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To my lovely family

To my parents

My husband and my dear sons

To my sister, brothers and their families

To my husband's family

To all my relatives

To all my friends and colleagues

Yasmine

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Abstract

The present study aimed to determine the phenolic profile and some biological activities of three species from arid and semi-arid areas namely: *Senecio hoggariensis*, *Senecio coronopifolius* and *Bunium incrassatum*. A variation in yield and total polyphenols and flavonoid content was recorded by methanolic extracts (ME) and their fractions: ethyl acetate (EAE) and butanol (BuE). The chromatographic analysis using HPLC-DAD confirmed the presence of eleven phenolic compounds, including *p*-coumaric acid, as a major component in both EAE extracts of the two *Senecio* studied. While rutin and chlorogenic acid were dominant in the ME of *S.coronopifolius* (10.29mg/g) and *S.hoggariensis* (5.97mg/g), respectively, BuE extracts contained low amounts of identified compounds. Rutin was the major phenolic compound in ME, EAE and BuE fractions of *B.incrassatum* (26.55 mg/g, 16.04 mg/g and 4.45 mg/g, respectively). Regarding biological activities, EAE of *B. incrassatum* displayed a considerable antioxidant potential in DPPH, ABTS, CUPRAC and β-carotene linoleic acid assays, whereas ME extracts of two *Senecio* plants exhibited the highest chelating activity. Furthermore, EAE of *S.hoggariens* and *S.coronopifolius* extract among all fractions had moderate inhibition against AChE activity with 45.99±1.81% and 42.42±1.39 %, At 200 µg/mL, respectively. All extracts showed weak inhibition against BChE enzyme but displayed moderate tyrosinase inhibitory capacity. In addition, the study of antimicrobial and antibiofilm formation showed that EAE and BuE extracts were active against all tested microorganisms at MIC and sub-MIC concentrations with different degrees of activity against biofilm attachment. EAE of *B.incrassatum* exhibited the highest antibiofilm activity on *S.aureus* ATCC 25923 (72.88±1.83%) at 5 mg/mL. The anti-quorum sensing of extracts was determined using various quorum quenching strategies by evaluation the QS inhibition on CV026, the violacein inhibition on CV12472, and the swarming motility inhibition in *P.aeruginosa* PA01. Results showed that the EAE extracts of the studied plants were the most powerful compared to BuE fractions. They provided a potential inhibition against both biofilm formation and microbial cell-to-cell communication (quorum sensing). These findings indicate that extracts of the studied plants could be further investigated in the treatment of numerous free-radical mediated cellular damage and various infectious diseases.

Keywords: *Senecio Sp*; *Bunium incrassatum;* Phenolic compounds; Antioxidant; Anticholinesterase; Anti-tyrosinase; Antibiofilm; Anti-quorum sensing.

Résumé

Le présent travail vise à établir le profil phénolique ainsi que quelques activités biologiques de trois espèces provenant des régions arides et semi-arides qui sont : *Senecio hoggariensis, Senecio coronopifolius* et *Bunium incrassatum*. Une variation en rendement et en teneur des polyphenols et des flavonoides a été enregistré par l'extrait méthanolique (ME) et ces fractions acétate d'éthyle (EAE) et l'extrait butanolique (BuE). L'analyse chromatographique par HPLC-DAD a confirmé la présence de 11 composés phénoliques dont *p*-coumaric acid est le composé majoritaire dans la fraction EAE des deux *Senecio* étudiés alors que le rutin et le chlorogenic acid sont dominants dans ME de *S.coronopifolius* (10,29mg/g) et *S.hoggariensis* (5,97mg/g), respectivement. les extraits BuE contiennent le contenu le plus faible en composés identifiés. Rutin représente le composé phenolique majoritaire dans les fractions ME,EAE et BuE de *B.incrassatum* (26,55 mg/g, 16,04 mg/g et 4,45mg/g, respectivement). Concernant les activités biologiques, EAE de *B.incrassatum* a révélé un potentiel antioxydant considérable en utilisant le test de DPPH, ABTS et CUPRAC et blanchissement de β-carotène, cependant, les fractions ME des deux plantes *Senecio* ont montré l'activité chélatrice la plus élevée. De plus, l'extrait de EAE de *S.hoggariens* et de *S.coronopifolius*, parmi toutes les fractions ont présenté une inhibition modérée contre l'AChE avec 45,99 ±1,81 % et 42,42 ±1,39 %, à 200 µg/mL respectivement. Tous les extraits ont montré une faible inhibition contre l'enzyme BChE mais une capacité d'inhibition modérée contre la tyrosinase. De plus, l'étude antimicrobienne et antibiofilm ont montré que les extraits EAE et BuE étaient actifs contre tous les microorganismes testés à des concentrations CMIs et sous-CMIs, avec divers degrés d'activité contre l'attachement des biofilms. L'extrait EAE de *B.incrassatum* a enregistré l'activité antibiofilm la plus élevé sur *S.aureus* ATCC 25923 (72,88 ±1,83%) à 5mg/ml. L'anti-quorum sensing des extraits a été déterminé à l'aide de diverses stratégies d'extinction du quorum en évaluant l'inhibition de la violaceine sur CV12472, l'inhibition de QS sur CV026 et l'inhibition de la motilité chez *P.aeruginosa* PA01. Les résultats ont montré que les extraits EAE des plantes étudiées ont été plus actifs par rapport aux fractions BuE. Ils ont fourni une inhibition potentielle de la formation de biofilm et de la communication microbienne d'une cellule à cellule (détection de quorum). Les résultats indiquent que les extraits des plantes étudiées pourraient être etudiés dans le traitement de nombreuses maladies à médiation par les radicaux libres et de diverses maladies infectieuses.

Mots clés: *Senecio Sp; Bunium incrassatum;* Composés phenoliques; Antioxydant Anticholinesterase; Anti-tyrosinase; Antibiofilm; Anti-quorum sensing.

الملخص

تهدف هذه الدراسة إلى تحديد المحتوى الفينولي و بعض الأنشطة البيولوجية لثلاثة أنواع نباتية من المناطق الجافة وشبه الجافة وهي *Senecio hoggariensis*، *Senecio coronopifolius* و *Bunium incrassatum*. تم تسجيل تباين في المردود والمحتوى الكلي البوليفينولي والفلافونويدي للمستخلص الميثانولي (ME)، مستخلص خلات الإيثيل(EAE) ومستخلص بيوثانول (Bu). أظهرت نتائج التحليل الكروماتوغرافي باستخدام كروماتوغرافيا السائلة ذات المردود العالي وجود 11 مركب بما في ذلك حمض*P*-comaric كمركب رئيسي في كل من مستخلصات EAE لنبتتي *Senecio* قيد الدراسة، بينما سجل Rutin وحمض كلوروجينيك كمركبين أعظميين في مستخلص ME لكل من *S.coronopifolius* (10،29 مغ/غ) و*S. hoggariensis* (5،97مغ/غ) على التوالي . احتوت مستخلصات البيوثانول على كميات قليلة من المركبات المحددة. تم تسجيل Rutin كمركب فينولي رئيسي في كل من مستخلصات ME وEAE و BuE لنبتة *B.incrassatum* بنسب (26،55مغ/غ)، (16،04مغ/غ) و (4،45مغ/غ) على التوالي. فيما يتعلق بالانشطة البيولوجية، أظهر مستخلص EAE لنبتة *B.incrassatum* نشاطا معتبرا مضادا للأكسدة في كل من اختبار CUPRAC, ABTS DPPH, و تبييضβ-caroten ، بينما سجلت مستخلصاتME لنبتتي *Senecio*قدرة عالية على استحلاب المعادن. علاوة على ذلك، أظهر مستخلص EAE لكل من *S. hoggariensis* و *S.coronopifolius* من بين كل المستخلصات تثبيط معتدل ضد انزيم Ache بنسبة 45،99±1،81% و 42،42±1،39% على التوالي،عند 200مكغ/مل. سجلت جميع المستخلصات فعالية منخفضة ضد انزيم Bche و لكن لها فعالية معتدلة على تثبيط انزيم التيروزيناز. بالإضافة الى ذلك أظهرت الدراسة المكروبية و النشاطية المضادة لتكوين البيوفيلم (الاغشية الحيوية) أن مستخلصات EAE و BuE تملك فعالية ضد جميع الميكروبات قيد الدراسة عند أدنى تركيز مثبط و تحت المثبط بدرجات متفاوتة في منع تكوين البيوفيلم . سجلEAE لنبتة *B.incrassatum* أعلى نسبة تثبيط لتكوين البيوفيلم ضد بكتيريا *S.aureus* ATCC 25923 بقيمة 72،88±1،83% عند تركيز 5مغ/مل. تم تحديد الاستشعار المضاد لنصاب المستخلصات باستخدام مختلف استراتيجيات QQمن خلال تقييم تثبيط النشاط على CV026، CV1247 وعلى حركة *P.aureuginosa* P01 .أظهرت النتائج أن مستخلصاتEAE للنباتات قيد الدراسة كانت أقوى من BuE كما سجلت تثبيطًا معتبرا لكل من تكوين الأغشية الحيوية و الاتصال الميكروبي من خلية إلى خلية (استشعارالنصاب). تشير النتائج إلى أن مستخلصات النباتات قيد الدراسة يمكن أن تخضع للمزيد من الابحاث عليها في علاج العديد من الأمراض التي تسببها الجذور الحرة ومختلف الأمراض المعدية.

الكلمات المفتاحية: المركبات الفينولية، النشاط المضاد لتكوين الاغشية الحيوية، الاستشعار المضاد للنصاب، النشاط المثبط للكوليناستيراز، المضاد للاكسدة، المضاد للتيروزيناز،*Senecio SP*، *Bunium incrassatum*.

List of Abbreviations

$A_{0.50}$: Concentration in which the absorbance is 0.50

ABTS: Acide 2,2’-azino-bis (3-ethylbenzothiazolin-6-sulfonic)

AChE: Acethylcholinesterase

BChE: Butyrylcholinesterase

BHA: Butylated hydroxyanisole

BHT: Butylated Hydroxytoluene

Bu: *n*-butanol extract

CUPRAC: cupric reducing antioxidant capacity

DAD: Diode array

DMSO: Dimethylsulfoxide

DPPH: 2,2 diphenyl-1-picrylhydrasyl

DTNB: 5, 5'-dithiobis (2-nitrobenzoïc) acid

EA: ethyl acetate extract

GAE: Equivalent of galic acid

HPLC-DAD: high-performance liquid chromatography- Diode Array Detector

IC_{50}: The half maximal inhibitory concentration

L-DOPA: L-3,4-dihydroxyphenylalanin

ME: methanol extract

QE: Equivalent of quercetin

TFC: Total flavonoid content

TPC: Total phenolic content

TR: Time of retention

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Introduction

Introduction

The use of products from natural sources affords excellent benefits to human health because of the significant prevention of various human illnesses. These benefits are attributed to their diverse pharmacological abilities, which include antioxidant, inflammatory and analgesic activities **(Carocho and Ferreira, 2013).** Plants are an excellent source of antioxidant activity compounds, such as phenolic acids, flavonoids, tannins, vitamins and carotenoids, which may be utilized as pharmacologically active products **(López*et al.*, 2007).** In this case, antioxidants derived from plants are highly recommended; they are safer for the body and can block oxidative damage through reduction with free radicals, capturing oxygen and forming chelates with catalytic metal compounds **(Sayuti and Yenrina, 2015)**. Oxidative stress has been considered one of the initial occurrences of Alzheimer's disease (AD) **(Barja, 2004)**.

Neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD), common in the elderly population over the 65, have become serious health problems, especially in industrialized countries. Hence, much research is being conducted to find new drugs and treatment strategies for these diseases. In this sense, natural products and medicinal herb extracts are attractive sources in the search for novel anti-AD and anti-PD drug candidates. Acetylcholine deficiency, hydrolyzed by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), has been proved in the brains of AD patients (**Orhan, 2012)**. On the other hand, the function of tyrosinase (TYR) is to catalyze the oxidation reaction of tyrosine to melanin, which is linked to hyperpigmentation of the skin, the occurrence of melanoma, unwanted browning of fruits and vegetables, and dopamine toxicity in PD **(Mendes et al.,2014).**

In recent years, the emergence of microbial resistance has increased significantly due to the uncontrolled use of antimicrobial drugs commonly employed in the treatment of infectious diseases **(Zampini *et al.,* 2005)**. Therefore, there is a great interest in medicinal plants and herbs due to their high antimicrobial properties. On the other hand, bacterial biofilms are another serious global health concern due to their ability to tolerate antibiotics, host defense systems and other external stresses, contributing to persistent chronic infections **(de la Fuente-Núñez *et al.,* 2013)**. The current antibiofilm study concentrates on preventing biofilm formation by various strategies, such as inhibition of adhesion, interfering with quorum sensing and promoting early cell detachment **(Kaplan, 2005)**. Quorum sensing is a

bacterial cell-cell communication process that controls collective behaviors **(Rutherford and Bassler, 2012)**.

QS relies on the production, accumulation, detection, and population-wide response to extracellular signaling molecules called autoinducers (AIs) **(Waters and Bassler, 2005)**. As a result, natural plant compounds might be an effective biological control agent targeting bacterial QS networks. *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PA01 are well-known bioindicators that can be used to identify substances that can inhibit the quorum sensing mechanism **(Noumi *et al.,* 2018)**.

Algeria benefits from a diverse range of climates and soils thanks to its distinctive geographical location in the southern Mediterranean basin, its vast surface, and its relief, allowing the development of a rich and diverse flora with over 3139 species of wild and naturalized plants growing in the country, which have become one of the hallmarks part of the community's identity **(Ababsa *et al.,* 2014)**. In this regard, the present work aimed to document some biological effects of various extracts from selected plants belonging to the Apiaceae family*: Bunium incrassatum* from semi-arid areas and to Asteraceae family: *Senecio coronopifolius* and *Senecio hoggariensis* from the desert area, The later one is an endemic species in the Sahara Mountains in Algeria, Egypt, Niger and Chad **(Lebrun *et al.,* 1981)**. One of the motives driving interest in this subject, particularly in the desert region, is the diversity of plants and their content in natural products that can have therapeutic benefits. In the present study, the following objectives are formulated:

- Qantification of the polyphenols and flavonoids contents;
- Characterization by HPLC-DAD of the phenolic compounds present in plant studied extracts;
- *In vitro* evaluation of antioxidant activity using $DPPH^{\bullet}$, $ABTS^{\bullet+}$ radical scavenging, β-carotene-linoleic acid, cupric reducing antioxidant capacity (CUPRAC) and metal chelating assays;
- Evaluation of anti-acetylcholinesterase and anti-tyrosinase properties;
- Evaluation of antimicrobial activity and antibiofilm potency;
- The inhibition of quorum-sensing in bacteria using *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PA01 as bacterial model.

Literature Review

I. The plant natural products

In general, natural products are chemical compounds or substances isolated from living organisms. This can be divided into two major classes, the primary and secondary metabolites. The primary metabolites are substances with essential functions to the survival of the organism that produces them. In contrast, the secondary are not essential to survival but to increase the competitiveness of the organism within its environment **(Karlovsky, 2008).**

Plant kingdom produce many types of secondary metabolites that have important ecological functions for plants, providing protection against attack by herbivores, microbes and serving as attractants for pollinators and seed-dispersing agents. They may also contribute to competition and invasiveness by suppressing the growth of neighbouring plant species **(Osbourn and Lanzotti, 2009).**

The secondary metabolites extracted from plants are subdivided in three major classes; terpenoids, alkaloids and phenolic compounds **(Kabera et al., 2014).**

I.1. Alkaloids

The name alkaloid comes from the Latin words alkali (meaning ash) and -oid (like). Alkaloids are a class of nitrogen-containing chemicals found primarily but not exclusively in plants. Over 27,000 distinct alkaloids have been identified, with plants accounting for 21,000 of them. The structures of alkaloids contain one or more nitrogen atoms in the form of primary, secondary, or tertiary amines. These nitrogen atoms give alkaloids basic characteristics and allow them to exist as water-soluble salts following interaction with acids. Nature also contains alkaloids with quaternary nitrogen atoms **(Dewick, 2009)**.

I.2.Terpenoids

Terpenoids are a huge and structurally diverse class of natural chemicals, with at least 35,000 distinct molecules identified **(Dewick, 2009)**. Terpenoids' carbon skeletons are made up of C5 isoprene units that are connected head to tail, while tail to tail junctions are also found in nature. Cyclisation reactions can modify the combination of isoprene units, however the isoprene unit is typically still immediately identifiable. Terpenoids are classified based on the number of isoprene units in their carbon skeleton. As a result, they are divided into: "hemiterpenes(C5), monoterpenes(C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25), triterpenes (C30), and tetraterpenes (C40)". Isopentenyl pyrophospate is the primary biosynthetic precursor for terpenoid production (IPP). IPP can be produced via two pathways:

the cytosolic mevalonic acid (MVA) pathway and the plastidic methylerythritol phosphate (MEP) pathway **(Cseke *et al*.,2006).**

I.3. Phenolic compounds

Polyphenols are compounds of the plant secondary metabolism that can accumulate in certain plant organs such as leaves, fruits, roots and stems. As a large group of bioactive chemicals, they have diverse biological functions. Because they are essential to plant life, they can provide defense against microbiological attacks and make food unpalatable to predators and other herbivores **(Vogt, 2010)**.

Polyphenols are divided into several classes according to the number of phenol rings and to the structural elements that bind these rings to one another. Although such structural diversity results in the wide range of phenolic compounds that occur in nature, they can basically be categorized into several classes **(Balasundram *et al*., 2006)**. They range from simple molecules (such as phenolic acids with a single aromatic structure), biphenols (such as ellagic acid) and flavonoids, which contains 2 to 3 aromatic rings **(Ignat *et al*., 2011)**, to polyphenols containing 12 to 16 rings. The main groups of polyphenols are flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and et lignans **(D'Archivio *et al*., 2007).**

I.3.1. Flavonoids

Flavonoids belong to a large group of phenolic plant constituents **(Erlund, 2004)**. Flavonoids are commonly found in green plants except the Anthocerotae as glycosides in leaves, flowers, stems and roots **(Harborne and Williams, 2000)**. They are presented as derivatives of 2-phenyl-benzo-γ-pyrone. The carbon atoms in flavonoid molecules are assembled in two benzene rings, commonly denoted as A and B, which are connected by an oxygen containing pyrene ring (C). A common part in the chemical structure of all flavonoids is carbon skeleton based on flavan system (C6-C3-C6) (Figure 1) **(Symonowicz and Kolanek, 2012)**. Flavonoid classes are distinguished by additional oxygens present as substituents of the heterocyclic ring and by hydroxyl groups as well as by the alkyl groups. Thus, the flavonoid classes are chalcones, flavones, flavonols, flavanones, anthocyanins, and isoflavones. Due to presence of conjugated double bonds, flavonoids are often brightly colored **(Harborne and Williams, 2000)**.

