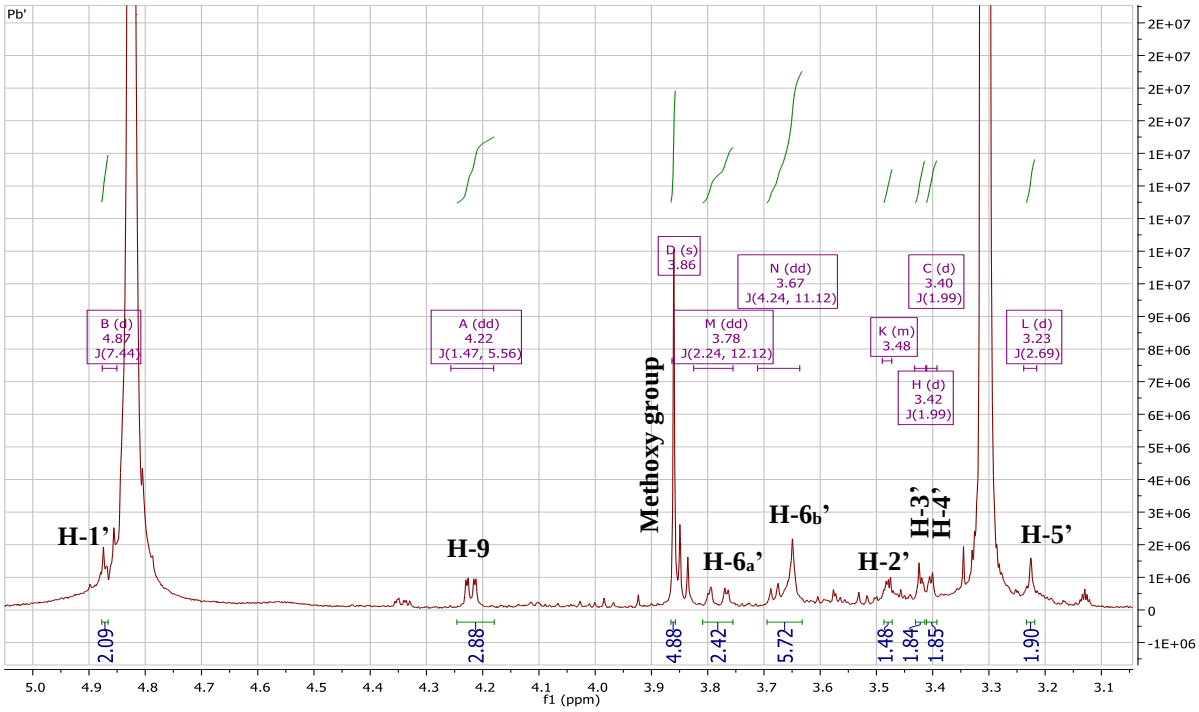


Figure.V.31. [1]H NMR spectrum spread out (3.1-5.0 ppm) of compound Pb (400 MHz, Methanol-*d4*).

By examining the complete analyses HSQC spectrum (Figure.V.33), it is feasible to identify the corresponding protons and carbons:

- C-7 to δ_C 130.22 ppm due to its correlation with H-7.
- C-8 to δ_C 128.58 ppm due to its correlation with H-8.
- C-9 to δ_C 62.07 ppm due to its correlation with H-9.
- C-1‘ to δ_C 104.18 ppm due to its correlation with H-1‘.
- C-2‘ to δ_C 81.43 ppm due to its correlation with H-2‘.
- C-3‘ to δ_C 76.43 ppm due to its correlation with H-3‘.
- C-4‘ to δ_C 70.03 ppm due to its correlation with H-4‘.
- C-5‘ to δ_C 76.96 ppm due to its correlation with H-5‘.
- C-6‘ to δ_C 61.10 ppm due to its correlation with H-6‘a and H-6‘b.

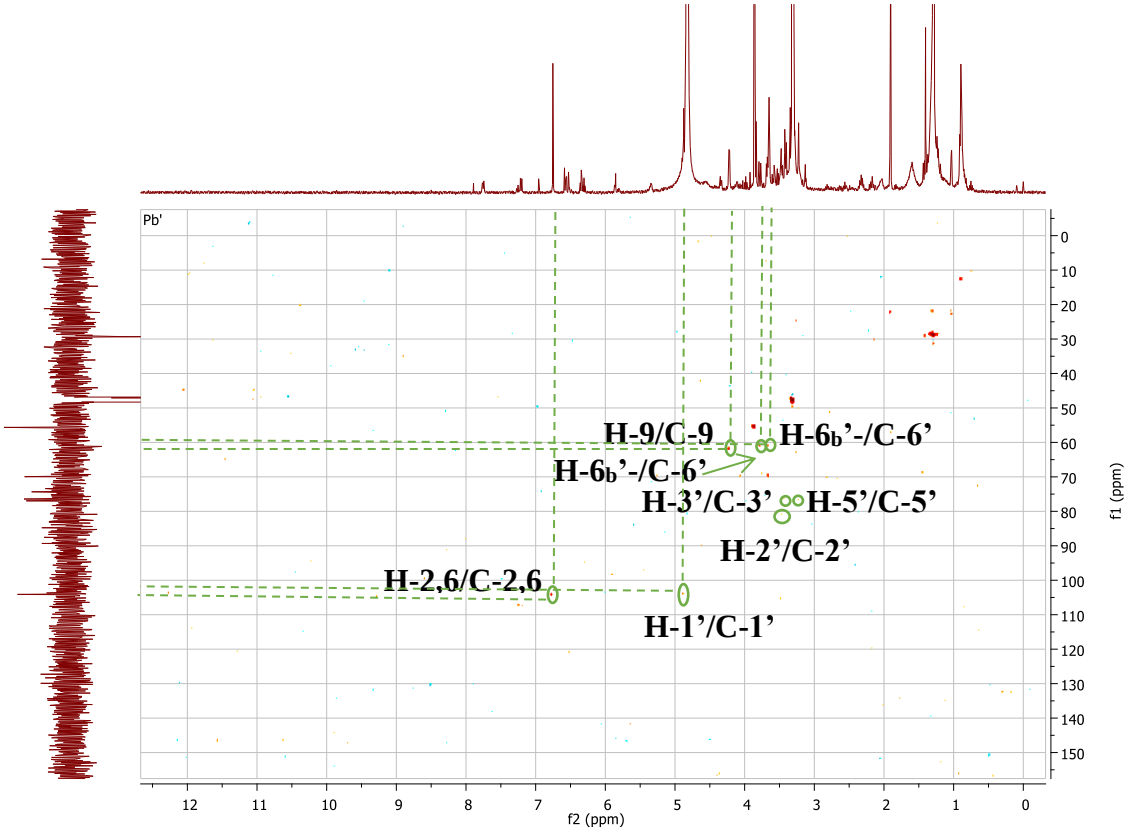


Figure.V.32. HSQC spectrum spread out of compound Pb.

These correlations allow for the identification of a hexose. It‘s a glucose of Configuration β. Configuration assumed out from the coupling constant $J_{1'\text{-}2'}$ = 7.4 *Hz*.

Starting from this first identification, the other protons and carbons are gradually identified by two-dimensional experiments COSY, and HMBC.

The COSY spectrum clearly highlights the expected correlations between (Figure.V.32):

- The anomeric proton H-1‘ (δ_H= 4.87; *J* =7.44 *Hz*; d) and the resonant proton H-2‘ at δ_H= 3.48, in multiplet form.
- The proton H-5‘ (δ_H= 3.23; J=2.96 *Hz*; d) and H-4‘ proton (δ_H =3.40; *J*=1.99 *Hz*; d).
- The proton H-5‘ (δ_H= 3.23; J=2.96 *Hz*; d) and H-6_a‘ proton (3.78; dd; *J* = 12.1;2.2 *Hz*).
- proton H-5‘ (δH= 3.23; J=2.96 *Hz*; d) and proton H-6_b‘ (3.67; dd; *J* = 11.1; 4.2 *Hz*).
- The proton H-4‘ (δ_H=3.40; J=1.99; d) and H-3‘ proton (δ_H=3.42; *J* =1.99; d).
- The proton H-8 (6.33; dt; J =15.8; 5.6 *Hz*) and proton H-7 (6.56; dd; *J* = 9.06; 14.05 *Hz*).
- The proton H-8 and proton H-9 (δ_H = 4.22; dd; *J* = 1.47; 5.56).

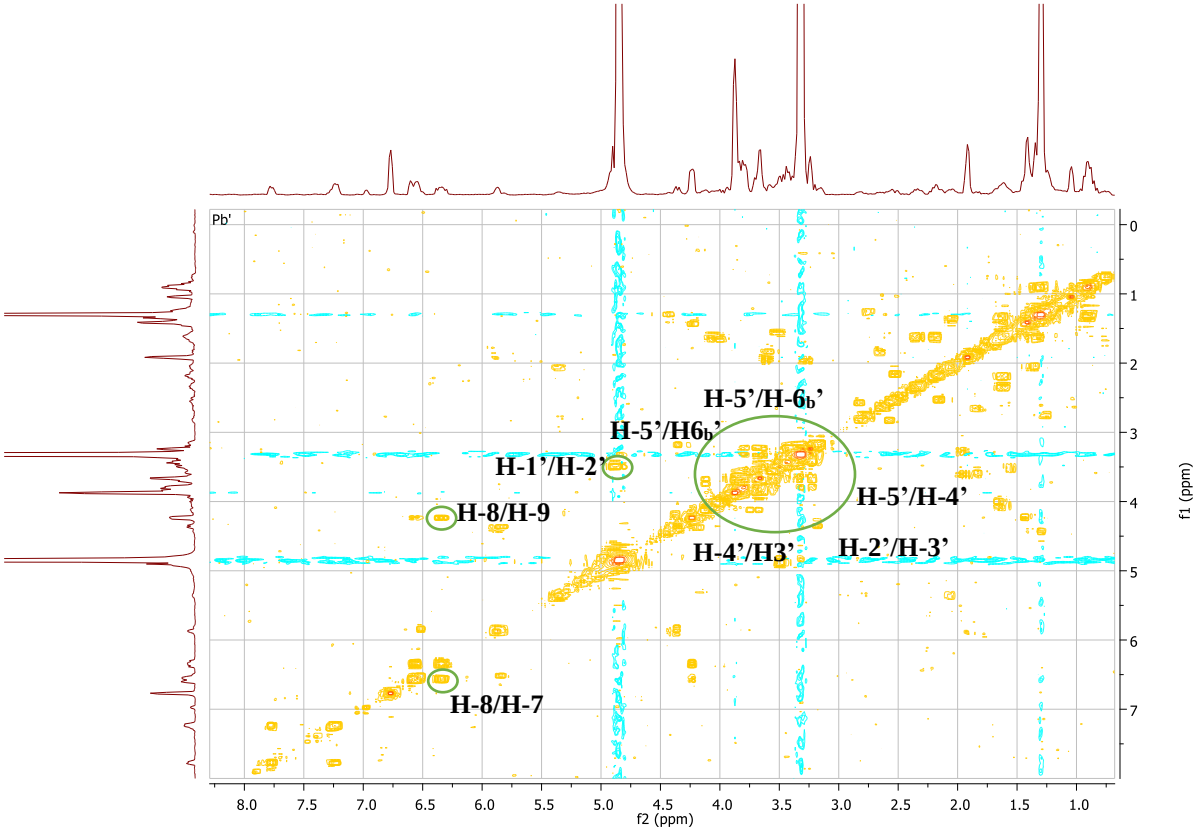


Figure.V.33. COSYspectrum spread out of compound Pb.

The analysis of the HMBC spectrum (Figure.V.34) allows for identifying additional carbons in the phenyl skeleton. Indeed, this spectrum also reveals:

- H-2 and H-3 (δ_H 6.75 ppm) shows two spot of correlations with
  - C-1 (δ_C 134.28 ppm).
  - C-3 and C-5 (δ_C 154.63 ppm).
- O-CH_3 (δ_H 3.86 ppm) shows one spot of correlations with
  - C-3/C-5 (δ_C 154.63 ppm).

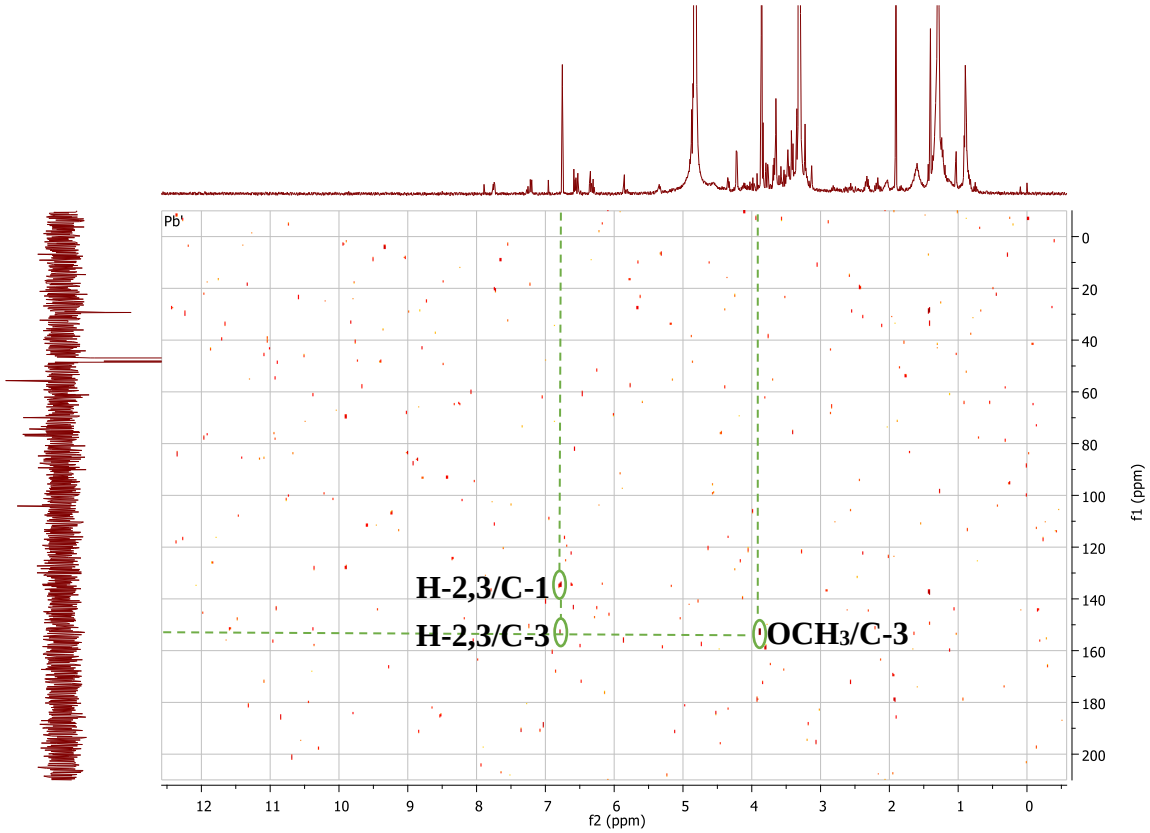


Figure.V.34. HMBC spectrum spread out of compound Pb.

The HMBC experiment confirms the C-1 glucosylation site by observing a $^3J_{C\text{-}H}$ coupling between the anomeric proton H-1' (4.87 ppm) of glucose and C-4 carbon (140.62 ppm).

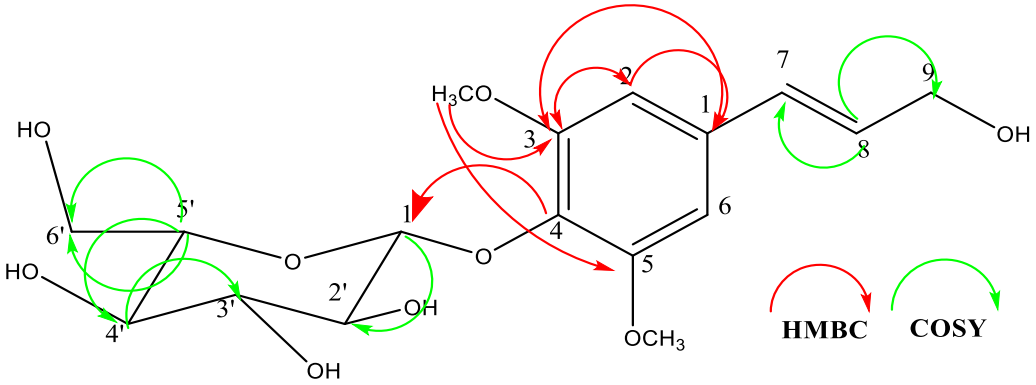


Figure.V.35. The HMBC and COSY correlations of compound Pb.

All these assignments by examining the ^{1}H-^{1}H COSY spectrum and the HSQC spectrum confirm the previous assignments that were made by examining the NMR spectrum. All of this information leads to the structure of phenylpropanoid glycoside as shown in Figure.V.36. The results are consistent with the Syringing data reported in the literature [394].

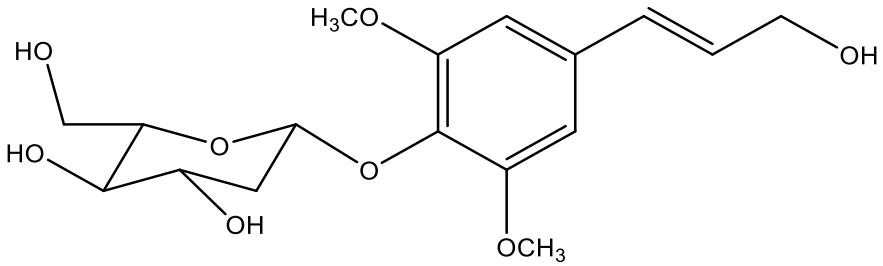


Figure.V.36. Structure of Syringing.

Table.V.13. ^{13}C NMR and ^{1}H NMR spectral data of compound Pb (400 M*Hz*, 125 M*Hz*, Methanol-d4).

Position	δ_C (ppm)	δ_H (ppm)	*multiplicity*	*J(Hz)*
1	134.28	-	-	-
2	104.15	6.75	s	-
3	154.63	-	-	-
4	140.62	-	-	-
5	154.63	-	-	-
6	104.15	6.75	s	-
7	130.22	6.56	dd	9.06; 14.05
8	128.58	6.33	dt	15.8; 5.6
9	62.07	4.22	dd	5.56; 1.47
1'	104.18	4.87	d	7.44

2'	81.43		3.48	m	-
3'	76.43		3.42	d	1.99
4'	70.03		3.40	d	1.99
5'	76.96		3.23	d	2.96
6'	61.10	H_a	3.78	dd	12.1; 2.2
		H_b	3.67	dd	11.1; 4.2
(2 -OCH_3)	56.07		3.86	s	-

V.5.2.2. Compound III: PV2

The compound was obtained as a white amorphous powder soluble in methanol. Using an acid solution and heating makes a purple spot. This product is visible under UV light as a dark spot.

The ^{13}C NMR (Figure.V.37) and DEPT 135° (Figure.V.38) spectra show the presence of the 13 carbon atoms of this molecule and allow them to be distributed in: 1 CH_2, 9 CH, and 3 Cq. This spectrum reveals multiple signals, including the following:

- δc=115.86 ppm and δc= 130.24 shows the presence of an aromatic ring.
- δc=163.68 ppm which indicates the presence one carbone engaged in link carbonyle (C=O).
- signals: δc= 100.20 ppm; δc= 73.40 ppm; δc= 76.91 ppm; δc= 69.89 ppm; δc= 76.54 ppm and δc= 61.05 ppm are present of the carbon of a sugar.

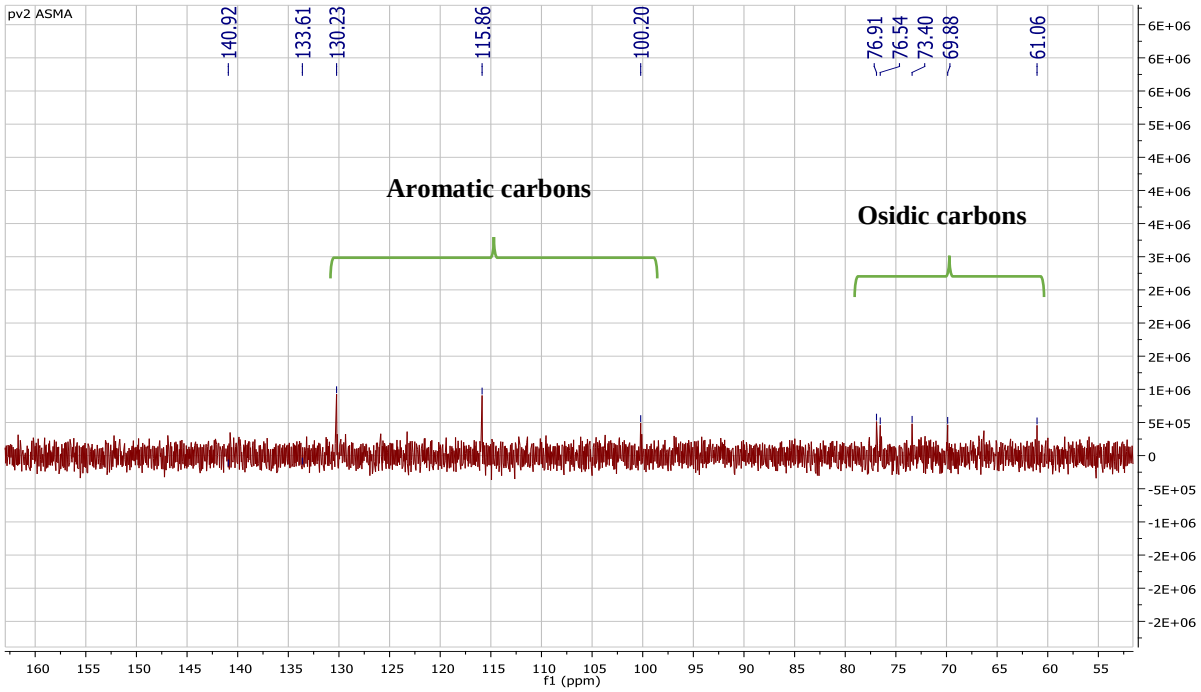


Figure.V.37. ^{13}C NMR spectrum of compound PV2 (100 MHz, Methanol-*d4*).

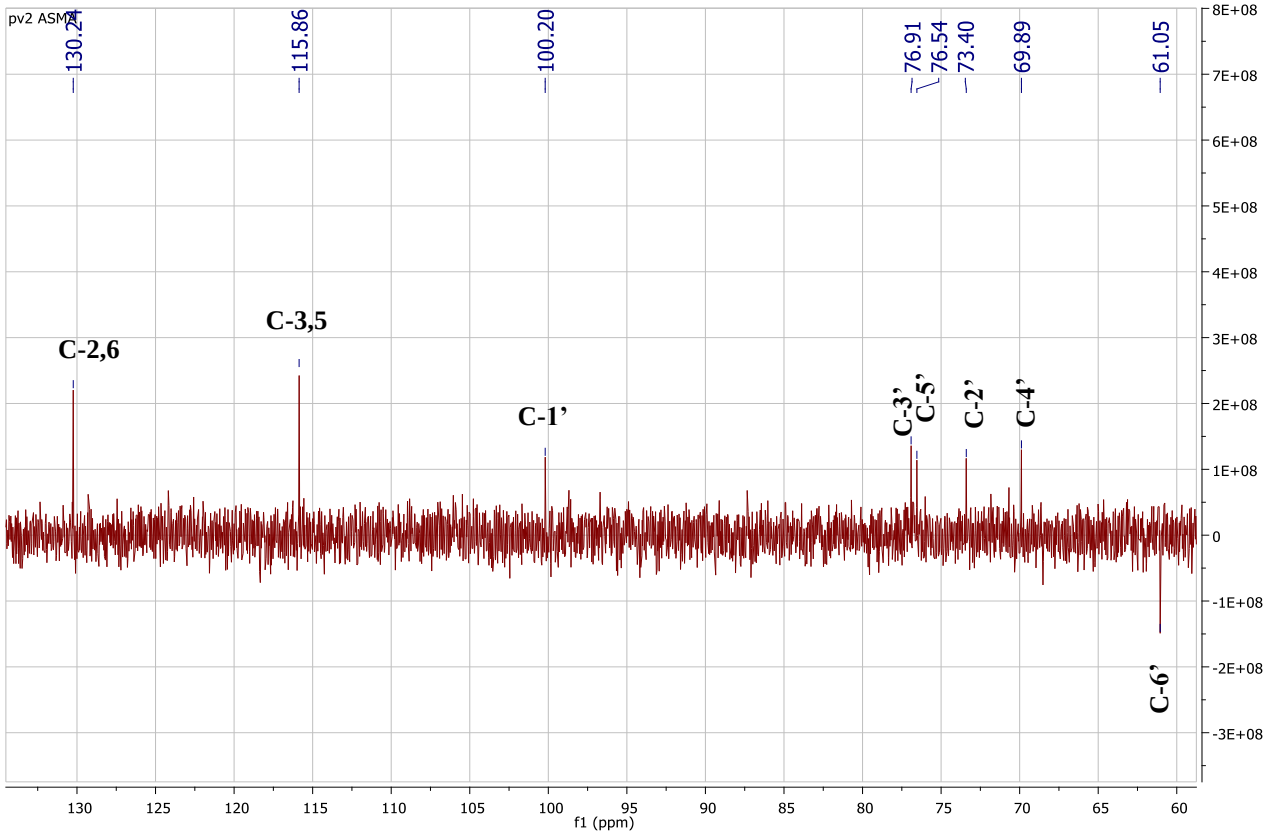


Figure.V.38. DEPT 135° spectrum of compound PV2 (100 MHz, Methanol-*d4*).

The ^{1}H NMR spectrum (400 M*Hz* in Methanol-*d4*) (Figure.V.39) shows the presence of a tetrasubstituted aromatic core in positions 2, 6, 3, and 5. Indeed, the four aromatic protons present as follows:

- A doublet signal with δ_H =7.88 ppm (J = 9.0 *Hz*) of 2H integration due to two equivalent protons of the aromatic nucleus (δ_C =130.24).
- A doublet signal with integration of 2H at δ_H = 7.07 ppm (J = 9.0 *Hz*) points towards a para-substituted aromatic ring.

The examination of the ^{1}H NMR spectrum (Figure.V.39→Figure.V.41) of this compound also reveals the presence of signals in the osidic region from δ_H 3.31 ppm to 4.93 ppm with intensities indicating that this sugar moiety is glucose, which distributed as follows:

- A doublets at δ_H = 4.93 ppm (d, *J*= 7.6 *Hz*) integrated 1H attributable to H-1‘ proton corresponds to the anomeric proton, with an intense coupling constant indicating the presence of sugar in the β configuration.
- Peaks at δ_H = 3.39 ppm (dd, *J*= 6.2; 2.1 *Hz*) integrated 1H attributable to H-2‘ proton.
- Peaks at δ_H = 3.31 ppm (dd, *J*= 6.0; 3.1 *Hz*) integrated 1H attributable to H-3‘ proton.
- A doublets at δ_H = 3.56 ppm (d, *J*= 5.6 *Hz*) integrated 1H attributable to H-4‘ proton.
- Peaks at δ_H = 3.39 ppm (dd, *J*= 6.2; 2.1 *Hz*) integrated 1H attributable to H-5‘ proton.
- A doublet of doublet integration at δ_H = 3.60 ppm (dd, *J*= 12.1; 5.6 *Hz*) attributable to H-6_a ‘ proton.
- A doublet of doublet integration at δ_H = 3.80 ppm (dd, *J*= 12.0; 2.2 *Hz*) attributable to H-6_b ‘ proton.

Figure.V.39. [1]H NMR spectrum of compound PV2 (400 MHz, Methanol-*d*4).

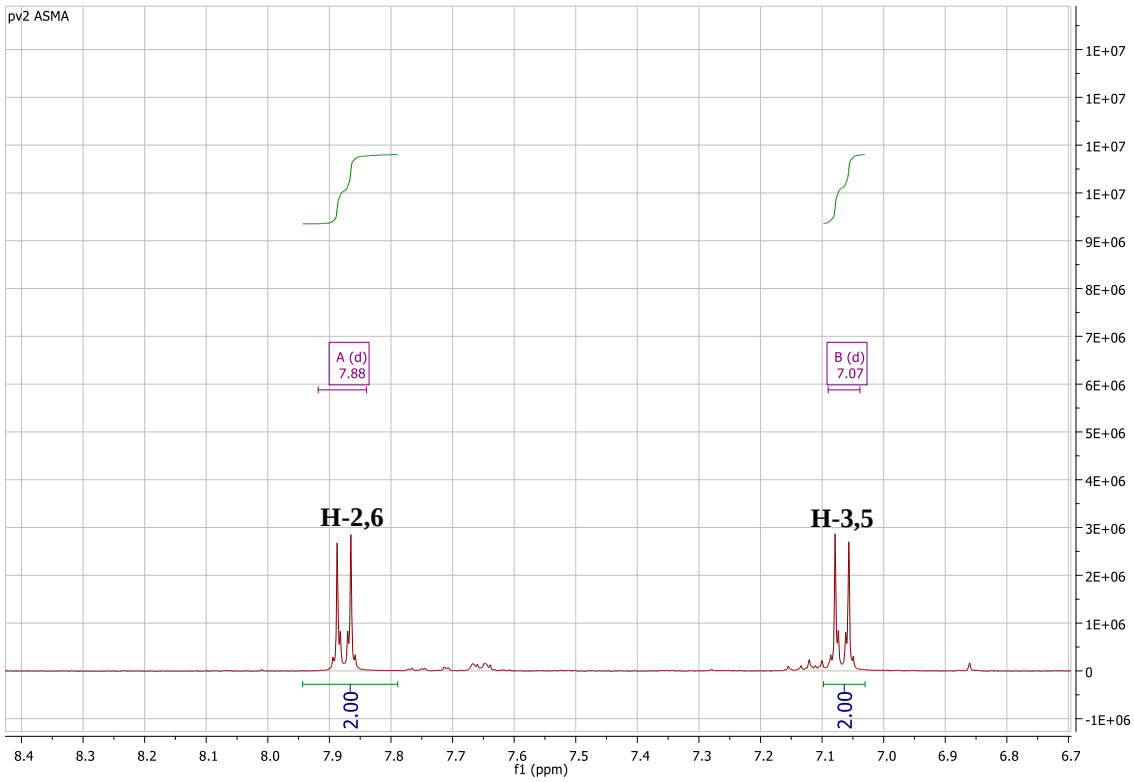


Figure.V.40. [1]H NMR spectrum spread out (6.7-8.4 ppm) of compound PV2 (400 MHz,Methanol-*d4*).