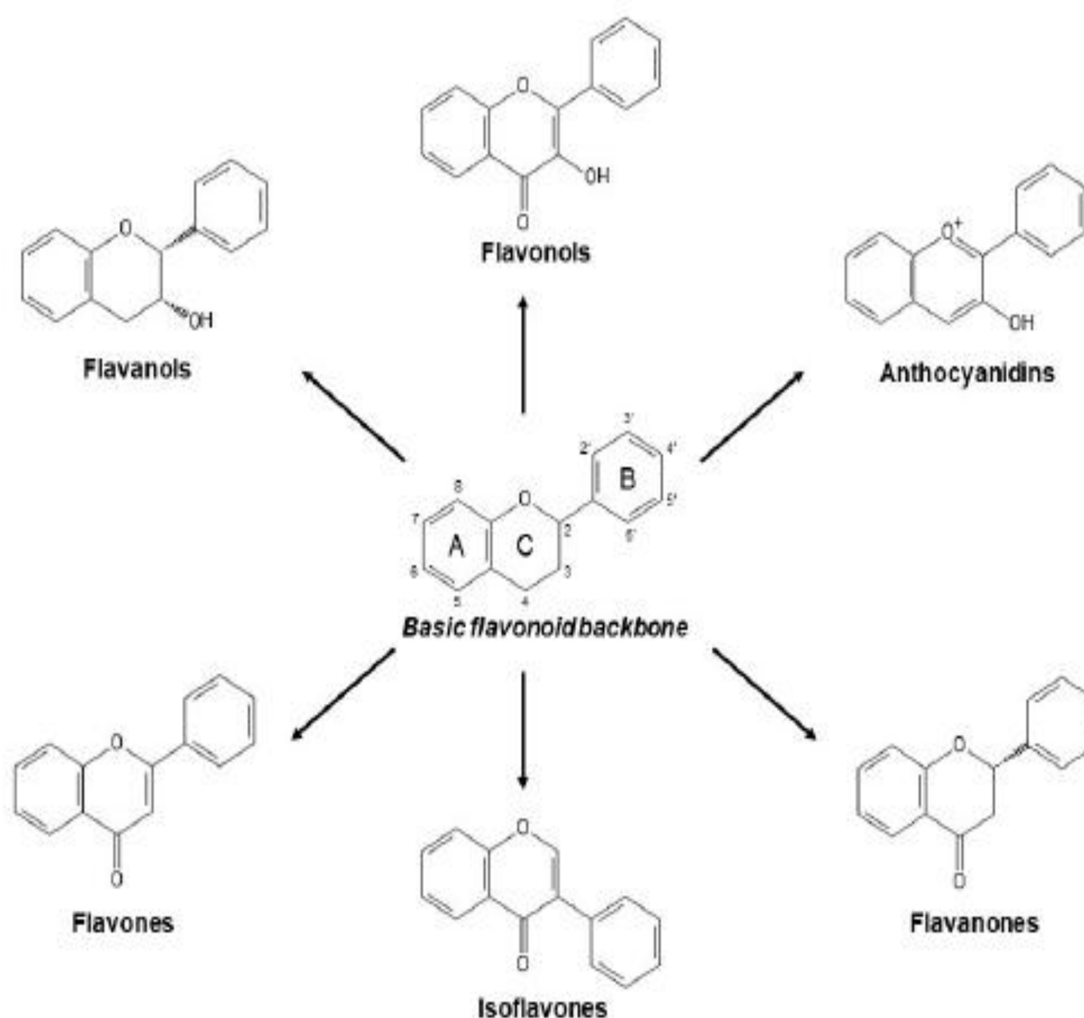


Figure 1. Chemical structures and examples of compounds for each group of flavonoids **(Caleja, 2017)**.

I.3.2. Non-flavonoid phenols

Compounds with smaller and simpler chemical structures than flavonoids belong to this class. However, there are also non-flavonoids with complex structures and high molar mass **(De la Rosa *et al*., 2018).** This group consists primarily of phenolic acids, coumarins, stilbenes, lignans, and tannins. Phenolic acids are aromatic acid compounds with a phenolic ring and an organic carboxylic acid function. The principal groups that make up the polyphenol class are hydroxycinnamic acids and hydroxybenzoic acids **(Del Rio *et al*., 2013)**.

II. Species under investigation

II.1. Classification of the studied species

Schema 1 shows the classification of Asteraceae family species **(Quezel and Santa, 1963)**.

Domain: Eukarya
Kingdom: plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida-Dicotyledonae

Order: Asterales	**Order:** Apiales
Family:Asteraceae	**Family:**Apiaceae
Genus: *Senecio*	**Genus**: *Bunium*
Species: *S.hogarriensis* Batt. &Trab; *S.gallicus* ssp *coronopifolius* (Desf.)M.	**Species:** *B.incrassatum*(Boiss.) Batt. & Trab.

Schema 01. Classification of the studied species.

II.2. Asteraceae family

Asteraceae, also called Compositae, is one of the largest angiospermic plant families among the dicotyledonous, based on the large number of species (1,620 genera and 23,600 species) that represen this plant family with cosmopolitan distribution **(Funk *et al.*, 2005)**. Constituting almost 10% of all flowering plants worldwide, Asteraceae is usually divided into 12 subfamilies **(Funk *et al.*, 2009).** Except for Antarctica, the family is most abundant in the sub-tropical and temperate latitudes, occurring commonly across meadows, valleys, grassy plains, rolling plateaus, and mountainous slopes **(Bayer *et al.*, 2007)**. It includes edible, medicinal, noxious, invasive and endangered species **(Heywood *et al.*, 2007)**. The majority of plant members representing this family are herbaceous in nature, but shrubs andtrees, as well as creepers and climbers, are also reported. They can easily be detected by several factors, such as fused anthers, single ovules in fruits, and their capitulum inflorescence **(Garcia *et al.*, 2010)**.

II.2.1 The genus *Senecio*

The genus *Senecio*, which belongs to the tribe Senecioneae, is the largest and most complex genus in the family of the Asteraceae and includes more than 1500 species with a worldwide distribution **(Loizzo *et al.*, 2004)**. In Algeria, it is represented by 18 species, 5 of which are endemic **(Quezel and Santa 1963)**. It is a well-known source of pyrrolizidine

alkaloids (PA), sesquiterpenes, in particular eremophilanolide derivatives and benzofurans **(Assem *et al*., 2002)**. More than 180 Senecio species contain pyrrolizidine alkaloids as the most characteristic secondary metabolites **(Liddell, 2000)**.

In traditional medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, eczema and wound healing have also been reported **(Burgueno *et al*., 2004)**. This species is used in traditional medicine for the treatment of skin, respiratory and osteoarticular diseases. Moreover, some studies have reported the cytotoxic activity of these species, while others were focused on the biological activities and more attention has been paid not only to antioxidant activity or finding some natural cholinesterases (ChEs) inhibitors for the management of cognitive/mental and memory disorders, but also to the antibacterial, antifungal properties of these species. Biosynthesis of algal pheromones, antiviral activity, anti-inflammatory effects, antiulcer activity, antimitotic effects, phytotoxic activity, insecticidal, neurotoxic, and glutathione depleting activities **(Yang *et al.,* 2011)**.

II.2.1.1. *Senecio hoggariensis* Batt. &Trab

II.2.1.1.1. Morphological description

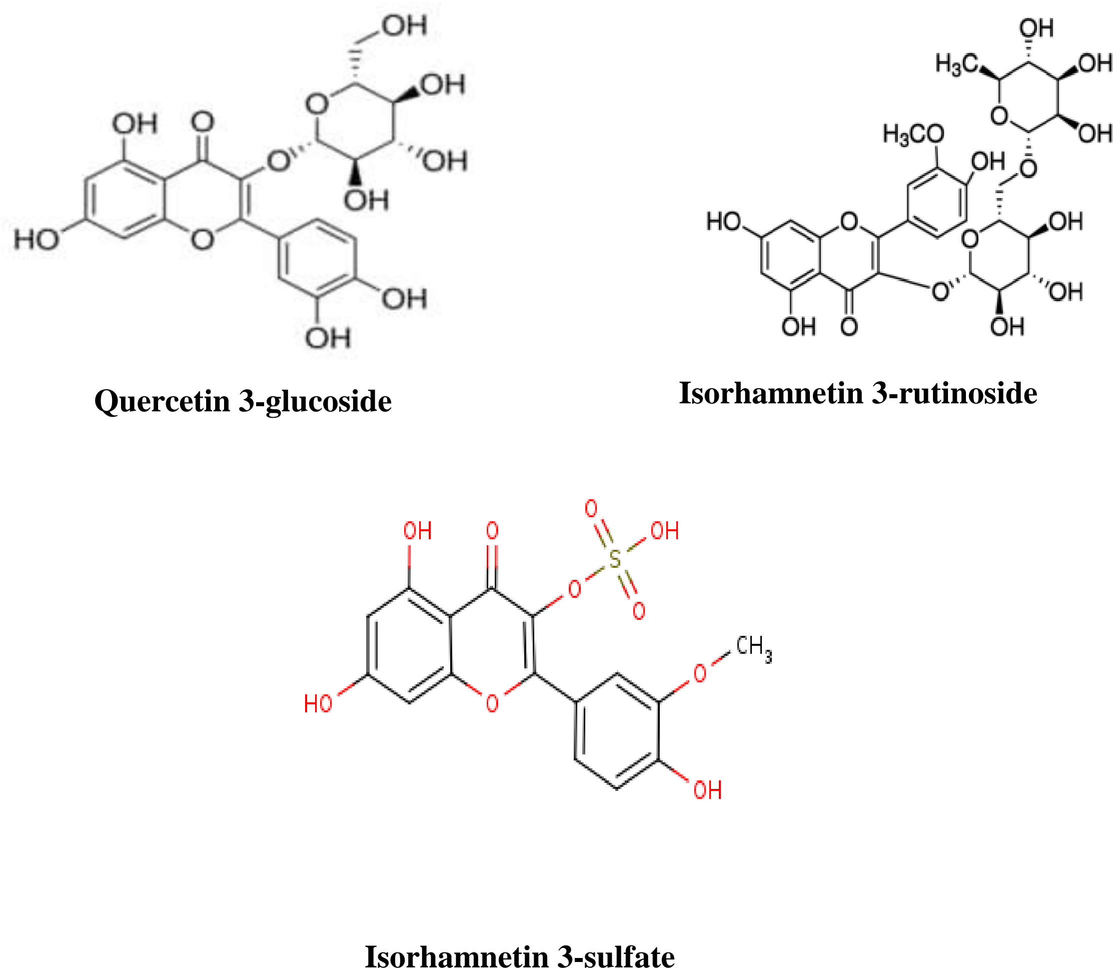
Annual or biennial plants with Ligules purplish, sometimes very short, barely radiating (var. eradiatus Maire). *S.hoggariensis* is a hairless plant with fleshy leaves, with linear straps. Flower heads in loose corymbs. Involucre cylindrical-campanulaceous, with 12-15 purple and linear bracts. Linear achenes, large (4 mm approximately) with crystalline hairs. Egret as long as the achene, deciduous -Rock gardens- above 1200 m **(Quezel and Santa, 1963)**.

II.2.1.1.2. Geographic distribution

S.hoggariensis is un endemic species found in Saharan of Algeria (Hoggar mountains), Egypt (Gebel Elba and Sinaï), Niger (Aïr) and Tchad (Tibesti) **(Lebrun, 1981)**.

II.2.1.1.3. Phytochemistry and pharmacological properties

Very little excited work on the phytochemical study of this plant, the first and the last work is reported by **Ragaa and Nabiel. (1981),** they allowed to identify the presence of Quercetin 3-glucoside, in the same time the presence of Isorhamnetin 3-rutinoside and Isorhamnetin 3-sulfate in traces. There is no study in the literature concerning biological activities about this plant.



Quercetin 3-glucoside **Isorhamnetin 3-rutinoside**

Isorhamnetin 3-sulfate

Figure 2. Chemical structures of some compounds founed in *Senecio hoggariensis*.

II.2.1.2. *Senecio gallicus* L *ssp. coronopifolius* (Desf.)M

Synonymes: *Senecio desfontainei* Druce.

Vernacular names: Qorreis, Omm Lonein, Loweinein

II.2.1.2.1. Morphological description

Stems are smooth and glabrous and measures about 2 or 3 feet high. Leaves are stalkless/sessile, whorled, fleshy, semi-cylindrical, awl-shaped and long. Flowers can be numerous and are arranged in corymb. The petalis yellow, calicle with straps, subulate can be with or without straps. Fruits are sometimes hairy but never glandular **(Frédiric, 1827)**.

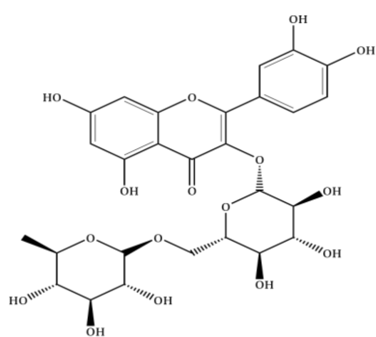
II.2.1.2.2. Geographic distribution

Senecio gallicus L. *ssp. coronopifolius* is a Saharan plant **(Quezel and Santa, 1963)**. It is most often restricted to southern North Africa and northern Asia. However, it grows in areas of the Eastern Province of Saudi Arabia especially along the Gulf Coast **(De Pooteret et *al*.,**

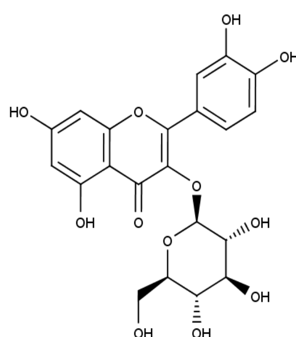
1986). *S.coronopifloius* grows on arid, acidic soils close to the coast, in desert wadis, and on agricultural borders **(Boulos, 2002)**.

II.2.1.2.3. Phytochemistry and pharmacological properties

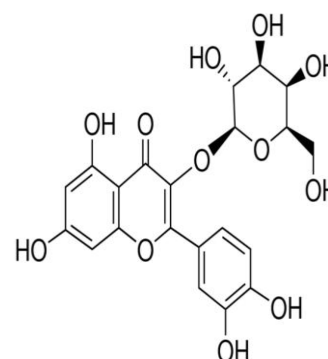
There are some studies on *S.coronopifolius* in the literature. In one study, from ethanolic extract from aerial parts of the plant, Three isorhamnetin glycosides, four quercetin glycosides and quercetin were isolated: Quercetin 3-*O*-β-rutinoside; Quercetin 3-*O*-β-D-galactopyranoside ;Isorhamnetin 3-*O*-β-rutinoside Isorhamnetin-3-*O*-β-D-glucopyranoside ;Isorhamnetin 3-*O*-β-D-galactopyranoside; Quercetin 3-*O*-robinobioside **(Nassar *et al.*, 2002).** In another study, GC/MS results of *Senecio glaucus* subsp. *coronopifolius* leaves revealed the presence of 6 identified hydrocarbons: Octacosane (11.85%) was the major identified compound followed by hexatriacontane (4.76%) while 4-ethyl-tetradecane (0.28%) was the minor identified hydrocarbon while results of the GC/MS analysis of the saponifiable matter of *Senecio glaucus* subsp. *coronopifolius* leaves indicated the presence of 12 identified fatty acid methyl esters. Linolenic acid methyl ester (31.07%) (Poly unsaturated fatty acid) was the major identified compound followed by palmitic acid methyl ester (saturated fatty acid) (22.11%), while Tetracosanoic acid methyl ester (0.3%) was in minor quantities **(Mohamed, 2015)**.



Quercetin 3-*O*-rutinoside



Quercetin 3-*O*-glucopyranoside



Quercetin 3-*O*-β D galactopyranoside

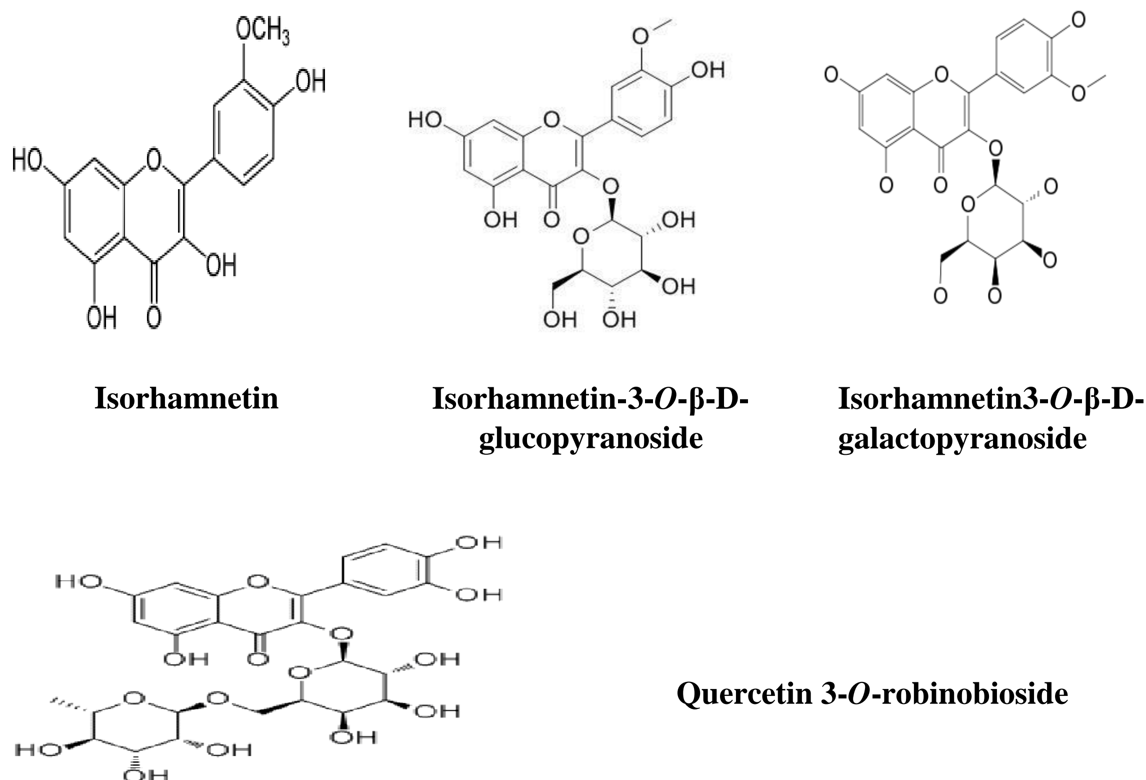


Figure 3. Chemical structures of major compounds of *S.coronopifolius*

Only few reports have appeared concerning the volatil oil chemistry of this species, In the study of **El- Shazly. (1999)** a total of 41 compounds were identified from different parts of the plant. In the flowers, β-myrcene (38.1%), cymene (19.7%), phellandrene (9.8%) and dehydrofukinone (10.1%) are the major constituents. In the leaves and stems, dehydrofukinone was the major component constituting 42.8% and 77.6%, of the oil respectively. The major components of the root oil were, eremophilane (dehydrofukinone 46.9% and euparin 20.9%), caryophyllene 8.5% and (Z)-β-farnesene (3.7%).

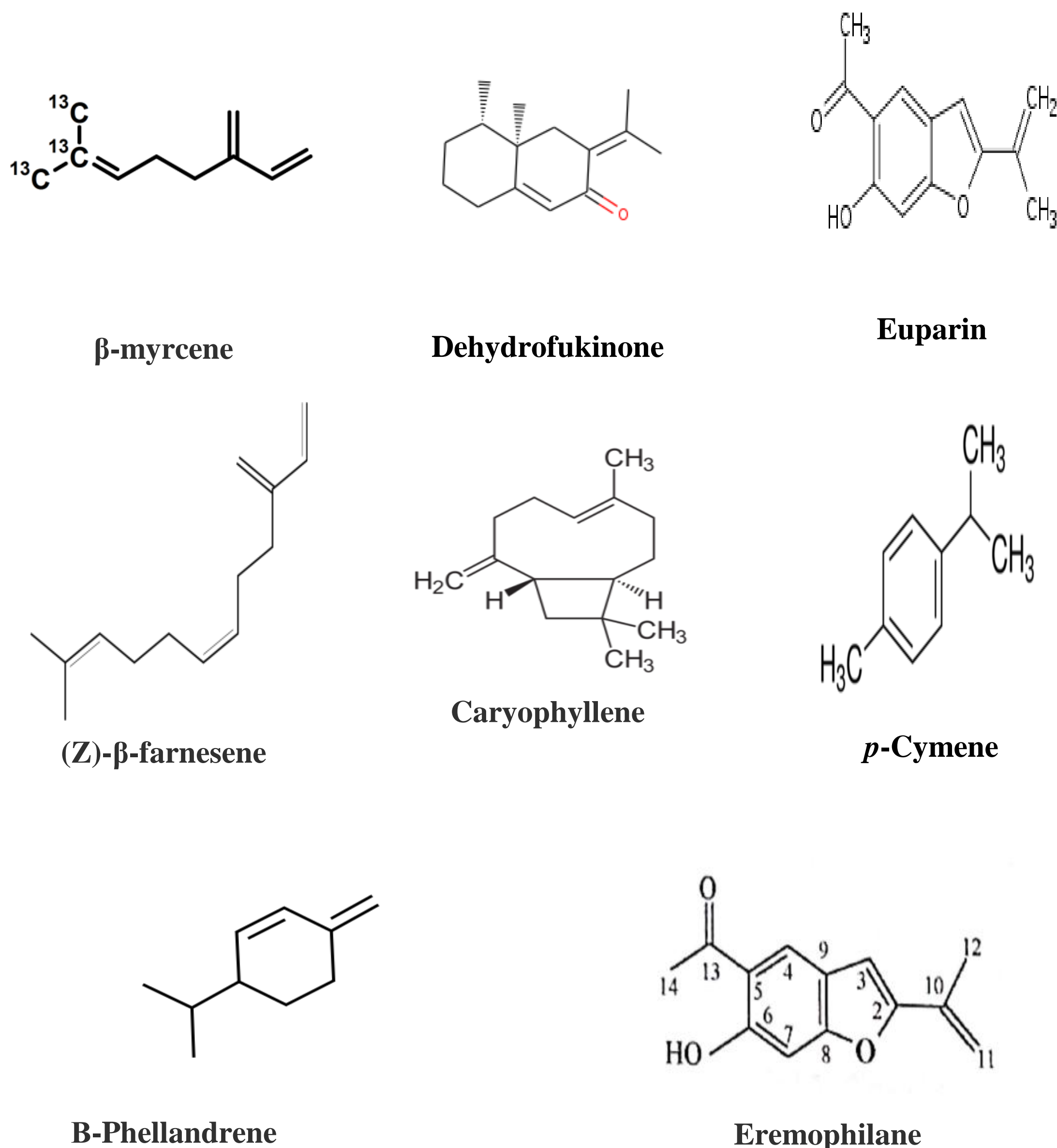


Figure 4. Chemical structures of some compounds identified in *Senecio coronopifolius* essential oil.