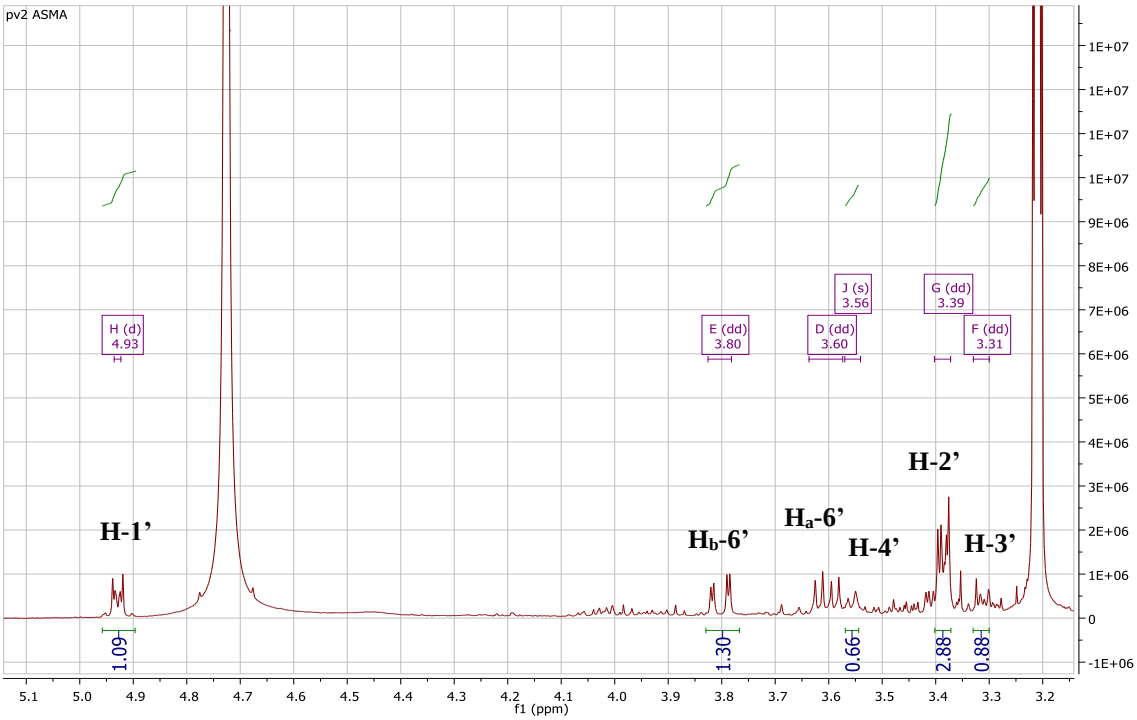


Figure.V.41. ^{1}H NMR spectrum spread out (3.2-5.1 ppm) of compound PV2 (400 MHz, Methanol-d_4).

Starting from this first identification, the other protons and carbons are gradually identified by two-dimensional experiments HSQC, COSY, and HMBC.

By examining the complete analyses HSQC spectrum (Figure.V.42), it is feasible to identify the corresponding protons and carbons:

- C-2 to δ_C 130.24 ppm due to its correlation with H-2.
- C-3 to δ_C 115.86 ppm due to its correlation with H-3.
- C-5 to δ_C 115.86 ppm due to its correlation with H-5.
- C-6 to δ_C 130.24 ppm due to its correlation with H-6.
- C-1‘ to δ_C 100.20 ppm due to its correlation with H-1‘.
- C-2‘ to δ_C 73.40 ppm due to its correlation with H-2‘.
- C-3‘ to δ_C 76.91 ppm due to its correlation with H-3‘.
- C-4‘ to δ_C 69.89 ppm due to its correlation with H-4‘.
- C-5‘ to δ_C 76.54 ppm due to its correlation with H-5‘.
- C-6‘ to δ_C 61.05 ppm due to its correlation with H_a -6‘ and H_b -6‘.

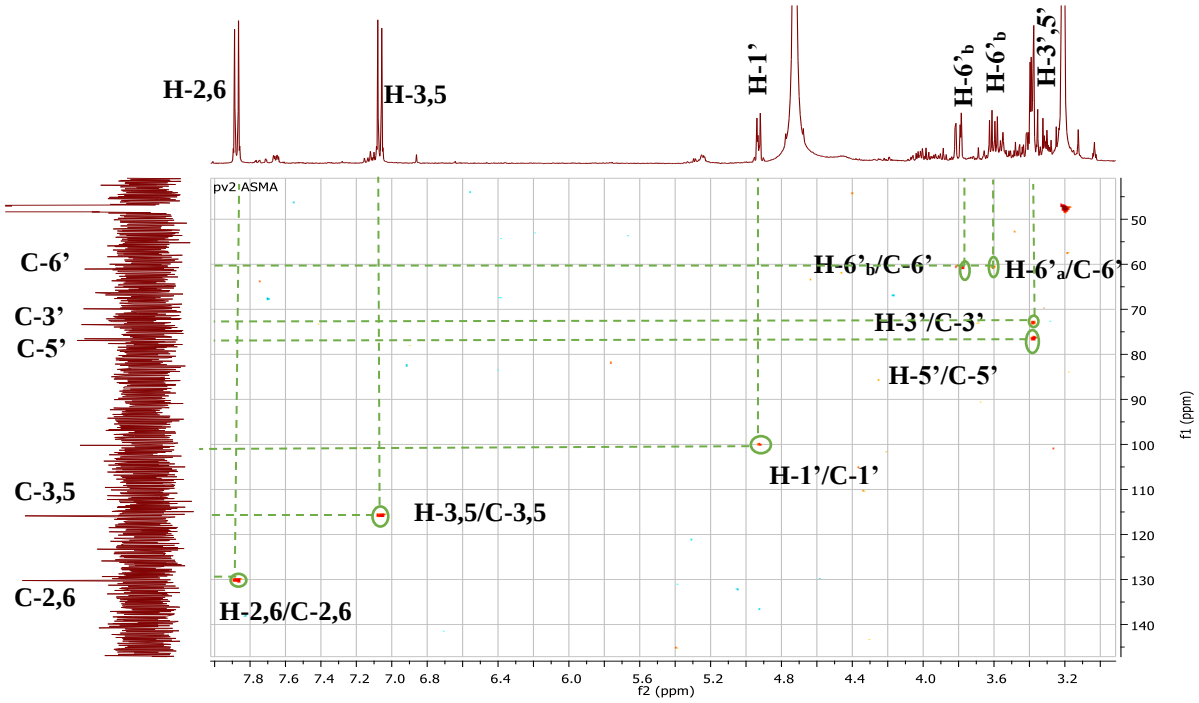


Figure.V.42. HSQC spectrum of spread out of compound PV2.

These correlations allow for the identification of a hexose. It's a glucose of Configuration β. Configuration assumed out from the coupling constant $J_{1'-2'}$ = 7.4 *Hz*.

The COSY spectrum (Figure.V.43) clearly highlights the expected correlations between:

- the aromatic protons:
  - H-2,6 (δ_H= 7.88; *J*=9 *Hz*; d) and the resonant proton H-3.5 at δ_H= 7.07; *J*= 9 *Hz*, in doublet form.
- The anomeric proton H-1' (δ_H= 4.93; *J* =7.6 *Hz*; d) and the resonant proton H-5' at δ_H= 3.39; *J* = 6.2; 2.1 *Hz*, in doublet of doublet form.
- The proton H-5' (δ_H= 3.39; J=6.2; 2.1 *Hz*; dd) and H-6'a proton (3.60; dd; *J* = 12.1; 5.6 *Hz*).
- The proton H_a -6' (δ_H = 12.1; *J* =5.6 *Hz* ; dd) and proton H_b -6' (3.80; dd; *J* = 12.0 ; 2.2 *Hz*).
- The proton H-2' (δ_H = 3.39; *J* = 6.2 ; 2.1 *Hz*; dd) and H-1' proton (δ_H= 4.93; *J*=7.6; d).

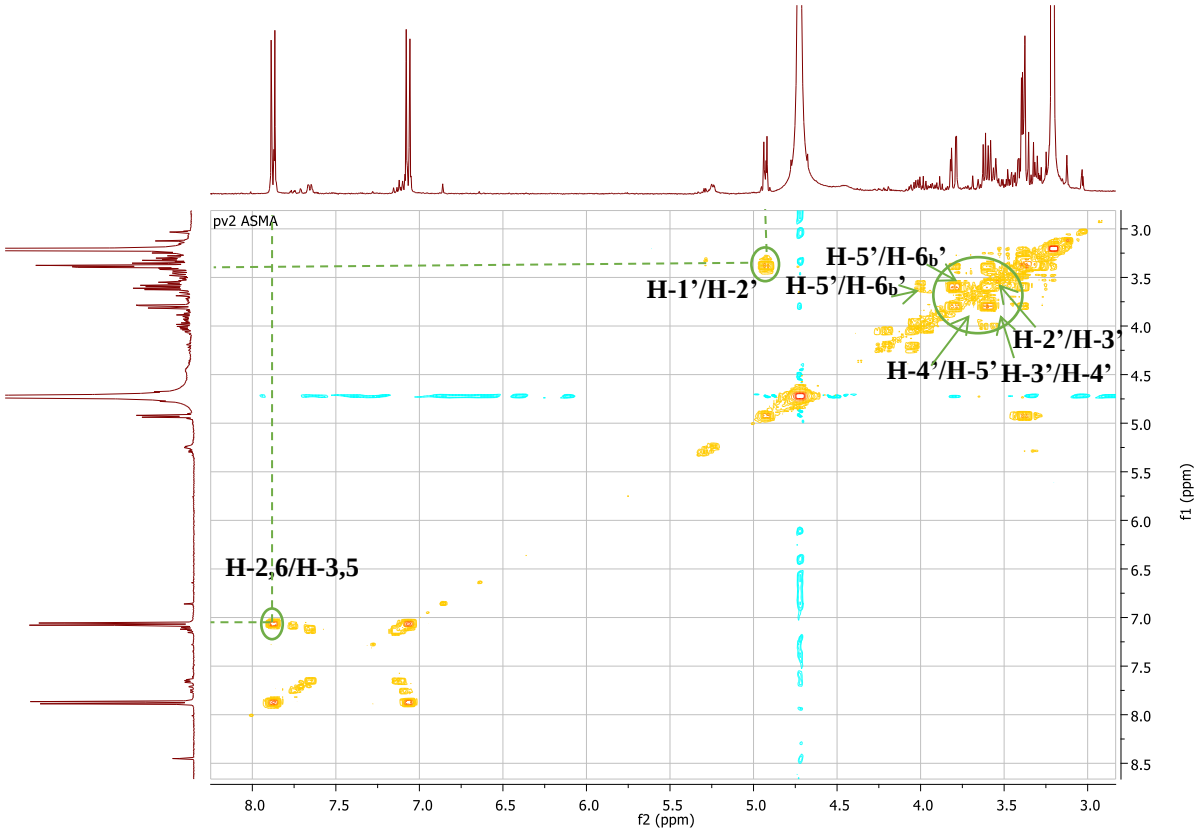


Figure.V.43. COSY spectrum of spread out of compound PV2.

The assignments of the remaining carbons were completed by the study of the correlation spectrum heteronuclear HMBC recorded in methanol, the HMBC correlation experiment makes it to identify the H/C correlation en ^{2}J and ^{3}J which are:

- The carbon C-1 at δ_C= 133.61 ppm correlates with H-2 and H-6 resonant at δ_H =7.88 ppm.
- The carbon C-4 at δ_C= 140.92 ppm shows a correlation spots with H-1'.
- The quaternary carbon C-7 at δ_C= 163.68 ppm shows a correlation spots with H-2 and H-6.

The positions for the carbonyl fonction and glucosyl moieties were confirmed by HMBC to be at C-1 and C-4, respectively (Figure.V.44).

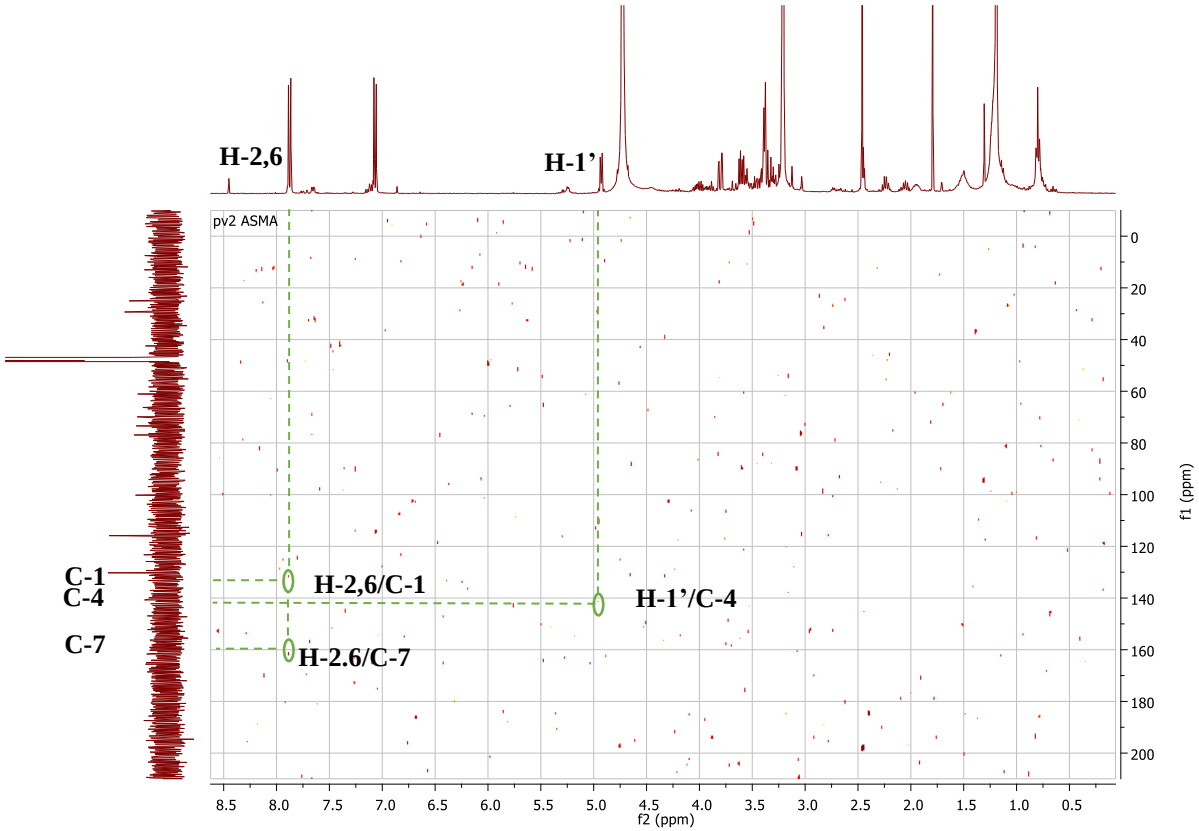


Figure.V.44. HMBC spectrum spread out of compound PV2.

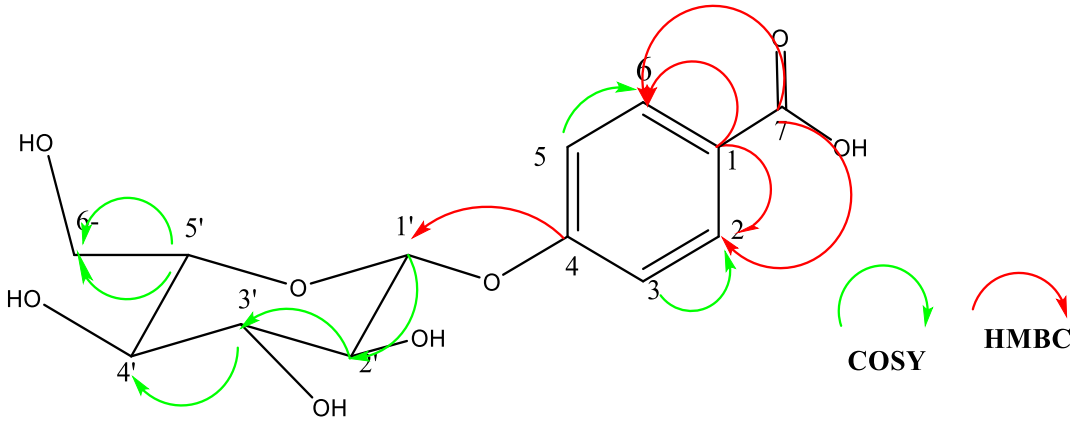


Figure.V.45. The HMBC and COSY correlations of compound PV2.

All these assignments by examining the ^{1}H-^{1}H COSY spectrum, HMBC spectrum and the HSQC spectrum confirm the previous assignments that were made by examining the NMR spectrum. All of this information leads to the structure of di-substitued benzoic acid and glucose moieties as shown in Figure.V.46.

the new benzoic acid derivative was identified as 4-O-β-D-glucopyranosyl benzoic acid by comparison of their NMR data with those reported in the literature [395-397]. Table.V.14 summarizes all NMR spectroscopy data.

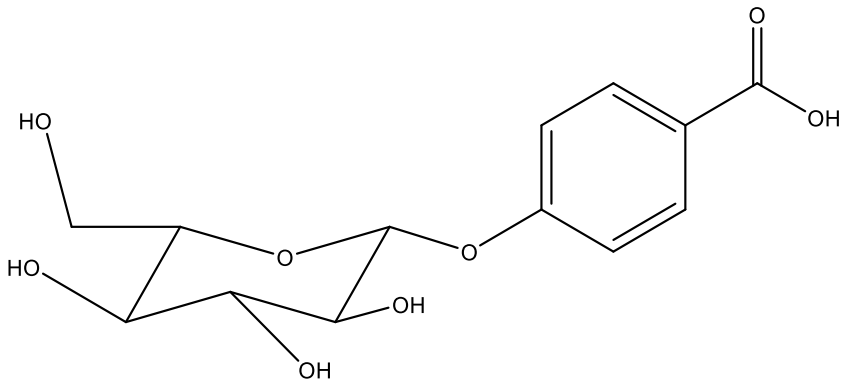


Figure.V.46. Structure of 4-*O*-β-D-glucopyranosyl benzoic acid.

Table.V.14. ^{13}C NMR and ^{1}H NMR spectral data of compound PV2 (400 M*Hz*, 125 M*Hz*, Methanol-d4).

Position	δ_C **(ppm)**	δ_H **(ppm)**	***Multiplicity***	***J(Hz)***
1	133.61	-	-	-
2	130.24	7.88	d	9.0
3	115.86	7.07	d	9.0
4	140.92	-	-	-
5	115.86	7.07	d	9.0
6	130.24	7.88	d	9.0
7	163.68	-	-	-
1'	100.20	4.93	d	7.6
2'	73.40	3.39	dd	6.2; 2.1
3'	76.91	3.31	dd	6.0; 3.1
4'	69.89	3.56	d	5.6
5'	76.54	3.39	dd	6.2; 2.1
6'	61.05	H_a 3.60	dd	12.1; 5.6
		H_b 3.80	dd	12.0; 2.2

Chapter VI : results and disscussion of Biological Activities

VI. Biological activities

VI.1. Total bioactive components assessment

In the current study, the amounts of bioactive components present in *A.campestris* and *F.bruguieri* extracts are shown from Table.VI.1 to Table.VI.4 .Total phenol and flavonoid contents were defined respectively in (µg GAE/mg) and (µg QE/mg) of extract by using calibration curve (Phenol: y = 0.0034x + 0.1044; R^2 = 0.9972, Flavonoid: y = 0.0048x; R^2 = 0.997).

Artemisa campestris L. extracts

For *A.campestris*, the highest successive contents of AcEAE for phenolic compounds and flavonoid were 527.333±0.61 µg GAE/mg and 203.4194±0.14 µg QE/mg, respectively. In contrast, when compared to the other extracts, AcEAE demonstrated that it contained a high concentration of phenolic and flavonoids components Figure.VI.1. It was followed by the AcDE, which showed high contents of phenolic compounds and flavonoids at 203.607±0.67 µg GAE/mg and 69.444±0.147 µg QE/mg, respectively.

Total phenolic content (TPC)

Table.VI.1. Total phenolics of different extracts of *A.campestris* L.

Extracts		Total phenolic compounds content (µg GAE/mg) *
AcME	*Artemisa campestris* Methanol Extract	135.37±1.35 [a]
AcPEE	*Artemisa campestris* Petroleum Ether Extract	30.27±0.33 [b]
AcDE	*Artemisa campestris* Dichloromethane Extract	203.60±0.67 [c]
AcEAE	*Artemisa campestris* Ethyl Acetate Extract	527.33±0.61 [d]
AcBE	*Artemisa campestris* Butanol Extract	130.27±0.33 [e]
AcAE	*Artemisa campestris* Aqueous Extract	141.64±1.52 [f]

Results are expressed as means ± SEM of three measures, Tukey test. Values with different letters in the same column are significantly different at $p<0.05$. *µg AGE/ mg: microgram Gallic acid equivalent/milligram of extract.

Total flavonoids content (TFC)

Table.VI.2. Total flavonoids contents of different extracts of *A.campestris* L.

	Extracts	Flavonoids content (µg QE/mg) *
AcME	*Artemisa campestris* Methanol Extract	61.59±0.58 [a]
AcPEE	*Artemisa campestris* Petroleum Ether Extract	65.69±0.29 [b]
AcDE	*Artemisa campestris* Dichloromethane Extract	69.44±1.47 [c]
AcEAE	*Artemisa campestris* Ethyl Acetate Extract	203.19±0.14 [d]
AcBE	*Artemisa campestris* Butanol Extract	66.87±0.29 [e]
AcAE	*Artemisa campestris* Aqueous Extract	63.4±0.14 [f]

Results are expressed as means ± SEM of three measures, Tukey test. Values with different letters in the same column are significantly different at $p<0.05$. * µg QE/ mg: microgram Quercetin equivalent/milligram of extract.

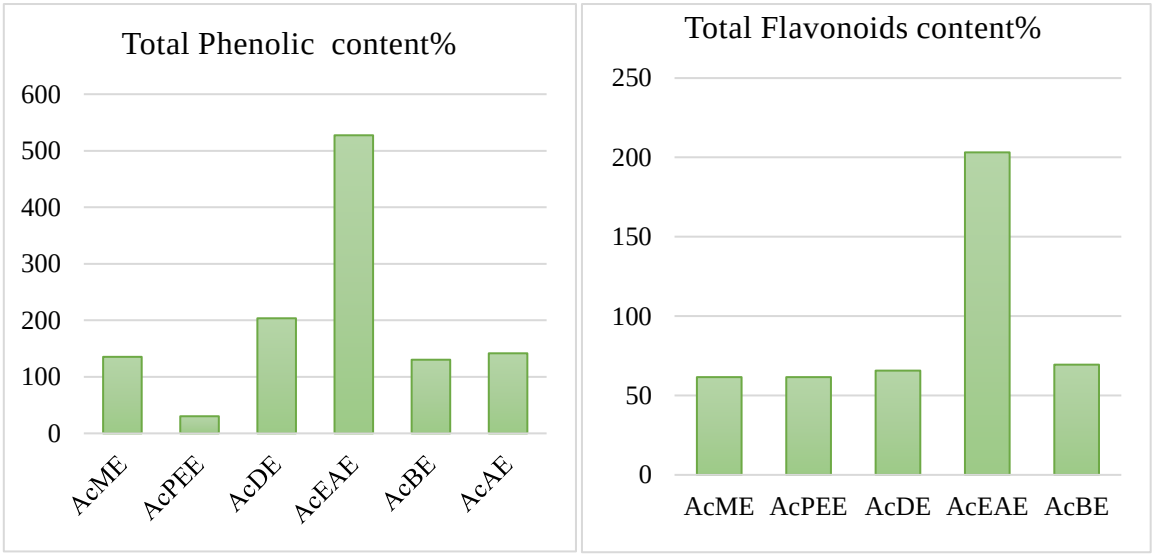


Figure.VI.1. Total phenolic and flavonoids contents of different extracts of *A.campestris.*

***Fagonia bruguieri* DC. Extracts**

Maximum phenolic and Flavonoid contents in *F.bruguieri* reached (444.49±0.84 µg GAE/mg) and (266.458±0.44 µg QE/mg) successively for FbBE. Whereas, the FbBE indicated that it was very rich in phenolic and flavonoid compounds relative to the other extracts, followed by FbEAE, which demonstrated a high content of phenolics, flavonoids with a value 178.902±0.74 µg GAE/mg and 116.25±0.88 µg QE/mg respectively Figure.VI.2. The FbDE also gave an interesting phenolic content with a 376.82±0.29 µg GAE/mg value.

Total phenolic content (TPC)

Table.VI.3. Total phenolics of contents of different extracts of *F.bruguieri* DC.

Extracts		Total phenolic compounds content (µg GAE/mg) *
FbPEE	*Fagonia bruguieri* Petroleum Ether Extract	57.62±0.44 [a]
FbDE	*Fagonia bruguieri* Dichloromethane Extract	376.82±0.29 [b]
FbEAE	*Fagonia bruguieri* Ethyl Acetate Extract	178.902±0.74 [c]
FbBE	*Fagonia bruguieri* Butanol Extract	444.49±0.84 [d]
FbAE	*Fagonia bruguieri* Aqueous Extract	147.52±1.060 [e]

Results are expressed as means ± SEM of three measures, Tukey test. Values with different letters in the same column are significantly different at p<0.05. *µg AGE/ mg: microgram Gallic acid equivalent/milligram of extract.

Total flavonoids content (TFC)

Table.VI.4. Total flavonoids contents of different extracts of *F.bruguieri* DC.

Extracts		Flavonoids content (µg QE/mg) *
FbPEE	*Fagonia bruguieri* Petroleum Ether Extract	53.611±0.24 [a]
FbDE	*Fagonia bruguieri* Dichloromethane Extract	91.73±0.73 [b]
FbEAE	*Fagonia bruguieri* Ethyl Acetate Extract	116.25±0.88 [c]
FbBE	*Fagonia bruguieri* Butanol Extract	266.458±0.44 [d]
FbAE	*Fagonia bruguieri* Aqueous Extract	72.638±0.14 [e]

Results are expressed as means ± SEM of three measures, Tukey test. Values with different letters in the same column are significantly different at p<0.05. * µg QE/ mg: microgram Quercetin equivalent/milligram of extract.

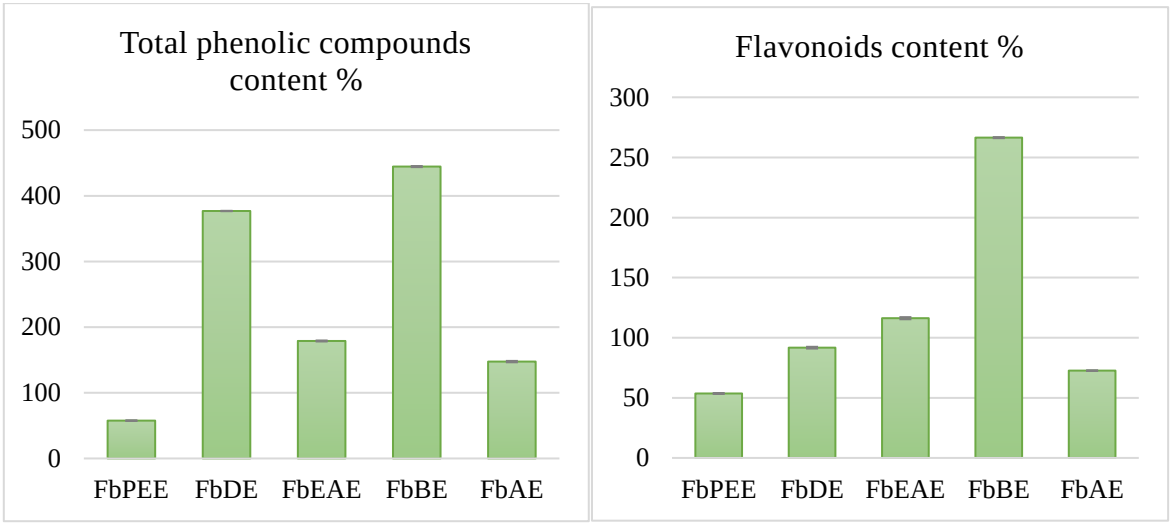


Figure.VI.2. Total phenolic and flavonoids contents of different extracts of *F.bruguieri* DC.

Discussion

Total phenolics and flavonoids represent a class of secondary metabolites widely spread in edible and inedible plants. The bioactivity of plant extracts is the common factor of these compounds [398]. They are of significant interest due to their staggering pharmacological influences on the human organism, including antioxidant; antimicrobial; antidiabetic; anti-inflammatory; anticancer, and numerous others [399].

The content of polyphenols in *A.campestris* extracts was determined by examining the extracts with solvents at different polarities. This quantitative analysis discovered various levels of polyphenols and flavonoids. The total phenolics and flavonoids produced by the AcEAE and AcDE were the greatest (Table.VI.1 and Table.VI.2). Our findings were significantly more remarkable when compared to the results obtained by Megdiche-Ksouri et al., 2015 and Djeridane et al., 2006 [400, 401].

The contents of the total phenolic flavonoids of *F.bruguieri* extracts are outlined in (Table.VI.3 and Table.VI.4). The FbBE extract registered the highest classes of phenolic and flavonoids (444.49±0.84 µg GAE/mg; 116.25±0.88 µg QE/mg, respectively). Compared to the literature, the TPC and TFC results of this study are more elevated than those obtained by Saleem *et al.*, (2019) in methanolic extract of *F.bruguieri* [215]. Other studies have reported higher findings in *F.bruguieri* and *F.Olivieri* by Alia Ahmed *et al.*, (2020) growing in Balochistan-Pakistan [402].

Our findings indicated that polar organic solvents were the most efficient in extracting phenolic flavonoid compounds. Several factors have been reported to make differences in phytochemical

content, including solvents (degrees of polarity) utilized, environmental and ecological conditions, extraction and quantification procedures, and geographic region.

VI.2. Antioxidant Activity

Phytochemicals are known for their complex nature, and the antioxidant activities of plant extracts cannot be assessed by a single method. The chemical nature of plant tissues creates a difference in antioxidant activity, so it is relatively difficult to identify each antioxidant component separately [403]. Table.VI.5 to Table.VI.18 resumed the antioxidant activity of *A.campestris* and *F.bruguieri* determined using the DPPH, ABTS, β-Carotene, Reducing Power, CUPRAC, GOR, and Phenanthroline tests. Figure.VI.3 to Figure.VI.16 illustrates the graphic of the percentage inhibition of the antioxidant activities of *A.campestris* and *F.bruguieri* to create inclusive results quickly to consider, more straightforward to comprehend and correspond with other compounds. The parameters $A_{0.5}$ and IC_{50} are employed to represent the antioxidant activity of the studied fractions. The more down value of $A_{0.5}$ or IC_{50} compromises that the fraction exhibited the most elevated antioxidant activity of a compound. Among the properties of polyphenols are their potent antioxidants, and the latter play a role as reducing agents; hydrogen donors; and singlet oxygen quenchers. They also may have a metallic chelating potential. Confirming the correlation between polyphenol concentration and antioxidant activity as reported by Rice-evans *et al.*, (1995) ([404].

VI.2.1. DPPH˙ Scavenging Activity

Artemisa campestris L. extracts

The DPPH test assesses a substance's antioxidant capacity by monitoring its ability to scavenge free radicals using the hydrogen proton transfer approach [405]. *A.campestris* extracts exhibited a significant capacity for scavenging the radical DPPH. The following decreasing effectiveness for both the standards and the plant extracts: BHA (IC_{50}: 5.73±0.41 µg/mL) > AcEAE (IC_{50}: 10,45±0,19 µg/mL) > BHT (IC_{50}: 22.32±1.19 µg/mL) > AcDE (IC_{50}: 73,82±1,98 µg/mL) > AcAE (IC_{50}: 126,09±1,63 µg/mL) > AcME (IC_{50}: 141,47±0,65 µg/mL) > AcBE (IC_{50}: 147,09±0,17 µg/mL). According to the results shown in Table.VI.5, AcEAE showed potent antioxidant activity in the DPPH method, in which the IC_{50} was relatively close to the IC_{50} stated by the BHA and more efficient compared with standard (BHT).