Chemical composition of oils were reported to be associated with the antimicrobial activity, it was showed broad and powerful activity against two Gram negatif (*K.pneumoniae* and *E.coli*), two Gram positif (*B.subtilis* and *S.aureus*) and two fungi (*A.flavus* and *C.albicans*) **(El Shazly, 1999)**. In another study, the major volatile constituents of *S. glaucus* subsp.*coronopifloius* from Belgium are myrcene (24.0%) and dehydrofukinone (21.0%) **(De Pooter *et al.*, 1986)**.

Other reports have been effected on *S.coronopifolus* and found to contain other classes of secondary metabolites including alkaloids. Table 1 gives informations about the compounds isolated from *Senecio coronopifolius*.

Table 1. Compounds isolated from *Senecio coronopifolius*.

Compound	References
Senecioside, ethyl 3,5-di-*O*-caffeoylquinic acid, ethyl 3,4-di-*O*-caffeoylquinic acid, quercetin-3-*O*-rutinoside, isorhamentin 3-*O*-β-D-rutinoside, 3β,23- dihydroxy-lup-20(29)-en-28-oic acid-3β-caffeate, 5,7,4′-trihydroxy-3,8-dimethoxyflavone, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, 2-(1,4-dihydroxy cyclohexanyl)- acetic acid, p-hydroxyphenylacetic acid, β-sitosterol-3-*O*-β-D-glucopyranoside.	**(Hussain *et al.*, 2013)**
Senecionine, sen-eciphylline riddelliine senecivernine, spartioidine, integerrimine, acetylseneciphylline and retrorsine	**(El-Shazly, 2002)**
Euparin and 9,10-dehydrofukinone	**(Ghazy and El-Masry, 1986).**
Senecionine, seneciphylline, senkirkine, 7-angelylretronecine, retrorsine, and an alkaloid (probably an isomer of senkirkine)	**(Rizk *et al.*, 1983).**
Senecionine, riddelliine and sen-eciphylline	**(Gharbo and Habib., 1969; Habib, 1974).**
Senecionine (I), riddelliine (III) and Fumaric acid	**(Klasek *et al.*, 1968).**

II.3. Apiaceae family

Apiaceae family is one of the most important familles of flowering plants, which consists of 3780 species in 434 genera. lt is distributed throughout the world, mostly in the northem temperate regions and high altitudes in the tropics. The main common features of Apiaceae species are: aromatic herbaceous nature, altemate leaves with sheathing bases, hollow stems, small flowers, inflorescences determined in simple or compound umbel, and indehiscent fruits or seeds with oil ducts **(Christensen and Brandt, 2006).** This family is well known for its distinctive flavors due to the secretory cavities consisting of schizo genous oil ducts with resin, oil, or mucilage and located in the fruits, stems, leaves and roots **(Berenba um, 1990).** Apiaceae family pro vides a large number of plants which are used for different purposes including nutrition, medicine, beverages, spices, repellents, staining, cosmetics, fragrances and industrial uses. Ethnomedically, several plants of this family are used as home based remedies to treat various illnesses re lated to digestive, endocrine, reproductive and respiratory systems **(Aéimovié and Kostadinovié, 2015).** This family is rich in phytochem icals and secondary metabolites which are potential source of drugs such as terpenoids, triterpenoid saponins, flavonoids, coumarins, polyacetylenes, and steroids. Furthermore, several species of this family are an excellent source of essential oils, more than 760 different corn ponents from different chemical classes with high pharmaceutical in terest are detected in the essential oils within this family.

II.3.1. Genus *Bunium*

Bunium L. is a genus of 212 geophytes in the Apiaceae family. From morphological standpoint, This genus' members are distinguished by: white petals with inflexed terminal lobes, although the fruits appear, homogeneous petioulate primary segments of bi- or tri-pinnatifid leaf blades, tuberiform storage roots, and calyces without teeth **(Degtjareva *et al.*, 2009).**

At morphological point, *Bunium* species are similary to those of the *Carum* L's genus. These two genus appear as aromatic plants and beneficial herbs **(Jassbi *et al.*, 2005).** Which frequently grow in temperate, warm and dry, arid and semi-arid climates, and mainly on mountain slopes **(Saeidnejad *et al.*, 2013).** In Algerian flora, the genus *Bunium* contains seven species, four of which are endemic **(Quezel and Santa, 1963).**

II.3.1.1. Ethnobotanical and medicinal application of *Bunium* species

A vast number of *Bunium* species are utilised in traditional folk medicine all over the world thought not all *Bunium*'s species have been studied in this regard. The most significant relevant species is *B. persicum*. Table 2 listed some popular ethnobotanical application of *Bunium* species.

Table 2. Ethnobotanical and pharmacological applications of some *Bunium* species.

Species/organ	Region	Folkloric applications	References
B. persicum (Boiss). whole plant	Iran	- To treat gastrointestinal diseases, as well as headaches, urinary and respiratory tract infections, and colic.	**(Hassanzadazar *et al*., 2018)**
B. persicum (Boiss). seeds	Kashmir (India),	- Diarrhea, indigestion and dysentery.	**(Bhardwaj *et al*., 2019)**
B. bulbocastanum	Morocco	- To treat musculoskeletal and gynecological problems.	**(Teixidor-Toneu *et al*., 2016)**
B. bulbocastanum whole plant	Algeria	- Against flatulence and intestinal worms	**Miara *et al*., 2018)**
B. cylindricum fruits	Pakistan, Iran	- Carminative treatment.	**(Mojahedi *et al*.,2014);**
B. elatum (Batt.) aerial parts	Algeria	- Against intestinal gas and stomach colic.	**(Miara *et al*., 2018)**
B. fontanesii	Algeria	- Treat allergy, bronchitis, and cough.	**(Benarba *et al*.,2015)**
B.macuca Boiss. tubers	Spain	- Against warts.	**(Benitez *et al*., 2010)**
B. paucifolium	Turkey	- As food.	**(Demirci *et al*., 2014).**

Previous phytochemical studies on *Bunium* genus revealed the existence as frequent metabolites of coumarins **(Appendino *et al.*, 1994)**, sesquiterpenes **(Appendino *et al.*, 1991)** and especially essential oils (monoterppenoids) **(Salehi *et al.*, 2008)**. Furthermore, essential oils and extracts from various *Bunium* sphave been shown to have antihistaminic, antibacterial and antifungal properties (**Boskabady and Moghaddas, 2004**), in addition to their antioxidant properties **(Shahsavari *et al.*, 2008**).

II.3.1.2. *Bunium incrassatum* (Boiss.) Batt. & Trab

Synonymes: *Bunium mauritanicum, Bunium bulbocastanum* L.

Vernacular names: Talghouda (التلغودة)

II.3.1.2.1. Morphological description

Bunium incrassatum is a 40-60 cm glabrous and perennial plant. Its root is are rounded with blackish bulb and grows about 5 dm long, cylindrical, striated and slightly branchy. Its leaves are two or three times winged, divided into narrow and linear cutouts. The lower parts are borne on long petioles, and the radicles have slightly wider and shorter cutouts. The flowers are white and form fairly large umbels. The general annulus is made up of seven to eight line arleafl ets, much shorter than the spokes The fruits are cylindrical, a little thickened at the top, ending in two styles, shiny and then deciduous. This plant is found in slightly wetfields and pastures. Its size varies from 1-5 dm; its root is good to eat and is called earth-nut **(Lariushin, 2014**).

II.3.1.2.2. Geographic distribution

This genus is often found in Spain, the Balearic Islands and northern Africa **(De Lamarck, 1805)**, its harvest period begins from the beginning of may and ends at the end of july.

II.3.1.2.3. Phytochemistry and pharmacological properties

In the study of **Bousetla *et al.* (2011)**, and from the methylene chloride: methanol extract of *B.incrassatum* roots two coumarins, β-sitosterol, sucrose and oleic acid are fouanded. Morover, the crude extract exhibited significant antimicrobial potential against all the tested microbes, particulary fungal strains.

Concerning the composition of the essential oils isolated from different parts of *B.incrassatum,*from ground fruit (A) 28 components (81.4%) (including caryophyllene oxide

(31.0%), (Z)-β-farnesene (8.7), β-caryophyllene (7.2), and germacrene B (5.8)) are presented as the principal constituents of sample A. *B.incrassatum* fruit-bearing branches Oils (B) included 40 constituents (85.2%) (caryophyllene oxide (26.8%), nonacosane (11.6), germacrene B (7.7), β-caryophyllene (5.8), (Z)- β-farnesene (5.1), caryophyllenol II (4.8), and spathulenol (2.5)) as the principal constituents in sample B. *B.incrassatum* thickened branches (C) provided 24 constituents (75.4%) including (nonacosane (44.7%), spathulenol (5.3), eudesm-4(15),7-dien-1β-ol (4.4), caryophyllenol II (4.1), (Z)-β-farnesene (2.3), germacrene B (1.2), and β-caryophyllene (1.0)) as the principal constituents of sample C **(Bousetla *et al.*, 2014)**.

In another study essential oil of *B. incrassatum* showed seven major compounds :palmitic acid (18.39%), caryophyllene oxide (17.36%), β-eudesmol (13.95%), *n*-pentacosane (5.13%), 10-epi-α-muurolol (4.36%), hedycaryol (4.14%) and spatuleneol (4.04%) **(El kolli *et al.*, 2017)**.

B. incrassatum essential oils and methanolic extract had moderate antioxidant activity; The methanol extracts were shown to have anti inflammatory properties by inhibiting denaturation of albumin, which contributes to their inflammation response **(El kolli *et al.*, 2017)**. Oils from aerial parts shown significant antibacterial potent against a wide rang of bacterial species and antifungal activities. It also had estrogenic properties **(Chentouh *et al.*, 2017)**.

The roots of this plant are very nutritious and are consumed like potato. There are various formulation in case it is employed as an astringent and diarrhea for its qualities, but most people prefer to consume it directly without mentioning that it has been properly cleansed and stripped of the parties. Dried and powdered tubers are considred astringent and anti diarrheic in traditional medecine, and have been shown to be effective against inflammatory hemorrhoids. Furthermore, this plant is employed to cure bronchitis and cough **(Bousetla *et al.*, 2011)**.

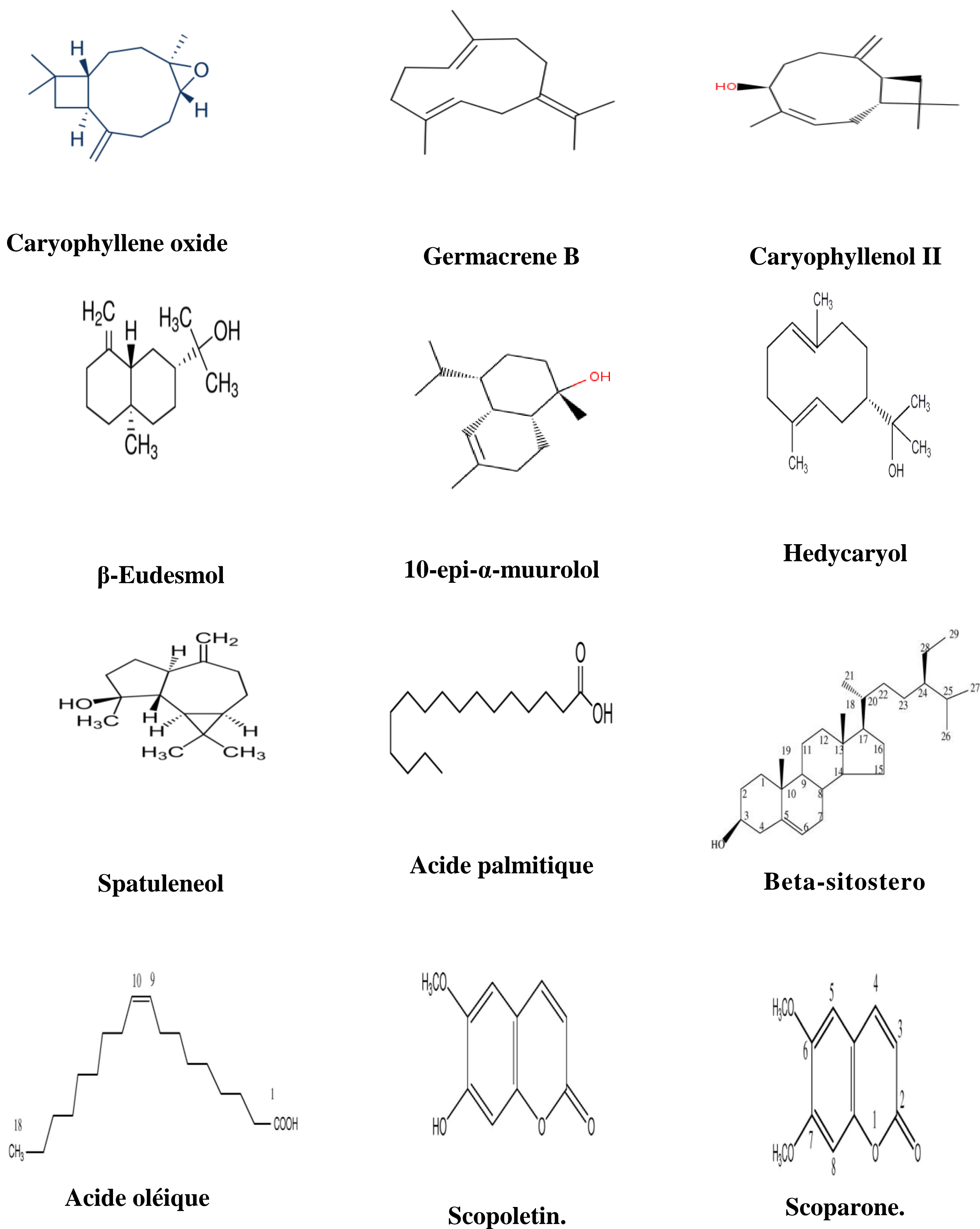


Figure 5. Chemical structures of some compounds identified in *Bunium incrassatum.*

III. Oxydative stress

III.1. What is oxidative stress ?

Oxidative stress has been defined by the imbalance between the production and degradation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) **(Fujii *et al.*, 2011)**. ROS are molecules whose chemical makeup gives them high reactivity and can come from the metabolism of oxygen or nitrogen. ROS and RNS can be free radicals such as the superoxide radical ($O2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), and nitric oxide ($NO^{\cdot}$). However, other non free radicals can also be found, such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) **(Phaniendra *et al.*, 2015)**. Overproduction of ROS results in cellular injury, including lipid peroxidation, protein oxidation and DNA damage **(Won *et al.*, 2013).** The oxidative damage created by free radical generation is a critical aetiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the aging process **(Inbathamizh *et al.*, 2013)**. The cells of the body are exposed to oxidants from endogenous and exogenous sources. Exogenous sources include heat, trauma, ionizing radiation, UV radiation, ozone, smoking, infection and metabolism of a broad spectrum of drugs and xenobiotic. Endogenous sources are mainly byproducts of metabolism by functional generation by host defense cells (phagocytes) and cells of connective tissues **(Dahiya *et al.*, 2013)**.The main endogenous source of ROS is the electron transport chain **(Ye *et al.*, 2015)**.

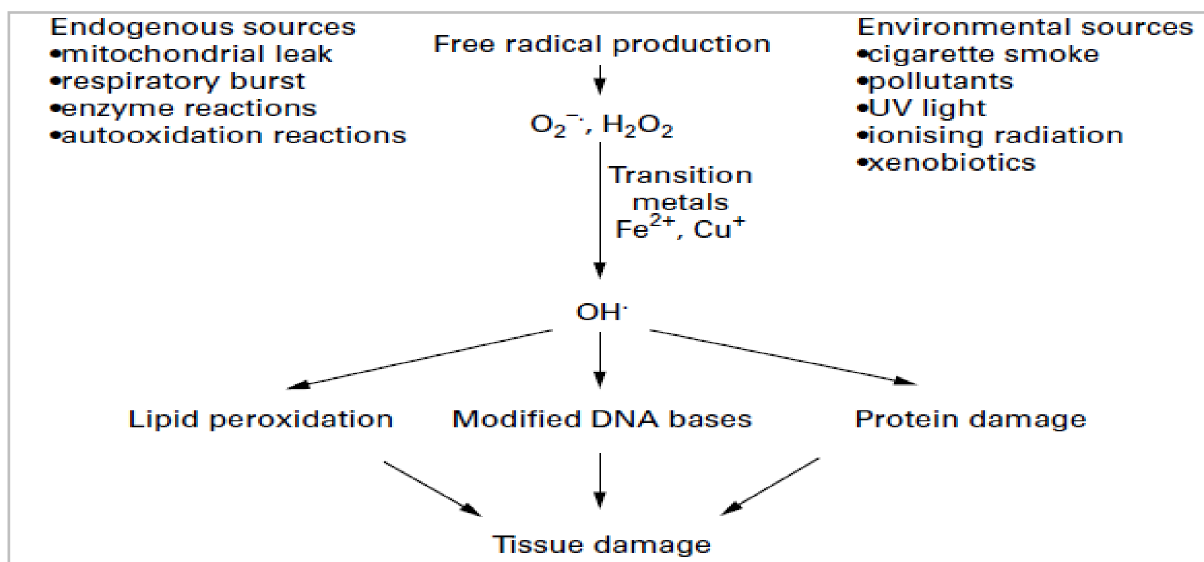


Figure 6. Main sources of free radicals and their consequences **(Young and Woodside *et al.*, 2001)**.

III.2. Antioxidant systems

Antioxidants are molecules, at low concentrations, slow or significantly inhibit the oxidation of an oxidizable substrate **(Pendyala *et al*., 2008)**. Antioxidants are diveded into two classes based on their mechanism of action to: antioxidants that break chains and preventive antioxidants **(Trivedi and Lal, 2017)**. Antioxidantsare also classed as enzymatic and non-enzymatic. Table 3 summarizes some enzymatic antioxidants, whereas table 4 includes some non-enzymatic antioxidants **(Menaca-Guerrero *et al*., 2020)**.

Table 3. Enzymatic antioxidants

Name	Function
Superoxide dismutase (SOD)	$O_2^- + O^-_2 + 2H^+ \rightarrow O_2 + H_2O_2$
Catalase (CAT)	$2 H_2O_2 \rightarrow 2 H_2O + O_2$
Glutathione peroxidase (GPx)	$H_2O_2 + 2 GSH \rightarrow H_2O + GSSG$
Glutathione reductase (GR)	GSSG+NADPH+H+ 2 GSH+NADP
Peroxiredoxin (Prx)	Catalyzes peroxides reduction . The peroxides oxidize the cysteine from the catalytic site, then reacts with another cysteine residue to form a disulfide, which is reduced by an electron donor.
Thioredoxin (Trx)	Reduces oxidized proteins by creating disulfide bonds and transferring electrons from their reactive cysteines. Thioredoxin reductase uses NADPH electrons to reduced the dithiol residues of Trx.