Table.VI.5. DPPH radical scavenging activity of different extracts of *A.campestris* L.

Extracts	% Inhibition in DPPH assay							IC_{50} µg/mL[a]
Concentration µg/mL	3.125	6.25	12.5	25	50	100	200	
AcME	1.01±0.65	1.22±0.33	3,94±0,49	35,77±1,34	75,69±0,12	82,78±1,34	84,82±0,37	141,47±0,65
AcPEE	10,96±0.53	11,01±0,18	11,73±0.70	13,37±0,69	13,62±1,15	18,08±1,58	24,29±0,89	>200
AcDE	10,80±1,60	14,19±0,55	20,18±0.70	24,85±1,15	39,15±0,89	60,43±0,62	87,59±0,44	73,82±1,98
AcEAE	22,90±0,09	35,15±0,98	57,71±1,07	79,55±0,53	90,21±0,09	90,41±0,09	92,77±0,15	10,45±0,19
AcBE	8,96±0,67	11,78±0,71	16,39±0.32	19,11±0,80	31,41±1,60	40,69±0,85	60,37±0,44	147,09±0,17
AcAE	NA	1,24±0,61	9,70±0,12	46,03±1,12	79,41±1,70	82,50±0,73	84,47±0,68	126,09±1,63
BHT*	11,69±1,88	22,21±1,30	37,12±1,80	52,63±2,70	56,02±0,53	83,60±0,23	87,28±0,26	22.32±1.19
BHA*	28,95±1,16	54,33±1,59	76,76±1,65	84,09±0,35	87,53±0,82	87,73±0,15	88,43±0,23	5.73±0.41

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

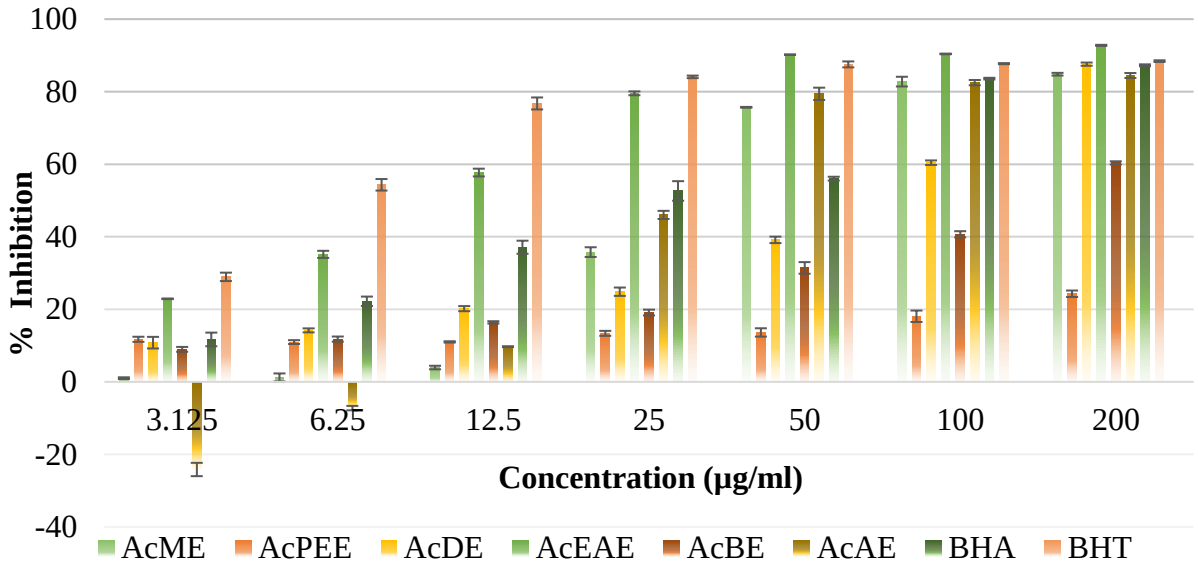


Figure.VI.3. Comparison of DPPH capacity between extracts of *A.campestris* L. and BHA and BHT.

Fagonia bruguieri DC. extracts

The DPPH scavenging effect of both standards and plants extracts decreasing in the order of BHA (IC_{50}: 6.14±0.41 µg/mL) > BHT (IC_{50}: 12.99±0.41 µg/mL) > α-Tocopherol (IC_{50}:

13.02±5,17 µg/mL) > FbBE (IC_{50}: 29,21±0,10 µg/mL) > FbEAE (IC_{50}: 92,05±0,38 µg/mL) > FbDE (IC_{50}: 128,65±0,83 µg/mL) > FbAE(IC_{50}: 493,53±6,52 µg/mL) respectively. Higher radical scavenging activity towards DPPH is evidenced by a lower IC_{50} value. According to the results presented in Table.VI.6. IC_{50} of BHA, BHT, α-tocopherol was found to be (6.14±0.41; 12.99±0.41; 13.02±5,17) *µ*g/mL successively whereas FbBE (29,21±0,10 *µ*g/mL) was the most active scavenger of DPPH radical than FbEAE (IC_{50} =92,05±0,38 *µ*g/mL).

Table.VI.6. DPPH radical scavenging activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in DPPH assay							IC_{50} µg/mL[a]
Concentration µg/Ml	12.5	25	50	100	200	400	800	
FbPEE	NA	0,72±0,35	3,47±1,15	5,91±0,89	10,64±0,18	15,83±0,77	26,61±0,95	>800
FbDE	12,98±0,11	19,85±0,09	30,88±0,70	46,45±0,79	64,91±0,31	78,08±0,54	81,84±0,26	128,65±0,83
FbEAE	11,71±0,88	20,05±0,00	34,49±0,18	52,65±0,15	74,88±0,18	86,42±0,31	87,39±0,35	92,05±0,38
FbBE	29,21±1,19	44,11±0,70	76,55±0,18	88,46±0,09	88,61±0,21	88,91±0,54	89,52±0,09	29,21±0,10
FbAE	NA	NA	NA	0,56±1,10	24,95±0,76	52,57±1,10	67,60±0,24	493,53±6,52
BHT*	49,09± 0,76	72,63± 2,06	88.73±0,89	94.00±0,31	94.97±0,08	95.38±0,41	95.02±0,23	12.99±0.41
BHA*	76,55± 0,48	79,89± 0,26	81,73±0,10	84,18±0,10	87,13±0,17	89,36±0,19	90,14±0,00	6.14±0.41
α-Tocopherol*	37,21±1,82	81,53±1,51	89,23±0,12	89,38±0,19	89,45±0,22	89,99±0,23	89,52±0,33	13.02±5,17

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

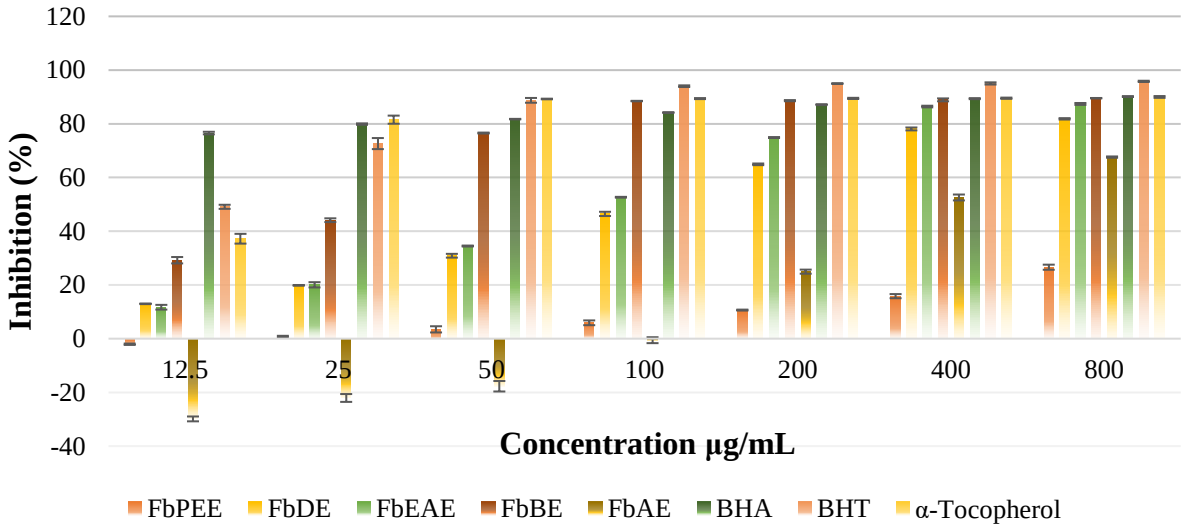


Figure.VI.4. Comparison of DPPH capacity between extracts of *F.bruguieri* DC. and BHA, BHT and α-Tocopherol.

VI.2.2. $ABTS^{\bullet+}$ Scavenging Activity

Artemisa campestris L. extracts

Another methodology for evaluating the anti-radical activity of *A.campestris* extracts is the ABTS radicals, which function similarly to the DPPH radicals by monitoring electron transfer in the medium. The $ABTS^{\bullet+}$ scavenging capacities among the various extracts were in the ensuing order: BHT (IC_{50}: 1.29±0.30 µg/mL) > BHA (IC_{50}: 1.81±0.10 µg/mL) > AcEAE (IC_{50}: 9,52±0,12 µg/mL) > AcDE (IC_{50}: 23,26±0,42 µg/mL) > AcME (IC_{50}: 26,04±0,39 µg/mL) > AcAE (IC_{50}: 58,67±0,58 µg/mL) > AcBE (IC_{50}: 66,52±0,94 µg/mL). The data was obtained from a study performed at the highest concentration (Table.VI.7). AcEAE demonstrated remarkable high capacity scavenging of $ABTS^{\bullet+}$ radical Figure.VI.5.

Table.VI.7. ABTS scavenging activity of different extracts of *A.campestris* L.

Extracts	**% Inhibition in ABTS assay**							**IC_{50} µg/mL** [a]
Concentration µg/Ml	3.125	6.25	12.5	25	50	100	200	
AcME	30,69±0,11	54,22±0,44	76,09±1,55	87,85±0,11	89,32±0,11	91,05±0,11	93,22±0,55	26,04±0,39
AcPEE	2,14±0,98	3,67±0,95	5,17±0,77	9,38±0,42	14,20±0,14	21,33±0,14	30,28±0,63	>200
AcDE	12,30±0,56	22,83±0,28	33,19±0,64	52,71±0,70	67,49±0,79	82,27±0,80	90,77±0,36	23,26±0,42
AcEAE	22,06±0,25	37,36±0,70	62,22±0,63	88,74±0,56	92,91±0,07	93,48±0,07	93,64±0,07	9,52±0,12
AcBE	8,01±0,07	11,45±0,63	20,52±0,70	31,77±0,35	46,88±0,60	61,90±0,43	77,81±0,77	66,52±0,94
AcAE	2,75±0,33	17,65±1,44	49,94±0,00	81,65±0,11	90,15±0,11	90,66±0,11	91,24±0,22	58,67±0,58
BHT*	59.22±0.59	78.55±3.43	90.36±0.00	92.18±1.27	93.37±0.86	94.87±0.87	96.68±0.39	1.29±0.30
BHA*	83.42±4.09	93.52±0.09	93.58±0.09	93.63±0.16	93.63±0.95	94.20±0.90	95.39±2.62	1.81±0.10

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

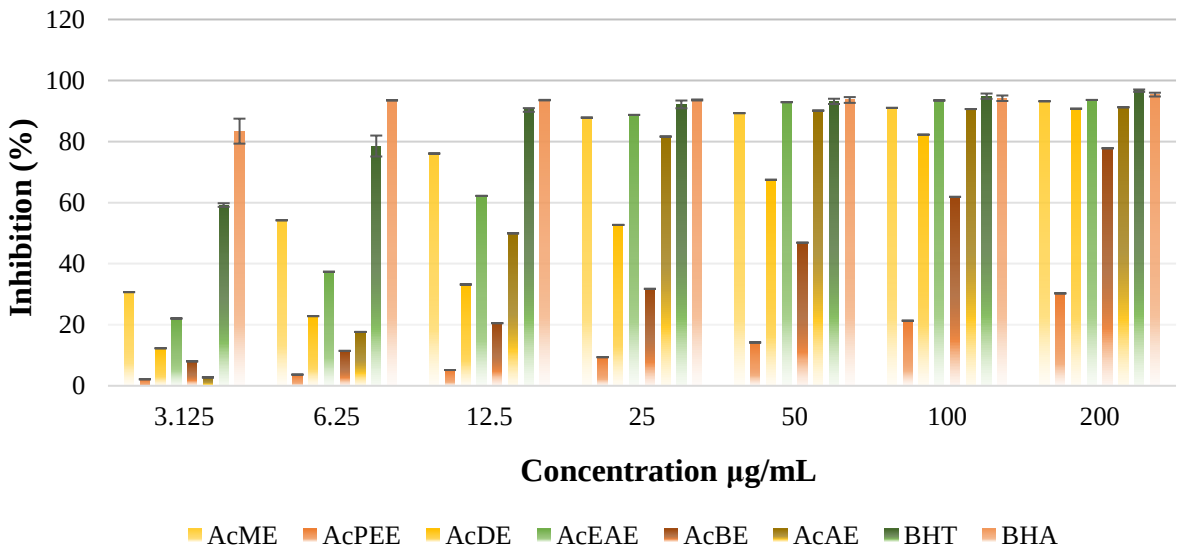


Figure.VI.5. Comparison of $ABTS^{\bullet+}$ capacity between extracts of *A.campestris* L. and BHA and BHT.

Fagonia bruguieri DC. extracts

As presented in Table.VI.8 and Figure VI.6, the parameter IC_{50} the inhibition concentration of ABTS, follows the increasing order: FbAE (IC_{50}= 200.38±1.23μg/mL) < FbDE (IC_{50}= 25.83±0.41 μg/mL) < FbBE (IC_{50}= 21.88±0.19μg/mL) < FbEAE (IC_{50}=14.03±0.47 μg/mL) < BHA (IC_{50}=1.81±0.10 μg/mL) < BHT (IC_{50}=1.29±0.30μg/mL) respectively. Similarly, According to the results FbEAE had the highest antioxydant activity.

Table.VI.8. ABTS scavenging activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in ABTS assay							IC_{50} µg/mL [a]
Concentration µg/Ml	12.5	25	50	100	200	400	800	
FbPEE	1,93±0,76	2,97±0,99	4,99±0,76	8,55±0,28	14,68±0,58	21,58±0,47	31,21±0,57	>800
FbDE	33,45±0,47	54,25±0,76	72,25±0,28	84,68±0,19	90,15±0,16	91,90±0,09	92,89±0,34	25.83±0.41
FbEAE	47,24±0,09	61,42±0,28	79,53±2,18	89,05±0,19	91,90±0,09	92,23±0,09	92,72±0,09	14.03±0.47
FbBE	28,03±0,66	66,29±0,38	89,05±0,09	91,19±0,09	91,63±0,28	91,68±0,09	91,90±0,09	21.88±0.19
FbAE	0.6,61±0,11	1,07±0,78	3,20±0,33	19,18±1,16	63,24±1,44	89,77±0,44	92,14±0,19	200.38±1.23
BHT*	69.21±0,40	78.23±1,34	88.12±1,28	88,76±3,07	90.85±1,74	90.95±0,51	96.68±0.39	1.29±0.30
BHA*	92.83±1,42	94.68±0,42	94.95±0,90	95.32±0,25	95.59±0,47	95.83±0,15	95,86±0,10	1.81±0.10

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

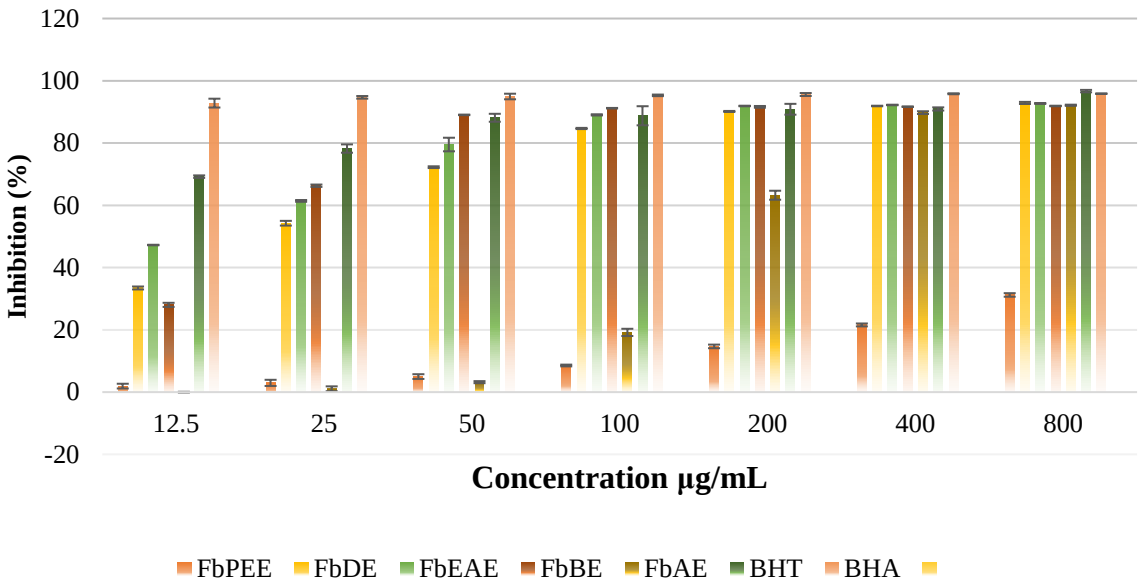


Figure.VI.6. Comparison of ABTS$^{\bullet+}$ capacity between extracts of *F.bruguieri* DC.and BHA and BHT.

VI.2.3. β-carotene-linoleic Acid Bleaching Activity

Artemisa campestris L. extracts

We used the β-carotene bleaching methodology to investigate the efficacy of *A.campestris* fractions in inhibiting lipid peroxidation. The outcomes in Table.VI.9 revealed that AcBE (IC_{50}: 183.87±1.30 µg/mL) had a moderate effect compared with BHT and BHA (IC_{50}: 1.05±0.01;0.90±0.02 µg/mL, respectively). In contrast, AcME, AcPEE, AcDE, AcEAE, and AcAE were inactive against -carotene bleaching.

Table.VI.9. β-carotene-linoleic acid bleaching activity of different extracts of *A.campestris* L.

Extracts	% Inhibition in β-carotene assay							IC_{50} µg/mL [a]
Concentration µg/Ml	3.125	6.25	12.5	25	50	100	200	
AcME	NA	NA	NA	NA	NA	NA	NA	>200
AcPEE	0,63±2,74	5,71±1,59	6,58±0,53	6,98±1,14	9,40±1,86	11,91±1,71	11,58±0,46	>200
AcDE	14,43±2,23	19,35±2,61	23,88±1, 66	25,79±0,17	28,88±0,52	29,26±0,34	32,06±0,35	>200
AcEAE	24,87±3,62	26,42 ±2,17	27,29±1,02	30,59±1,31	31,65±1,22	32,91±1,84	32,76±2,23	>200
AcBE	36,40±1,70	39,84±0,73	42,74±2,03	45,74±1,90	50,82±0,55	51,55±0,27	52,70±0,58	183,87±1,30
AcAE	34,80±1,39	40,30±2,18	41,39±2,56	42,41±2,12	42,42±0,88	43,53±1,97	45,53±2,70	>50

BHT*	81.14±0.84	86.0.9±1.04	87.52±4.24	91.67±0.52	94.11±0.42	94.41±0.32	95.28±3.25	1.05±0.01
BHA*	84.23±1.14	90.11±0.68	94.59±0.77	96.09±0.02	97.35±1.08	99.59±0.14	99.76±05	0.90±0.02

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

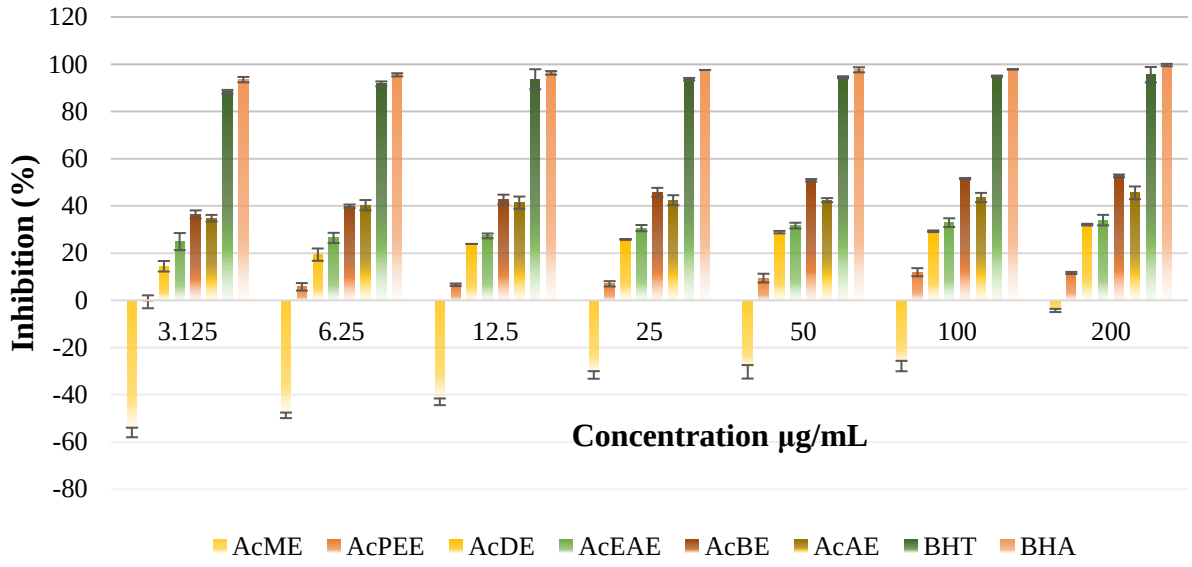


Figure.VI.7. Comparison of β-carotene capacity between extracts of *A.campestris* L. and BHA and BHT.

Fagonia bruguieri DC. extracts

The Activity antioxidant of the *F.bruguieri* DC. extracts was assessed by the beta-carotene bleaching, as illustrated in Figure.VI.8 and Table.VI.10. According to the parameter IC_{50} the inhibition concentration of β-carotene, follows the increasing order: FbPEE (IC_{50}=174.52±1.94 μg/mL) < FbDE (IC_{50}=66.99±2.39 μg/mL) < FbEAE (IC_{50}=34.55±2.85 μg/mL) < FbBE (IC_{50}=32.30±1.89 μg/mL) < BHA (IC_{50}=1.05±0.03 μg/mL) < BHT (IC_{50}=0.91±0.01 μg/mL) respectively. Interestingly, FbBE extract had the most substantial antioxidant effect.

Table.VI.10. β-carotene-linoleic acid bleaching activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in β-carotene assay							IC_{50} µg/mL [a]
Concentration µg/Ml	12.5	25	50	100	200	400	800	
FbPEE	26,68±0,50	30,98±0,37	32,85±1,39	48,70±1,06	50,58±0,27	56,58±1,34	57,49±0,99	174,52±1,94
FbDE	45,98±0,64	48,39±0,91	48,88±0,68	52,18±0,55	51,41±0,15	52,88±0,99	53,62±1,57	66.99±2.39
FbEAE	48,39±0,88	50,24±1,03	50,66±0,00	50,93±0,62	52,10±1,03	50,64±2,30	54,26±0,39	34.55±2.85
FbBE	44,96±1,58	47,82±0,65	52,91±0,72	55,95±1,01	56,88±1,50	57,77±0,17	59,15±1,41	32.30±1.89
FbAE	32,09±1,08	38,85±2,28	38,66±1,57	40,90±1,56	44,84±2,31	44,37±1,87	47,52±0,18	>800
BHT*	88.29±0.10	91.70±0.36	93.65±0.30	93.68±0.46	94.49±0.07	94.88±0.10	95.58±0.19	0.91±0.01
BHA*	93.48±0.44	95.52±0.33	96.34±0.55	97.56±0.19	97.64±2.22	97.85±0.32	99.66±0.52	1.05±0.03

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

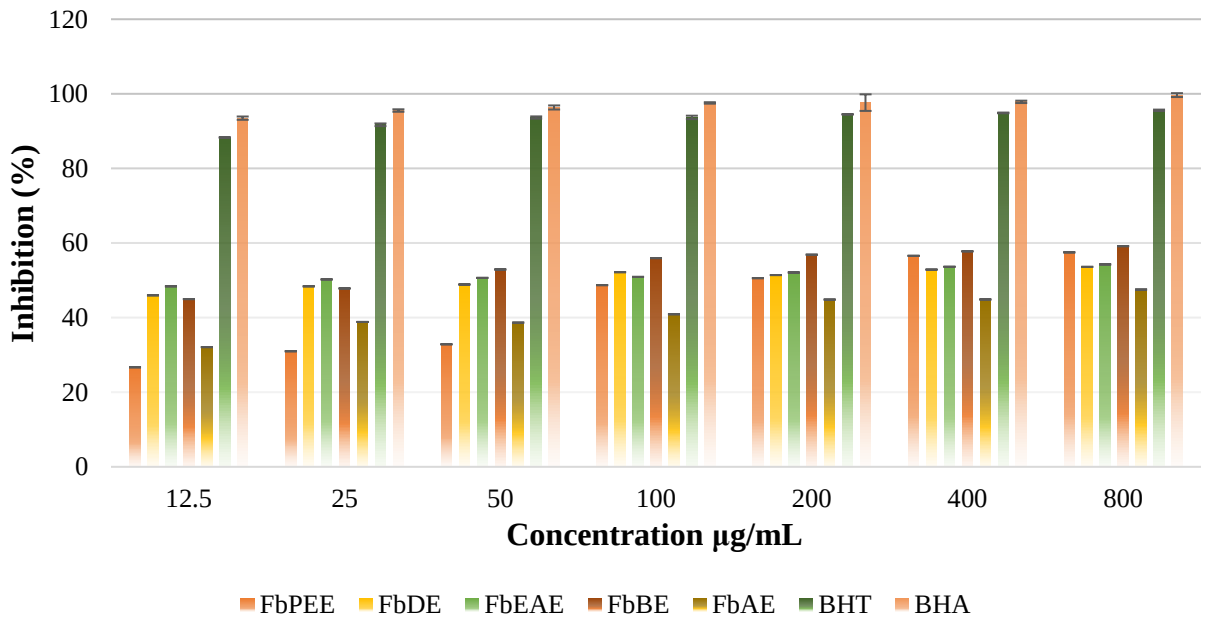


Figure.VI.8. Comparison of β-carotene capacity between extracts of *F.bruguieri* DC. and BHA and BHT.

VI.2.4. Cupric Reducing Antioxidant Capacity (CUPRAC) Activity

Artemisa campestris L. extracts

According to Table.VI.11, AcEAE exhibited high antioxidant activity in a CUPRAC assay, with an $A_{0.5}$ value of 9,94±0,21 µg/mL, which is very close to the value presented by BHT ($A_{0.5}$:

9.62±0.87 µg/mL). the parameter IC_{50} the inhibition concentration of CUPRAC, follows the increasing order: AcME ($A_{0.5}$ = 449,57±4,87 μg/mL) < AcAE ($A_{0.5}$ = 233,33±0,58 μg/mL) < AcBE ($A_{0.5}$ = 91,58±2,67μg/mL) < AcDE ($A_{0.5}$ =56,44±1,11 μg/mL) < BHT ($A_{0.5}$ =9.62±0.87 μg/mL) < BHA ($A_{0.5}$ =3.64±0.19 μg/mL) respectively (Figure.VI.9).

Table.VI.11. Cupric reducing antioxidant capacity activity of different extracts of *A.campestris* L.

Extracts	Absorbances in CUPRAC assay							$A_{0.50}$ µg/mL [a]
Concentration µg/Ml	3.125	6.25	12.5	25	50	100	200	
AcME	0,10±0,01	0,11±0,00	0,14±0,00	0,19±0,01	0,24±0,01	0,40±0,00	0,78±0,00	449,57±4,87
AcPEE	0,10±0,01	0,11±0,01	0,13±0,01	0,13±0,01	0,19±0,01	0,27±0,00	0,41±0,01	>200
AcDE	0,13±0,00	0,15±0,00	0,21±0,00	0,32±0,01	0,45±0,01	0,76±0,01	1,38±0,00	56,44±1,11
AcEAE	0,22±0,00	0,31±0,01	0,62±0,01	1,20±0,00	2,36±0,01	3,65±0,00	3,97±0,01	9,94±0,21
AcBE	0,12±0,02	0,13±0,00	0,15±0,01	0,22±0,01	0,37±0,01	0,54±0,00	0,98±0,01	91,58±2,67
AcAE	0,11±0,00	0,14±0,01	0,19±0,01	0,30±0,00	0,46±0,01	1,02±0,03	1,27±0,01	233,33±0,58
BHT*	0.19±0.01	0.33±0.04	0.66±0.07	1.03±0.07	1.48±0.09	2.04±0.14	2.32±0.28	9.62±0.87
BHA*	0.46±0.00	0.78±0.01	1.34±0.08	2.36±0.17	3.45±0.02	3.76±0.03	3.93±0.01	3.64±0.19

[a] **$A_{0.5}$**: the concentration at the 0.50 absorption. **$A_{0.5}$** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at p<0.05. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

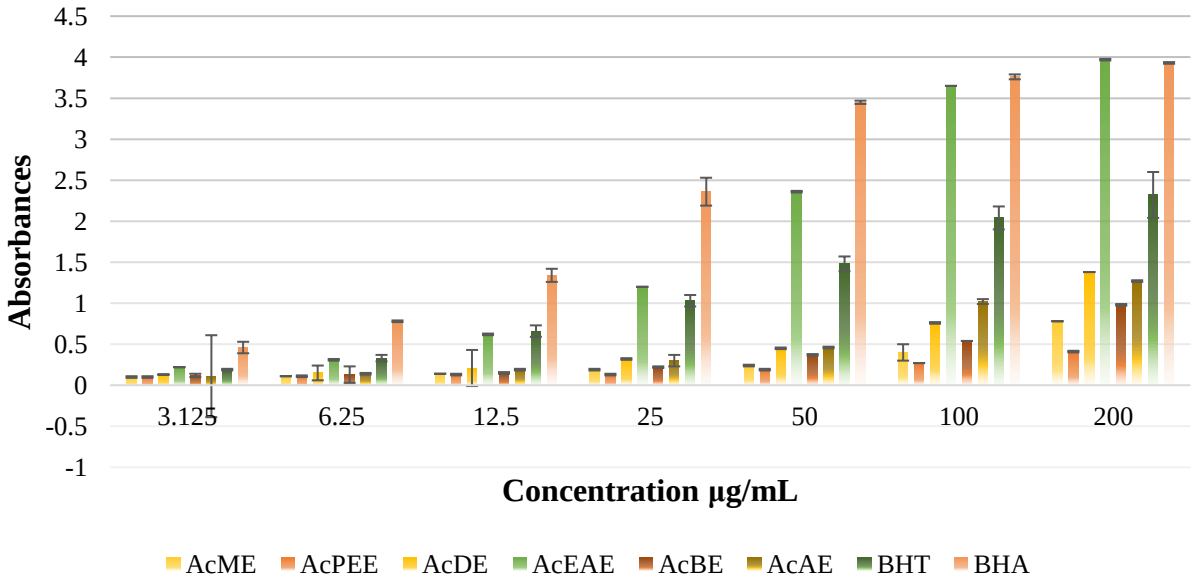


Figure.VI.9. Comparison of CUPRAC capacity between extracts of *A.campestris* L. and BHA and BHT.

***Fagonia bruguieri* DC. extracts**

The $A_{0.5}$ findings of each of the various extracts are presented in Table.VI.12 and Figure.VI.10. FbBE and FbEAE, FbDE presented the highest Antioxidant activity (CUPRAR) with ($A_{0.5}$=38.60±1.48 ; 47.52±0.79 ; 67.22±0.51 µg/mL, respectively) compared with BHA, BHT as the positive control (A0.5= 5.35±0.71 and 8.97±3.94 µg/mL respectively).

Table.VI.12. Cupric reducing antioxidant capacity activity of different extracts of *F.bruguieri* DC.

Extracts	Absorbances in CUPRAC assay							$A_{0.50}$ µg/mL [a]
Concentration µg/Ml	12.5	25	50	100	200	400	800	
FbPEE	0,11±0,00	0,13±0,00	0,15±0,01	0,23±0,03	0,28±0,00	0,34±0,00	0,49±0,01	>800
FbDE	0,18±0,01	0,24±0,01	0,45±0,01	0,74±0,01	1,41±0,00	2,22±0,01	2,93±0,01	67,22±0,51
FbEAE	0,20±0,00	0,29±0,00	0,56±0,01	0,83±0,01	1,61±0,00	2,46±0,00	2,93±0,02	47,52±0,79
FbBE	0,25±0,01	0,37±0,01	0,64±0,01	1,04±0,00	1,98±0,01	3,41±0,00	3,86±0,01	38,60±1,48
FbAE	0,13±0,00	0,18±0,00	0,22±0,00	0,29±0,00	0,46±0,01	0,78±0,01	1,30±0,01	367,33±1,53
BHT*	1.41±0.03	2.22±0.05	2.42±0.02	2.50±0.01	2.56±0.05	2.86±0.07	3.38±0.13	8.97±3.94
BHA*	1,12±0,05	1,95±0,31	3,14±0,46	3,35±0,42	3,58±0,20	3,77±0,19	3,92±0,13	5,35±0,71

[a] **$A_{0.5}$**: the concentration at the 0.50 absorption. **$A_{0.5}$** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

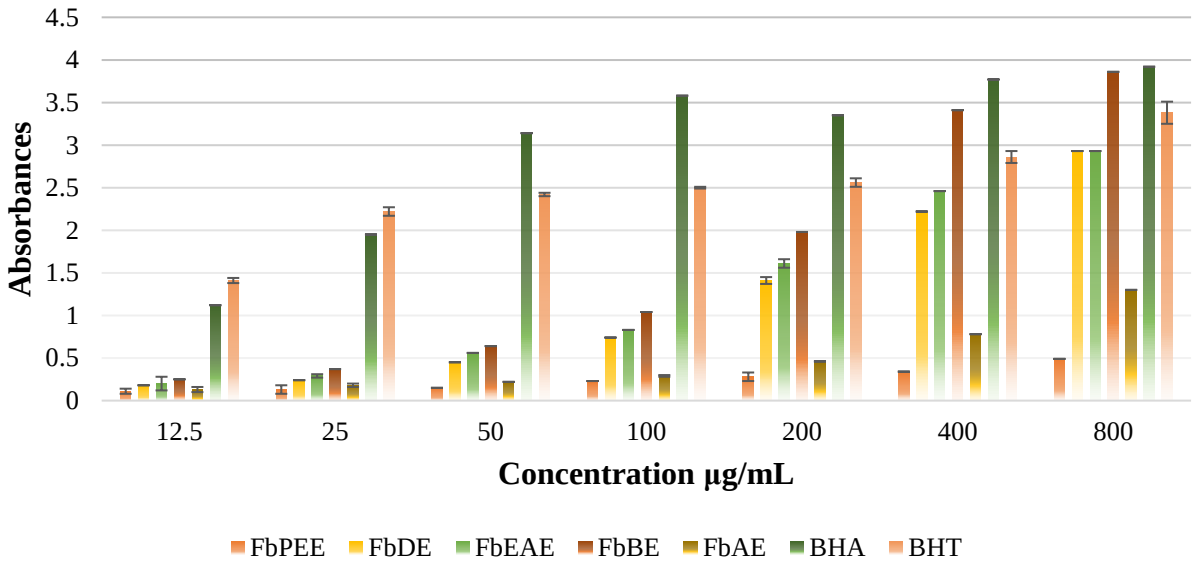


Figure.VI.10. Comparison of CUPRAC capacity between extracts of *F.bruguieri* DC. and BHA and BHT.

VI.2.5. Reducing Power Assay

Artemisa campestris L. extracts

The reducing power approach depends on the transition of Fe^{3+} to Fe^{2+} [406]. AcEAE was the most effective in reducing iron ions, with the lowest $A_{0.5}$ value ($A_{0.5}$: 16.05±0.16 µg/mL) (Table.VI.13). The effective concentrations at which the absorbance was 0.5 in decreasing order were as follows: BHA (8.41±0.67 µg/mL) > Ascorbic acid (9.01±1.46 µg/mL) > AcEAE (16,05±0,16 µg/mL) > AcME (54,00±0,33µg/mL) > AcDE (96,58±1,51 µg/mL) > AcAE (103,25±1,09 µg/mL).

Table.VI.13. Reducing power assay of different extracts of *A.campestris* L.

Extracts	**Absorbances in Reducing power assay**							**$A_{0.50}$ µg/mL** [a]
Concentration µg/Ml	0.781	1.562	3.125	6.25	12.5	25	50	
AcME	0,11±0,00	0,18±0,01	0,19±0,00	0,27±0,00	0,51±0,00	0,56±0,00	0,84±0,02	54,00±0,33
AcPEE	0,05±0,00	0,06±0,00	0,07±0,01	0,09±0,00	0,10±0,00	0,16±0,01	0,24±0,00	>200
AcDE	0,10±0,00	0,11±0,00	0,13±0,03	0,20±0,01	0,33±0,01	0,51±0,00	0,59±0,01	96,58±1,51
AcEAE	0,20±0,01	0,27±0,00	0,45±0,01	0,70±0,00	1,11±0,01	1,27±0,21	1,42±0,01	16,05±0,16
AcBE	0,07±0,00	0,08±0,00	0,11±0,01	0,13±0,01	0,18±0,00	0,31±0,01	0,43±0,01	>200
AcAE	0,10±0,01	0,13±0,00	0,16±0,01	0,19±0,00	0,33±0,00	0,53±0,01	0,72±0,01	103,25±1,09
BHT*	0.07±0.00	0.08±0.00	0.10±0.01	0.13±0.02	0.22±0.04	0.28±0.05	0.43±0.02	>200
BHA*	0.09±0.00	0.11±0.01	0.18±0.02	0.36±0.04	0.78±0.07	1.74±0.07	3.53±0.19	8.41±0.67
Ascorbic acid*	0.09±0.00	0.11±0.00	0.16±0.01	0.33±0.04	0.76±0.16	2.02±0.23	3.87±0.27	9.01±1.46

[a] **$A_{0.5}$**: the concentration at the 0.50 absorption. **$A_{0.5}$** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

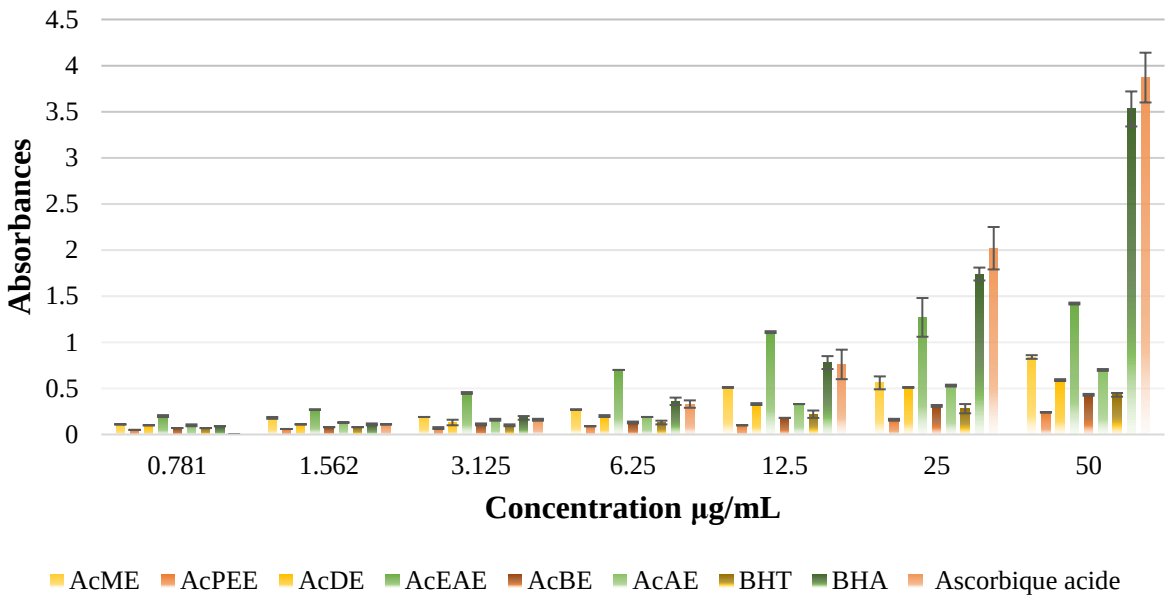


Figure.VI.11. Comparison of reducing power capacity between extracts of *A.campestris* L. and BHA, BHT and ascorbic acid.

Fagonia bruguieri DC. extracts

The effective concentrations at which the absorbance was 0.5 were in decreasing order: FbDE (105.58±0.88 µg/mL) > FbEAE (86.08±0.95 µg/mL) > FbAE (76±0.83 µg/mL) > FbBE (57.48±1.40 µg/mL) > α-tocopherol (34.93±2.38 µg/mL)> Ascorbic acid (6.77±1.15 µg/mL). According to the parameter $A_{0.5}$ (µg / mL). In terms of antioxidant capability, FbBE showed the most extraordinary reducing power (Table.VI.14, Figure.VI.12).

Table.VI.14. Reducing power assay of different extracts of *F.bruguieri* DC.

Extracts	Absorbances in Reducing power assay							$A_{0.50}$ µg/mL [a]
Concentration µg/Ml	3.125	6.25	12.5	25	50	100	200	
FbPEE	0,07±0,00	0,07±0,00	0,07±0,00	0,08±0,00	0,09±0,00	0,10±0,00	0,16±0,00	>200
FbDE	0,09±0,00	0,10±0,00	0,18±0,00	0,20±0,00	0,27±0,00	0,53±0,00	0,65±0,03	105.58±0.88
FbEAE	0,11±0,00	0,14±0,00	0,18±0,00	0,25±0,00	0,37±0,01	0,56±0,00	0,64±0,01	86.08±0.95
FbBE	0,09±0,00	0,10±0,00	0,18±0,01	0,27±0,00	0,45±0,00	0,78±0,02	1,01±0,00	57.48±1.40
FbAE	0,06±0,00	0,11±0,00	0,11±0,01	0,14±0,01	0,21±0,00	0,57±0,01	0,59±0,01	76±0.83
α-Tocopherol*	0.11±0,00	0.16±0.00	0,21±0,03	0,35±0,03	0,73±0,03	1,37±0,08	1,81±0,09	34.93±2.38
Ascorbic acid*	0,35±0,05	0,46±0,03	0,84±0,12	0,93±0,30	1,18±0,34	1,37±0,20	1,44±0,21	6.77±1.15

[a] **$A_{0.5}$**: the concentration at the 0.50 absorption. **$A_{0.5}$** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

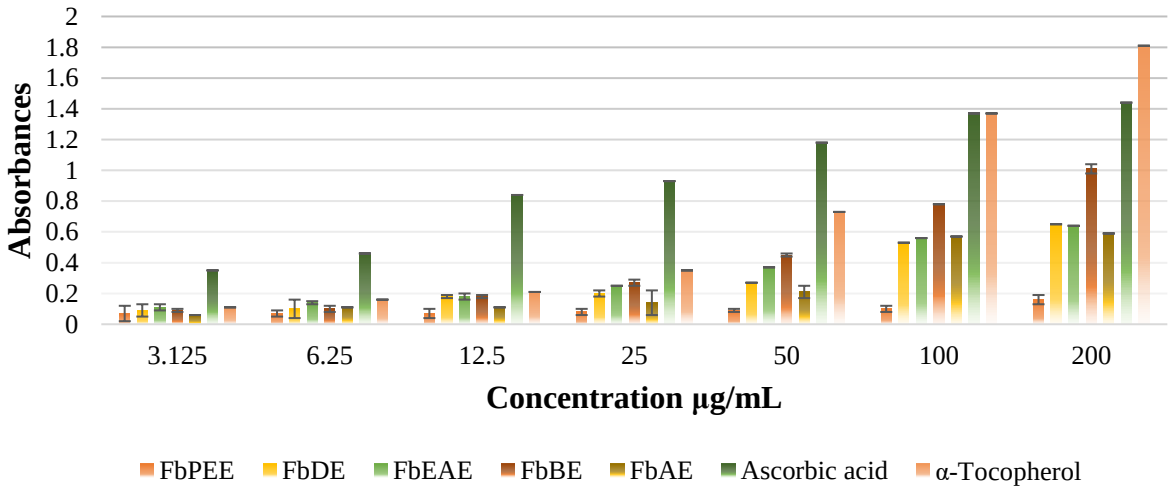


Figure.VI.12. Comparison of reducing power capacity between extracts of *F.bruguieri* DC. and alpha-tocopherol and ascorbic acid.

VI.2.6. Galvinoxyl (GOR) Scavenging Activity

Artemisa campestris L. extracts

Table.VI.15 demonstrated that AcEAE had high antioxydant activity for the GOR assay, where the IC_{50} (IC_{50}: 2.45±0.03 µg/mL) was more potent than the values shown by the standards, followed by BHT and BHA (IC_{50}: 3.32±0.18 ; 5.38±0.06 µg/mL, respectively), and the rest in the following order: AcDE (IC_{50}: 16.11±0.02 µg/mL) > AcBE (IC_{50} : 62.37±0.16 µg/mL) > AcME (IC_{50}: 68.21±0.13 µg/mL) > AcAE (IC_{50}: 152.18±0.47 µg/mL).

Table.VI.15. Galvinoxyl scavenging activity of different extracts of *A.campestris* L.

Extracts	% Inhibition in Galvinoxyl radical (GOR) scavenging assay							IC_{50} µg/mL [a]
Concentration µg/mL	1.5625	3.125	6.25	12.5	25	50	100	
AcME	6.54±0.37	13.63±0.35	50.55±0.56	66.61±0.52	69.31±0.33	70.63±0.04	71.11±0.14	68.21±0.13
AcPEE	1.91±0.22	2.89±0.14	6.36±0.23	9.40±0.31	11.86±0.03	14.82±0.25	23.67±0.08	>100
AcDE	14.26±0.39	22.54±0.11	38.87±0.09	75.15±0.02	75.56±0.03	77.50±0.09	78.23±0.10	16.11±0.02
AcEAE	68.82±1.14	75.03±0.25	78.28±0.11	81.43±0.05	84.47±0.08	86.83±0.06	87.87±0.05	2.45±0.03
AcBE	4.50±0.17	9.45±0.23	20.64±1.39	26.16±0.06	47.30±0.17	69.80±0.31	76.00±0.09	62.37±0.16
AcAE	NA	NA	8.60±0.33	41.66±0.19	63.35±0.29	66.27±0.54	73.48±1.86	152.18±0.47
BHT*	34,66±2.62	49,23±0.77	61,29±0.69	68,89±0,26	70,02±0.50	70,49±0,55	71,13±0,74	3.32±0,18
BHA*	25.99±2,56	39.15±0.88	54.16±0.27	65.02±1,26	70,19±0.51	70.32±0.65	70.60±0,10	5.38 ±0,06

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

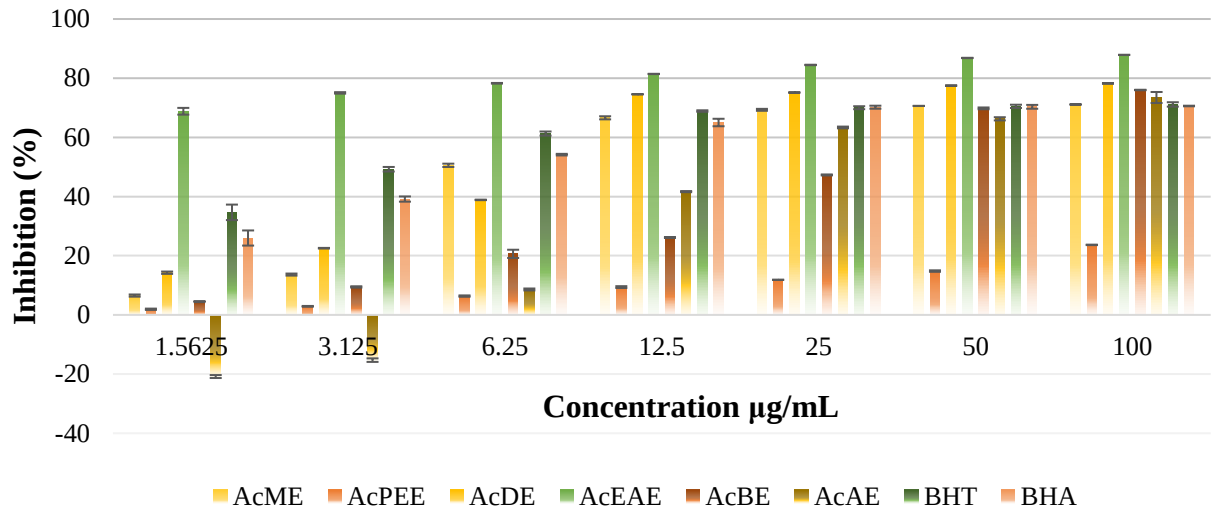


Figure.VI.13. Comparison of galvinoxyl radical (GOR) scavenging capacity between extracts of *A.campestris* L. and BHA and BHT.

***Fagonia bruguieri* DC. extracts**

Using the GOR method to measure antioxidant activity, Table.VI.16 and Figure Figure.VI.14 show that the following are in order from most to least antioxidant activity: FbAE (IC_{50}=478.67±3.27 μg/mL) < FbDE (IC_{50}=195.99±027 μg/mL) < FbEAE (IC_{50}=83.75±0.49 μg/mL) < FbBE (IC_{50}=33.39±0.11 μg/mL) < BHA (IC_{50}=5.38 ±0,06 μg/mL) < BHT (IC_{50}=3.32±0,18 μg/mL) respectively. In GOR scavenging activities, the FbBE exhibited the highest antioxidant capacity.

Table.VI.16. Galvinoxyl (GOR) scavenging activity of different extracts of *Fagonia bruguieri* DC.

Extracts	**% Inhibition in Galvinoxyl radical (GOR) scavenging assay**							**IC_{50} µg/mL** [a]
Concentration µg/mL	1.5625	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	
FbPEE	2,65±0,19	2,98±0,19	3,41±0,52	4,39±0,08	4,91±0,05	6,03±0,24	11,02±0,13	>100
FbDE	6,83±0,14	9,66±0,24	11,99±0,11	28,00±0,19	50,37±0,13	68,43±0,25	72,10±0,05	195.99±027
FbEAE	9,58±0,24	18,87±0,19	34,50±0,27	57,77±0,43	74,09±0,16	76,77±0,14	82,77±0,20	83.75±0.49
FbBE	18,28±0,21	41,28±0,19	70,89±0,19	72,35±0,03	74,94±0,02	75,93±0,03	76,21±0,25	33.39±0.11
FbAE	NA	NA	NA	1,22±0,23	19,11±0,14	63,82±0,14	64,88±1,06	478.67±3.27
BHT*	34,66±2.62	49,23±0.77	61,29±0.69	68,89±0,26	70,02±0.50	70,49±0,55	71,13±0,74	3.32±0,18
BHA*	25.99±2,56	39.15±0.88	54.16±0.27	65.02±1,26	70,19±0.51	70.32±0.65	70.60±0,10	5.38 ±0,06

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at p<0.05. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

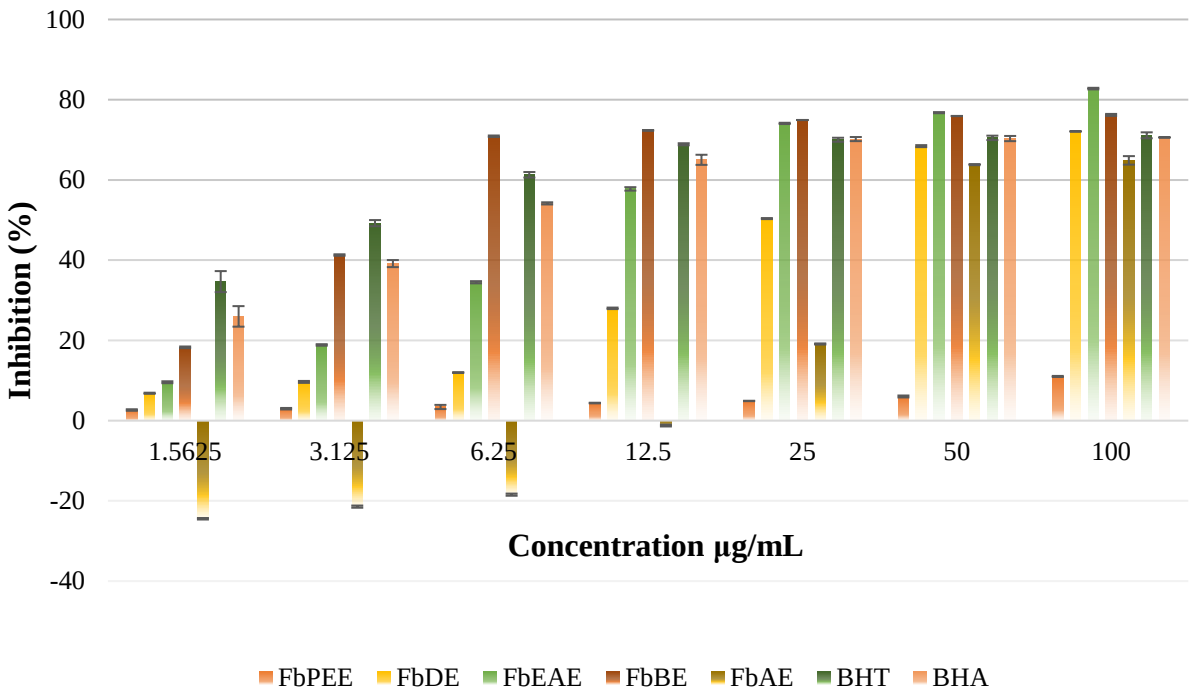


Figure.VI.14. Comparison of galvinoxyl radical (GOR) scavenging capacity between extracts of *F.bruguieri* DC. and BHA and BHT.

VI.2.7. O-Phenanthroline Activity

Artemisa campestris L. extracts

Based on the o-phenanthroline method, Table.VI.17 shows that *A.campestris* is able to lower the amount of the Fe^{3+} ion [338]. Likewise, AcEAE displayed the greatest chelating activity ($A_{0.5}$: 7.12±0.15 µg/mL). According to the results of the O-phenanthroline assay, the order of decreasing antioxidant activity is as follows: BHA > BHT > AcEAE > AcDE > AcBE > AcAE (Figure.VI.15).

Table.VI.17. O-Phenanthroline Activity of different extracts of *A.campestris* L.

Extracts	Absorbances in phenanthroline assay							$A_{0.5}$ µg/mL [a]
Concentration µg/mL	0.78125	1.5625	3.125	6.25	12.5	25	50	
AcME	0,23±0,00	0,24±0,01	0,25±0,01	0,26±0,01	0,27±0,01	0,28±0,01	0,29±0,00	>50
AcPEE	0,22±0,00	0,24±0,00	0,24±0,00	0,25±0,00	0,27±0,00	0,28±0,00	0,33±0,00	>50
AcDE	0,26±0,00	0,31±0,00	0,35±0,02	0,48±0,00	0,65±0,01	1,02±0,02	1,47±0,01	31,95±0,22
AcEAE	0,34±0,00	0,47±0.03	0,72±0,02	1,38±0,00	1,98±0,01	3,85±0,00	4,25±0,02	7,12±0,15
AcBE	0,28±0,00	0,29±0,01	0,38±0,01	0,44±0,00	0,62±0,00	0,88±0,01	1,42±0,00	35,56±1,51
AcAE	0,24±0,01	0,26±0,01	0,28±0,01	0,30±0,00	0,37±0,01	0,52±0,00	0,62±0,01	136,67±1,53
BHT*	0,47±0,01	0,47±0,01	0,53±0,03	1,23±0,02	1,84±0,01	3,48±0,03	4,84±0,01	2,24±0,17
BHA*	0,49±0,01	0,59±0,01	0,73±0,02	0,93±0,01	1,25±0,04	2,10±0,05	4,89±0,06	0,93±0,07

[a] $A_{0.5}$: the concentration at the 0.50 absorption. $A_{0.5}$ values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

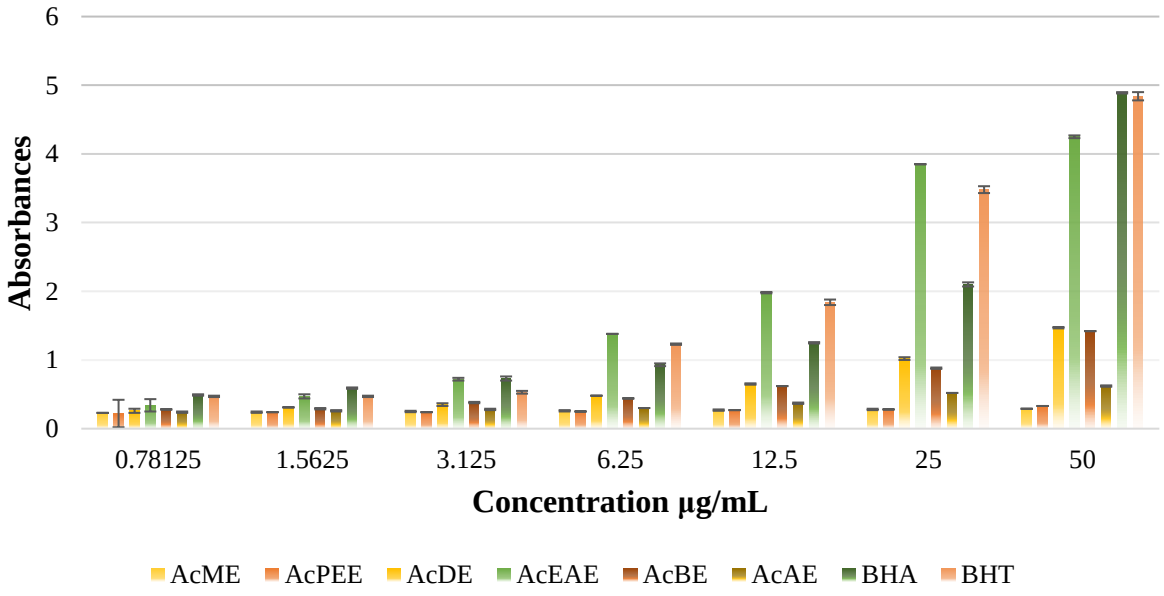


Figure.VI.15. Comparison of O-Phenanthroline capacity between extracts of *A.campestris* L. and BHA and BHT.

Fagonia bruguieri DC. extracts

Table.VI.18 shows *F.bruguieri's* capacity to reduce Fe^{3+} ions via O-Phenanthroline. The antioxidant activity measured by the o-phenanthroline assay is listed below in decreasing order: BHA ($A_{0.5}$: 0,93±0,07 µg/mL) > BHT ($A_{0.5}$: 2,24±0,17 µg/mL) > FbBE ($A_{0.5}$: 20,52±1.72

µg/mL) > FbDE ($A_{0.5}$: 21,14±1,76 µg/mL) > FbEAE ($A_{0.5}$: 21.91±1.97 µg/mL) > FbAE ($A_{0.5}$: 74,00±1,33 µg/mL) respectively. Even when compared with BHA and BHT standards, the chelation activity level shown by FbBE ($A_{0.5}$: 20,52±1.72 µg/mL) was the highest achievable (Figure.VI.16).

Table.VI.18. O-Phenanthroline Activity of different extracts of *F.bruguieri* DC.

Extracts	**Absorbances in phenanthroline assay**							
Concentration µg/mL	0.78125	1.5625	3.125	6.25	12.5	25	50	**$A_{0.5}$ µg/mL** [a]
FbPEE	0,24±0,00	0,24±0,00	0,25±0,01	0,24±0,00	0,25±0,01	0,26±0,01	0,26±0,01	>50
FbDE	0,29±0,01	0,32±0,00	0,42±0,03	0,53±0,02	0,89±0,02	1,13±0,14	1,61±0,34	21,14±1,76
FbEAE	0,29±0,00	0,33±0,01	0,38±0,01	0,59±0,01	0,79±0,03	1,42±0,09	2,75±0,34	21.91±1.97
FbBE	0,28±0,00	0,33±0,01	0,42±0,01	0,58±0,01	0,82±0,05	1,36±0,05	2,42±0,27	20,52±1.72
FbAE	0,28±0,01	0,28±0,00	0,29±0,03	0,36±0,01	0,47±0,00	0,63±0,03	1,02±0,08	74,00±1,33
BHT*	0,47±0,01	0,47±0,01	0,53±0,03	1,23±0,02	1,84±0,01	3,48±0,03	4,84±0,01	2,24±0,17
BHA*	0,49±0,01	0,59±0,01	0,73±0,02	0,93±0,01	1,25±0,04	2,10±0,05	4,89±0,06	0,93±0,07

[a] **$A_{0.5}$**: the concentration at the 0.50 absorption. **$A_{0.5}$** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

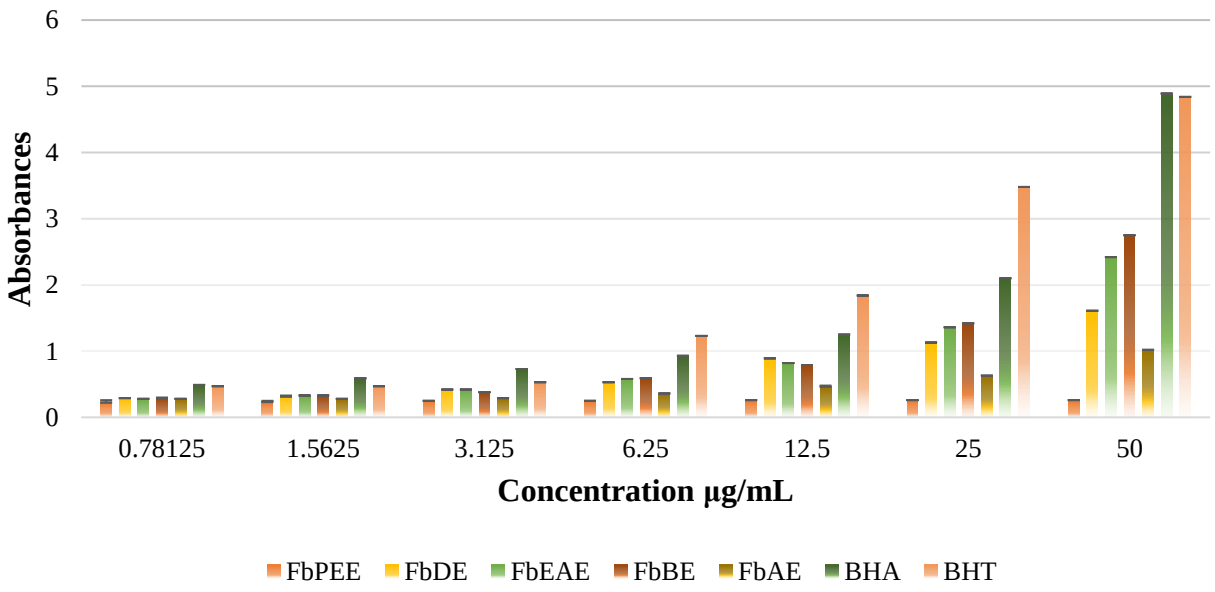


Figure.VI.16. Comparison of O-Phenanthroline capacity between extracts of *F.bruguieri* DC. and BHA and BHT .