Table 4. Non-enzymatic antioxidants

Name	Function
Vitamin C	It act in combinition with vitamin E to quench free radicals. In the same , it regenerate the reduced form of vitamin E.
Vitamin E	It prevent the cell membrane from lipid peroxidation by removing peroxyl radicals.
Carotenoids	β carotene is effective at removing singlet oxygen. In addition, it protect cell membranes and lipoproteins against peroxyl radicals damage.
Glutathione	Direct ROS purification, as well as, the reduction of other antioxidants such as vitamin E and ascorbic acid. It also work in a cyclical manner with GPx / GR and NADPH

III.3. Plant secondary metabolites as powerful antioxidant agents

Plants possess miraculous antioxidant effects because of their high oxygen exposure physiology. In fact, plants may have more sites of ROS generation. Therefore, they could evolve more proficient non-enzymatic antioxidant systems than humans (**Kasote *et al.*, 2015**). Plants synthesize several enzymatic and non-enzymatic antioxidants to avoid free radicals' toxic effects, in addition to be able to synthesize and accumulate a wide variety of low and high molecular weight secondary metabolites, which play important roles in ROS metabolism and effectively avoid the uncontrolled oxidation of essential biomolecules, thus, acting as antioxidants (**Williams *et al.*, 2004**). There is a broad diversity of naturally-occurring antioxidants found in plants, differing in their composition, physicochemical properties, site, and mechanism of action. The major antioxidant plant secondary metabolites are phenolic compounds, and they can be divided into five general groups, namely, phenolic acids, flavonoids, lignans, stilbenes, and tannins **(Blokhina *et al.*, 2002)** Briefly, they provide protection through scavenging numerous ROS, including hydroxyl radicals, peroxyl radicals, hypochlorous acids, superoxide anions and peroxynitrite **(Halliwell *et al.*, 2007)**.

III.4. Polyphenols as antioxidante agent

The most prominent activity of polyphenols is expressed to be their antioxidant activity against oxidative stress by scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals (**Celep and Rastmanesh, 2013**). Polyphenols have been found to be strong antioxidants that can neutralize free radicals by donating an electron or hydrogen atom. Polyphenols suppress the generation of free radicals, thus reducing the rate of oxidation by inhibiting the formation of or deactivating the active species and precursors of free radicals. More frequently, they act as direct radical scavengers of the lipid peroxidation chain reactions (chain breakers). In addition to radical scavenging, polyphenols are also known as metal chelators. Chelation of transition metals such as Fe^{2+} can directly reduce the rate of Fenton reaction, thus preventing oxidation caused by highly reactive hydroxyl radicals **(Tsao, 2010)**.

The chemical structures of flavonoids are predictive of their antioxidant potential in terms of their radical scavenging, hydrogen- or electron-donation, and metal-chelating capacities by the scavenging of free radicals or ROS, metal chelating, inhibition of enzymes associated with free radicals generation (eg oxidase) and the activation of antioxydant enzymes (**Trouillas *et al.*, 2006**).

IV. Alzheimer and anticholinesterase activity

Alzheimer's disease (AD), one of the leading causes of dementia, is an overwhelming neurodegenerative disease that particularly affects brain function, resulting in memory loss and impairment of language, emotional disturbance, personality changes, depression, behavioral problems, and judgment capacity **(Ali Reza *et al*., 2018)**.

The pathological hallmark of Alzheimer's disease is widespread neuritic plaques which are accumulations of amyloid beta protein (Aβ) and neurofibrillary tangles. However, several factors, including aging **(Tan *et al*., 2014)** and some pathological conditions, such as impaired mitochondrial function (**Johry, 2012**), aggregated proteins deposit (**Takalo *et al*., 2013**). neuroinflammation (**Chen *et al*., 2016**), cholinergic deficit **(Schliebs and Arendt, 2011)** and oxidative stress **(Butterfield *et al*., 2013)** have been associated with NDs.

According to the cholinergic hypothesis, which based on the finding that in AD there is impaired synthesis and secretion of acetylcholine in cholinergic neurons in the cerebral cortex of the AD sufferer and that cholinergic enhancement improves memory in people with AD **(Bartus 1982)**. This has been the rationale behind treatment of the cognitive impairment in AD with drugs that enhance cholinergic transmission **(Wilkinson, 2001)**. The use of acetylcholinesterase inhibitors (AChE-Is) has been the most consistent and successful of the potential strategies for enhancing cholinergic transmission AChE-Is' main mode of action is to inhibit the breakdown of acetylcholine by the enzyme acetylcholinersterase (Figure 7) **(Holmes and Wilkinson, 2000)**.

In the brain, the ChEIs act via raising ACh level (Figure 7) through inhibition of AChE and/or BuChE. AChEIs bind to enzyme and indirectly provide a cholinergic action by preventing its degradation resulting in the accumulation of ACh and provokes a response that is helpful in ameliorating the symptoms of AD **(Kabir *et al*., 2019)**.

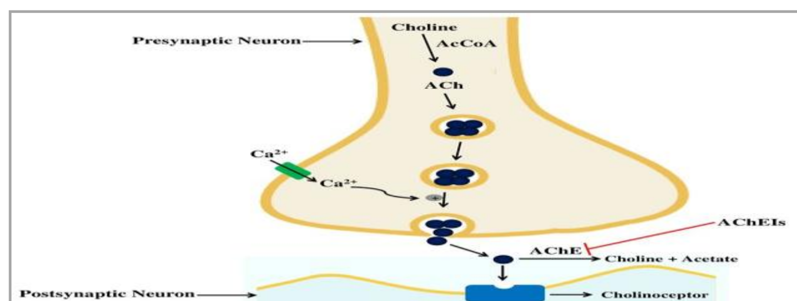


Figure 7. Mechanism of action of acetylcholinesterase inhibitors. AcCoA, Acetyl coenzyme A; ACh, Acetylcholine **(Kabir *et al*., 2019)**.

IV.1. Cholinesterases enzymes

Acetylcholinesterase "AChE" and butyrylcholinesterase "BChE" are hydrolases that the neurotransmitter acetylcholine (ACh) to choline and acetate, in the synaptic cleft. Both enzymes have been found in neuritic plaques and neurofibrillary tangles in the brain. It has been proposed that AChE predominates in the healthy brain, with BChE playing a minor role in regulating ACh'slevels. However, BChE activity progressively increases in patients with AD, while AChE activity remains unchanged or declines. Both enzymes are indeed legitimate medicinal targets for treating the cholinergic deficiency responsible for the declines in cognitive, in behavior and to global aspects associated with Alzheimer's disease **(Greig *et al.*, 2002)**.

Despite the unknown etiology of AD, these findings suggest the need to regulate the of tcholinesterase enzyme activity at various phases of the 's progress. Therefore, decreasing level of AChE and BChE considred as one of the most effective therapy for constraining cholinergic function in the treatment of patient with AD **(Mohamed and Rao, 2010)**.

IV.2. Relationship between ROS and alzheimer's disease

The production of reactive oxygen species (ROS) seems to be involved in triggering and maintaining the degeneration cycle of AD, causing the damage of mitochondrial DNA and of the electron transport chain, which leads to an increased production of ROS **(Patten *et al.*, 2010)**. The free radicals thus generated are known to attack macromolecules such as deoxyribonucleic acid, proteins, lipids, and carbohydrates. This leads to either onset or acceleration of degenerative disorders. The main damage occurs for integration with cellular macromolecules essential to survival, such as DNA, proteins, and polyunsaturated fatty acids (which make up the cell membrane **(Sultana *et al.*, 2009)**. Thus, ROS have been shown to trigger a variety of damage to cellular DNA and RNA, causing peroxidation of membranes and neuronal damage. In addition, the alterations of oxidative metabolism may render the brain more susceptible to further damage from Aβ, which in turn has a prooxidant action **(Jimenez-Del-Rio and Velez-Pardo, 2012)**. Accumulating evidence suggests that brain tissues in AD patients are exposed to oxidative stress during the development of the disease **(Nunomura *et al.*, 2006)**.

The vicious cycle of damage caused by OS promotes plaque and tangle pathology in addition to synaptic dysfunction and inflammatory responses. Reactive oxygen species (ROS) released by metabolically active neuronal mitochondria can promote the excess generation of

amyloid-beta (Ab) peptide. Ab oligomer accumulates extracellularly as plaques and can further interfere with neurotransmission in the synaptic microenvironment. Activation of microglia and astroglia might additionally enhance the expression of proinflammatory cytokines, further augmenting damage caused by OS. Prolonged OS can subsequently lead to hyperphosphorylation of tau protein and disorganization of microtubules, thus leading to the development of neurofibrillary tangle (NFT) pathology **(Bhatt *et al.*, 2020)**.

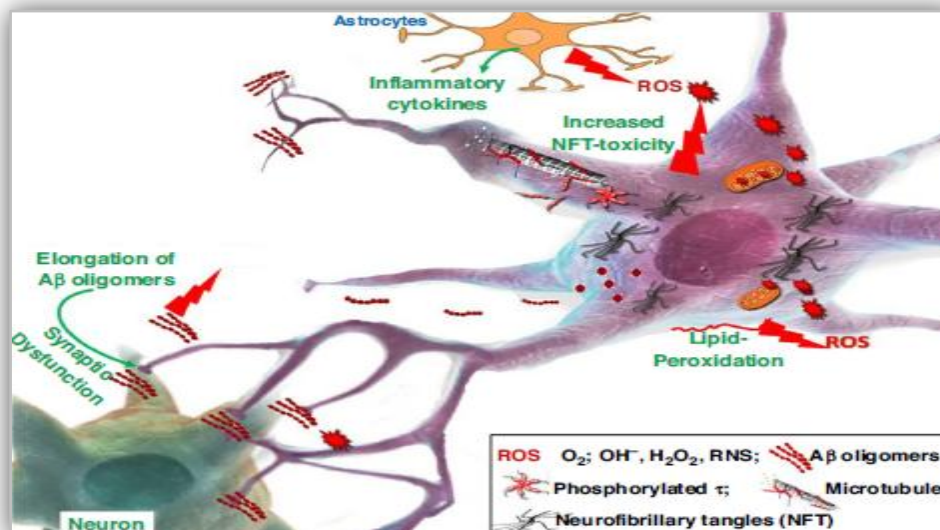


Figure 8. Role of oxidative stress(OS) in the pathogenesis of Alzheimer's disease (AD) (**Bhatt *et al.*, 2020**).

IV.3. Cholinesterases inhibitors

IV.3.1. Drugs

A number of ChE inhibitors have been developed: Galanthamine (1), donezepil (2), tacrine (3), rivastigmine (4) of which galanthamine is a natural alkaloid. It was first obtained from Galanthus spp. Donezepil and tacrine were made synthetically and are approved by US-FDA. Rivastigmine was designed from a natural alkaloid, physostigmine. Another natural alkaloid, Huperzine A is AChEI and is used in dietary supplement for memory support (**Ahmed *et al.*, 2021**). However, the efficacy of these drugs is limited, and these drugs have shown various dose associated side-effects, particularly at higher doses Galantamine and donepezil are AChE inhibitors whereas rivastigmine is a reversible inhibitor of both AChE and butyrylcholinesterase (BChE). Notably, donepezil is highly selective for AChE compared with BChE (**Ogura *et al.*, 2000**).

IV.3.2. Natural sourse

Terpenoids can inhibit cholinesterases throught many ways: camphor, 1,8-cineole, and α-pinene could reversibly inhibit Acetylcholinesterase enzyme **(Perry *et al.*, 2000).** Some tanshinone derivatives may be non competitive inhibitors of AChE and BChE enzymes in humans and can bind to their allosteric site of specially into hydrophobic interactions as well as hydrogen bonding with AChE's Tyr337 and Gly120 **(Wong *et al.*, 2010)**. Monoterpenoids are the most promising terpenoids because their inhibition capacity of AChE. It has been shown to treat AD by inhibiting and clearing amyloid-beta-induced neurotoxicity, tau-protein phosphorylation, and oxidative stress by increasing antioxidant defenses, neuro-inflammation, mitochondrial function restoration, processes initiation with simultaneous inhibition of pro-apoptotic genes and proteins **(Wojtunik-Kulesza *et al.*, 2021).**

IV.3.3. Phenolic compounds as cholinesterase inhibitors

Inhibitory activity in the phenolic compounds is related to position, number of hydroxyl methoxyl groups bonded to the phenol ring, and to methoxy substitution on the phenol ring Furthermore, phenolic acids can inhibit amyloid β-peptide (Aβ) fibrils formation **(Szwajgier *et al* [a]., 2018)**. Phenolic compounds exhibit neuroprotective effects, even though it is considered that the polyphenols transfer across the blood–brain barrier is limited. Similary, various studies have been contected on the presence absorption and availability of phenolic acids in the brain **(Szwajgier *et al* [b] ., 2018)**. Phenolic compounds can inhibit AChE or BChE enzymes throught their binding to their active site **(Jabir *et al.*, 2018)**. The selection and stabilization of the (+) charge of the quaternary group in the acetylcholine may related to aromatic ring moieties fuction. Phenolic compounds that are structurally similar to caffeic acid can fit into the gorge of AChE's active site and therefore be more potent **(Roseiro *et al.*, 2012)**.

The maximum AChEI activity of most of flavonoids is due to the presence and position of hydroxyl (OH) group at ring A and ring B, and due to the unsaturation of ring C. The inhibitory activity of flavonoids may also be enhanced by the presence of double bond between carbon 3 and 4 of ring C **(Khan *et al.*, 2018)**. Similarly, the inhibitory activity may also be improved by increasing the gallation of catechin, for example, (-)-epigallocatechin gallate is more potent than (-)-epigallocatechin **(Balkis *et al.*, 2015)**.

V. Melanogenesis and tyrosinase activity

The skin has epidermal units that are responsible for melanin production and distribution, a process called melanogenesis. These units are composed of a melanocyte surrounded by keratinocytes and regulated by a closed paracrine system. Melanin is the primary determinant of skin, hair, and eye color. **(Lin and Fisher, 2007)**. There are two types of melanin pigments that can be produced by melanocyte cells, namely, eumelanin which is black or brown, and pheomelanin which is red or yellow and alkali soluble **(Summers, 2006)** Melanogenesis is a complex process with different stages. When disturbed, it may determine different types of pigmentation defects, which are classified as hypo or hyperpigmentation and which may occur with or without an altered number of melanocytes **(Fistarol and Itin, 2010)** The key enzyme that is responsible for melanin production is tyrosinase **(Nerya *et al*., 2003)**.

V.1. Tyrosinase enzyme

Tyrosinases have been isolated and purified from different sources such as some plants, animals and microorganism **(Zolghadri *et al*., 2019)**. The enzyme tyrosinase (EC 1.14.18.1) is known to be a multifunctional copper-containing enzyme from the oxidase superfamily. This is the key enzyme which is involved in the biosynthesis of the large biological pigment, melanin. This enzyme catalyzes two types of reactions of melanin biosynthesis, the hydroxylation of L-tyrosine to 3-4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to o-dopaquinone. This o-quinone is a highly reactive compound and can polymerize spontaneously to form the pigment melanin, which causes a serious aesthetic problem in human beings **(Prashar *et al*., 2006)**. overactivity of tyrosinase enzyme occurs due of the hyperpigmentation of the skin and its underactivity leads to hypopigmentation of hair. Overactivity of the enzyme is associated with ageing while under-activity can occur in any age group depending on a person's heredity **(Sharma, 2005)**.

V.2. Relationship between ROS and melanogenesis

Skin is the largest organ that interfaces with the environment, and a major source of ROS that are induced by sun exposure. Epidermal melanocytes are particularly vulnerable to excessive ROS production owing to their specialized function: melanin synthesis, which is stimulated by sun exposure, during the process of tanning, and by inflammation that results in postinflammatory hyperpigmenta (Figure 9,10).

Oxidative stress can disrupt the homeostasis of melanocytes, compromising their survival or leading to their malignant transformation **(Laurence *et al*.,2014)**. Oxidative stress has important role in in melanoma, based on different findings demonstrated that mutations in multipe melanoma-associated genes result exacerbate, or from, oxidative stress. This later may activate V600EBRAF mutation, (a somatic mutation frequently seen in nevi and melanoma) **(Landi *et al*., 2006)**. Furthermore, oxidative stress can affect nucleotide excision repair, the primary repair pathway for UV-induced DNA photoproducts, throught lipid peroxidation products that inhibit DNA repair enzymes **(Feng *et al*., 2004)**.

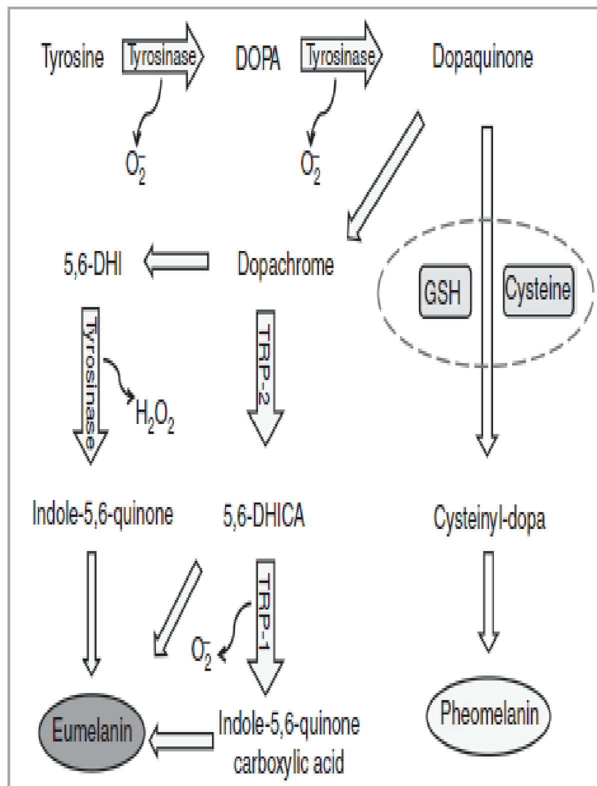


Figure 9. Generation of reactive oxygen species (ROS) by the various steps in the melanin synthetic pathway **(Laurence *et al*.,2014)**

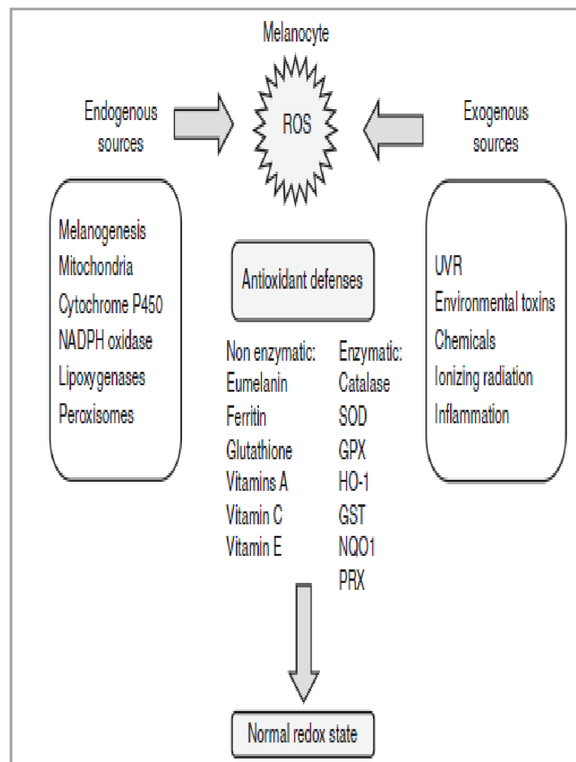


Figure 10. Induction of reactive oxygen species (ROS) by endogenous and exogenous sources and antioxidant defenses that restore normal redox state in melanocytes **(Laurence *et al*.,2014)**

Tyrosinase inhibitors is an important end eavor to treat hypopigmentary disorders. Several effective inhibitors have been identified and developed for use in the medical and cosmetic products, as well as, food bioprocessing, agricultural and environmental industries. However, tyrosinase inhibitors are a class of important clinical antimelanoma medicine, althought only few substances are known to be efficient and safe tyrosinase inhibitors **(Zolghadri *et al*., 2019).**

V.3. Tyrosinase inhibitors

As tyrosinase is the key enzyme in melanogenesis in melanine production, it has been the main successful target for melanogenesis inhibitors that directly reduce tyrosinase catalytic activity. Cosmetics or skin whitening products available are tyrosinase inhibitors. Since tyrosinase is particulary produced only by melanocytes, his inhibitors can be specifically, by inhibiting the melanogenesis in cells without causing side effects. Many tyrosinase inhibitors have been used as skin-whitening agents with some drawbacks including hydroquinone, kojic acid, arbutin, L-ascorbic acid, ellagic acid, azelaic acid, and tranexamic acid **(Pillaiyar *et al.*, 2017)**.