Discussion

Different mechanisms considering the multifaceted in the antioxidant activity, these assays can roughly be divided into different mechanisms, those that evaluate lipid peroxidation radical scavenging, binding of transition metal ion catalysts, hydrogen abstraction, chain initiation prevention, and reducing capacity [401]. Accordingly, we combined a variety of methods assays complementary to evaluate the antioxidant effect.

Due to the high levels of phenolics and flavonoids in AcEAE, the above techniques show that this extract has a strong ability to scavenge free radicals, which gives AcEAE its distinctive properties. In fact, phenolic compounds, which include hydroxyl groups in their structure that enable hydrogen or electrons to be transferred to a reactive molecule, are considered the most potent antioxidants.

Similarly, for *F.bruguieri*, the results obtained by the overhead strategies demonstrated that FbBE and FbEAE extracts have a potential antioxidant activity due to the presence of phenolics and flavonoids in elevated amounts. Subsequently, the close relationship between antioxidant activity and phenol content became widely comprehended [401].

DPPH and ABTS are synthetic free radicals frequently employed to assess plant extracts and pure compounds for their potential anti-radical effects [407]. Both the AcEAE and the FbEAE have shown significant antioxidant activity against the radical DPPH as well as the radical ABTS. Electrons' reception leads to a change from a purple colour ($DPPH^{-}$) to a yellow colour (DPPH-H), which reveals properties measured at 515 nm. The blue-green chromophore known as 2.2'-azinobis-3 ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) is subjected to the ABTS decolourization procedure to assess which it is colour is changed as a result of the addition of an antioxidant.

The outcomes obtained for *A.campestris* appear to be in better correlation with the findings that Akrout *et al.,* (2009) reported in *A.campestris* [408], using infusion and ethanol 50%. On the other hand, the findings acquired using DPPH are similar to those reported by Megdich *et al*., (2015), which used the decoction of *A.campestris* from southern Tunisia [400].

The antioxidant activity of *F.bruiguieri* extracts, notably FbEAE and FbBE, was significant, with DPPH and ABTS findings greater than those reported by Satpute *et al*., 2012, for the alcoholic and acetone extract of *Fagonia arabica* growing in India [409].

Recently, Saleem *et al*., (2019) demonstrated that the methanol fraction of *F. bruguieri* exhibited powerful activity with an IC_{50} value of 32.733 mM [215]. Furthermore, El-Amier and Aisha (2019) reported high DPPH antioxidant activity of the *F. mollis* species (IC_{50} = 0.74 mg/mL) was followed by *F. arabica* and *F. cretica* (IC_{50} = 0.76 and 0.82 mg/mL, respectively) [410].

The antioxidant activities of AcBE were the only ones that moderately reduced the oxidation of β -carotene due to free radicals generated as a by-product of linoleic acid oxidation in *A.campestris*. The capability of acting on lipid peroxidation products is a feature that gives a significant benefit and is thus very important [330]. Compared to the findings published in the previous studies, ours show that AcBE is astoundingly effective in β–carotene [125].

The beta-carotene and linoleic acid system are now being reported for *F.bruguieri* for the first time. The lack of antioxidants is responsible for the fast discolouration of beta-carotene. As observed, our results showed that these obtained IC_{50} values of FbBE and FbEAE presented a powerful protector on β-carotene. This may be due to various antioxidants; those neutralizing radicals peroxyl generated the free radical of linoleate and other compounds in the system. The results of the present study demonstrated that FbBE and FbEAE exhibited a significant effect on beta-carotene, which delayed and reduced lipoperoxidation and, consequently, the oxidation of beta-carotene. These results could be explained that these compounds are drawn to solvents with high polarity.

AcEAE and FbBE showed exceptional effectiveness in reducing iron and copper ion, which may be due to their ability to rebuild oxidized molecules and therefore broke the hydrogen subtract. The O-phenanthroline test can be assessed by the reduction of Fe(III), resulting in a bright red colour. This applied methodology has been exposed to an oversized field of relevancy and applied to fruit juices, vinegar, and wines [411]. The AcEAE and FbBE were identified to have the most outstanding efficiency, which aids in understanding why the highlights of the Fe^{3+} ions reducing donor potency were found in these fractions. For the first time, we evaluated antioxidant activity utilizing CUPRAC and O-Phenanthroline for *A.campestris* and *F.bruguieri* extracts.

Table.VI.13 shows the ferric reducing power ($A_{0.5}$) of *A.campestris* extracts. It explains the rate at which $Fe^{3+}(CN)_6$ is reduced to $Fe^{2+}(CN)_6$ by direct electron donation. Then the complexes of ferric ferrous from the reaction mixture were accompanied by a high absorption at 700 nm [412]. Comparable to that reported by Boulanouar *et al.*, (2013), *A.campestris* oil has a strong

reducing power activity [101], with an IC_{50} of 0.305±0.006 mg/mL^{-1}, which is higher than our findings.

Saleem *et al.*, (2019), claimed that the methanol extract of *F.bruguieri* utilizing total reducing power assay (TRP) was lower than our results [215]. However, FbBE also demonstrates a remarkable capacity to donate an electron for *F.bruguieri.*

Galvinoxyl free radical scavenging experiment is a novel technique applied for the first time to *A.campestris* and *F.bruguieri*. Similarly, Both AcEAE and FbBE exhibit strong antioxidant activity, which is higher compared to the standards. Consequently, when hydrogen donors interact with the free radical Gox•, it is reduced to GoxH [336]. This reduction was monitored spectrophotometrically as a disappearance of colour when they were quenched.

VI.2.8. Antioxidant Activity Evaluation via Electrochemical Method

VI.2.8.1. $O_2^{\bullet-}$ free radical scavenging assay

Electrochemical tests are a relatively new approach that has been selected to determine the extract's antioxidant capacity. A sweeping examination of the relevant literature revealed that no previous investigation focused on the antioxidant activity of the cyclic voltammetry test of *A. campestris* and *F. bruguieri* extracts. In the current study, the antioxidant activity of *A. campestris* and *F. bruguieri* extracts was evaluated based on their ability to scavenge $O_2^{\bullet-}$ radicals By electrochemical method.

Voltamograms were obtained in the absence of an antioxidant in order to determine the values of the i_{pa0} current, which is used to determine the anodic $O_2^{\bullet-}$ oxidation current. This current is directly related to the concentration of $O_2^{\bullet-}$ on the electrode surface, the oxygen solubility in the DMF, and the selected experimental parameters.

The Voltammogram and a calibration curve of the Ascorbic Acid (standard antioxidant) were recorded to assess the antioxidant's ability to research when it reacts against the form of $O_2^{\bullet-}$. the first step is to achieve various concentrations at each injection into the cell for the purpose of plotting their calibration curve; the standard antioxidant is dissolved in DMF in varying concentrations (Figure.VI.17).

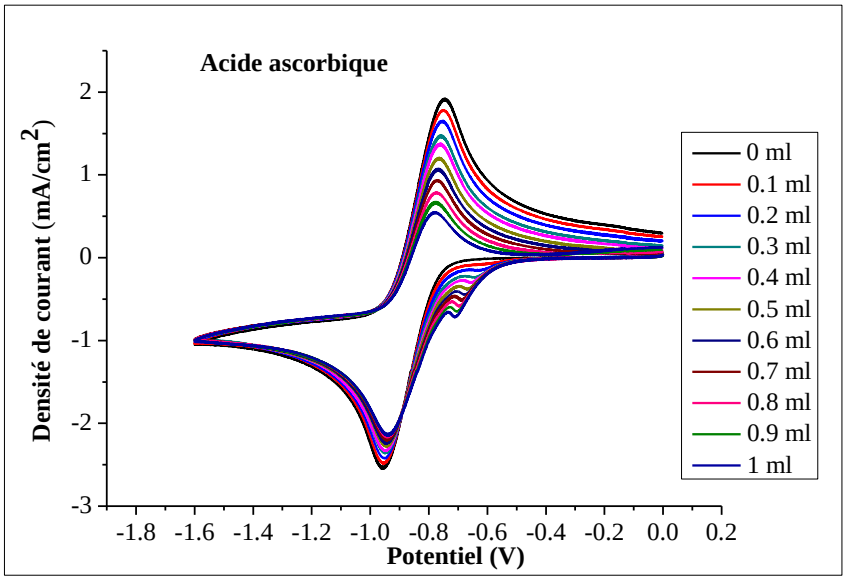
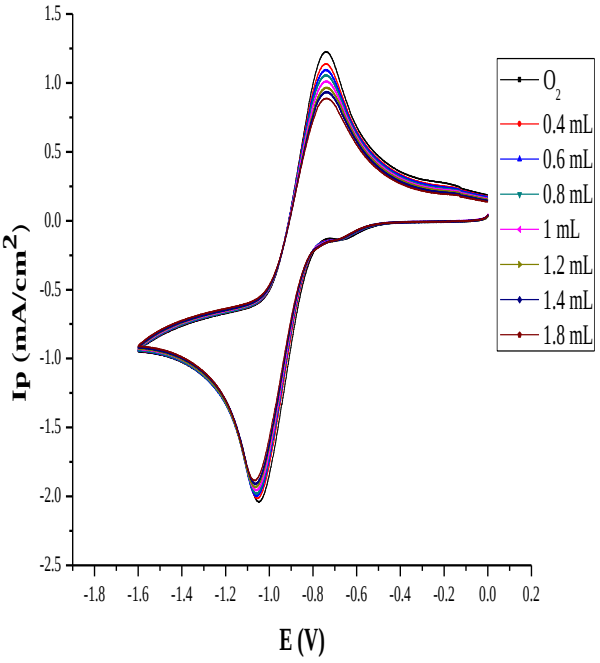


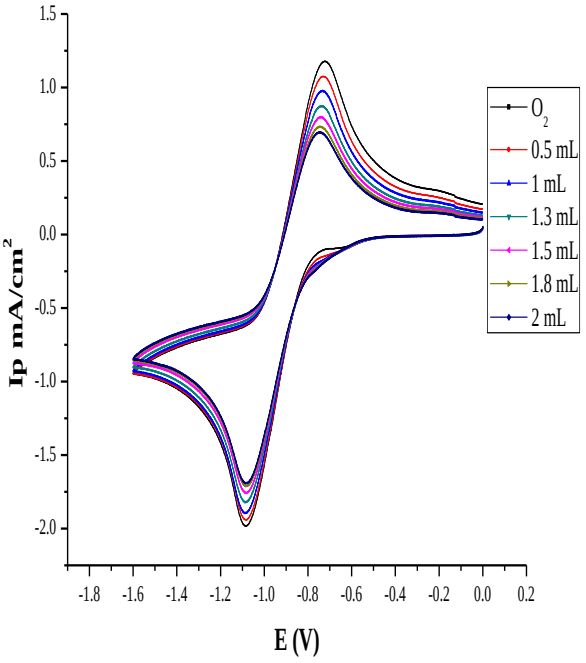
Figure.VI.17. Cyclic voltammograms of an oxygen-saturated cell in the presence of various concentrations of Ascorbic acid , recorded at 0.1V s^{-1} potential sweep rate on GC disk electrode at 298K in DMF/nTBuNBF$_4$ 0.1 M.

All voltammograms recorded to reduce the superoxide anion radical (Figure.VI.18 and Figure.VI.19) in the presence of samples of different extracts of *A.campestris* and *F.bruguieri* to assess antioxidant activity for free radical scavenging reveal obvious oxidation and reduction peaks. Sample extracts decreased anode current peaks but did not affect cathode current peaks.

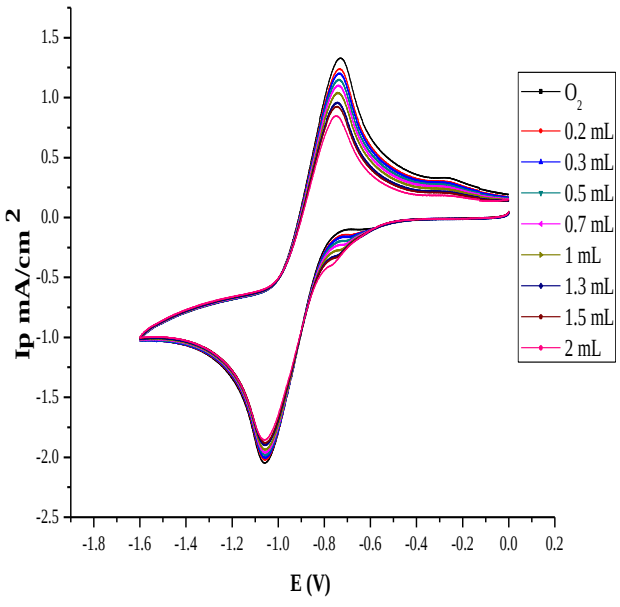
***Artemisa campestris* L. extracts**



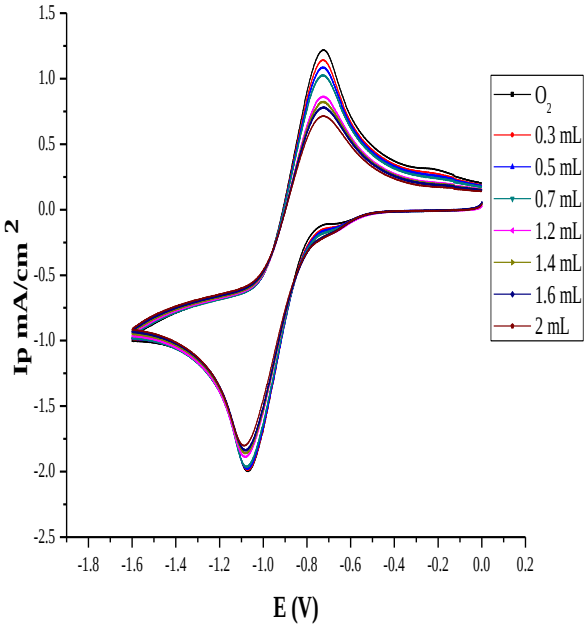
A



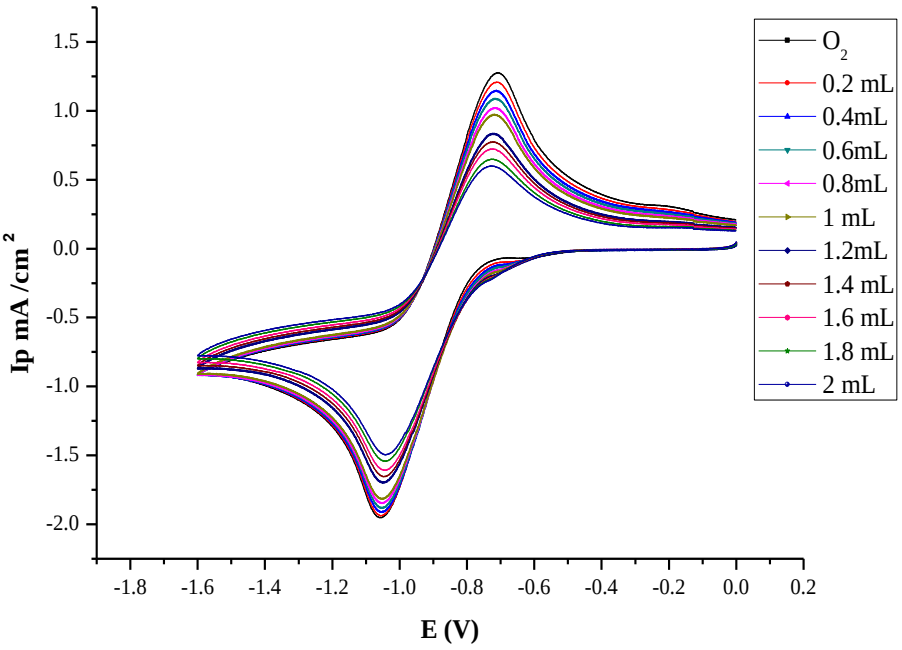
B



C



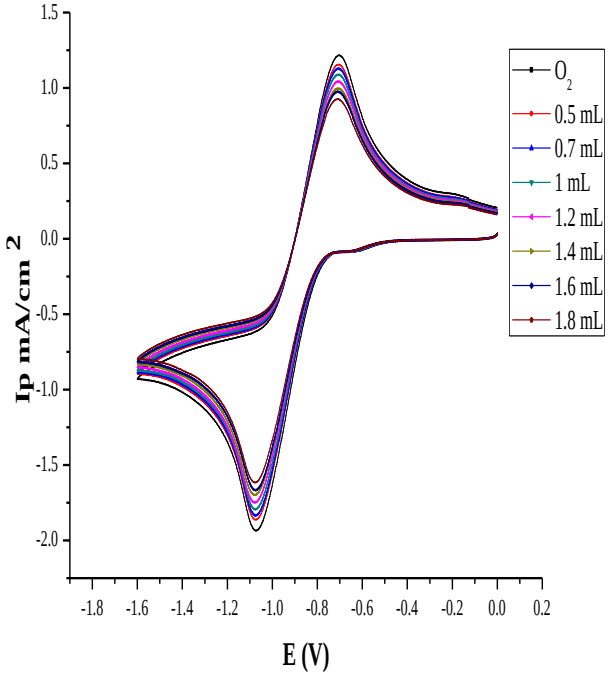
D



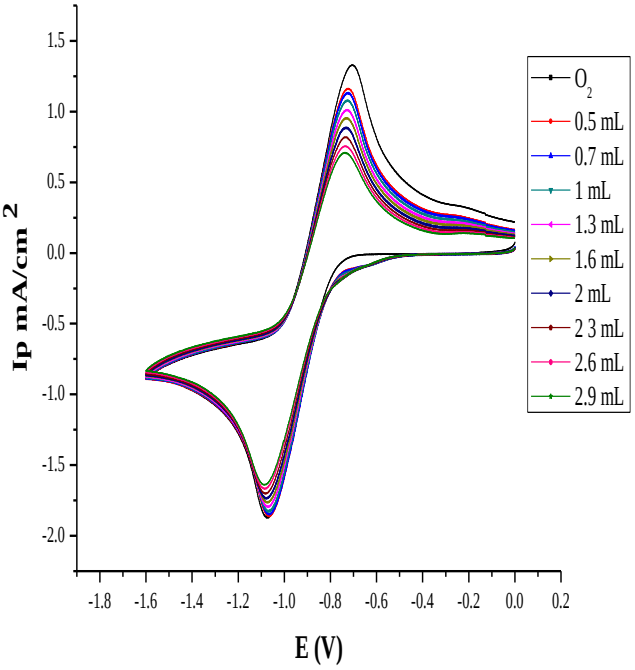
E

Figure.VI.18. Cyclic voltammograms of an oxygen-saturated cell in the presence of various concentrations of *A. campestris* L. extracts **(A):** AcPEE; **(B):** AcDE; **(C):** AcEAE **(D):** AcBE; **(E):** AcAE, recorded at 0.1V s^{-1} potential sweep rate on GC disk electrode at 298K in DMF/nTBuNBF4

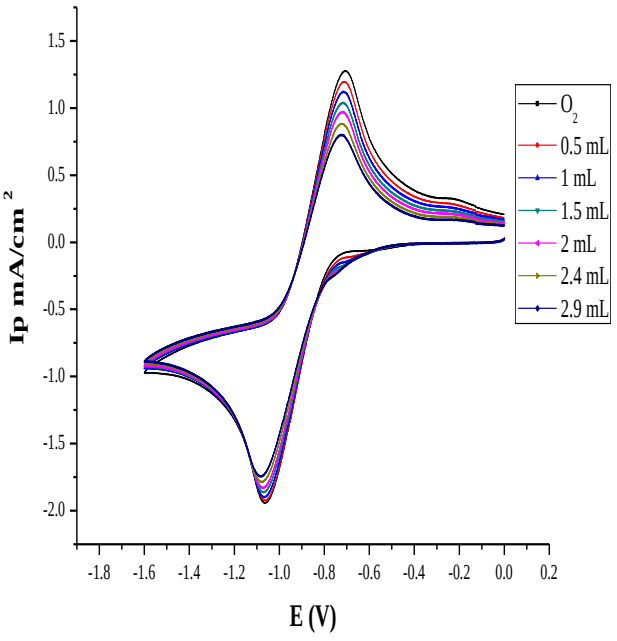
***Fagonia bruguieri* DC. extracts**



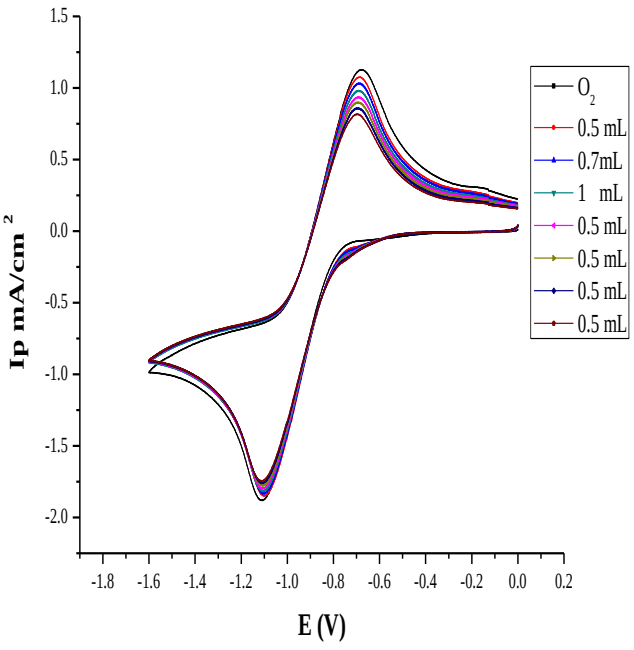
A



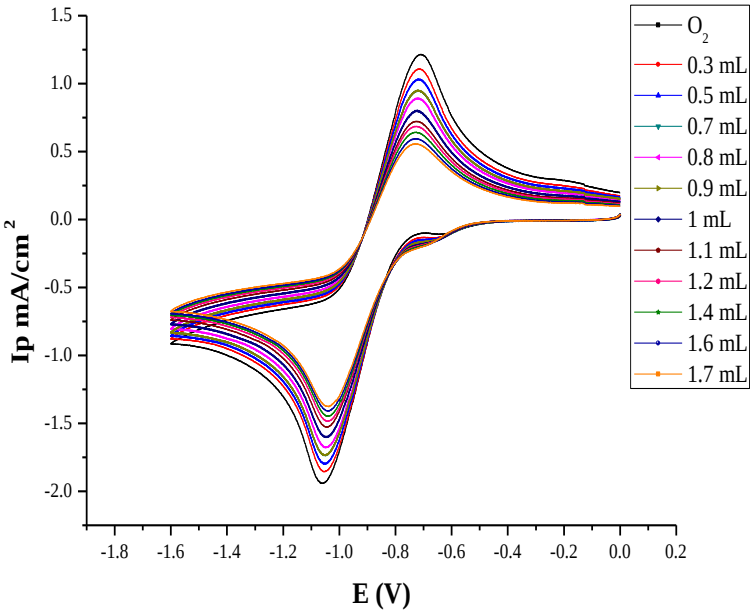
B



C



D



E

Figure.VI.19. Cyclic voltammograms of an oxygen-saturated cell in the presence of various concentrations of *F.bruguieri* DC. extracts **(A):** FbPEE; **(B):** FbDE; **(C):** FbEAE **(D):** FbBE; **(E):** FbAE, recorded at 0.1V s^{-1} potential sweep rate on GC disk electrode at 298K in DMF/nTBuNBF$_4$ 0.1 M.

The anodic peak current was found to have significantly decreased, as shown by the experimental data for each investigated sample.

- **Half-maximal inhibitory concentration (IC_{50})**

Inhibition curves expressed as a percentage (%) are derived from the concentrations of the tested extracts to get IC_{50} values (Figure.VI.20 and Figure.VI.21).

These values represent the concentration of the studied extracts needed to reduce the free radical concentration table by 50 %. The equation obtained from the linear calibration graph shown in Figure.VI.122, where y represents the value of the oxidation peak current density of $O_2^{\bullet-}$ and x represents the value of the concentration of the extracts expressed in mg/mL.

***Artemisa campestris* L. extracts**

Using the Superoxide Anion $O_2^{\bullet-}$ method to measure antioxidant activity, Table.VI.19 shows that the following are in order from most to least antioxidant activity: AcEAE (IC_{50}= 0.27 mg/mL) < AcME (IC_{50}= 0.22 mg/mL) < AcPEE (IC_{50}= 0.18 mg/mL) < AcAE (IC_{50}= 0.12 Mg/mL) < Ascorbic acid (IC_{50}= 00.05 mg/mL) respectively. In Superoxide Anion $O_2^{\bullet-}$ Scavenging activities, the AcAE exhibited the highest antioxidant capacity.

Table.VI.19. Superoxide Anion $O_2^{\bullet-}$ Scavenging of different extracts of *A.campestris* L.

Superoxide anion $O_2^{\bullet-}$ scavenging assay			
Extracts	**Equation**	**R^2 Value**	**IC_{50} mg/mL** [a]
AcME	NT	NT	NT
AcPEE	y = 222.42x + 1.0188	0.9991	0.22
AcDE	y = 291.96x - 2.5847	0.9958	0.18
AcEAE	y = 200.07x - 5.3199	0.997	0.27
AcBE	y = 290.36x - 3.0766	0.9968	0.18
AcAE	y = 393.95x - 1.1461	0.9963	0.12
Ascorbic acid*	y = 234.11x + 0.6611	0.9903	0.0596

[a] **IC_{50}**: the concentration at the 50 of inhibition. *Standard compounds. **NT**: Note Tested.

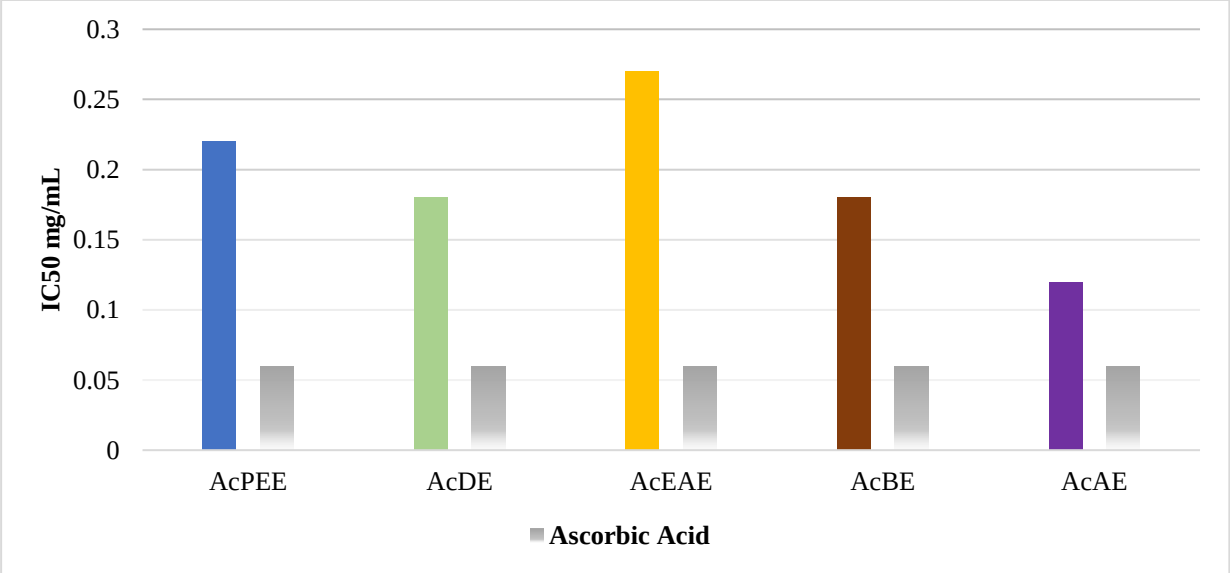


Figure.VI.20. Comparison of superoxide anion radical $O_2^{\bullet-}$according to I % values between extracts of *A.campestris* L. and Ascorbic acid.

***Fagonia bruguieri* DC. extracts**

Table.VI.20 shows *F.bruguieri's* capacity to reduce O_2 ions via superoxide anion $O_2^{\bullet-}$. The antioxidant activity measured by the electrochemical assay is listed below in decreasing order: Ascorbic acid (IC_{50} : 0.0596 mg/mL) > FbAE (IC_{50}: 0.43 mg/mL) > FbDE (IC_{50}: 0.89 mg/mL) > FbPEE (IC_{50}: 0.96 mg/mL) > FbBE (IC_{50}: 1.08 mg/mL) > FbEAE (IC_{50}: 1.13 mg/mL) respectively. Even when compared with Ascorbic acid standards, the $O_2^{\bullet-}$ radical scavenging activity level shown by FbAE (IC_{50}: 0.43 mg/mL µg/mL) was the highest achievable.

Table.VI.20. Superoxide Anion $O_2^{\bullet-}$ Scavenging of different extracts of *F.bruguieri* DC.

Superoxide anion $O_2^{\bullet-}$ scavenging assay			
Extracts	**Equation**	**R^2 Value**	**IC_{50} mg/mL** [a]
FbPEE	y = 53.077x - 1.4839	0.9971	0.96
FbDE	y = 55.507x + 0.4354	0.998	0.89
FbEAE	y = 47.853x - 4.1945	0.9921	1.13
FbBE	y = 47.395x - 1.3803	0.9977	1.08
FbAE	y = 119.15x - 1.3448	0.9955	0.43
Ascorbic acid*	y = 234.11x + 0.6611	0.9903	0.0596

[a] **IC_{50}**: the concentration at the 50 of inhibition. *Standard compounds. **NT**: Note Tested.

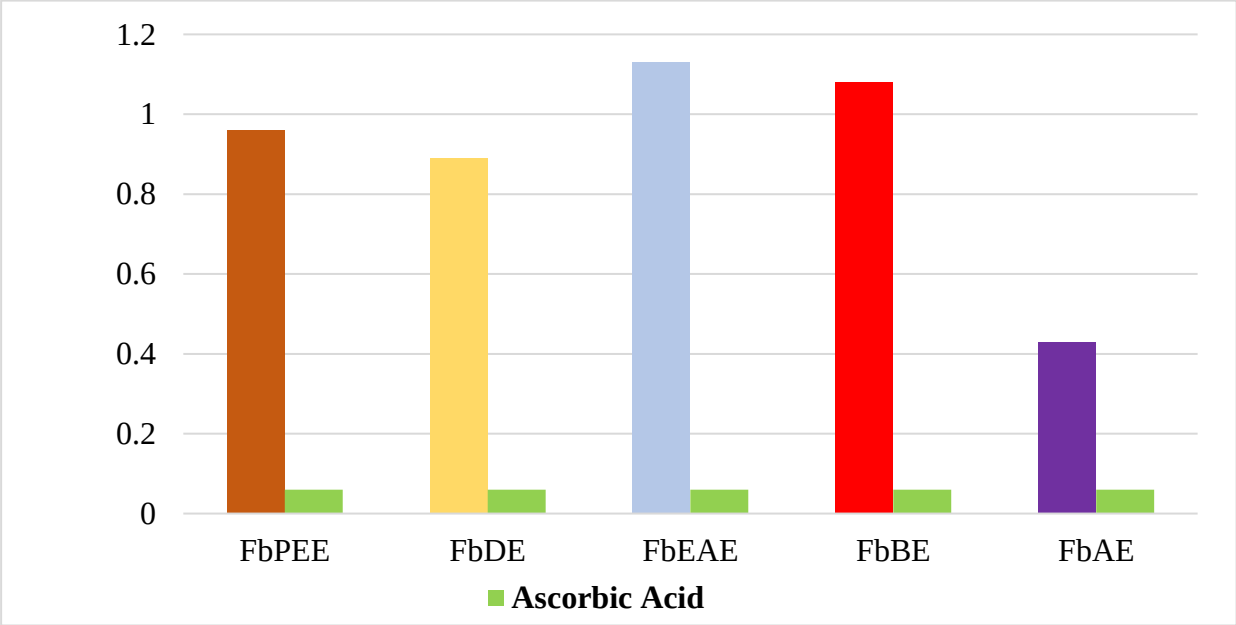
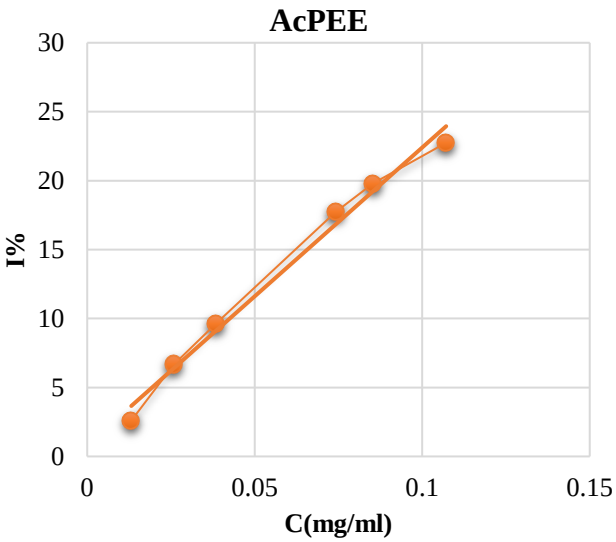
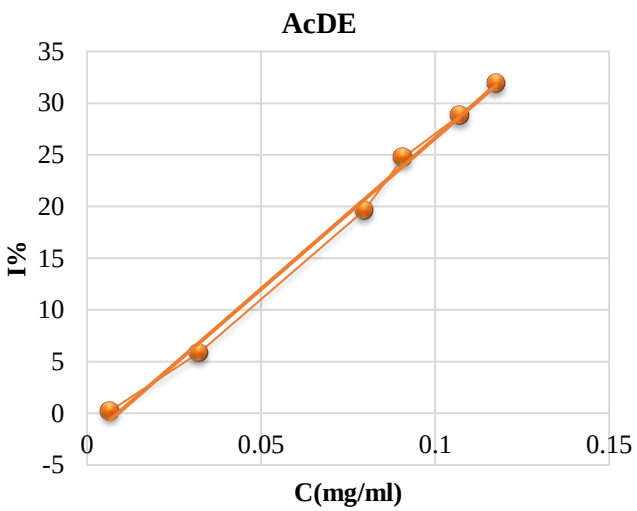
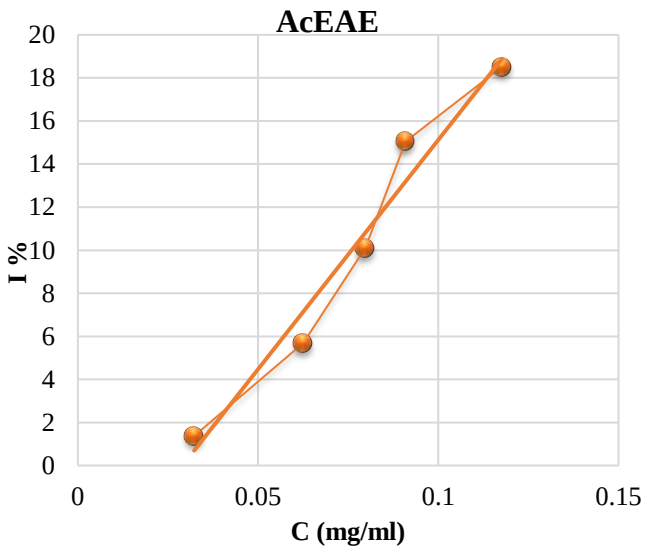
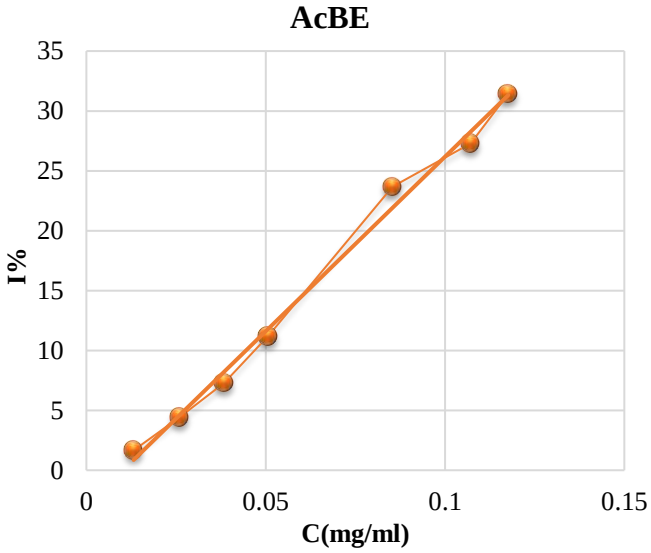


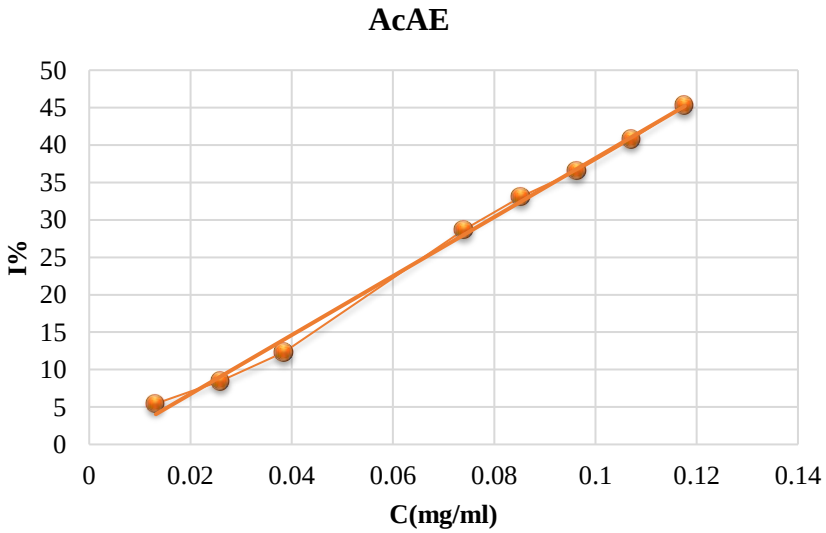
Figure.VI.21. Comparison of superoxide anion radical $O_2^{\bullet-}$according to I % values between extracts of *F.bruguieri* DC. and Ascorbic acid.


AcPEE
I%
C(mg/ml)


AcDE
I%
C(mg/ml)


AcEAE
I %
C (mg/ml)


AcBE
I%
C(mg/ml)


AcAE
I%
C(mg/ml)

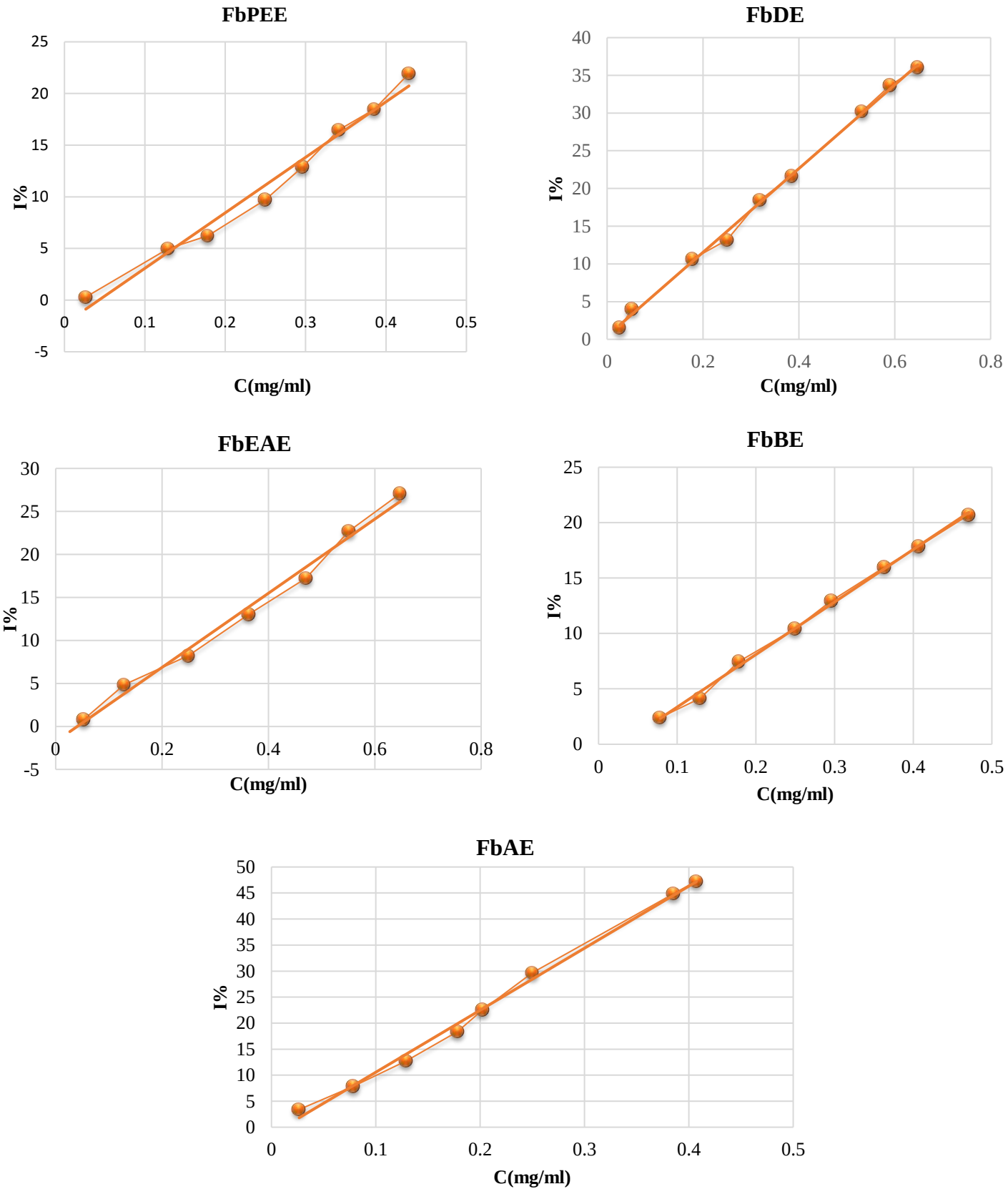


Figure.VI.22. Plots of (A_0 -A) / A_0 *100 verses the concentration for diffrents extracts of *A.campestris* L. and *F.bruguieri* DC.

The IC_{50} values show that antioxidants are a significant component of the extracts' protection against oxidants. The ability of phenolic compounds to act as antioxidants is determined not only by the amount of hydroxyl groups present but also by the configuration of those groups and the existence of substitute electron donors in the cycle structure.

VI.3. Enzymes Inhibitory Activity

VI.3.1. Cholinesterase Inhibitory Activity

The anticholinesterase activities were determined for all fractions employing two enzymes: AChE and BChE, which reduce neurotransmitter Ach involved in Alzheimer's illness (Table.VI.21→ Table.VI.24).

VI.3.1.1. Acetylcholinesterase

***Artemisa campestris* L. extracts**

AcEAE (IC_{50}= 23,16±0,19 µg/mL) showed a significant inhibitory effect for AChE Table.VI.21; it was more effective than AcPEE (IC_{50}=59.03±0.58 µg/mL), whilst AcDE showed the weakest inhibitory effectiveness (IC_{50} >200 µg/mL).

Table.VI.21. Acetylcholinesterase inhibitory activity of different extracts of *A.campestris* L.

Extracts	**% Inhibition in Acetylcholinesterase inhibitory activity**							**IC_{50} µg/mL** [a]
Concentration µg/mL	3.125	6.25	12.5	25	50	100	200	
AcME	NA	NA	NA	NA	NA	NA	NA	NA
AcPEE	6,87±0,12	14,07±0,12	14,77±0,09	34,95±0,58	41,15±0,32	72,83±0,09	88,01±0,68	59.03±0.58
AcDE	1,32±0,84	1,58±0,24	1,92±0,59	2,41±0,16	2,78±0,23	3,48±0,28	4,94±0,50	>200
AcEAE	6,34±0,96	6,51±0,33	33,84±0,60	51,54±0,56	60,11±0,24	70,83±0,27	83,25±0,39	23,16±0,19
AcBE	NA	NA	NA	NA	NA	NA	NA	NA
AcAE	NA	NA	NA	NA	NA	NA	NA	NA
Galantamine	35,93 ± 2,28	43,77 ± 0.00	68,50 ± 0,31	80,69 ± 0,41	85,78 ± 1,63	91,80 ± 0,20	94,77 ± 0,34	6.27±1.15

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

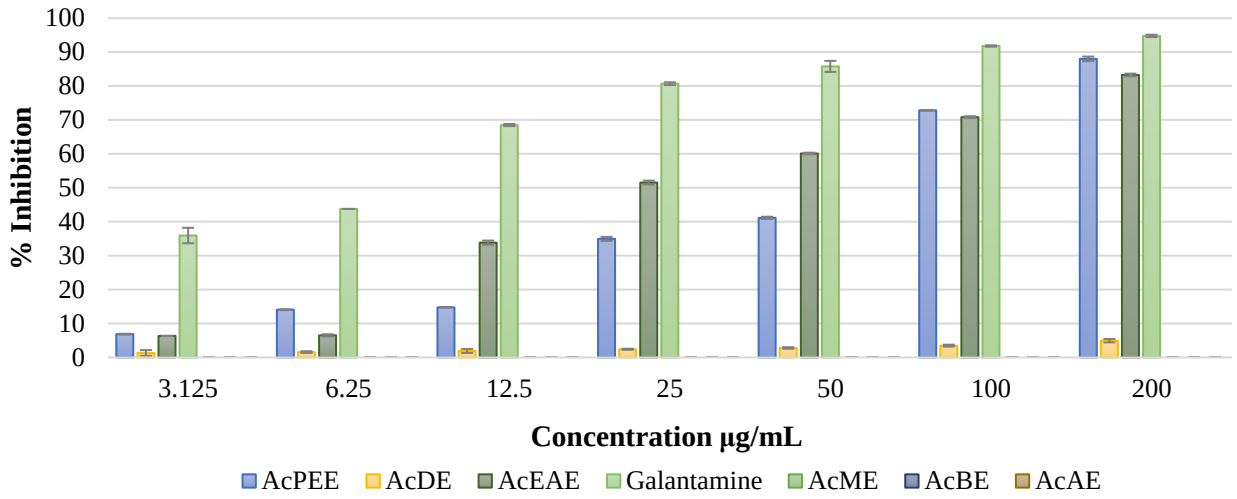


Figure.VI.23. Comparison of acetylcholinesterase inhibition according to I % values between extracts of *A.campestris* L. and Galantamine.

Fagonia bruguieri DC. extracts

Extracts of *F.bruguieri* showed a significant inhibitory effect for ACHE it was more effective with the FbPEE (IC_{50}=27.41±1,03 µg/mL) followed by FbBE (IC_{50}=154,56±3,03 µg/mL) ; FbDE (IC_{50}=163,42±1,76 µg/mL) whilst the FbEAE shown the weakes tinhibitory effectiveness (IC_{50}>200 µg/mL) (Table.VI.22, Figure.VI.24).

Table.VI.22. Acetylcholinesterase inhibitory activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in Acetylcholinesterase inhibitory activity							IC_{50} µg/mL
Concentration µg/mL	3.125	6.25	12.5	25	50	100	200	
FbPEE	18,47±0,35	31,07±0,88	34,51±0,20	51,28±1,50	62,08±0,50	82,14±0,77	96,50±1,15	27.41±1,03
FbDE	NA	NA	NA	0,90±1,17	7,38±0,50	35,84±0,23	55,15±0,82	163,42±1,76
FbEAE	18,82±0,40	19,92±0,56	20,50±1,14	27,78±0,74	28,33±0,28	37,58±0,88	38,73±0,70	>200
FbBE	3,33±1,20	9,06±0,76	15,56±1,04	15,58±0,43	32,46±2,05	43,01±1,44	50,90±0,70	154,56±3,03
FbAE	NA	NA	NA	NA	NA	NA	NA	NA
Galantamine	35,93 ± 2,28	43,77 ± 0.00	68,50 ± 0,31	80,69 ± 0,41	85,78 ± 1,63	91,80 ± 0,20	94,77 ± 0,34	6.27±1.15

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at p<0.05. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

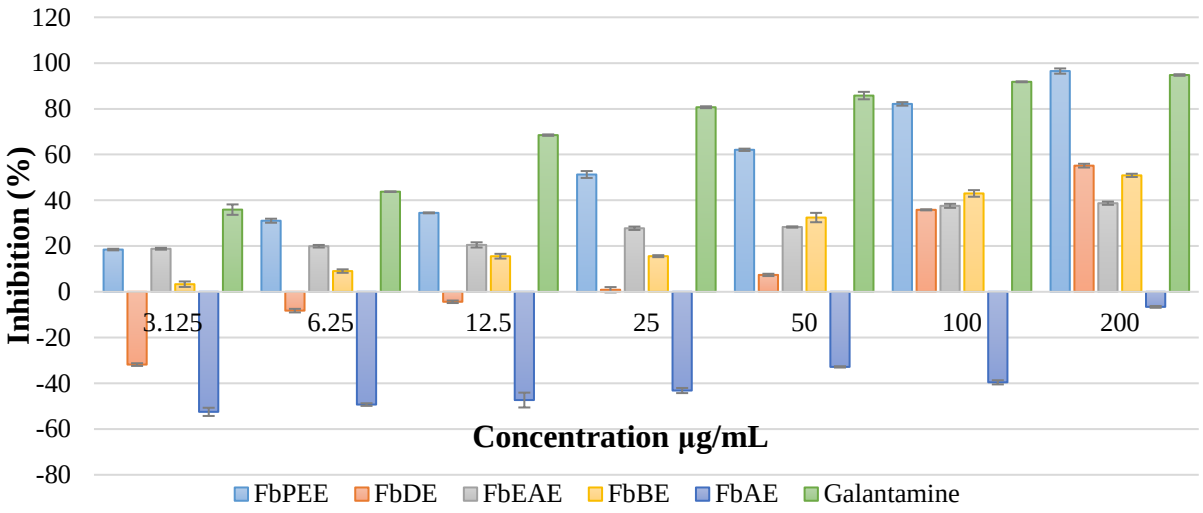


Figure.VI.24. Comparison of acetylcholinesterase inhibition according to I % values between extracts of *F.bruguieri* DC. and Galantamine.

VI.3.1.2. Butyrylcholinesterase

Artemisa campestris L. extracts

On the other hand, significant inhibition was shown against BChE, where the AcPEE (IC_{50}=93.50±1.60 µg/mL) declared the most heightened inhibitory effect; AcBE and AcAE did not affect the activities of BChE (Figure.VI.25) .

Table.VI.23. Butyrylcholinesterase inhibitory activity of different extracts of *A.campestris* L.

Extracts	% Inhibition in Butyrylcholinesterase inhibitory activity							IC_{50} µg/mL [a]
Concentration µg/mL	3.125	6.25	12.5	25	50	100	200	
AcME	NA	NA	NA	1,08±0,26	9,10±1,63	20,03±1,52	30,51±0,29	>200
AcPEE	11,52±2,32	18,45±0,22	22,40±0,34	35,43±1,56	38,48±1,23	51,74±0,77	56,13±1,49	93.50±1.60
AcDE	NA	NA	NA	7,88±2,47	15,55±0,59	27,61±0,22	52,49±1,52	185.11±2.51
AcEAE	NA	NA	NA	NA	NA	10,26±0,56	26,49±1,55	>200
AcBE	NA	NA	NA	NA	NA	NA	NA	NA
AcAE	NA	NA	NA	NA	NA	NA	NA	NA
Galantamine*	3,26± 0,62	6,93± 0,62	24,03± 2,94	45,13± 2,60	63,87± 2,85	73,57± 0,77	78,95± 0,58	34.75±1.99

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

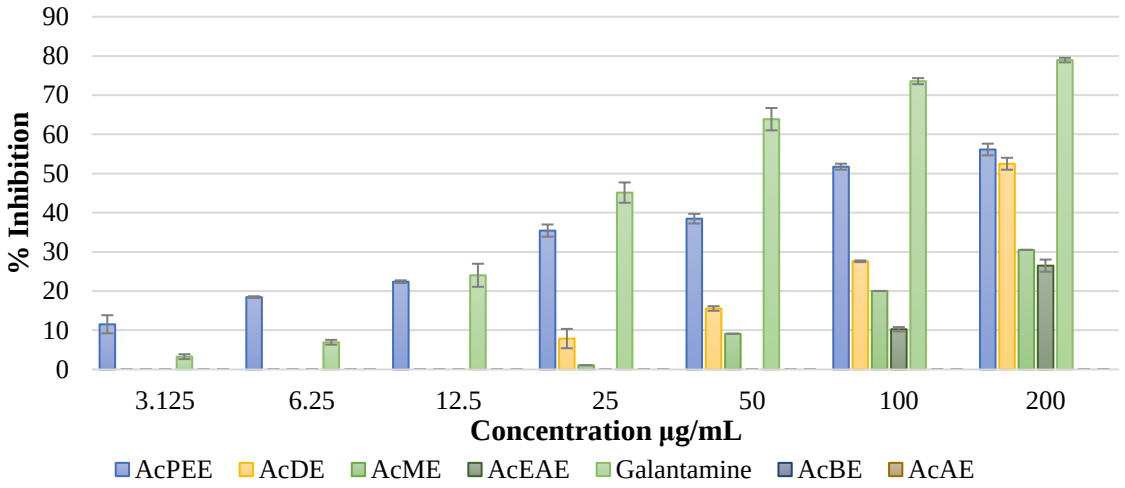


Figure.VI.25. Comparison of Butyrylcholinesterase inhibition according to I % values between extracts of *A.campestris* L. and Galantamine.

***Fagonia bruguieri* DC. extracts**

The anti BChE activity (IC_{50}) of different extracts of *F.bruguieri* was obtained as follows : FbEAE (IC_{50}=171,94±3,25 µg/mL) > FbBE (IC_{50}=119,55±1,66 µg/mL) > FbDE (IC_{50}=69,97±0,70 µg/mL) > FbPEE (IC_{50}=46,14±0,78 µg/mL) > Galantamine (IC_{50}=34.75±1.99 µg/mL).

Table.VI.24. Butyrylcholinesterase inhibitory activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in Butyrylcholinesterase inhibitory activity							IC_{50} µg/mL [a]
Concentration µg/mL	3.125	6.25	12.5	25	50	100	200	
FbPEE	29,67±0,43	29,72±0,11	32,24±0,80	40,77±1,95	51,53±0,71	53,60±0,00	57,34±0,21	46,14±0,78
FbDE	24,01±0,56	24,57±0,56	32,12±1,49	43,78±0,65	50,04±0,74	50,71±1,70	71,89±1,84	69,97±0,70
FbEAE	16,53±1,03	19,71±1,03	20,02±0,71	22,69±0,39	32,46±0,94	39,60±0,88	53,79±0,94	171,94±3,25
FbBE	25,53±1,22	32,09±0,48	35,71±0,93	39,26±0,74	43,11±0,80	47,50±0,46	58,60±0,61	119,55±1,66
FbAE	NA	NA	NA	NA	NA	NA	NA	NA
Galantamine*	3,26± 0,62	6,93± 0,62	24,03± 2,94	45,13± 2,60	63,87± 2,85	73,57± 0,77	78,95± 0,58	34.75±1.99

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

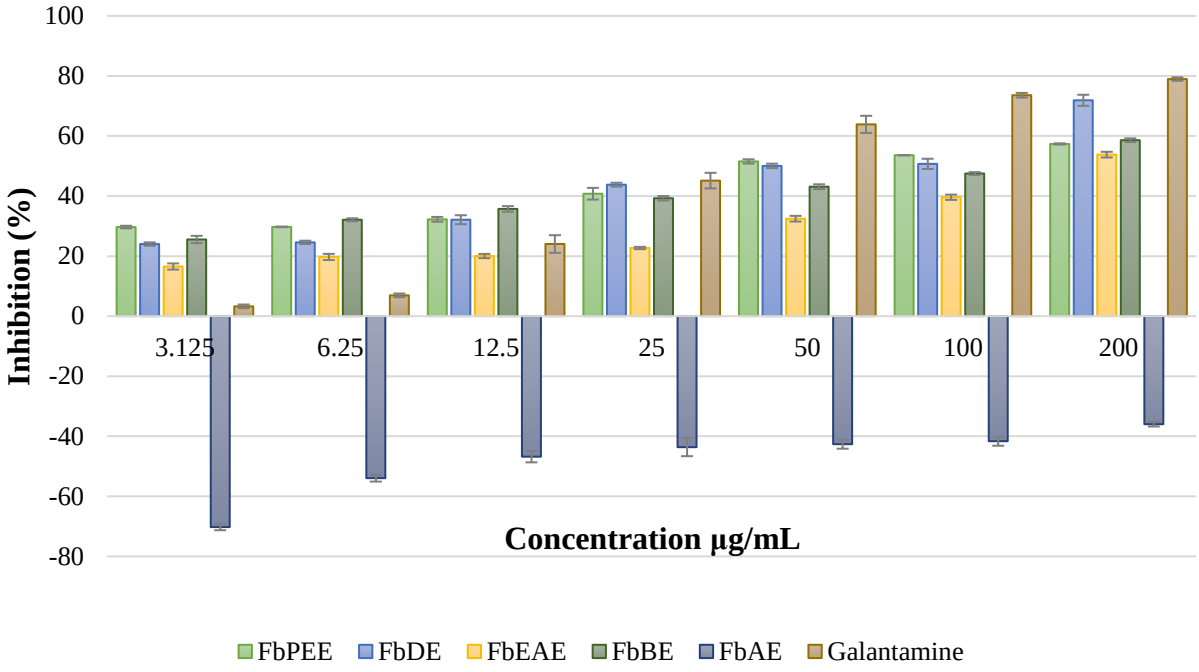


Figure.VI.26. Comparison of Butyrylcholinesterase inhibition according to I % values between extracts of *F.bruguieri* DC. and Galantamine.

Discussion

Alzheimer's disease, such as neurodegenerative diseases, concerns public health priorities. It is a fatal neurodegenerative disease that is characterized by progressive damage to the brain and increased mental disorder [413]. Several illnesses require enzyme therapy to be effectively cured. Cholinesterase inhibitors are the main enzyme in Alzheimer's disease pathologies as the key to inhibiting the enzymes impacted in acetylcholine hydrolysis, acetylcholinesterase and butyrylcholinesterase by trying to extend the availability of acetylcholine to create the receptors in the brain [414, 415]. Galantamine is a cholinesterase inhibitor widely used in treating AD, and it represents a positive control for comparison purposes.

According to the findings shown in Table.VI.21, *A.campestris* revealed good inhibitory effectiveness for the AcEAE against AChE (IC_{50} = 23.16±0.19 µg/mL) and then AcPEE (IC_{50} = 59.03±0.58 µg/mL).The AcPEE, nonetheless, it moderately inhibited BChE activity (IC_{50} =93.50±1.60 µg/mL). In comparison with the findings obtained for the essential oil of *A.campestris* reported in 2020 by Cherif. K *et al.*, [416], the AcPEE and AcEAE demonstrated a strong inhibitory effect on the AChE and BChE enzymes. For this reason, we may conclude that the chemical components are capable of acting as antagonists of both AChE and BChE. Szwajgier *et al*. (2015) have proven that some flavonoids and phenolic acids play this function,

such as acacetin and apigenin have a substantial effect on inhibiting activity towards AChE and BChE and whose presence has already been established according to the LC-MS/MS results [417].

Murray *et al.* (2013) have shown that naturally occurring substances, such as terpenoids and alkaloid chemicals, can inhibit the enzymes AchE and BChE. Such compounds originate from a variety of multiple sources [418]. It is conceivable that the activity of acetyl and butyrylcholinesterase in the AcPEE is due to secondary metabolites. Recently, Boukhalkhal *et al*. (2020) have previously identified these terpenoids in *A.campestris*: OH-Rubescensin BI, BII and Carnosic acid [419]. These compounds might have produced the anticholinesterase effect that was studied in this approach. In light of this, we have highlighted our results, which suggest that *A.campestris* might very well have an anti-Alzheimer effect that could be helpful in the treatment of AD.

Additionally, the extracts of *F.bruguieri* showed a considerable inhibitory effect on both AChE and BChE, as shown in Table.VI.23 and Table.VI.24. It has been found that the FbPEE exhibits the most significant activity against both BChE and AChE. As was discussed earlier, natural chemicals derived from various resources such as terpenoids, phenolic compounds, and alkaloids, can inhibit the enzymes AchE and BChE [418]. We suggest that secondary metabolites in the FbPEE may be responsible for the acetyl- and butyrylcholinesterase activity. Abdel-Kader *et al*., (1994) demonstrated a new ent-erythroxane diterpene:15,16-dihydroxy-7-hydroxy-cis-ent-erythrox-3-ene present in *F.bruguieri* [245]. In 1993 the same authors also afforded the first reported diterpenes: 15,16-dihydroxy-7-oxo-cis-ent-erythrox-3-ene (fagonone) with an ent-erythroxan structure16-*O*-acetylfagonone in aerial parts of *F.bruguieri* [229]. Morever, Maksoud & El-Hadidi., (1987) have previously identified in *F.bruguieri* seven flavonol glycosides of these Quercetin 3-rhamnogalactoside, kaempferol 3-rhamno-galactoside, and Quercetin 3-galactoside [420]. These chemicals may provide the foundation for the anticholinesterase effect investigated above. Several monoterpenes obtained from essential oils are potent inhibitors of AChE activity in vitro [421]. Cinéole, Camphre, trans-géraniol, o-Cymène, and α-citral have all been found in the aerial fractions of *Fagonia cretica* L. in Previous research [247]. Preliminary in vivo studies showed that *F.bruguieri* extracts strongly inhibited AchE activity in the haemolymph of freshly emerging S. gregaria adults [214]. In light of this, we've consolidated our findings that the anti-Alzheimer activity in *F.bruguieri* may be beneficial in the treatment of AD.