V.4. Tyrosinase inhibition mechanisms

It has been proved that presently known inhibitors possess different mechanisms for expressing their tyrosinase inhibitory activity. Some of these mechanisms are:

a) Reducing agents causing chemical reduction of dopaquinone such as ascorbic acid, which is used as a melanogenesis inhibitor because of its capacity to reduce back odopaquinone to DOPA, thus preventing dopachrome and melanin formations.

b) o-dopaquinone scavenger such as most thiols are well-known melanogenesis inhibitors, reacting with dopaquinone and preventing the formation of melanins. The melanogenetic process is therefore slowed until all the scavenger is consumed, and then it goes at its original rate.

c) Alternative enzyme substrates such as some phenolic compounds, whose quinoid reaction products absorb in a spectral range different from that of melanins. When these phenolics show a good affinity for the enzyme, dopachrome formation is prevented. These compounds can be mistakenly classified as inhibitors of tyrosinase.

d) Nonspecific enzyme inactivators such as acids or bases, which nonspecifically denature the enzyme, thus inhibiting its activity.

e) Specific tyrosinase inactivators such as mechanism-based inhibitors or suicide substrates. These inhibitors can act as tyrosinase substrates and form covalent bond with the enzyme, thus irreversibly inactivating the enzyme.

f) Specific tyrosinase inhibitors which bind reversibly to tyrosinase and reduce its catalytic capacity **(Chang, 2009)**.

V.5. Inhibitors from natural sources

There is an ongoing effort to search for tyrosinase inhibitors from natural sources particularly from plants as they are a rich source of bioactive chemicals and are mostly free from harmful side effects. A number of research have been dedicated to identify tyrosinase inhibitors from plants, fungal metabolites and marine algae. Polyphenols are widely distributed in nature and are the largest groups in tyrosinase inhibitors. Best-studied polyphenols are flavonoids, that may be subdivided into seven major groups, including lavones, flavonols, flavanones, flavanols, isoflavonoids, chalcones, and catechin. In addition to flavonoids, stilbenes and coumarin derivatives; long-chain lipids and steroids; benzaldehyde and benzoate derivatives also identified as tyrosinase inhibitors. A large number of compounds have been identified from the natural products and investigated for mushroom tyrosinase inhibitory activity; these compounds differ from one another in the potency and type of inhibition imposed on the enzyme.zzz. **(Masum *et al*., 2019)**.

V.6. Phenolic compounds as tyrosinase inhibitors

Flavonoids are a group of naturally occurring antioxidants, and are proposed to act as metal chelators of the copper at the tyrosinase active site forming the copper-flavonoid complexes **(Jacob *et al*., 2011)**. Some flavonoids, such as kaempferol, quercetin and morin, show the inhibitory activity of tyrosinase, while others, e.g. catechin and rhamnetin, act as cofactors or substrates of tyrosinase **(Gómez-Cordovés *et al*., 2001)**. All flavanoids inhibit the enzyme due to their ability to chelate copper in the active site. However, this condition is applicable only if the 3-hydroxy group is free. They further elucidated that the 3-hydroxy group is not an essential requirement for inhibition as other types of flavonoids such as luteolin 4′-O-glucoside and luteolin 7-O-glucoside, lacking this 3-hydroxy group, still showed inhibitory activity **(Kubo *et al*., 1995)**.

Badria and el Gayyar. (2001) found that flavonoids containing a keto group possess potent tyrosinase inhibitory activity. This may be explained in terms of the similarity between the dihydroxyphenyl group in L-DOPA and the keto group in flavonoids.

Another important inhibitor is gallic acid, which occurs as multiple esters with D-glucose, and their esters are widely used as additives in food industries. Gallic acid and other gallates have been shown to inhibit the oxidation of L-DOPA by tyrosinase, however, gallic acid itself acts as a substrate **(Kim and Uyama, 2005)**.

VI. Antimicrobial activity, Biofims and quorum sensing

In recent years, the control and prevention of bacterial illnesses has seen significant rise in the use of antibiotics and different antimicrobial agents. Traditional antimicrobial agents kill and inhibit bacteria by disrupting their structure and functions as well as their metabolism **(Marinelli *et al*., 2018)**. Numerous host-associated bacteria monitor their own population density and regulate the expression of particular genes in response to population density, using chemical signals, which is termed on quorum sensing "QS" **(Williams and Camara, 2009)**.

Important bacterial activities including virulence gene expression and biofilm formation are regulated by quorum sensing process. An antipathogenic approach has been recently considered as an alternative to combat the biofilm development due to antibiotic-resistant bacteria, and suppression of quorum sensing is attractive target. As an alternative to the less effective antibiotics, scientists have focused on plant-derived antimicrobials **(Husain and Ahmad, 2013)**.

VI.I. What is biofilms

Biofilms are bacterial populationin that concict of which cells embedded in a matrix of extracellular polymeric substances adhering to a surface **(Branda *et al*., 2005)**. biofilms may form on a wide different types of surfaces including living tissues, indwelling medical iquipement, natural aquatic systems and on piping in potable or industrial water system **(Donlan, 2002)**.

Bacteria in biofilms communities are protect from harmful conditions. Moreover, biofilms appears to be the main factor contribuating in the graviness of infections disease, in both plants and animals **(Davey and O'Toole, 2000)**.

VI.1.1. Biofilms composition

Biofilms are communities of microorganisms, in which microbes secreted an extracellular polymeric substances (EPS) including: polysaccharides (1-2%), proteins (<1-2% with enzymes), DNA (<1%) and RNA (<1%), and water (up to 97%), in addition to these components, which represent the major componenet of biofilm responsible for the mouvement of nutrients inside biofilm matrix **(Lu and Collins, 2007).** The biofilm architecture is based on water channel (for nutrients transport) and on densely packed cells with no prominent pores in it **(Muhsin *et al*., 2015).**

VI.I.2. Biofilms formation

The formation of biofilm is a complex process in which microorganism cells change from planktonic to sessile growth **(Okada *et al*., 2005)**. It has been proposed that this formation is based on specific genes expression that control the growth of biofilm **(Sauer *et al*., 2004)**. The production of biofilm is resulting from series of events that lead to adaptability under a variaty of environmental and nutritional conditions **(Hentzer *et al*., 2005)**. It is a multi-step procedure, in which microorganisms transform after attaching to a surface. Planktonic bacteria, reversible attachment, irreversible attachment, micro-colony, macro-colony and dispersion are the six stages of biofilms development (Figure 11) **(Xin *et al*., 2010)**.

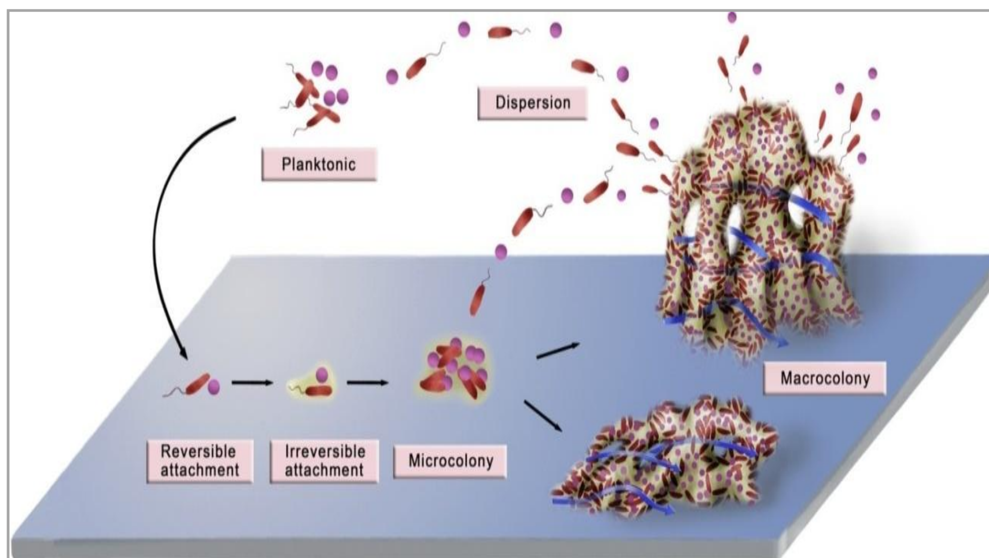


Figure 11. Diagram of biofilm development **(Xin *et al*., 2010)**.

VI.1.3. Biofilm resistance to antimicrobial agents

The bacterial cells present in the biofilm are very resistant (10-1000 times) to the antimicrobials in comparison to the bacterial cells present outside of biofilm **(Mah and O'Toole, 2001)**.

Mechanisms of resistance in biofilms communities differ from those in planktonic forms including, decreased cell permeability, target site mutations, efflux pumps, drug neutralizing proteins, and drug modifying enzymes **(Walsh, 2000)**. Antibiotics resistance in biofilms appeared to different mechanisms such as incomplete or slow penetration of the antibiotics into their communities **(Mah and O'Toole, 2001)**, Alteration of their chemical microenvironment **(Xu *et al*., 2000)** and a subpopulation of micro-organisms (a sort of cell differentiation similar

to spore formation) **(Cochran *et al*., 2000).** These mechanisms are the results of biofilms multicellular nature, which leads to the antibiotics resistance in biofilms **(Davies, 2003).**

Other researchers reported that extracellular polymetric substances"EPS" conferred resistance toaminoglycosides **(Khan, 2010)**. EPS may quench diffusion of antibiotics inside biofilms throught the phenomen of diffusion–reaction inhibition, in which chelating of antibiotics may by complex formation or degrading them via enzymatique reactions **(Billings *et al*., 2015)**.

During biofilm formation many species of bacteria are able to communicate with one another through a mechanism called quoreum sensing **(Preda and Săndulescu, 2019)**.

VI.2. Quorum sensing (QS)

Quorum sensing (QS) is a chemical mechanism by which bacteria respond to external environmental changes promptly and effectively by using their chemical languages **(Asfahl and Schuster, 2017)**.Upon reaching a threshold bacterial population density, diffusible signal molecules trigger the expression of genes involved in biofilm formation , virulence factor production, motility, bioluminescence, antibiotic production sporulation, and nitrogen fixationBoth gram-negative and gram-positive bacteria are known to have QS mechanism, but there are differences between them **(Kim *et al*., 2007)**.

VI.2.1. QS Regulation of biofilm formation

The QS involves cell-to-cell communication among bacteria using small diffusible chemical signaling molecules called autoinducers (AIs) **(Waters and Bassler, 2005)**. The signaling molecules accumulate in the surrounding environment with an increase of bacterial density. When the concentration of signaling molecules reaches a minimal threshold, they bind to receptor proteins, thereby activating the expression of genes associated with biofilm formation **(Williams, 2007)**.

According to the chemical kinds of signal molecules, The quorum sensing system can be classified to 3 types, **(Wu *et al*., 2020)**:

(1) N-acyl-homoserines (AHLs)-mediated quorum sensing system in Gram negative (-) bacteria; (2) Auto-inducing peptide (AIP)-mediated quorum sensing system exists in Gram (+) positive bacteria; (3) autoinducer 2 (AI-2)-mediated quorum sensing system exists in both Gram negative and positive .

VI.2.2. Quorum quenching (QQ)

Quorum quenching (QQ) refers to all processes involved in the disturbance of QS **(Dong *et al*., 2001)**. QQ molecular actors are diverse in nature (enzymes, chemical compounds), mode of action (QS-signal cleavage, competitive inhibition, and so on) and targets, as all main steps of the QS pathway that are synthesis, diffusion, accumulation and perception of the QS signals may be affected. Usually, the enzymes that inactivate QS signals are named QQ enzymes, while the chemicals disrupting QS pathways are called QS inhibitors (QSIs).

Quorum quenching effect can occur at different stages of the Quorum sensing pathway, which mainly includes 4 mechanisms **(Palush *et al*., 2020):**

1) Inhibition of the transport of signal molecules

2); Direct inhibition of the synthesis of signal molecules;

3) Chemical or biological degradation of signal molecules;

4) Competitive inhibition of the combination of signal molecules and receptor (Figure 12).

An attractive aspect of QQ is that it does not kill pathogens and does not cause harsh selective pressures, thereby minimizing the production of drug resistance **(Von Bodman et *al*., 2008).** Therefore, QQ is regarded as a promising biological control strategy, and is expected to become a new approach for antibacterial treatment and biological control.

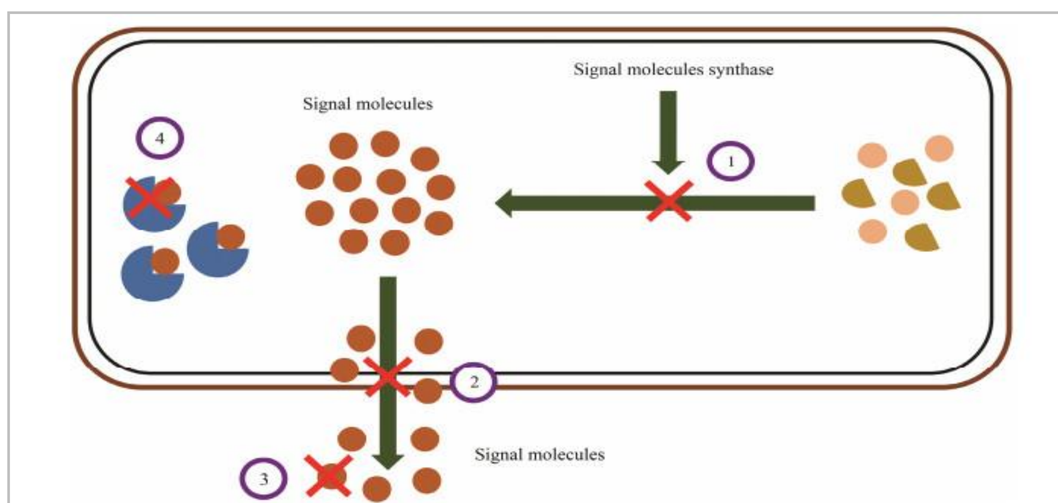


Figure 12. Inhibition mechanisms of quorum sensing system. 1, Inhibition of signal molecule synthesis; 2, Inhibition of signal molecule transport; 3, Degradation of signal molecules; 4, Competitive inhibition in the combination of signal molecules and receptors.

VI.2.3. Medicinal plants QS inhibitors

Medicinal plants contain several bio-active molecules such as terpenoids, polyphenols, flavonoids, tannins and anthocyanins, polyamines, cytokinins and polysaccharides that can be useful severely to counterbalance the bacteria resistance by targeting QS signaling pathways **(Packiavathy *et al.*, 2014)**.

The mechanisms of action of these products have been suggested against numerous bacterial targets including the membrane, the wall and the respiratory chain **(Bouhdid *et al.*, 2010)**. Table 4below describes some of the polyphenols from natural sources that act as QSI.

Table 5. Some of the polyphenols that act as QSI.

Components	Mechanism	Bacterial strain inhibition	References
Furocoumarins	Inhibits both AI-1 and AI-2 signaling as well as biofilm formation	*E.coli*, *S. typhimurium*, and *P.aeruginosa*	**(Ganin *et al.*, 2013)**.
Malabaricone C	Affects lasR and rhlR signaling system	*P.aeruginosa*, *C.violaceum*	**(Jakobsen *et al.*, 2012)**.
Curcumin	Inhibition of virulence genes expressio.	*P.aeruginosa*	**(Kalia, 2013)**.
Urolithin A and urolithin B	Decreases QS processes and levels of AHLs	*Y. enterocolitica*	**(Giménez-Bastida *et al.*, 2012)**.
Rutin	Reduction in biofilm production (concentrationdependent)	*S. aureus* and *E. coli*	**(Al-Shabib *et al.*, 2017)**.
Epigallocatechin gallate	Biofilm inhibition by suppressing gtf genes and disrupting the initial attachment	*S. mutans*	**(Xu *et al.*, 2012)**.

Experimental study

Materials and Methods

I. Materials and methods

I.1. Plant material

The flowring aerial parts of *Senecio coronopifolius*, *Senecio hoggariensis* and *Bunium incrassatum* were collected from different regions of algeria. Taxonomic identification of the plants were confirmed by Dr. Youcef Halis in the Scientific and Technical Research Centre for Arid Areas (CRSTRA). A voucher specimens were deposited in the herbarium of the Laboratory of Biomolecules and Plant Breeding, university of Larbi Ben Mhidi Oum El Bouaghi, Algeria (Table 6). Collected plant materials, in the absence of direct sunlight, were air-dried in darkness at room temperature, and then grouned to powder.

Table 6. Location of studied plants

Area	Plants	Station	Voucher number
Semi- arid	*Bunium incrassatum*	Oum El Bouaghi	ZA 103
Arid	*Senecio gallicus* ssp *coronopifolius*	El Oued	ZA66
	Senecio hogariensis	El Hoggar	ZA67

Bunium incrassatum ***Senecio coronopifolius*** ***Sencio hoggariensis***

Figure 13. Representation of studied plants.

I.2. Chemicals and instrumentation

All the chemicals and solvents were of the analytical grade of HPLC grades as required. The optical densities for bioassays were measured using SpectraMax340PC384 (Microplate reader by Molecular Devices, Silicon Valley, USA). The phenolic profiling of the sample was done using Shimadzu 20AT series HPLC-DAD (Shimadzu Corporation, Japan). The solvents and other chemicals, including quercetin, ethylenediaminetetraacetic acid (EDTA), sodium chloride, ferrous chloride,and copper (II) chloride dihydrate ($CuCl_2.2H_2O$), were acquired from Merck (Germany). DPPH (1,1-diphenyl-2-picrylhydrazyl), butylated hydroxytoluene (BHT), β-carotene, α-tocopherol, neocuproine, polyoxyethylene sorbitan mono palmitate (Tween-40), ferrene, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), linoleic acid, kojic acid, BChE (butyrylcholinesterase) from horse serum (EC 3.1.1.8, 11.4 U/mg) and AChE (acetylcholinesterase) from electric eel (Type-VI-S, EC 3.1.1.7, 425.84 U/mg), mushroom tyrosinase (EC 232-653-4, 250 KU, ≥1,000 U/mg), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), galantamine, butyryl-thiocholine chloride, acetylthiocholine iodide,L-DOPA (3,4-dihydroxy-D-phenylalanine), the certificated reference compounds used to screen the phenolic ingredients of both species were purchased from Sigma-Aldrich GmbH (Steinheim, Germany).

Biomonitor strains used in the anti-QS studies were growth on Luria–Bertani broth. Other bacteria were fed on Nutrient Broth, Luria Bertani Broth, Sabouraud Dextrose Broth (SDB), and Mueller Hinton Broth over agar (Merck). Kanamycin sulfate (Sigma-Aldrich), D-(+)-glucose (≥99.5%, Sigma-Aldrich), sodium chloride (Sigma-Aldrich), proteose peptone (Sigma-Aldrich), dimethyl sulfoxide (DMSO, Sigma-Aldrich), Tryptone (Sigma-Aldrich), and N-hexanoyl-l-homoserine lactone (C6-HSL) (Sigma-Aldrich, Germany), were used in anti-QS activities. The minimal inhibitory concentration (MIC) and biofilm essay of extracts against the strains was calculated by using 96-well microplate reader "type Greiner Bio-One, 125 sterile, PP, U-bottom".

I.3. Microorganisms and conditions for cultivation

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644 and *Candida albicans* ATCC 10239 were obtained from the American Type Culture Collection. The bacteria mentioned above were cultured in Nutrient Broth (NB), while, *Candida albicans* was cultivated in Sabouraud Broth (SB) at 28 ± 0.1° C. Inoculate was

prepared by adjusting the turbidity of the medium to match the 0.5 Mcfarland Standard. The cultures of bacteria were maintained in their appropriate agar slants at 4C° throughout the study and used as stock cultures.

Chromobacterium violaceum ATCC 12472, *Chromobacterium violaceum* CV 026 and *Pseudomonas aeruginosa* PA01 were used for the assay of quorum sensing inhibition assays of the extracts. CV 026 and CV 12472 cultures were grown in Luria Bertani (LB) broth at 30° C with shaking. PA01 cultures were grown on LB agar plates at 37° C.