VI.3.2. Evaluation of anti-diabetic activity

VI.3.2.1. Alpha -amylase Inhibitory activity

In order to evaluate the antidiabetic efficient of *A.campestris* and *F.bruguieri* extracts was determined by estimating the potential of the different extracts to inhibit the α-amylase enzyme.

***Artemisa campestris* L. extracts**

Based on the findings in Table.VI.25, the reaction with *Artemisa* extracts provided the ideal response with an IC50 value that was significantly lower than that of acarbose, except that the AcAE was not active against beta-amylase. The most heightened α–amylase inhibitory effect was recorded for AcPEE (IC_{50}=11.97± 0.14 µg/mL) (Figure.VI.27) . This activity is three hundred and thirty-third times (333x) higher than acarbose (IC_{50}=3650.93±10.70 µg/ml).

Table.VI.25. Alpha-amylase inhibitory activity of different extracts of *A.campestris* L.

Extracts	% Inhibition in α-amylase inhibitory assay							IC_{50} µg/mL [a]
Concentration µg/mL	62,5	125	250	500	1000	2000	4000	
AcME	37,44±0,30	38,16±0,31	39,04±0,13	39,73±0,24	40,58±0,36	41,27±0,27	45,76±0,31	>4000
AcPEE	47,42±0,00	50,21±0,27	56,46±0,18	59,40±0,45	68,75±0,39	71,95±0,72	97,91±0,63	11.79±0.14
AcDE	46,49±0,00	47,57±0,81	48,81±0,00	53,67±0,59	73,50±0,45	80,62±0,45	92,10±0,36	28.33±1.35
AcEAE	42,05±0,70	43,80±0,09	43,80±0,18	45,51±0,54	46,95±0,15	48,35±0,15	51,91±0,62	284.33±3.91
AcBE	40,54±0,30	44,90±0,07	51,99±0,07	62,32±0,15	71,34±0,63	87,57±0,15	88,98±0,07	21.55±0.66
AcAE	37,40±0,27	38,20±0,52	38,78±0,30	39,64±0,25	39,91±0,21	39,99±0,31	40,46±0,13	>4000
Acarbose *	7,76±0,17	8,08±0,30	9,46±0,11	10,70±0,96	31,81±2,89	37,21±3,54	53,05±1,59	3650,93±10,70

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

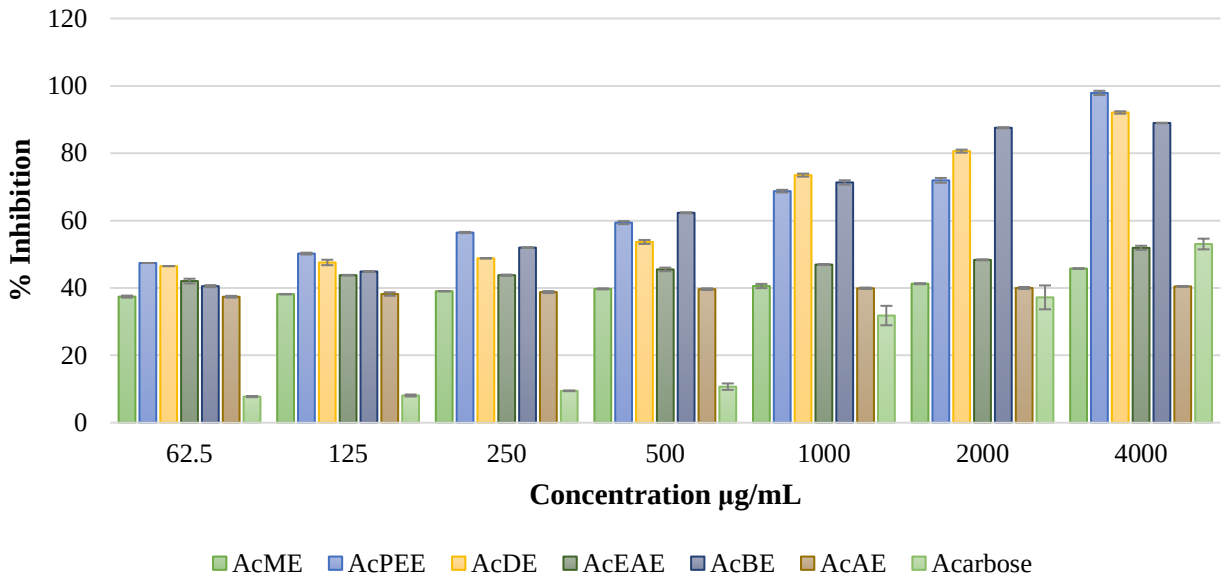


Figure.VI.27. Comparison of alpha-amylase inhibition according to I % values between extracts of *A.campestris* L. and Acarbose.

***Fagonia bruguieri* DC. extracts**

The α–amylase inhibitory activity of several *F.bruguieri* extracts was determined as follows: FbDE (IC_{50}=7,12±0,89 µg/mL) > FbEAE (IC_{50}=119,55±1,66 µg/mL) > FbBE (IC_{50}=43.55±0.33 µg/mL) > FbPEE (IC_{50}=68,86±2,91 µg/mL) > FbAE (IC_{50}=97.67±0.33 µg/mL) > acarbose (IC_{50}=3650.93±10.70 µg/ml). The highest α–amylase inhibitory activity was showed in FbDE . This activity is (520 x) higher than acarbose (Table.VI.26 and Figure.VI.28).

Table.VI.26. Alpha -amylase inhibitory activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in α-amylase inhibitory assay							IC_{50} µg/mL [a]
Concentration µg/mL	62,5	125	250	500	1000	2000	4000	
FbPEE	45,56±0,66	46,42±0,75	47,56±0,98	48,48±0,72	51,18±0,40	62,52±0,49	82,11±0,72	68,86±2,91
FbDE	47,84±0,55	54,92±0,63	56,54±0,35	59,88±0,44	68,64±0,55	76,13±0,38	89,70±4,13	7,12±0,89
FbEAE	45,76±0,53	48,09±0,35	51,83±0,23	54,92±0,32	62,82±0,23	80,44±0,61	104,99±0,49	19,12±0,33
FbBE	27,17±0,26	33,28±0,07	42,25±0,52	52,76±0,07	63,95±0,22	74,75±1,18	87,10±0,52	43.55±0.33
FbAE	26,38±2,04	32,86±0,71	33,95±0,20	38,72±0,34	50,71±0,53	55,18±0,27	76,66±0,60	97.67±0.33
Acarbose*	7,76±0,17	8,08±0,30	9,46±0,11	10,70±0,96	31,81±2,89	37,21±3,54	53,05±1,59	3650,93±10,70

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

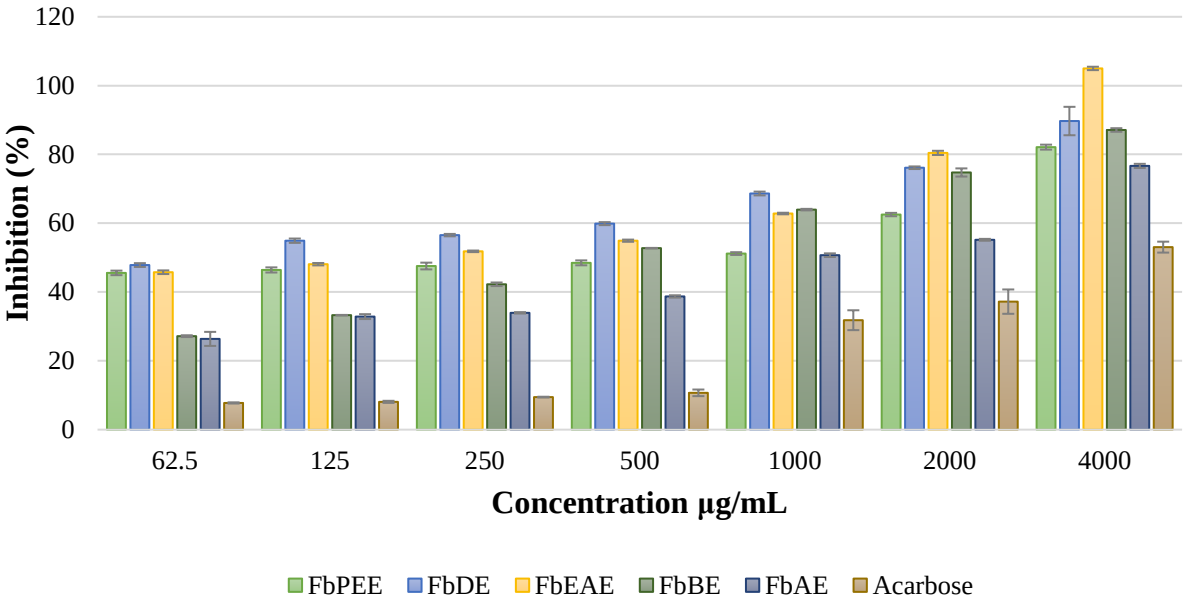


Figure.VI.28. Comparison of alpha-amylase inhibition according to I % values between extracts of *F.bruguieri* DC. and Acarbose.

Discussion

Diabetes, also known as hyperglycemia chronic, is a symptom of the metabolic. Diabetes Type 2 can be treated with a variety of medicinal approaches. Inhibiting alpha-amylase in the small intestine, which inhibits complex carbohydrates from being broken down and inhibits the body from absorbing them, is one of the strategies by which plants exert their anti-hyperglycemic impact on the body. Starch and other glucose polymers in diabetic individuals hydrolyze (1.4)-D-glycosidic linkages in the presence of this enzyme's inhibitors [422].

Both *A.campestris* and *F.bruguieri* extracts had a remarkable anti-diabetic action that was significantly more effective than acarbose (Table.VI.25; Table.VI.26 and Figure.VI.27; Figure.VI.28). Furthermore, we underlined a substantial difference in their IC_{50} values. The ability of polyphenols to inhibit the enzymes alpha-glucosidase and alpha-amylase was investigated through molecular docking simulations, and it was expected that compounds such as caffeic acid, naringin, chlorogenic acid, quercetin and Rutin might significantly inhibit the enzymes [423]. This includes all Phenolic chemicals and flavonoids discussed in the profile phytochemicals of *A.campestris* and *F.bruguieri* extracts, which are a crucial tool in the treatment of diabetes type II in this study. On the other hand, the presence of some minerals may favourably contribute to the anti-diabetic benefits of plants, by enhancing the effects of insulin, this could be the case as specific minerals have been shown to have anti-diabetic

properties [424]. Myricetin, a common flavonol and one of the *A.campestris* and *F.bruguieri* components (Table.V.8 and Table.V.9 Part 2: Chapter V), has been validated as a potent flavonoid inhibitor of amylase, and the possibility of delivering it in concentrated form to lower postoperative hyperglycaemia has been investigated in 2015, by Gu *et al*. [425]. Our study is highlighting demonstrates that *A.campestris* and *F.bruguieri* have anti-diabetic potential in vitro by inhibiting alpha-amylase.

VI.3.3. Evaluation of anti-obesity activity

VI.3.3.1. Pancreatic Lipase Inhibitory Activity

***Artemisa campestris* L. extracts**

The data on the Pancreatic Lipase inhibiting activity of extracts from *A.campestris* aerial parts is shown in Table.VI.27 and Figure.VI.29. *A.campestris* extracts were shown to significantly inhibit anti-lipase activity, with the AcPEE (IC_{50}=40.15±1.36µg/mL) proving to be more effective compared to the reference compound. The inhibitory activity follows the decreasing order: orlistat (IC_{50}=0.06± 0.001 µg/mL) > AcPEE (IC_{50}= 40.15±1.36µg/mL) > AcDE (IC_{50}= 86.29±2.60 µg/mL) > AcEAE (IC_{50}=155.47±3.44 µg/mL), respectively. While the AcME did not affect pancreatic lipase.

Table.VI.27. Pancreatic lipase inhibitory activity of different extracts of *A.campestris* L.

Extracts	**% Inhibition in Antilipase inhibitory activity**							**IC_{50} µg/mL** [a]
Concentration µg/mL	15.62	31.25	62.5	125	250	500	1000	
AcME	NT	NT	NT	NT	NT	NT	NT	NT
AcPEE	23.59±0.15	35.04±0.23	35.46±0.07	44.15±0.15	54.37±0.96	56.71±0.09	75.72±1.34	40.15±1.36
AcDE	32.68±0.30	36.71±0.03	36.90±0.24	42.91±0.35	43.94±0.14	52.29±0.38	62.49±0.38	86.29±2.60
AcEAE	24.55±1.39	27.75±0.08	29.57±0.86	34.14±0.20	32.92±0.11	46.21±0.42	55.04±0.58	155.47±3.44
AcBE	13.61±0.20	17.98±0.07	19.71±0.09	23.78±0.47	25.14±0.14	27.31±0.25	31.56±0.49	>1000
AcAE	NA	NA	NA	1.01±0.22	1.22±0.94	2.43±1.06	8.98±0.50	>1000
Orlistat*	-	21,66±0,87	68,59±1,60	74,72±0,88	76,80±0,19	77,48±0,87	79,84±1,07	0,06±0,01

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

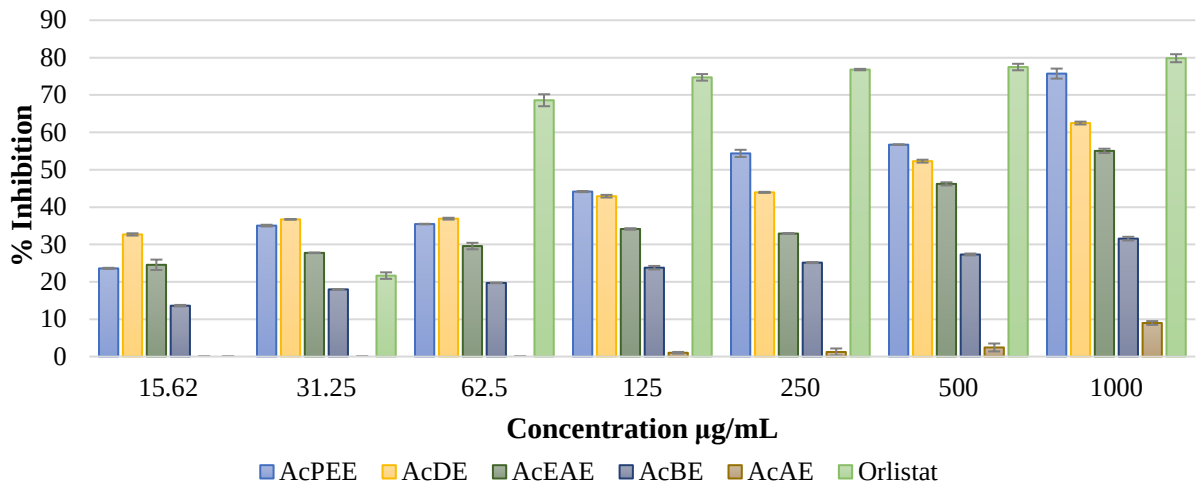


Figure.VI.29. Comparison of Antilipase inhibitory according to I % values between extracts of *A.campestris* L. and Orlistat.

***Fagonia bruguieri* DC. extracts**

Table.VI.28 and Figure.VI.30 show data on the pancreatic lipase inhibitory activity of extracts from *F.bruguieri* aerial parts. The inhibitory activity follows the decreasing order: orlistat (79.84%)> Dichloromethane (56.76%) > Petroleum Ether (48.28%) > Butanol (34.81%) > Ethyl acetate (32.21%) respectively.

As compared to the reference (IC_{50}=0,06±0,001µg/mL), FbBE have significant anti-lipase (IC_{50} = 122.5±0.93 µg/mL) activity to inhibit the Pancreatic Lipase.

Table.VI.28. Pancreatic lipase inhibitory activity of different extracts of *F.bruguieri* DC.

Extracts	% Inhibition in Antilipase inhibitory activity							IC_{50} µg/mL [a]
Concentration µg/mL	15.62	31.25	62.5	125	250	500	1000	
FbPEE	21,20±0,39	26,01±0,24	27,91±1,02	35,62±0,58	38,58±0,65	45,96±1,29	48,28±0,39	> 1000
FbDE	18,35±0,30	21,50±1,31	26,08±0,51	39,34±0,46	44,48±0,21	47,48±0,26	56,76±0,10	122.5±0.93
FbEAE	NA	6,41±0,31	7,91±0,12	16,15±0,56	23,39±1,81	27,94±0,04	32,21±0,36	> 1000
FbBE	NA	5,99±0,75	11,30±0,26	13,66±0,37	17,43±0,39	24,93±0,14	34,81±0,55	>1000
FbAE	NA	NA	5.66±1.01	9.12±0.32	14.12±0.65	18,95±0,76	29,88±1,50	>1000
Orlistat*	-	21,66±0,87	68,59±1,60	74,72±0,88	76,80±0,19	77,48±0,87	79,84±1,07	0,06±0,001

[a] **IC_{50}**: the concentration at the 50 of inhibition. **IC_{50}** values represent the means ±SEM of three measures. Tukey test. The differences in values were considered at $p<0.05$. *Standard compounds. **NT**: Note Tested. **NA**: Note Active.

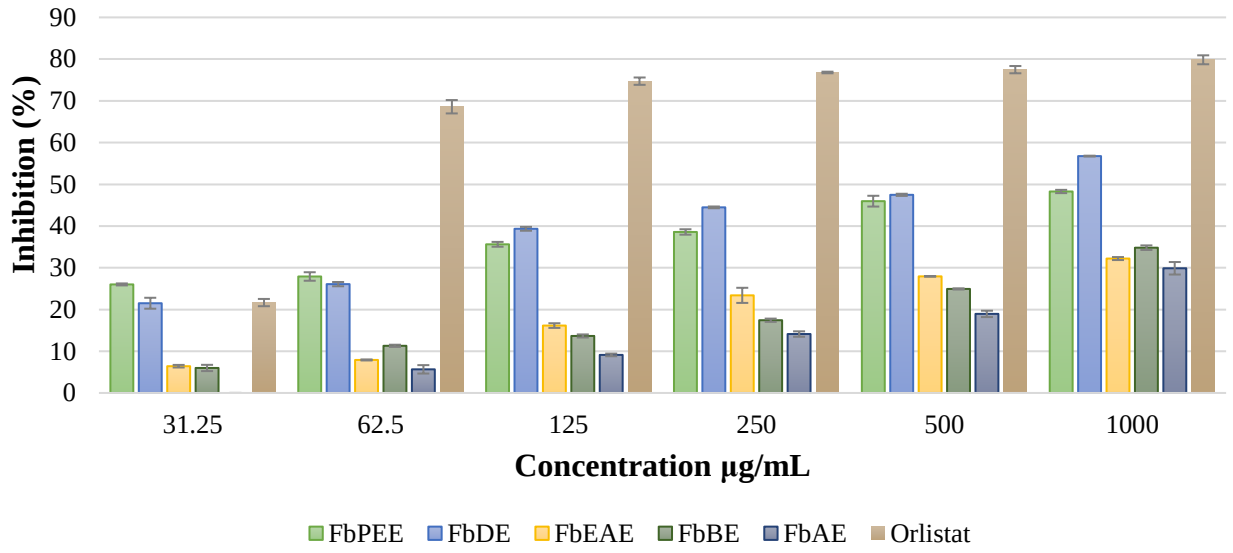


Figure.VI.30. Comparison of Antilipase inhibitory according to I % values between extracts of *F.bruguieri* DC. and Orlistat.

Discussion

Since pancreatic lipase is the primary source of excess calories, recent advancements in absorption inhibitors and nutritional digestion are crucial for treating this disease. The search for therapeutic drugs has become an urgent matter [426]. Among the most important and latest treatment for obesity is Orlistat, a pancreatic lipase inhibitor with a remarkable effect (IC_{50}= 0.061 ± 0.001µg/mL) due to its long-term effectiveness. The enzyme lipase breaks down dietary triglycerides into diacylglycerol, monoglycerides, glycerol, and polyunsaturated fatty acids [427].

Our study reports for the first time the anti-lipase activity of the aerial parts of the *A.campestris* extracts. According to our results (Table.VI.27), AcPEE is more effective, providing a real chance of discovering new antiobesity drugs. Natural compounds obtained from plants have been shown to have biological activity and potential as anti-obesity drugs. In effect, saponins, polyphenolics, terpenes, and triterpenes are abundant lipase inhibitors. Thus many plants have been studied for their antilipase activity [426, 427]. In particular, terpenes, such as carnosic acid; carnosol; and oleanolic acid were reported to inhibit pancreatic lipase [428, 429], previous research has proven the existence of the mentioned substances in *A.campestris* [419, 430].

The remarkable inhibitory activity of the FbDE was the most remarkable (Table.VI.28), which presents an exciting opportunity to discover newer anti-obesity factors. As was already declared, terpenes and triterpenes are sources of lipase inhibitors, which are thought to be

promising treatments for obesity [426, 427]. The *Fagonia* plant is well-known for its abundance of terpenes, although previous studies are isolated from *F.bruguieri* DC: oleanolic acid and its derivatives (terpenes) [229, 245]. Furthermore, an important study on Wistar rats found that methanol extract of *Fagonia cretica* L. protected Wistar rats from several obesity markers produced by a cafeteria diet [431]. Additionally, it was reported that methanol/water extract of *Fagonia arabica* L., another species of *Fagonia*, possesses pancreatic lipase inhibitory effects [432]. A recent study by Patel *et al.* (2020), conducted with ethyl acetate fraction of *Fagonia cretica* L. (IC_{50} = 83.02 ± 2.47 %) [433]. Nevertheless, this is the first research to investigate the anti-lipase activity of the aerial parts of *F.bruguieri* extracts.

VI.4. Anti-inflammatory Activity

We selected to use the anti-denaturation model of BSA (bovine serum albumin) as the basis for our in vitro evaluation of the various extracts' potential anti-inflammatory effects. The effect that each of our different extracts of *A. campestris* L. and *F.bruguieri* DC. had on the thermal denaturation of BSA was measured at various concentrations, and the findings are presented in Figure.VI.31; Figure.VI.32;Table.VI.29 and Table.VI.30 below.

***Artemisa campestris* L. extracts**

As shown in Figure.VI.31 and Table.VI.29, *A. campestris* L. extracts were evaluated in comparison to the reference compounds used in this investigation (Diclofenac). It is worth noting that extracts have a concentration dependent inhibitory effect on protein denaturation that can be influenced by high temperatures. The AcBE extract had the highest percentage (94.47%) of anti-inflammatory action at a 250 µg/mL concentration, which is very close to that of Diclofenac (99.23%).

The order of the various extracts of *A. campestris* L. in terms of their ability to inhibit was: AcBE (94.47%) >AcDE (91.58%) >AcEAE (84.78%) >AcPEE (58.72%) >AcAE (24.17%).

Table.VI.29. Inhibition percentage in Anti-Inflammatory activity of different extracts of *A.campestris* L.

Concentration (µg/mL)	% Inhibition percentage in Anti-Inflammatory inhibition					
	AcPEE	**AcDE**	**AcEAE**	**AcBE**	**AcAE**	**Dichlofenac***
250	58.72±0.86 [a]	91.58±1.00 [a]	84.78±0.26 [a]	94.47±0.27 [a]	24.17±2.82 [a]	99.23±0.41 [a]
125	34.56±1.47 [b]	82.22±0.56 [b]	83.16±1.68 [b]	85.76±0.37 [b]	6.02±0.80 [b]	76.87±1.24 [b]
62.5	14.65±1.82 [c]	53.95±0.79 [c]	73.89±0.84 [c]	29.06±0.53 [c]	NA	51.37±0.91 [c]
31.25	4.78±0.21[d]	28.31±1.17 [d]	43.85±2.57 [d]	12.47±1.07 [d]	NA	34.11±0.50 [d]

% Inhibition values are expressed as means ±SD of three replicates. The values with different superscripts ([a, b, c, d]) in the same columns are significantly different ($p < 0.05$). *Standard compounds, NA: Note Active.

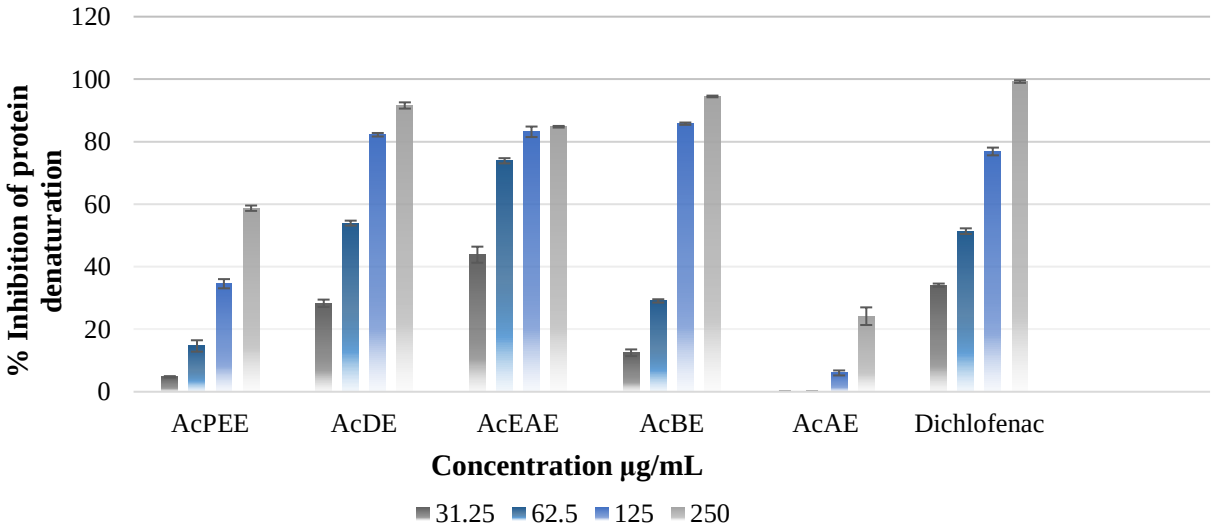


Figure.VI.31. Effect of different extracts of *Artemisia campestris* L. and Diclofenac sodium on the inhibition of protein denaturation.

***Fagonia bruguieri* DC. extracts**

The protein denaturation inhibition percentages provided by the various concentrations of the several extracts of the plant *F.bruguieri* DC. ranged from 10.78% to 92.67%. These results were not significant (p<0.05). The anti-inflammatory activity of diclofenac was used as a

standard to compare to our extracts; it showed maximum inhibition of 99.23% at 250 µg/mL, demonstrating that it is more potent than the extracts investigated. At a concentration of 250 µg/mL, maximal inhibition of 92.76% for FbBE and 90.32% for FbEAE were recorded, both of which are close to the diclofenac standard. Figure.VI.31 shows that the lowest inhibition percentages are seen for FbPEE and FbDE, from 31.25 to 125 g/mL. From this concentration onwards, the percentages of inhibition for the various extracts increase steadily. We observe that the effect of the extracts depends on the concentration and that diclofenac's effect is significantly more effective than that of our extracts.

The order of the various extracts of A. campestris in terms of their ability to inhibit was:
FbBE (92.76%) >FbEAE (80.38%) >FbDE (39.91%) >FbPEE (36.20%) >FbAE (14.81%)

Table.VI.30. Inhibition percentage in anti-inflammatory activity of different extracts of *F.bruguieri* DC.

Concentration (µg/mL)	% Inhibition percentage in anti-inflammatory inhibition					
	FbPEE	**FbDE**	**FbEAE**	**FbBE**	**FbAE**	**Dichlofenac***
250	36.20±0.28 [a]	39.91±1.66 [a]	80.38±0.19 [a]	92.76±0.8 [a]	14.81±0.12 [a]	99.23±0.41 [a]
125	23.24±0.55 [b]	26.63±1.03 [b]	76.15 ±1.02 [b]	84.88±0.87 [b]	NA	76.87±1.24 [b]
62.5	12.11±0.83 [c]	17.91±2.03[c]	60.64±0.78 [c]	66.47±1.76 [c]	NA	51.37±0.91 [c]
31.25	NA	10.78±0.20 [d]	44.04±0.19 [d]	64.18±0.53 [d]	NA	34.11±0.50 [d]

% Inhibition values are expressed as means ±SD of three replicates. The values with different superscripts ([a, b, c, d]) in the same columns are significantly different ($p < 0.05$). *Standard compounds, NA: Note Active.

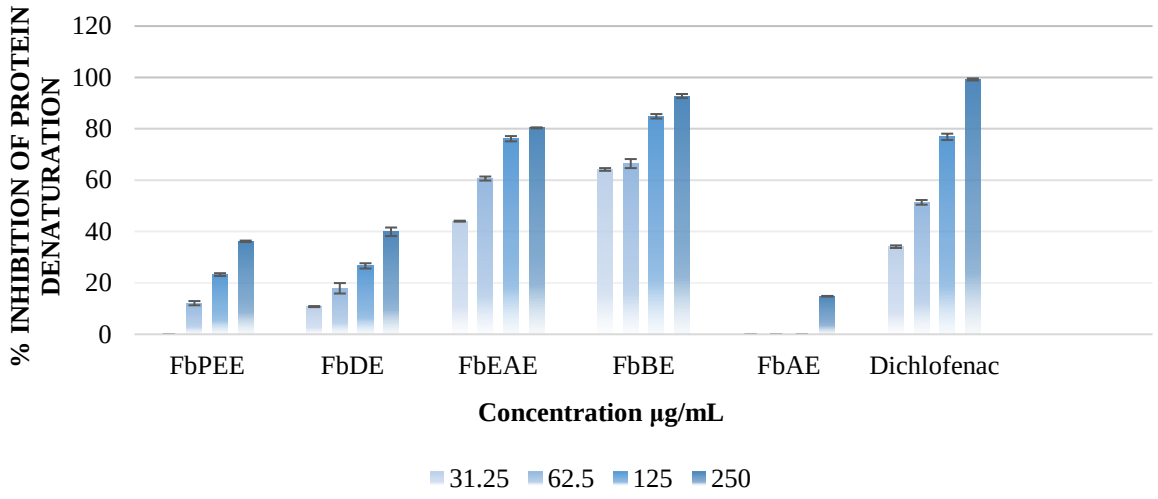


Figure.VI.32. Effect of different extracts of *Fagonia bruguieri* DC. and Diclofenac sodium on the inhibition of protein denaturation.