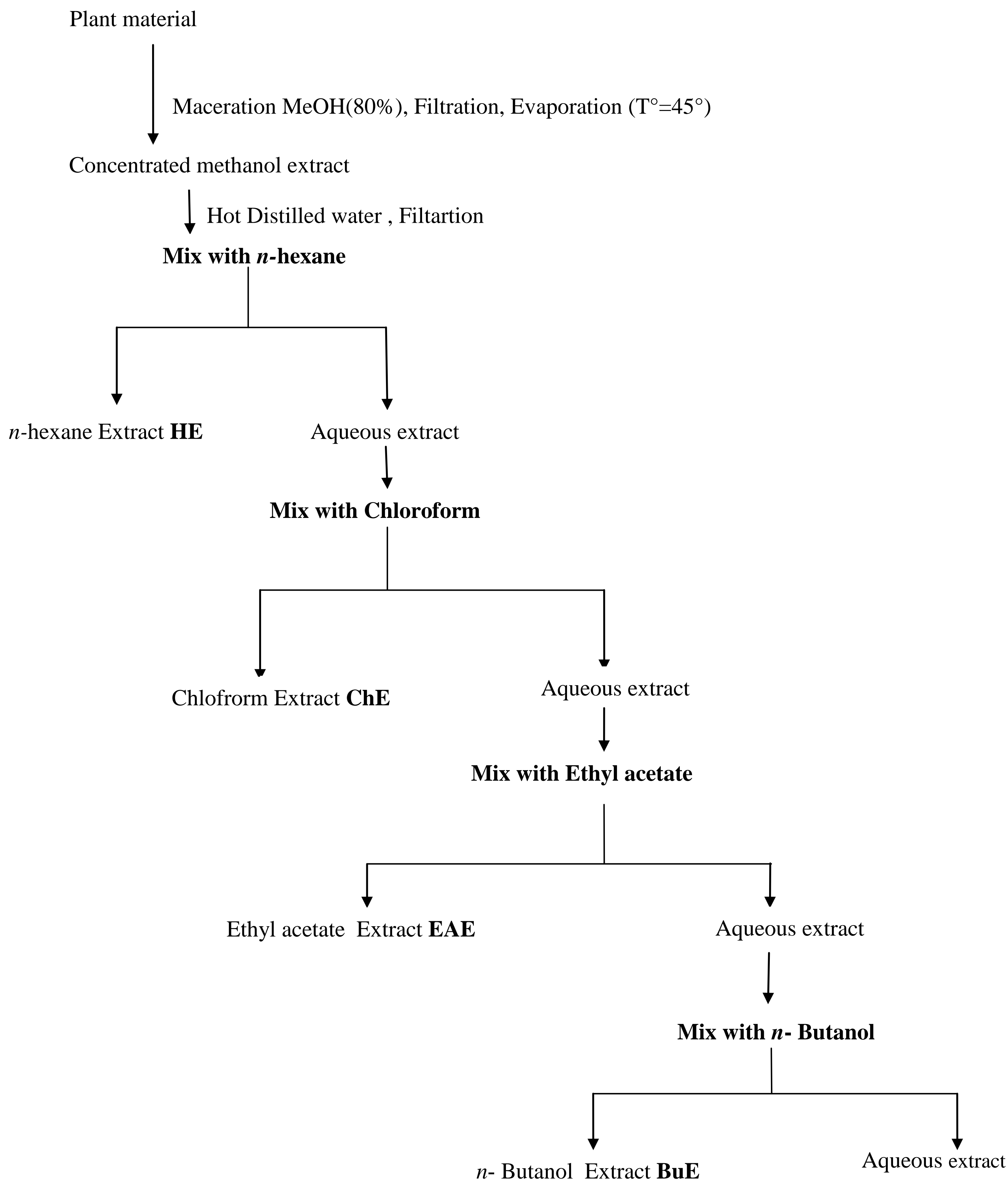
II. Phytochemical investigation

II.1. Extraction procedure profile

The methanolic extract was obtained by maceration in methanol/water mixture (20:80, V/V) for 24 h. This step was repeated for two more time with solvent replacement. The resultant extract was filtered through Wattman filter paper n°3 and the solvent was removed by rotary evaporator under reduced pressure at 45°C. The resulting crude extract was then stored until further analysis. Fractionation of the crude extract using liquid- liquid method is performed according to the method of **Cetkovic *et al.* (2007)** with slight modifications, using a series of solvents increasing in polarity (Shema 2). The crude extract was initially mixed with the hexane (V/V) to eliminate lipids and pigments, and after separation the upper organic phase was recovered. This step is repeated several times with renewal of the solvent until it becomes transparent. The lower aqueous phase was subjected to another fractionation with chloroform to give the chloroform extract (ChE),than with ethyl acetate to give the fraction of ethyl acetate (EAE) and finally with *n*- butanol to give *n*- butanol extract (BuE).

The percentage of the extract yield was calculated as follows:

Y (%) = (Mass of extract / Mass of plant powder) x 100



Shema 2. A sequential extraction procedure to prepare the sub-fractions **(Cetkovic *et al.*, 2007).**

II.2. Quantitative analysis of phenolic compounds by HPLC–DAD

The chemical constituents of sample extracts were determined by the reverse-phase HPLC-DAD system using a validated method against 27 standards **(Tokul-Ölmez *et al.*, 2020; Çayan *et al.*, 2020)**. The ODS-3 column (Inertsil, 150 mm × 4.0 mm i.d, 4 μm film thickness) was used to separate the compounds. The column oven temperature was set to 40 °C. Each extract's stock solution (8 mg/mL) was prepared in methanol/water (80/20,v/v).The stock solutions were pre-filtered using a disposable LC diskfilter (Agilent 0.45 µm). The mobile phases used are 0.5% acetic acid in water (A) and methanol (B). The gradient elution program was of 40 minutes, as 0–0.01 min (0–20% B); 0.01–2 min (20–60% B); 2–15 min(60–80% B); 15–30 min(100% B); 3–35 min (100–10% B); and 35–40 min (10–0% B). The flow was1.5 mL/min, and a 20 µL sample was introduced. The inherent compounds were detected using a photodiode array detector (PDA) in the range of 230–350 nm with reference to the UV data and retention timeof each commercial standard. Each analysis was performed in triplicate.

II.3. Determination of total phenolic content

The concentration of phenolic content in extracts was determined by using Folin–Ciocalteu reagent (FCR) colorimetric method, according to the method of **Müller *et al.* (2010)** slightly modified. Briefly, 20 µL of the extract (or gallic acid), 100 µL of Folin-Ciocalteu reagent (FCR) (1/10 in distilled water) and 75 µL of sodium carbonate were added to a 96-well microplate. After 2 hours, the absorbance was taken at 765 nm in a microplate reader. The total phenolic content represented in µg equivalents of gallic acid per mg extract. The amount was calculated using the calibration regression equation (Annexe 1).

II.4. Determination of total flavonoids

Measurement of flavonoid concentration of the extract was based on aluminium nitrate method described by **Toprçu et al. (2007)**. Briefly, In each well of a microplate, 50 µL of extract (or quercetin), 130 µL MeOH, 10 µL of potassium acetate(1M), and 10 µl of aluminium nitrate (10%), and were mixed. After 40 min, the absorvance was measured in a microplate reader. Flavonoid content was expressed as µg of quercetin equivalent (QE)/mg of extract. The amount was calculated from the regression equation of calibration curve (Annexe 2).

III. Biological investigations

III.1. Screening of *in vitro* antioxidant activity

III.1.1. Free radical-scavenging activity (DPPH assay)

The antiradical activity of plant extracts were tested by the DPPH free radical **(Blois, 1958; Sabudak *et al.*, 2009)**. DPPH is a clour radical that has and maximum absorbance at 517 nm, and upon reduction, its absorption decreases. Briefly, the prepared 0.1 mM DPPH (160 µL) was mixed with 40µL of the sample solution of various concentrations and incubated for 30 minutes in the dark, and the absorbance was measured at the same wavelength. The antioxidant activity of studied plant extracts was compared with the known standards, i.e.,α-tocopherol and butylatedhydroxyanisole. The DPPH radical scavenging was calculated using the equation given below:

$$DPPH\ Free\ radical\ scavenging\ ativity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times\ 100$$

From the inhibitory activity versus concentration graph, the IC_{50} (µg/mL) values were calculated.

III.1.2. ABTS cation radical scavenging activity

$ABTS^{•+}$ scavenging activity assay has some superiorities to DPPH scavenging assay. The antiradical activity of water insoluble compounds or having bulky structures cannot be tested with DPPH assay. Therefore, the $ABTS^{•+}$ scavenging activity of the extracts was also tested **(Re *et al.*, 1999)**. Briefly, 7 mM ABTS and 2.45 mM potassium persulfate were dissolved in water, kept for 16 hours in the dark to obtain $ABTS^{•+}$ solution. The tested $ABTS^{•+}$ solution was prepared by diluting it with ethanol to get an absorbance of 0.700±0.025 at 734 nm in a one cm pathway. To each well containing 40 µL of the extracts in methanol of various concentrations, 160 µL diluted $ABTS^{•+}$ solution was added and incubated for ten minutes. Then the absorbance was measured at 734 nm. For comparison, quercetin, BHT, and α-tocopherol were used, each assay was performed in triplicate. The sample's capability to scavenge $ABTS^{•+}$ was calculated using the formula given above for the DPPH assay. The results of $ABTS^{•+}$ scavenging activity were presented as IC_{50}, showing the concentration scavenging 50% of radicals.

III.1.3. Lipid peroxidation inhibitory activity

The inhibitory activity of lipid peroxidation of studied plants was estimated using the β-carotene-linoleic acid test **(Miller, 1971; Öztürk *et al.*, 2014)**. β-carotene (0.5 mg) was dissolved in chloroform (1 mL), 25 µL of linoleic acid, and 200 mg of Tween-40 emulsifier were mixed in a flask. The mixture was evaporated under a vacuum to remove the chloroform, and 100 mL oxygen saturated water was added by vigorous stirring. The prepared reagent (160 µL) was added separately into wells containing 40 µL of extracts. The absorbance at zero time of each reaction was recorded at 470 nm using a 96-well microplate reader. Each reaction was incubated at 50 ℃ for 2 hours. α-tocopherol and BHT were the standards used. The equation below was used to estimate the bleaching rate (R) of β-carotene.

$$R = \frac{ln\frac{a}{b}}{t}$$

Where ln = natural logarithm, a is the absorbance at 0 time while bis absorbance after two hours. t is the total time in minutes.

The following equation was used to calculate the percent lipid peroxidation inhibitory activity (AA):

$$AA(inhibition\ \%) = \left(\frac{R_{control} - R_{sample}}{R_{control}}\right) \times 100$$

The graph presenting inhibitory activity against concentration was used to calculate the IC_{50} (µg/mL) value.

III.1.4. Cupric reducing antioxidant capacity (CUPRAC)

The standard CUPRAC method with slight modifications was adopted **(Apak et al., 2004; Tel *et al.*, 2013)**. and the absorbance was recorded using an Eliza reader. The aqueous solution including 50 µL's of each $CuCl_2.2H_20$ (10 mM), neocuproine (7.5 mM), and pH 7.0 NH_4Ac buffer (100 mM,) in each well was reacted with 50 µL sample extract of various concentrations to make 200 µL final volume and incubated for one hour at room temperature. The absorbance was recorded at 450 nm. The blank was used as a solution of the same reactants except for the plant extract. The antioxidant standards, including BHT and α-tocopherol, were for comparison. The results showing the concentration exhibiting 0.500 absorbances were expressed as $A_{0.5}$.

III.1.5. Iron chelating assay

The ferrous ion chelating potential of extracts was measured using the following standard procedure with slight modifications **(Decker and Welch, 1990; Sabudak *et al*., 2009)**. To each well containing 40 µL of the extracts in methanolof various concentrations, 40 µL of $FeCl_2$ (0.2 mM) and 40 µL of ethanol wereadded. Then 80 µL of ferrene (0.5 mM) was added to initiate the reaction, incubated at room temperature for 10 minutes, and absorbance at 593 nm was measured. The EDTA was used as a chelating standard. The results are presented as inhibition (%) at 100 µg/mL concentrations.

III.2. Screening of enzyme inhibitory properties

III.2.1. Cholinesterase inhibitory assay

The inhibition of acetylcholinesterase (AChE; 5.32 × 10−3U) and butyrylcholinesterase (BChE; 6.85 × 10−3U) of each sample wastested using Elman's method **(Ellman *et al*., 1961; (Öztürk *et al*., 2014)**. In a 96-well microplate reader. In a 96 well plate, each concentration (25- 200 µg mL-1) of the sample (10 µL) in ethanol were incubated at 25°C for 15 min with 20 µL of enzyme solution and 150 µL sodium phosphate buffer (100 mM, pH=8). After incubation, Ellman's reagent, DTNB (0.5 mM, 10 µL), and substrates (10 µL) were added to each well to make 200 µL final volume. Then measurement was done at 412 nm for 10 minutes and galantamine was used as a standard. The percent of both enzymes inhibition was calculated using the following formula:

$$AChE/BChE\ inhibiton\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{\ control}} x100$$

Where A control is the enzyme inhibitory activity of blank and A sample is the enzyme inhibitory activity with the sample. Each test was conducted in triplicate.

The results are presented as inhibition (%) for 200 µg/mL extract concentrations.

III.2.2.Tyrosinase inhibitory activity

In vitro tyrosinase inhibitory potential of studied plant extracts was assessed using mushroom tyrosinase by following the Hearing method **(Khatib *et al*., 2005)**. The L-Dopa was employed as a tyrosinase substrate. Kojic acid as a standard was used to compare the activity. The tyrosinase inhibition (%) by each sample concentration (µg/mL) was calculated that of used for AChE and BChE assay.

III.3. Screening of antimicrobial Activity

III.3.1. Determination of minimum inhibitory concentrations (MIC)

The lowest concentration of extract showing no visible bacterial growth is called MIC. It was determined by a microtiter broth dilution assay **(CLSI, 2006)**. using five bacterial strains including *P.aeruginosa* ATCC 27853, *E.coli* ATCC 25922, *S.aureus* ATCC 25923, *E. faecalis* ATCC 29212*, Listeria monocytogenes* ATCC 7644 and one yeast *C.albicans* ATCC 10239. The Mueller-Hinton Broth (MHB) was used as a test medium, whereas inoculum density was 5×10^5 CFU/mL. Before read, the cell suspensions (100 µL) were incubated separately under aseptic condition with extracts of various concentrations (0.625, 1.25, 2.5, 5 and 10 mg/mL) at 37°C for one day.

III.3.2. Effect of extract on bacterial biofilm formation

Tested microorganisms biofilm-forming ability was tested using a microplate biofilm assay with extracts at 1/1, 1/2, 1/4 and 1/8 minimum inhibitory concentration **(Merritt *et al.*, 2005)**. Briefly, the tested microbes as mentioned for MIC were incubated at static in glucose (0.25%) containing sterile Tryptose-Soy Broth (200 µL) at 37 °C for 48 hours. After incubation, the wells were drained and washed with water. The crystal violet solution (0.1%) was used to stain the remaining bacteria and washed after 10 minutes with water to eliminate the crystal violet solution. The biofilm formed in each well was suspended with 33% glacial acetic acid (200 µL) and was shaken for 5 minutes. To a sterile tube, the solution (125 µL) was transferred separately from each well. The volume was completed to 1 mL using sterile distilled water. The absorbance was recorded at 550 nm, and % biofilm Inhibition was calculated using the equation below

$$Biofilm\ inhibition\ (\%) = \left(\frac{OD_{550\ contol} - OD_{550\ sample}}{OD_{550\ control}}\right) \times 100$$

III.3.3. Bioassay for quorum-sensing inhibition (QSI) activity using CV026

Method of **Koh and Tham, (2011)** was used to perform quorum sensing inhibition (QSI) extracts activity. The bacterial CV026 culture (100 µL) was transferred to the warm molten Soft Top Agar (5 mL). Then, the exogenous AHL source 20 µL of C6-HSL (100 µg/mL) was gently mixed and overlayed onto the solidified Luria Bertani Agar (LBA) plate. After solidification, the 50 µL extracts (sub-MIC concentrations) were transferred to each well and incubated for three days at 30 °C. The QSI was visualized bymonitoring a cream or

white-colored halo around each well against tested CV026 bacteria. Thus, the inhibition zones could be measured exhibiting antimicrobial activity.

III.3.4. Violacein pigment inhibition assay

QSI potential of extracts was qualitatively determined against *Chromobacterium violaceum* ATCC 12472 **(Sybiya Vasantha Packiavathy *et al.*, 2012)**. The overnight culture (10 µL) of *C. violaceum* with 0.400 optical density at 600 nm was transferred to plates that contains LB media (200 µL). The prepared samples with and without sub-MICs of extracts were incubated for twenty-four hours at 30 °C. The absorbance was recorded at 585 nm to assess the reduction in the violacein pigments. The formula presented below was used to determine the violacein percent inhibitory activity.

$$Violacein\, pigment\; inhibition\; (\%) = \left(\frac{OD_{585\; control} - OD_{585\; sample}}{OD_{585\; control}}\right)$$

III.3.5. Swarming motility assay

The effect of studied plants extracts on the inhibition of *Pseudomonas aeruginosa* was assessed by following the as described protocol**(Sybiya Vasantha Packiavathy *et al.*, 2012)** with few modifications. The swarming plates were prepared by using D-glucose (0.5%),agar (0.5%), NaCl (0.5%), and peptone (1%). The plates were treated with extracts (50, 75, and 100 µg/mL) followed by inoculation with a fresh culture of *P.aeruginosa* PA01. A control medium without extract was also prepared for comparison. All the plates were incubated at 37°C in an upright position for eighteen hours. The plate without the extract was maintained as a control. The bacterial growth and extension were measured as swarm motility.

VI. Statistical analysis

Results are reported as means value ±SD of three measurements; the IC_{50} and $A_{0.50}$ values were calculated by linear regression analysis. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisions. We employed GraphPad Prism-software (version 8.0.1) to analyze data obtained from this investigation. Differences were considered significant at $p \leq 0.05$.

Results and Discussion

I. Phytochimical invertigation

I.1. Extraction yield

Extraction is the main step for recovering and isolating phytochemicals from plant materials, extraction yield is affected by the chemical nature of phytochemicals, the extraction method used, the solvent used, as well as the presence of interfering substances **(Stalikas, 2017).** In this study, we partitioned four fractions (*n*-hexane, chloroform, ethyl acetate and *n*-butanol) from 80% MeOH extract to evaluate the extraction yield of different extract of studied plants using the extraction method described by **Cetkovic *et al.* (2007)**. This method is based on the degree of solubility of polyphenols in organic solvents. It takes place in four stages:

1)- Solubilization of polyphenols in methanol.

2)- Defatting of the extract by adding *n*-hexane and chloroform .

3)- The addition of ethyl acetate to obtain the aglycons flavonoid.

4)- The addition of *n*-butanol to obtain the glucoflavonoids.

The extraction yields and actual dry weights after concentration are shown in Table 7.

Table 7. Extractive values of plants extracts.

Extract	*S.coronopifolius*	*S.hoggariensis*	*B.incrassatum*
ME	15%	10%	9%
EA	0.23%	0.28%	0.97%
Bu	0.97%	1.33%	1.00%

The ME fractions showed the highest extraction yields and the Bu fractions performed with the second strongest recovery rate. However, compared to the fractions mentioned above, the EA fractions demonstrated relatively lower extraction yields.

Using dynamic maceration 80% MeOH, dray aerial parts of *S.coronopifolius* were extracted. The crude extract yielded a value of 15%, which was higher than previous reports. According to **Mohamed. 2015**, the extraction yield from root methyl alcohol of the same species growing in Egypt was 5% and 12% in Saudi Arabia **(Alqahtani *et al*., 2020).**

Compared to other *Senecio*'s species, it found 5.60% in *S. biafrae* (with 95% ethanol) **(Lienou *et al*., 2010)** and 12.57% in *S.aegyptius* **(Hassan *et al*., 2012),** 13.85% in *S.gibbosus* **(Conforti *et al*., 2006)** and 8.1% in *S.stabianus lacaita* **(tundis *et al*., 2012),** this finding were considerably lower than values obtained in our study. Conversely, the crude extract of *S.clivicolus* 96% ethanol (27.06%) exhibited the highest yield compared with our results **(Faraone *et al.,* 2018).**

Then, compounds were separated depending on their affinity to the solvent used, Bu fractions (0.97%) produced higher yields than EA fractions. Our results displayed lower values compared to that found in *S.glaucus* from Saudi Arabia (yield of 4.4%) **(Alqahtani *et al*., 2020)** and in *S.clivicolus* (13.53%;%) **(Faraone *et al*., 2018).** Instead, EA fraction demonstrated lower extraction yields (0.23%), these findings are lower that found in *S.glaucus* growing in Egypt (1.75%), in *S.stabianus Lacaita* (0.4%) **(Tundis *et al*., 2012)** and in *S. clivicolus* (6.87%) **(Faraone *et al*., 2018).**

Our results indicate that the extraction yield in the methanolic extract of *B.incrassatum* (9%) was substantially larger than that obtained by the study of **El Kolli *et al.* (2017)** (1.82 %). Also, it was larger than that obtained from the seeds (7.1%) **(Toul *et al*., 2022)** and from tubers (3.36%) **(Dehimi *et al*., 2020).** By comparing to the same genus from aerial parts in four *Bunium* species, the yield was 5.79%, 6.21%, 2.31% and 8.62% in *B.sayai, B.pinnatifolium, B.brachyactis* and *B.macrocarpum,* respectively *(***Zengin *et al*., 2019),** in *B.alpinum* (0.89 %) **(El Kolli *et al*., 2017).** However, our result was disagreed with the results of **Souilah *et al.* (2021**), the percentages yield from *B.crassifolium* of pure methanol and hydro-methanolic extracts were found to be 23.55 and 28.50% , respectively. EAE yield of *B.incrassatum* was lower than that obtained from seeds (2.2%), at the same time Bu fraction was lower than that obtained by **(Lefahal *et al*., 2017)** in *Bunium alpinum* (2%).

Based on this comparison, fraction yield varies with the nature of the solvent used. In the present study, ME and Bu fractions produced higher yields than the EA fractions among the solvents used. This slight difference in the extraction rate can be due to several parameters, such as the duration of the extraction, the nature of the compounds in the extract and the temperature, which favors the extraction by increasing the diffusion coefficient **(Oroian and Escriche, 2015).** Also, as **Stalikas. (2007)** indicated that extraction yield is strongly affected not only by the polarity of the solvent, but also by other parameters such as temperature, plant parts, and storage times,. Although several factors must be taked in

consideration: - the extraction method (directly extracted or partitioned), the typeof plant, and the intrinsic substances accumulating in the plant tissues.

I.2. Determination of total phenolic and flavonoid contents

Polyphenols and flavonoids were quantified using spectrometric methods. The total phenolic contents (TPC) and total flavonoids contents (TFC) of different extracts were evaluated employing the Folin-Ciocalteu reagent, aluminum chloride methods, respectively (Table 8).

The total phenolic content (TPC) of extracts was assessed by reacting samples with Folin-Ciocalteu reagent, which produces a blue color, where intensity was proportional to their amount. Gallic acid was used as the standard, and the results are reported in table 8 were expressed as microgram of gallic acid equivalents per milligram of dry extract (µg GAE/mg E). All extracts contain a considerable amount of phenolic metabolites. Statistically, differences among total phenolic contents of extracts are significant. The highest TPC was found in EA of *B.incrassatum* (392.92±1.50 µg EAG/ mg E), whereas ME of *S.hoggariensis* had the lowest value (47.13±0.44 µg GAE/mg E). Results of TFC contents in various solvent extracts displayed significant differences depending on the polarity of the solvent ($p<0.05$) (Table 8).

Table 8. Total phenolic content (TPC) and total flavonoid content (TFC) of studied extracts.

Extract	***S.coronopifolius***		***S.hoggariensis***		***B.incrassatum***	
	Polyphenol[a]	**Flavonoids**[b]	**Polyphenol**[a]	**Flavonoids**[b]	**Polyphenol**[a]	**Flavonoids**[b]
ME	62.72±0.33[c]	32.43±1.18[b]	47.13±0.44[c]	39.29±2.05[c]	103.31±0.8[c]	52.15±1.06[c]
EA	185.66±1.1[a]	78.05±1.27[a]	100.27±0.74[a]	80.06±1.14[a]	392.92±1.5[a]	85.06±1.57[a]
Bu	161.45±0.6[b]	73.61±1.02[c]	70.27±1.03[b]	67.70±0.62[b]	166.25±2.39[b]	77.70±1.08[b]

[a] Total phenolic content (µg GAE/mg extract); [b] Total flavonoids content(µg QE/mg extract); Results are expressed as means ± SD (n = 3).

Literature reports that there is no study that has been done on TPC and TFC of *S.hoggariensis*. Correspondingly in *S.coronopifolius*, Our results were higher than those obtained by **(Mohamed *et al*., 2022).** In case of methanolic extract (TPC: 0.11µg/g GAE; TFC: 0.256µg/g QE). Also, our results are greater ther than that founed in the root methyl alcohol extract for the same species growing in Egypt (TPC: 98.23±0.28 mg/gm E, TFC: 35.9 ±0.17 mg/gm E) **(Mohemed *et al*., 2015),** a comparable values for the same species growing in Saudi Arabia **(El Kahtani *et al*., 2020).** While **(Albayrak *et al*., 2014)** reported that several species of *Senecio* genus growing in Turkey contained high total phenolic contents that ranged from 11.63±2.1 mg GAE/g extract in *S.viscosus* to 117.45±1.8 mg GAE/g in *S.cilicius*. The result of the determination of flavonoids in the methanolic extract of *S.cineraria* was estimated at a significant content in the order of 60.16 mg EQ / g E **(Ababsa *et al*., 2014).**

In order to evaluate the total bioactive compounds of *B.incrassatum*, Total phenolic content (TPC) and total flavonoid (TFC) revealed that EA extract has a higher concentration than Bu and ME extracts or than the two studied *Senecio* (TPC: 392.92±1.5 µg GAE/mg E; TFC: 85.06±1.57 µg QE/mg E). In contrast to our results, Only one study carried out by **El Kolli *et al*. (2017)** reported that the phenolic content of *B.incrassatum* in aerial parts of the plant (236.6µg GAE/mg E) is much considerable lower in comparison with our finding. Regarding *B.incrassatum* tuber's, Methanolic extract displayed low values as reported by **Dehimi *et al*. (2020)** (TPC: 13.01 µg GAE/mg E; TFC: 16,32 ± 0.05 µg QE/mg E) and by **Aiouaz and Arezki. (2022)** (TPC: 37.37 ± 0.46 mg GAE/gE. TFC: 2.36 ± 0.06 mg QE/gE). To compare to the same genus, our results are higher than that founed in the methanol extract from the aerial parts in four *Bunium* species (*B.sayai, B.pinnatifolium, B.brachyactis* and *B. macrocarpum*) **(Zengin *et al*., 2019)**, in *B.crassifolium* **(Souiah *et al*.,2021),** in methanol extracts of different organs, including seeds prepared from four different *Bunium* species (*B.cylindricum, B.paucifolium, B.persicum*, and *B.wolffii)* **(Adelifar *et al*., 2021).** These noted differences may be due to the variability in phenolic compounds depending on a number of factors related to the plant it self (the vegetation phase and the organ) as well as on its exposure to diverse environmental conditions in which the plants have grown (climatic factors, altitude, and soil properties) **(Yang *et al*., 2018; Ribeiro *et al*., 2019).**

In general, our work shows elevated levels of phenolic compounds (TPC) and flavonoids (TFC), especially in the EA fraction, followed by the Bu fractions and then crude extracts wich contained the lowest rate. Our results confirmed the previous studies which showed that the solvents used for extraction have significant effects on the content of phenolic

compounds (**Huang *et al.* 2011**) and the solubility of those bioactive compounds **(Naczk and Shahidi 2006).** Our results clearly demonstrated that the ethyl acetate is the most suitable solvent to attain the highest amount of TPC and TFC. The ethyl acetate solvent was frequently used for the extraction of phenolic compounds with low and high molecular weight **(Mariod *et al*., 2009).** Considering the low extraction yield and high total phenol content of the ethyl acetate fraction, it could be predicted that phenol compounds mostly occupy the ethyl acetate fraction as compared to the other fractions, or some phenolic entity reacts strongly with the Foline Ciocalteu reagent, resulting in a solution more deeply discolored than other phenolic molecules. Generally, molecules with lower polarity have a propensity to be dissolved more effectively with lower polarity solvents **(Yu *et al*., 2002).**.

I.3. Identification and quantification of compounds by HPLC-DAD

High performance liquid chromatography with diode array detection (HPLC-DAD) constitutes a crucial, reliable technique for the characterization of phenolic compounds due to its versatility, precision and relatively low cost **(Parejo *et al*., 2004),** by achieving their separation, identification and quantification from natural complex samples. The chromatographic separation depends on several factors such as stereochemistry, molecular weight, polarity, degree of polymerization of polyphenols **(Alonso-Carrillo *et al*., 2017).** A total of 27 phenolic standards were used. The compounds were identified by comparing their spectroscopic characteristics and retention times (RT) with reference compounds (standards) and quantified in micrograms per gram of extract (mg/g).

The HPLC profile of ME, EA and Bu extracts of *S.coronopifolius* and *S.hoggariensis* extracts showed several peaks corresponding to different phenolic compounds with quantitative and qualitative difference of identified compounds, results are shown in table and chromatograms recorded at 254 nm for all fractions are shown in Figure 14 and 15.

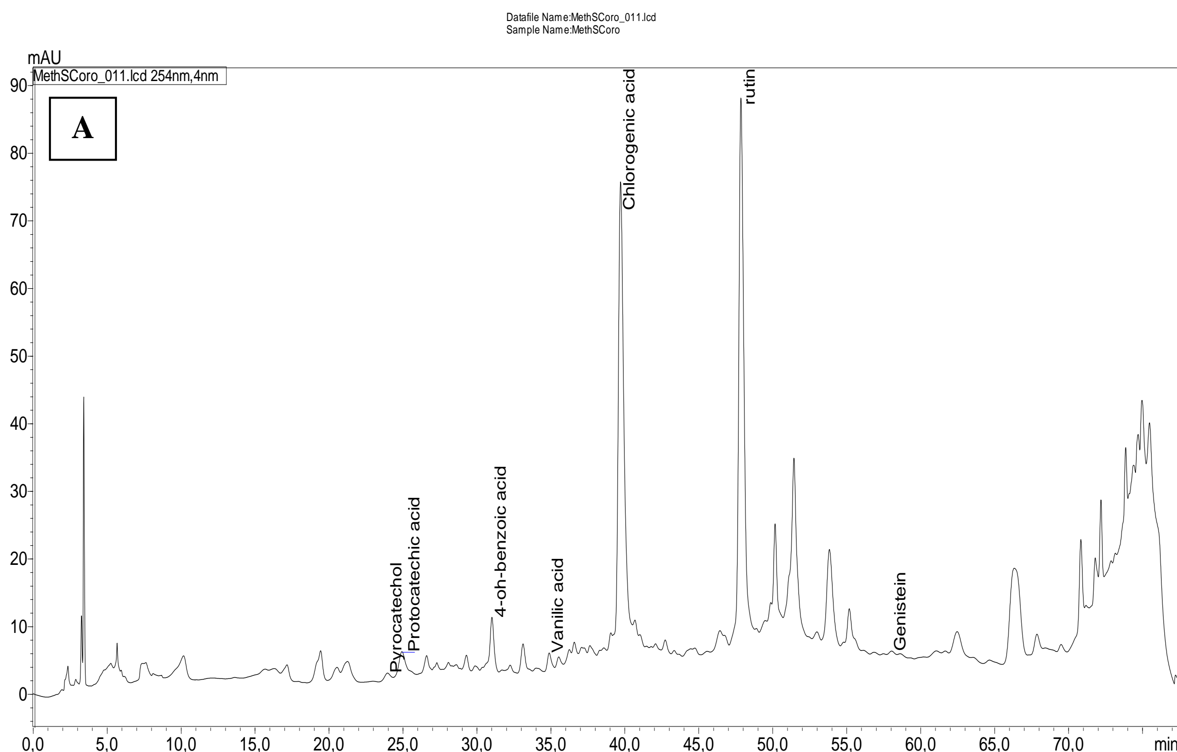
Nine and seven phenolic compounds were identified in the crude extracts of *S.hoggariensis* and *S.coronopifolius*, respectively. As seen in the table below. In the methanolic extract of *S.hoggariensis*, chlorogenic acid (5.97 mg/g) was the most abundant compound found, followed by curcumin (3.39 mg/g), 4-hydroxylresorkinol (2.85mg/g) and rutin (1.29mg/g), some compounds like ellagic acid, protocatechuic acid, 4-hydroxy benzaldehyde, pyrocatechol, 4-hydroxybenzoic acid were also detected in small amounts. in the crude extract of *S.coronopifolius*, as can be see, rutin (10.29 mg/g) was the most abundant compound found, followed by chlorogenic acid (5.61mg/g). Some others compounds like

protocatechuic acid, 4-hydroxybenzoic, genistein and vanilic acid, (0.27 mg/ml; 0.09 mg/ml; 0.03mg/ml and 0.01mg/ml, repectively) were found in small amount and pyrocatechol was found in trace.

Table 9. Composition of methanol extracts determined by HPLC-DAD (mg of pheolic coumpound/g extract

Compounds	Rt* (min)	*S.coronopifolius*	*S.hoggariensis*
Pyrocatechol	24.65	Tr	0.24
Protocatechic acid	24.68	0.27	0.10
4-oh-benzoic acid	31.69	0.09	Tr
4-Hydroxy benzaldehyde	33.36	Nd	0.35
Vanilic acid	34.68	0.01	nd
Chlorogenic acid	38.88	**5.61**	**5.97**
Rutin	47.52	**10.29**	**1.29**
Ellagic acid	50.00	Nd	0.50
Genistein	57.53	0.03	nd
Curcumin	72.89	Nd	**3.39**
4-Hydroxylresorcinol	73.06	Nd	**2.85**

nd: not determinated ; Tr : Trace



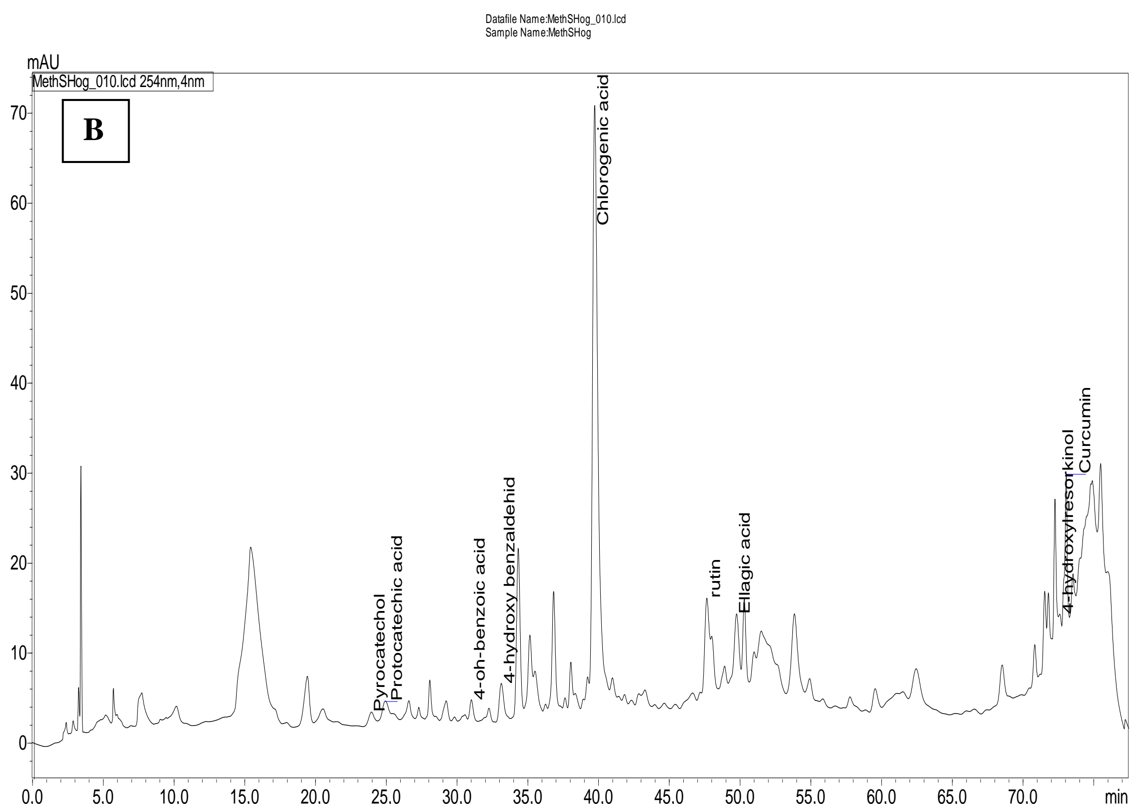


Figure 14. HPLC chromatograms of the total polyphenols of **(A)** ME *S.coronopifolius*; **(B)** ME *S.hoggariensis* at 254 nm.

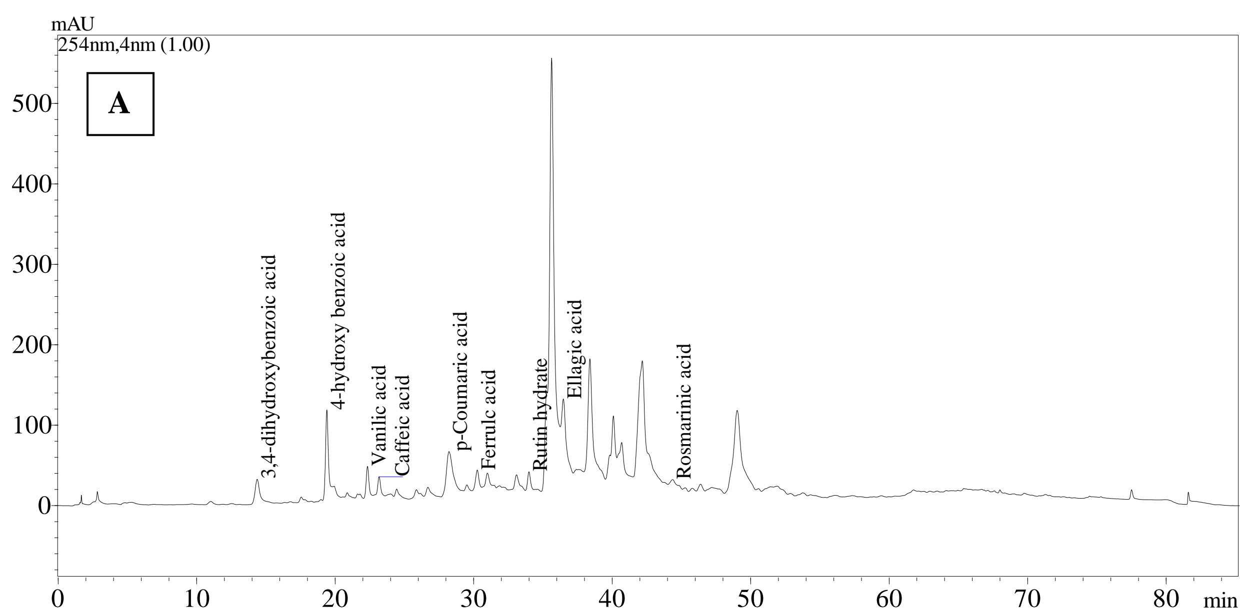
A total of nine and eight compounds were detected in EA of *S.coronopifolius* and *S.hoggariensis*, respectively; meanwhile, the Bu extracts contained low amounts or almost void of target compounds as shown in Table 10. Though in different amounts, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, caffeic acid, and *p*-coumaric acid and rosmanic acid were detected in both plants while some compounds were exclusively detected in *S.coronopifolius* (ferulic acid, rutin and ellagic acid) and *S.hoggariensis* (6,7-Dihydroxy coumarin, quercetin).