Discussion

Denaturation of proteins is a process in which proteins lose their tertiary and secondary structures due to exposure to external stress such as heat or by certain compounds such as acids or strong bases and organic solvents. This results in proteins losing their ability to perform their biological function [434]. The mechanism that leads to this denatured state involves a rupture of the electrostatic, hydrogen, or hydrophobic interactions as well as the disulfide bridges responsible for maintaining the three-dimensional structure of the [435]. Some forms of arthritis may be triggered by autoantigens, which can lead to the denaturing of tissue proteins. Therefore, it is possible to conclude that arthritis is characterized by the denaturing of tissue proteins [434].

The denaturation of cell-building proteins or intercellular substances is the source of a significant number of lesions that occur in the tissue. Nonsteroidal anti-inflammatory medications are widely utilized in clinical settings to treat inflammatory disorders due to their ability to reduce or prevent protein denaturation [436].

Inhibition of protein denaturation may play a significant part in NSAID (nonsteroidal anti-inflammatory drug) antirheumatic effectiveness. Still, the unavoidable and potentially fatal side effects are sometimes disastrous [437]. Mizushima & Kobayashi (1968) used protein denaturation as an in vitro screening model for potentially anti-inflammatory compounds [438]. In addition, agents that are able to inhibit or prevent the denaturation of proteins of plant origin are suitable for the development of new anti-inflammatory agents due to the fact that they are

safe, efficacious, and cost-effective as we mentioned previously, flavonoids and phenolic acids reduce inflammation [439].

In the present investigation, the protein denaturation biological test was used to evaluate the anti-inflammatory effects in vitro of the various extracts of *A. campestris* L. and *F. bruguieri* DC. Based on the results obtained, it is reasonable to assume that our extracts can prevent the formation of autoantigens by preventing the denaturation of proteins and consequently prevent the formation of autoantibodies associated with autoimmune diseases.

The increased absorbances of the test samples compared to the control show that the extracts and the reference drug diclofenac sodium stabilize the proteins by inhibiting the heat-induced protein denaturation (BSA) [440]. According to the percentages of inhibition, it is evident that AcBE, AcDE, FbBe and FbEAE were more active when compared to other extracts and effective at lower concentrations compared to diclofenac sodium.

Duganath *et al.,* (2010) found that the molecules in natural products interact with certain amino acids that make up proteins, in this case, albumin, to provide an anti-denaturation effect (model studied) [441]. Aliphatic chains at tyrosine and threonine would be the main sites of such interactions. In addition, they revealed that therapeutic compounds could reactivate the pattern-rich tyrosine receptor in conjunction with threonine, both of which regulate the biochemical mechanisms of signal transduction that are necessary for their overall biological function.

Artemisia campestris L. has been used to treat inflammatory diseases in Tunisia, Morocco, and Algeria for centuries, although its pharmacological properties are unclear [112].

In our study, an extremely high inhibition rate was recorded in the AcBE (94.74±0.27%), and even at the concentration (250 µg /mL), it had identical findings to those achieved with the anti-inflammatory drug that served as a reference (Diclofenac), and this was especially relevant at the same concentration. We suggest that this interesting response is influenced by the presence of the various bioactive substances mentioned in the preceding studies. Effectively, in 2017 Metoui *et al.* demonstrated that flavones tetrahydroxy-5'.6-dimethoxyflavone isolated from *Artemisia campestris* L. in the leaves had the best anti-inflammatory effect [115], which confirmed its existence in AcDE, AcEAE, AcBE via LC-MS/MS profil. In addition, the right hind paw oedema was successfully treated with the ethanolic extract of *A. campestris* L. leaves at doses of 150 and 300 mg/kg in rats [442]. In general, other studies, have also shown that plant extracts can have a vast variety of advantageous biological characteristics, notably properties that reduce the body's inflammatory response.

To our knowledge, no previous studies have provided findings on evaluating *Fagonia bruguieri* DC. on anti-inflammatory efficacy in vitro using the protein denaturation inhibition test in order to draw comparisons, however several researchers have reported that polyphenols and terpenes from *F.bruguieri* have anti-inflammatory effects.

Maximum inhibition of FbBE was observed at 92.76% and FbEAE at 90.32%, and the main components of the most effective FbBE and FbEAE remain phenolic acids, flavonoids, flavones, terpenes, and lignans (Table.V.9 and Table.V.11 Part 2: Chapter V). The anti-inflammatory effect may be due to a constituent or several phytochemical constituents' synergistic effect.

To validate the traditional use of *F.bruguieri* in treating inflammatory illnesses, the studies evaluated the anti-inflammatory activity of the methanol extract of the aerial part in an animal model. Irshad *et al*., (2022) reported the in-vivo (Carrageenan-induced paw oedema in adult male albino Wistar rats model) to assess the anti-inflammatory effects of the methanol extract. In effect, the methanolic extract of *F. bruguieri* DC. at 750 mg/kg showed anti-inflammatory effects in carrageenan-induced oedema after 2, 3, and 4th hours [211].

This preliminary research suggests that the plants *A.campestris* L. and *F.bruguieri* DC. contains components that exhibit the aforementioned pharmacological (anti-inflammatory) properties and can produce bioactive molecules for the development of novel drugs.

VI.5. *Sun protection factor activity (SPF)*

Artemisa campestris L. extracts

The Sun Protection Factor (SPF) was determined as an indicator of the photoprotective efficiency of *A.campetris* L. As illustrated in Table.VI.31 SPF values of the extracts of *A.campestris* varied from 24.79 ±0.07 to 40.76 ±0.1. Based on these findings, it was determined that all of the extracts displayed indications of having photoprotective properties. Furthermore, the outcomes demonstrate that AcME, AcDE, AcEAE, and AcBE extracts all had high photoprotective effects, with SPF values: 42,07±0,17; 40.76±0.11; 39.51±0.09; 38±0.05, respectively.

Table.VI.31. Photoprotective Activity of different extracts of *A.campestris* L.

	Nivea*	Vichy*	AcME	AcPEE	AcDE	AcEAE	AcBE	AcAE
SPF	50.11±0.53	44.22±0.35	42,07±0,17	24.79±0.07	40.76±0.11	39.51±0.09	38.00±0.05	26.07±0.22
Protection	High	High	High	Medium	High	High	High	medium

*Reference compounds.

Fagonia bruguieri DC. extracts

As presented in Table.VI.32, SPF values of the studies extracts of *F.brugueri* DC., ranging from 11.25±0.1 to 43.86±0.12. These findings showed that all fractions had evidenced photoprotective activity. The FbDE, FbBE, and FbEAE revealed a high photoprotective with SPF [43.86±0.12 ; 43.73±0.06; 43.07±2.06 respectively].

Table.VI.32. Photoprotective Activity of different extracts of *F.bruguieri* DC.

Extracts	Nivea*	Vichy*	FbPEE	FbDE	FbEAE	FbBE	FbAE
SPF	50,11 ±0,53	44,22 ±0,35	11.25 ±0.1	43.86 ±0.12	43.07 ±2.06	43.73 ±0.06	34.72 ±0.06
Protection	High	High	Minimum	High	High	High	High

*Reference compounds.

Discussion

The application of sunscreen as a photoprotective agent for protection against UV rays is rapidly gaining popularity. The ability of molecules to absorb, reflect, or scatter the ultraviolet light produced by the sun is essential to its functioning. Sunscreens with a higher SPF are more effective at preventing sunburn and ensure a high level of protection. [443].

Long-term exposure to ultraviolet radiation, which may be divided into three categories: UVA, UVB, and UVC, has been related to various damaging health consequences, including an increased risk of developing skin cancer [444].

In recent years, natural compounds have attracted significant attention as potential protective agents [445]. Phenolic compounds can reduce DNA damage by acting as oxy-reduction-sensitive signalling pathways. Effectively, phenolic compounds have the potential to be

effective in the prevention of UV-induced free oxygen radicals and lipid peroxidation, both of which are processes that are implicated in the development of pathological disorders such as photoaging and skin cancer [446]. Natural compounds are often used in conventional medicine and the cosmetics industry; several flavonoids, in particular, have been demonstrated to protect against the damaging effects of UV radiation [350].

Therefore, these herbal formulations, could produce several beneficial effects for the skin in addition to functioning as UV filters, which is one of the objectives of this study.

The sun protection factor, abbreviated SPF, is a quantitative rating of a sunscreen's efficacy. Considering this concept of SPF, it has newly been established that, according to the SPF ratings. The values of SPF [2 →12], [12 →30], [30 → 50] and >50 are classified as possessing a minimum, moderate and strong sun protection action, respectively.

A.campestris and *F.bruguieri* have so far not been reported to have photoprotective properties. Therefore, the facts reported in this contribution could constitute the first literature report on this species.

According to the study made by Mishra *et al.,* (2012), flavonoids and phenolic compounds possess excellent antioxidants and photoprotective properties [443]. It is common knowledge that flavonoids contain a significant amount of hydroxyl groups. Due to this, studying the phenolic components and flavonoids of plant species is essential for developing topical sunscreen formulations from these extracts. Both *A. campestris* and *F. bruguieri*, the studied plants, include phenolic compounds and flavonoids that can trap free radicals, which is directly related to their antioxidant capacity.

According to the Commission of European Communities 2006 recommendation [447], the AcME, AcDE, AcEAE, AcBE, FbDE, FbEAE, and FbBE belong to the high protection class (30→49.9). The outstanding photoprotective capacities of *A.campestris* and *F.bruguieri* extracts might well be particularly accountable to the presence of flavonoids and phenolics, which have a specific and significant feature: their ability to absorb solar radiation in the ultraviolet range.

Phytochemical analysis of *A. campestris* by LC-MS/MS and HPLC-UV described the presence of flavonoids (rutin, quercitin, acacetin, apigenin,..) and other constituents such as anthocyanidins and coumarins in the most active AcDE, AcEAE, and AcBE extracts, which are substances described with photoprotective action [448]. In contrast, triterpenoid saponins (

Oleanolic acid-3 O-β-D-glucopyranosyl-(1→3)-[(β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranoside) was exclusively detected in FbDE, FbEAE, and FbBE based on the results of the phytochemical profiles in this study. The terpenes and its derivatives are potential agents for treating burns and accelerating skin regeneration [449].

Based on the results of this examination and what was discussed concerning the phytochemicals profiling and its richness with phenolics and flavonoid substances before, *A.campestris* and *F.bruguieri* have a substantial affinity between its photoprotective effect and its total phenolic content. The extraordinary photoprotective effects of *Fagonia bruguieri* DC. and *Artemisia campestris* L. extracts may be due to the presence of these compounds.

On the other hand, the climate and geographical region Saharan and the daily exposure of the plant to sunlight contributed to the exhibition of more bioactive compounds to protect the plant from UV damage, which enhances the photoprotective activity. Consequently, the results obtained exhibited significant antioxidant activity as a promising source for sunscreen in the cosmetic industry or pharmaceutics formulations.

CONCLUSION

Conclusion

Our study focuses on evaluating and promoting Saharan plants to detect novel molecules with therapeutic and industrial uses.

At the outset of this study, we established for ourselves the goals of extraction, characterization, and determination of mineral elements concentration, and assessing the biological activities of extracts of two different plants: *Artemisia campestris* L. and *Fagonia bruguieri* DC from the Algerian Sahara that are utilised in traditional medicine for the treatment of serval diseases. Maceration in solvents of increasing polarity was the method used to extract phenolic compounds from the two plants. Each extract was characterized by its colour and its performance.

INAA and ICP-OES were the methodologies that were utilised in our work in order to carry out the analysis of the concentrations of various mineral elements present in the plants, the outcomes from the two techniques were extremely comparable. Recently, the INAA technique has been used for the purposes of analysis of traces and ultra-traces. In effect, the primary purpose of this approach is to calibrate and validate the data produced by other methods, such as ICP and XRF, which are distinguished by their accessibility and rapidity.

The analysis of minerals carried out on two plants that were investigated using these methods revealed the richness of *Artemisia campestris* L. and their abundance in the minerals required by humans, such as calcium (10538.1mg/kg), iron (1264.2366mg/kg), sodium (311.7137 mg/kg), magnesium (990.7mg/kg), manganese (41.06mg/kg)...etc. According to the results of the analyses carried out on two plants, *Fagonia bruguieri* DC. has the highest concentration of sodium (1762.55 mg/kg). In addition, the concentrations of potentially toxic elements were significantly lower than the toxicological reference values established by the World Health Organisation (WHO), which would confer unique pharmacological and therapeutic capabilities onto these plants.

The chemical profiles of *A. campestris* L. and *F.bruguieri* DC. extracts have been examined by HPLC- UV/Vis and LC-MS/MS analysis. Identified compounds by HPLC- UV/Vis in *A. campestris* L. extracts were mainly flavonoids and phenolic acids: Gallic Acid, Chlorogenic Acid, and Vanillic Acid. Caffeic Acid, Vanillin, p-Coumaric Acid, Rutin, Naringin, and Quercetin. While for *F.bruguieri* DC., the predominantly phenolic compounds present were: Chlorogenic Acid, Vanillin, and Caffeic Acid. The outcomes of LC-MS-MS analysis based

phytochemical investigation on the aerial parts of *A. campestris* L. conducted to the identification of 11 compounds mainly flavones, anthocyanidins, coumarins…etc. For *Fagonia bruguieri* DC. The findings of a phytochemical investigation based on LC-MS-MS analysis led to the identification of 13 constituents such as flavonoids, C-glycosylated flavones, flavan 3-ol...

Upon concluding our investigations, the utilisation of various chromatographic methodologies, including an open column in normal silica gel, reverse phase, Sephadex LH-20, and TLC, guided the isolation and purification of secondary metabolites from the butanolic extract of *Artemisia campestris* L. and *Fagonia bruguieri* DC., through a process of separation and purification.

The elucidation of the structures of these compounds was accomplished through the examination and interpretation of their NMR spectra, with a particular focus on the analysis of their one-dimensional NMR: ^{1}H and ^{13}C, DEPT 135° NMR and two-dimensional NMR, including HSQC, HMBC, and COSY.

Eleven secondary metabolites have been identified, out of which three have been comprehensively characterised, among which:

- ✓ Polysccharide: α-D-glucopyranosyl-(1↔2)-β-D-fructofuranoside (Saccharose) isolated for the first time from *A.campestris* L.
- ✓ Phenylpropanoid glycoside: 4-O-β-D-glucopyranosyloxyl benzoic acid isolated for the first time.
- ✓ Phenolic acids: Syringing isolated for the first time from *Fagonia bruguieri* DC.

In pharmacological terms, various activities have been conducted to assess the antioxidant, anti-inflammatory, anti-acetylcholinesterase, anti-diabetic, anti-obesity, and photoprotective effects of different extracts of *Artemisia campestris* L. and *Fagonia bruguieri* DC.

A series of antioxidant tests were carried out using DPPH, ABTS, beta-carotene, GOR, RP,

CUPRAC, and O-Phenanthroline assays. The results showed that *Artemisia campestris* L. extract has strong antioxidant activity in all the test samples except for the carotene bleaching assay. The heightened concentration of phenolics and flavonoids in AcEAE and FbBE confer an outstanding antioxidant potential, consequently providing a distinctive feature to this extract, as evidenced by the abovementioned techniques.

The interaction of the various extracts of *Artemisia campestris* L. and *Fagonia bruguieri* DC. with the relatively stable superoxide anion radical $O_2^{\bullet-}$ was used to assess the antioxidant activity using cyclic voltammetry techniques. All of the fractions had an remarkable antioxidant activity comparable to that of AcAE (IC_{50} = 0.12 mg/mL) and FbAE (IC_{50} = 0.43 µg/mL), and their IC_{50} values were extremely near to those of the reference antioxidant Ascorbic acid (IC_{50} = 0.0596 mg/mL).

The anti-Alzheimer action was characterized by inhibiting of the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). At IC_{50} = 23.16±0.19 µg/mL, AcEAE showed the most powerful inhibitory effects on acetylcholinesterase. As to BChE, AcBE showed a significant butyrylcholinesterase inhibitory effect (IC_{50} = 93.50±1.60 µg/ mL). The FbPEE extract shows a potent AChE and BChE activity with IC_{50} = 27.41±1.03 µg/ mL and 46.14±0.78 µg /mL, respectively.

Based on our results *Artemisia campestris* L. and *Fagonia bruguieri* DC. extracts showed an outstanding anti-diabetic action that was more efficient than acarbose, The most powerful inhibitor of α-amylase was AcPEE and FbDE (IC_{50} = 11.79±0.14 ; 7,12±0,89 µg/mL, respectively).

Regarding the anti-lipase activity, AcEAE and FbDE demonstrated a potent inhibition against the pancreatic lipase enzyme.

The anti-inflammatory effects of various extracts of *Artemisia campestris* L. and *Fagonia bruguieri* DC. were evaluated for their efficacy in treating inflammation through the anti-denaturation model of BSA. The experimental results indicate that AcBE and FbBE exhibited a statistically significant impact compared to the reference drug Diclofenac sodium.

As for the photoprotective effect of the plant, it was measured by the sun protection factor (SPF). The species *Artemisia campestris* L. and *Fagonia bruguieri* DC. were approved to possess a robust photoprotective capacity, effectively absorbing ultraviolet radiation.

A conclusion that can be drawn from all of these relevant results is that the presence of compounds that are endowed with promissing and promising biological activities within *Artemisia campestris* L. and *Fagonia bruguieri* DC. Would justifying their diverse medicinal uses.

Chemical structure of the compounds Isolated from *A.campestris* L. *& F.bruguieri* DC.

Species	Compound	Family of compound	Chemical structure	Name of compound	Report
Artemisia campestris L.	PN	Polysaccharide		*α*-D-glucopyranosyl-(1↔2)-*β*-D-fructofuranoside (saccharose)	Isolated for the first Time from *A.campestris* L.
Fagonia bruguiri DC.	Pb	phenylpropanoid glycoside		Syringing	Isolated for the first Time from *F.bruguieri* DC.
Fagonia bruguiri DC.	Pv 2	Phenolic Acid		4-O-β-D-glucopyranosyl benzoic acid	Isolated for the first Time

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ANNEX

Annex I

The gamma-ray spectra were acquired through medium neutron activation analysis and long periods of the *Artemisia campestris* L. and *Fagonia bruguieri* DC.samples.

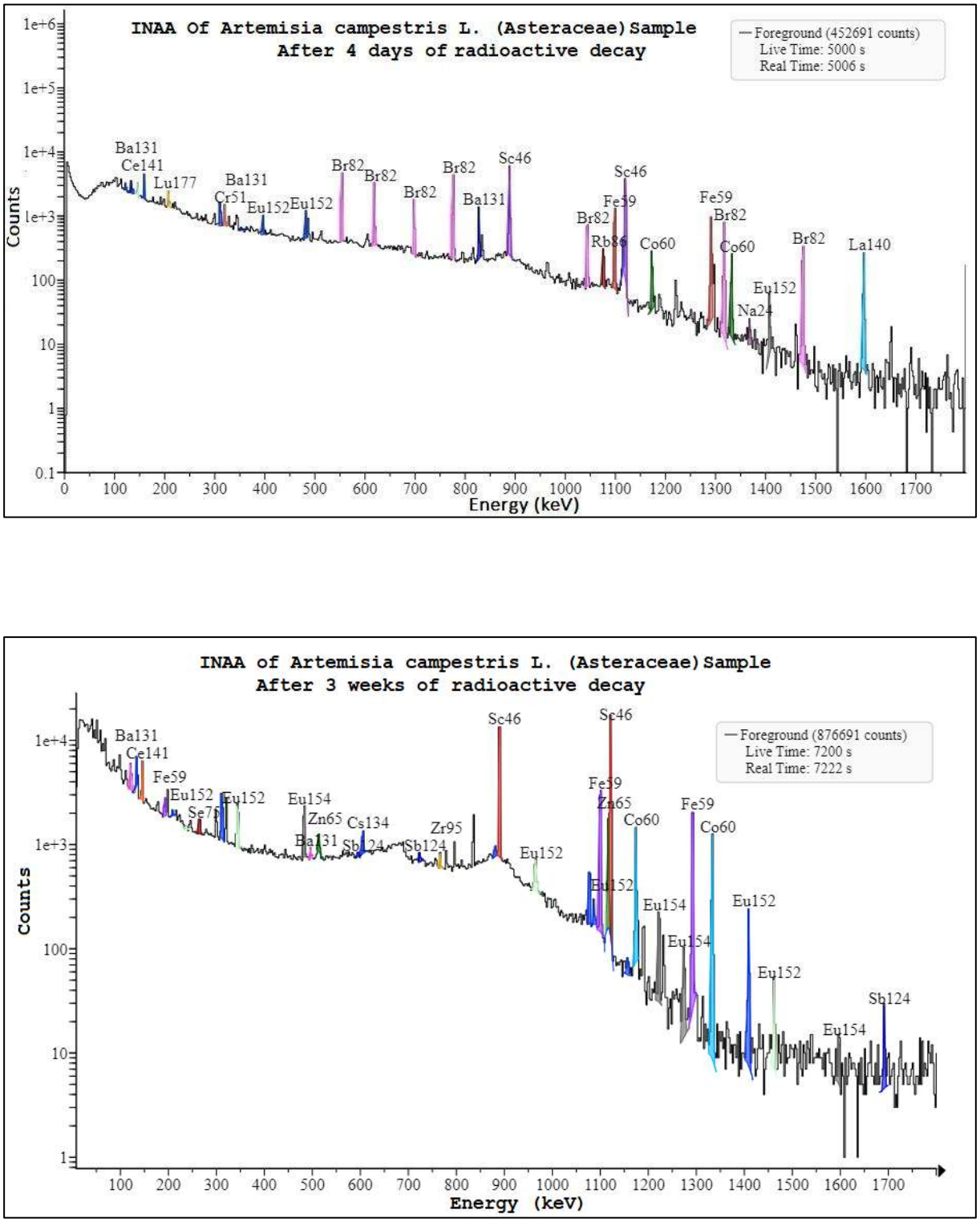

Figure S1. The medium and long-period gamma-ray spectra of *Artemisia campestris* L.

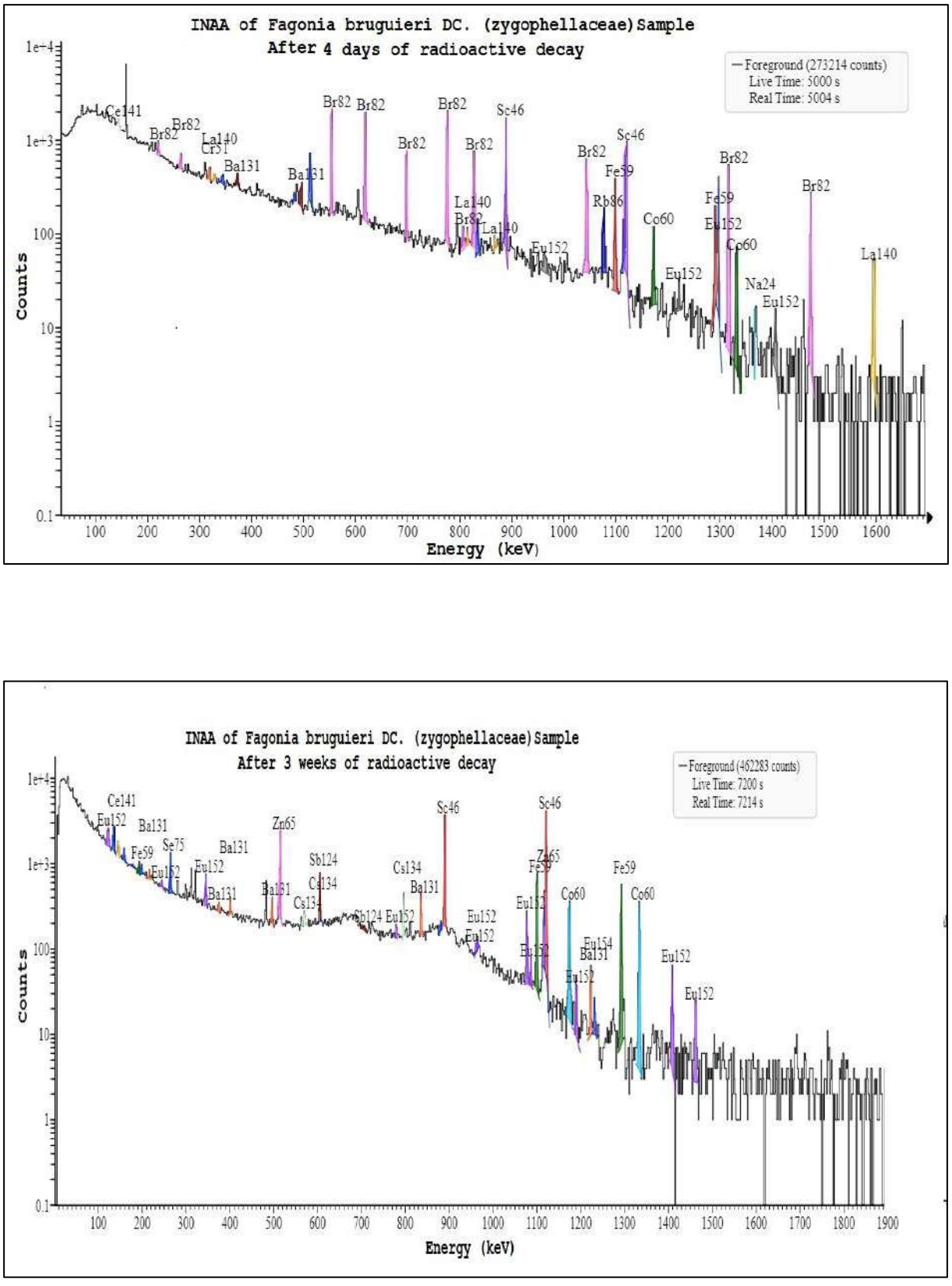

Figure S1. The medium and long-period gamma-ray spectra of *Fagonia bruguieri* DC.

ANNEX II

Figure N.1. DPPH Assay

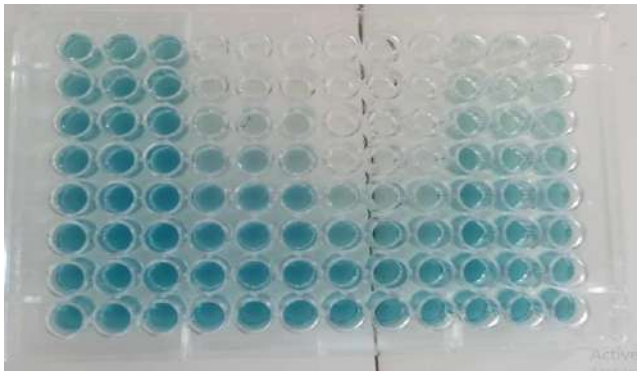

Figure N.2. ABTS Assay

Figure N.3. β- Carotene Assay

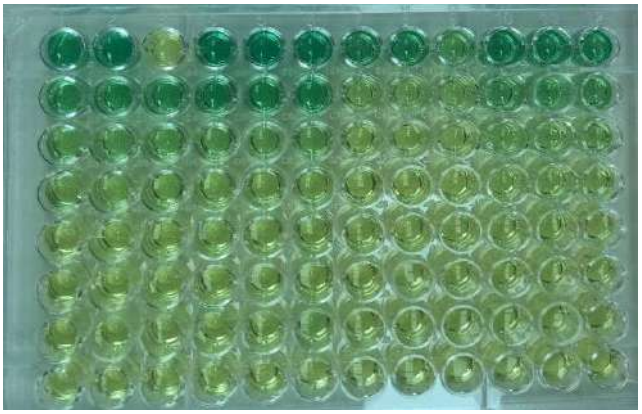

Figure N.4. Reducing Power Assay

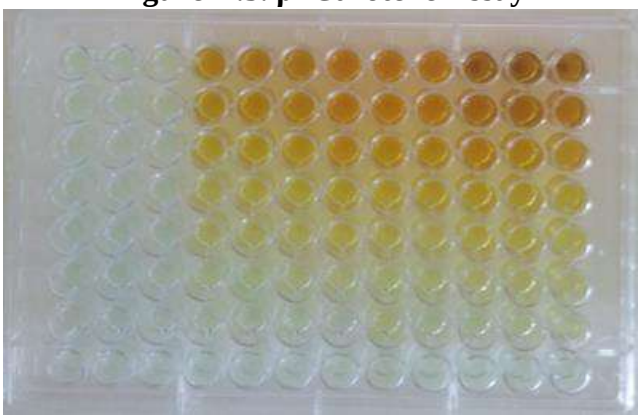

Figure N.5. CUPRAC Assay

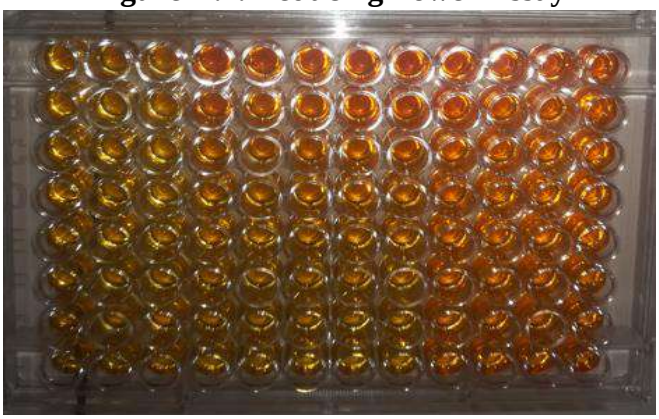

Figure N.6. Phenantroline Assay

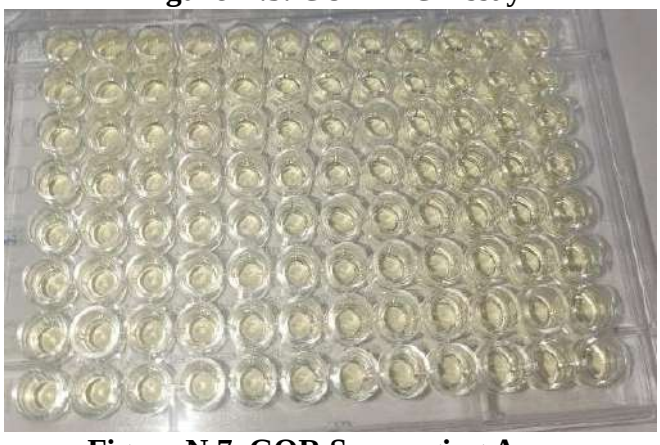

Figure N.7. GOR Scavenging Assay

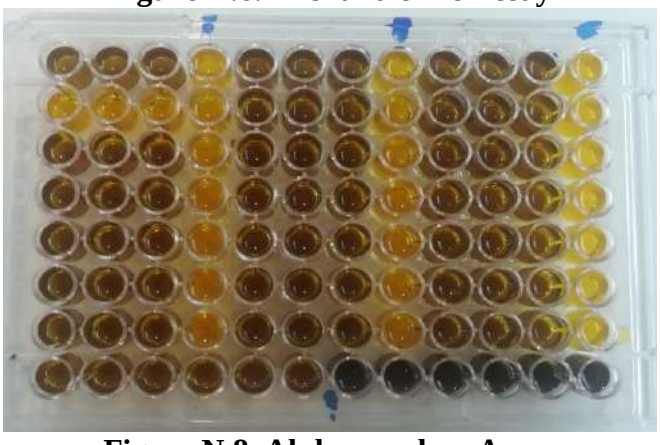

Figure N.8. Alpha-amylase Assay