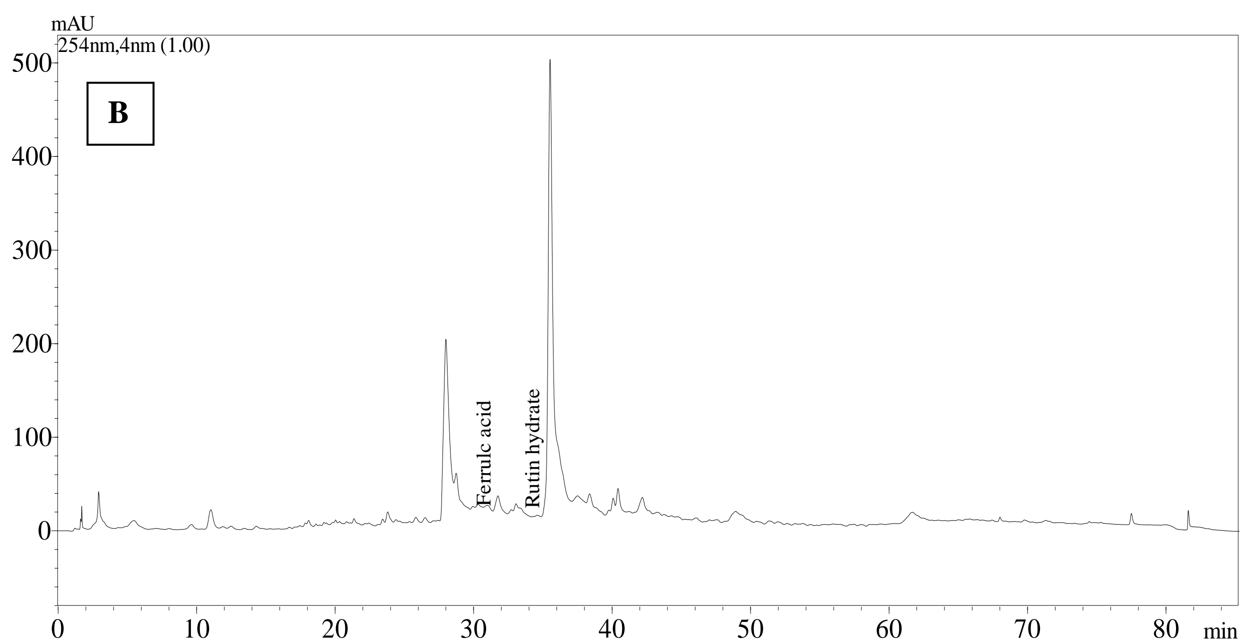
Table 10. Composition of ethyl acetate and *n*-butanol extracts determined by HPLC-DAD (mg of pheolic coumpound/g extract

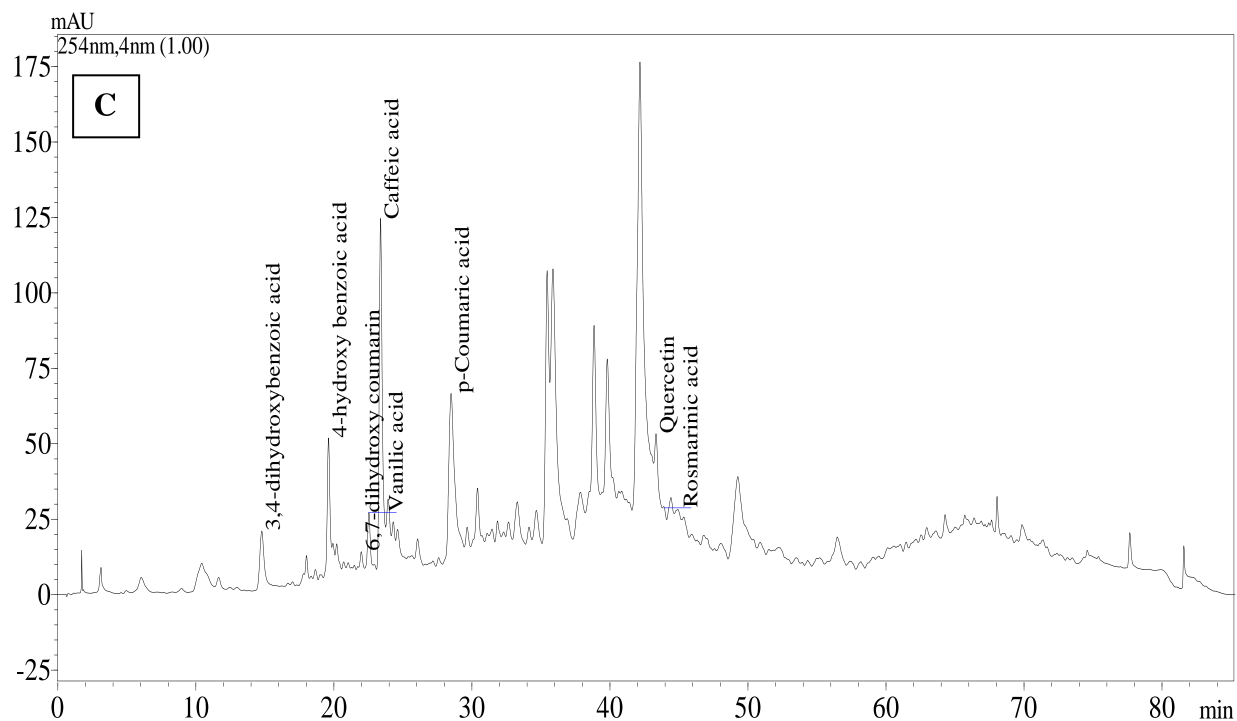
Compounds	RT*(min)	*S.coronopifolius*		*S.hoggariensis*	
		EA	**Bu**	**EA**	**Bu**
3,4-dihydroxybenzoic acid	14.10	1.24	nd	0.78	nd
4-hydroxy benzoic acid	19.50	1.52	nd	0.68	nd
6,7-dihydroxycoumarin	21.99	nd	nd	0.32	nd
Vanilic acid	22.37	0.88	nd	0.38	0.06

Caffeic acid	22.94	0.64	nd	**3.38**	nd
***p*-coumaric acid**	28.43	**11.67**	nd	**10.85**	nd
Quercetin	43.49	nd	nd	0.93	nd
Ferrulic acid	29.93	1.27	0.06	nd	nd
Rutin	35.02	1.10	0.52	nd	nd
Elagic acid	37.61	**4.14**	nd	nd	nd
Rosmarinic acid	44.20	Tr	nd	Tr	nd

nd: not determine; Tr: Trace







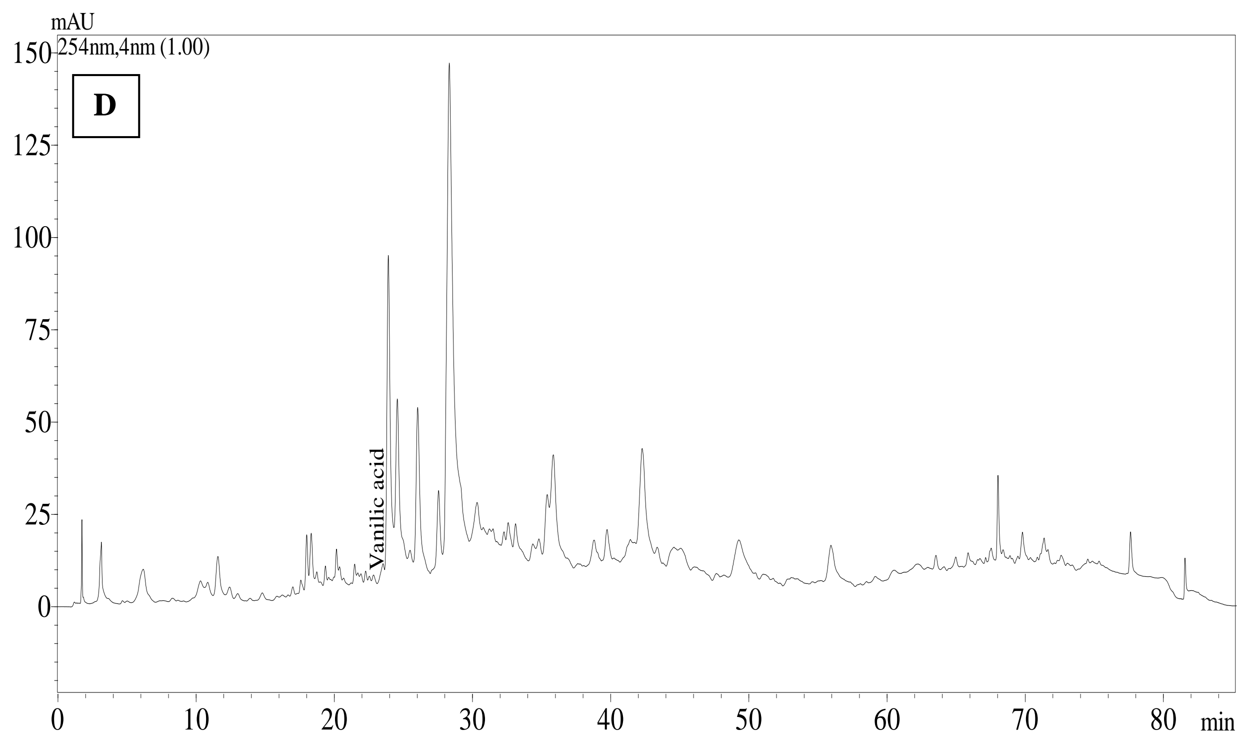


Figure 15. HPLC chromatograms of the total polyphenols of **(A)** EAE *S.coronopifolius*, **(B)** BuE *S.coronopifolius*, **(C)** EAE *S.hoggariensis* and **(D)** BuE *S.hoggariensis* at 254nm.

Though phenolics are not famous in *Senecio* plants, nevertheless, they have been found in *Senecio* plant species **(Yang *et al*., 2011)** and contribute to the biological activities of these species. Notably, *p*-coumaric acid is the major component in ethyl acetate extracts of both plants extract.

It has been noted that chlorogenic acid was the main component of crude extract of *S.hoggariensis*. Our results were in accordance with the study of A**lbayrak *et al*. (2014)** who demonstrated the dominance of chlorogenic acid in the all nine *Senecio* species tested (*S.mollis*, *S.othonnae, S.cilicius, S.inops subsp. karamanicus, S.olympicus*, *S.sandrasicus, S.salsuginea, S.tauricolus* and *S.viscosus*) using the same solvent in the studied extract. According to a research conducted by **Ajiboye *et al*. (2018)** concentration of chlorogenic acid in the crude extract of *S.Biafra* was estimated at 2.73 ± 0.03 mg/g of extract. Our study are in agreement with the study of **Balpinar and Okmen. (2019)** wich they detected the presence of protocatechic acid, chlorogenic acid, rutin in the methanolic leaf extract of *S.vernalis*, Also caffeic acid (2.17 mg/g), ellagic acid (2.13 mg/g), quercetin (1.65 mg/g), gallic acid (1.62 mg/g) and chlorogenic acids (0.59 mg/g) were detected in crude extract of *S.abyssinicus* from Nigeria **(Odubanjo *et al*., 2017).**

At present, the composition of phenolic compounds in *S.hoggariensis* is still limited and only one study was effected by **Ragaa *et al*. (1980)** which allowed to identify the presence of certain flavonoids like Q- 3-glucoside ,I 3 -rutinoside and I 3-monosulphatet in traces.

Previous studies reported similar results on the phenolic composition of *S.glaucus* sub *coronopifolius*. However, some differences depend on the extraction procedure as well as the place where or when the plants are harvested. A previous study on the methanolic root extract of *S.glaucus* sub *coronopifolius* growing in Egypt showed the presence of some phenolic compounds detected in our study, in which protocatechic acid (66.036) vanilic acid (4.578) chlorogenic acid (13.808) ellagic acid (3.258) and rutin (152.565) are detected. Moreover, **Alqahtani *et al*. (2020)** showed that gallic acid and vanilic acid were abundant compounds in *S glaucus* chloroform fraction.

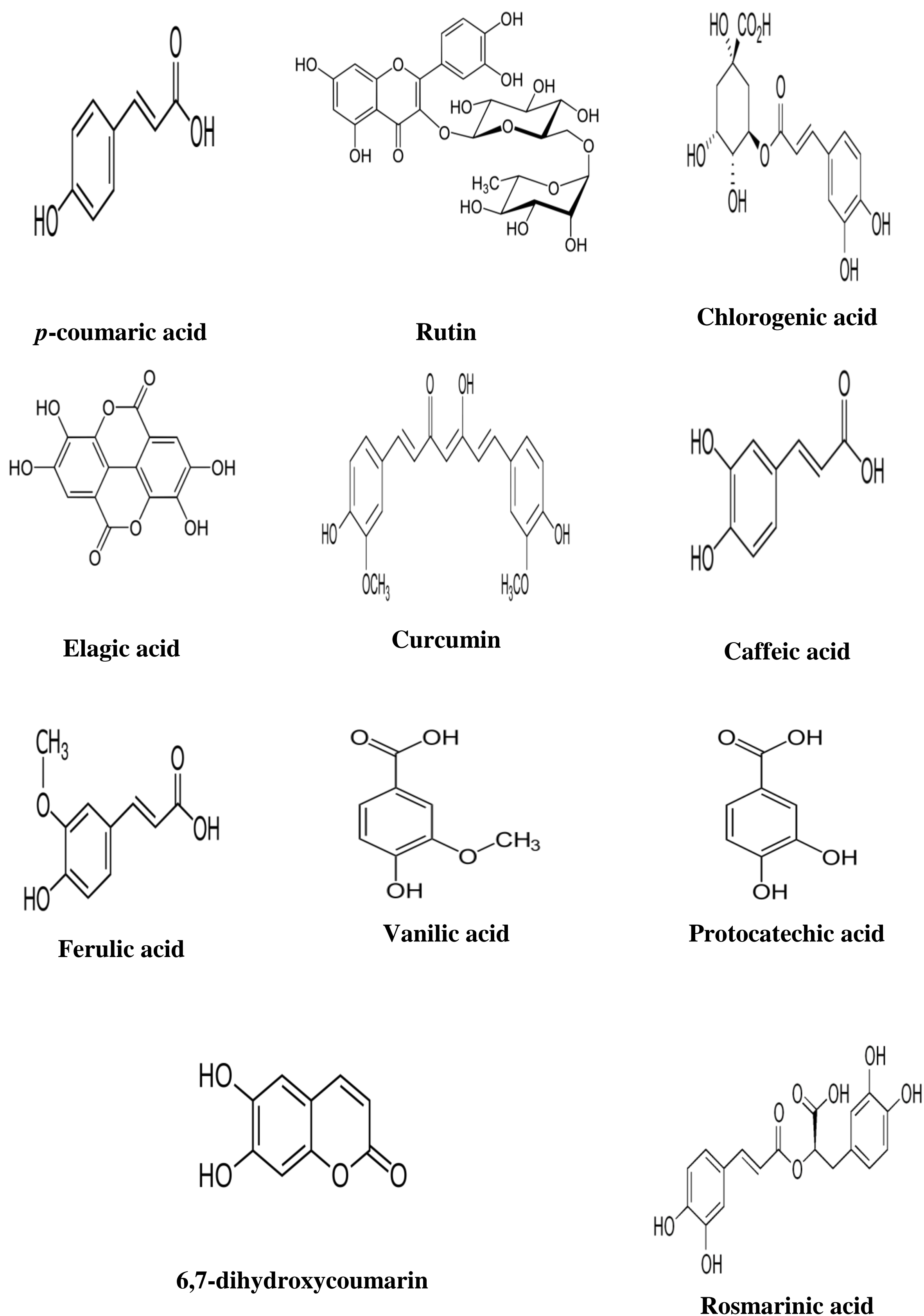


Figure 16. Chemical structures of some compounds identified from *S.coronopifolius* and *S.hoggariensis* ME, EA and Bu Extracts.

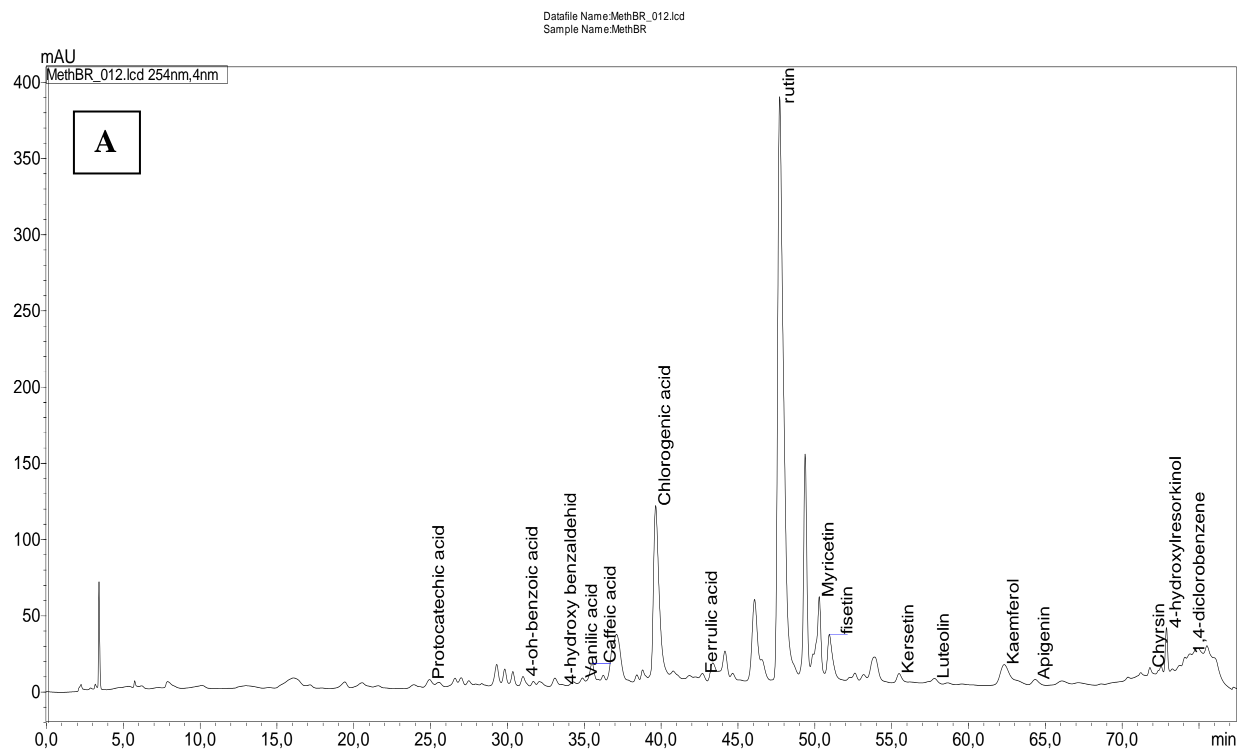
HPLC profile of *B.incrassatum* extracts is summarized in Table 11 and Table 12, chromatograms recorded at 254 nm for all fractions are shown in figure 17. A total of 17 compounds were detected in ME extract of *B.incrassatum*, while 7 compounds were detected in EA and Bu fractions as shown in Table 1. Rutin (16.04 mg/g, 4.45 mg/g) and chlorogenic acid (7.30, mg/g 2.63 mg/g) were found to be major phenolic compounds of Bu and EA extracts of *B.incrassatum* respectively, while rutin (44.40 mg/g), chlorogenic acid (10.67 mg/g) and 1,4-diclorobenzene (6.25 mg/g) were found in the ME extract. Also, 4-hydroxylresorkinol (2.31 mg/g) and chyrsin (1.05 mg/g) were found as other predominant phenolic compounds of ME extract. Besides that pyrocatechol (0.62 mg/ml) were exclusively detected in Bu fraction . Ferrulic acid, vanilic acid and 4-hydroxy benzoic acid were also detected in small amounts in all tested extracts of *B.incrassatum*.

Table 11. Composition of Methanol extracts determined by HPLC-DAD (µg of pheolic coumpound/g extract

Compounds	Rt* (min)	ME
Protocatechic acid	24.68	0.31
4-oh-benzoic acid	31.69	0.07
4-Hydroxybenzaldehyd	33.36	0.11
Vanilic acid	34.68	0.15
Caffeic acid	35.19	0.90
Chlorogenic acid	38.88	**10.67**
Ferrulic acid	42.92	Tr
Rutin	47.52	**44.40**
Myricetin	50.36	0.66
Fisetin	51.24	0.75
Quercetin	55.44	0.29
Luteolin	57.87	0.23
Kaepmferol	62.48	0.84
Apigenin	64.07	0.09
Chyrsin	72.77	**1.50**
4-hydroxylresorkinol	73.06	**2.31**
1,4-diclorobenzene	73.81	**6.35**

Table 12. Composition of ethyl acetate and n-butanol extracts determined by HPLC-DAD (µg of pheolic coumpound/g extract).

Compounds	Rt*(min)	EA	Bu
Pyrocatechol	13.51	nd	0.62
3,4-dihydroxybenzoicacid	14.10	0.57	0.38
4-hydroxy benzoicacid	19.50	0.29	0.39
Vanilic acid	22.37	0.17	0.12
Caffeic acid	22.94	**1.05**	nd
Chlorojenic acid	25.65	**2.63**	**7.30**
Ferrulic acid	29.93	0.55	0.48
Rutin	35.02	**4.45**	**16.04**



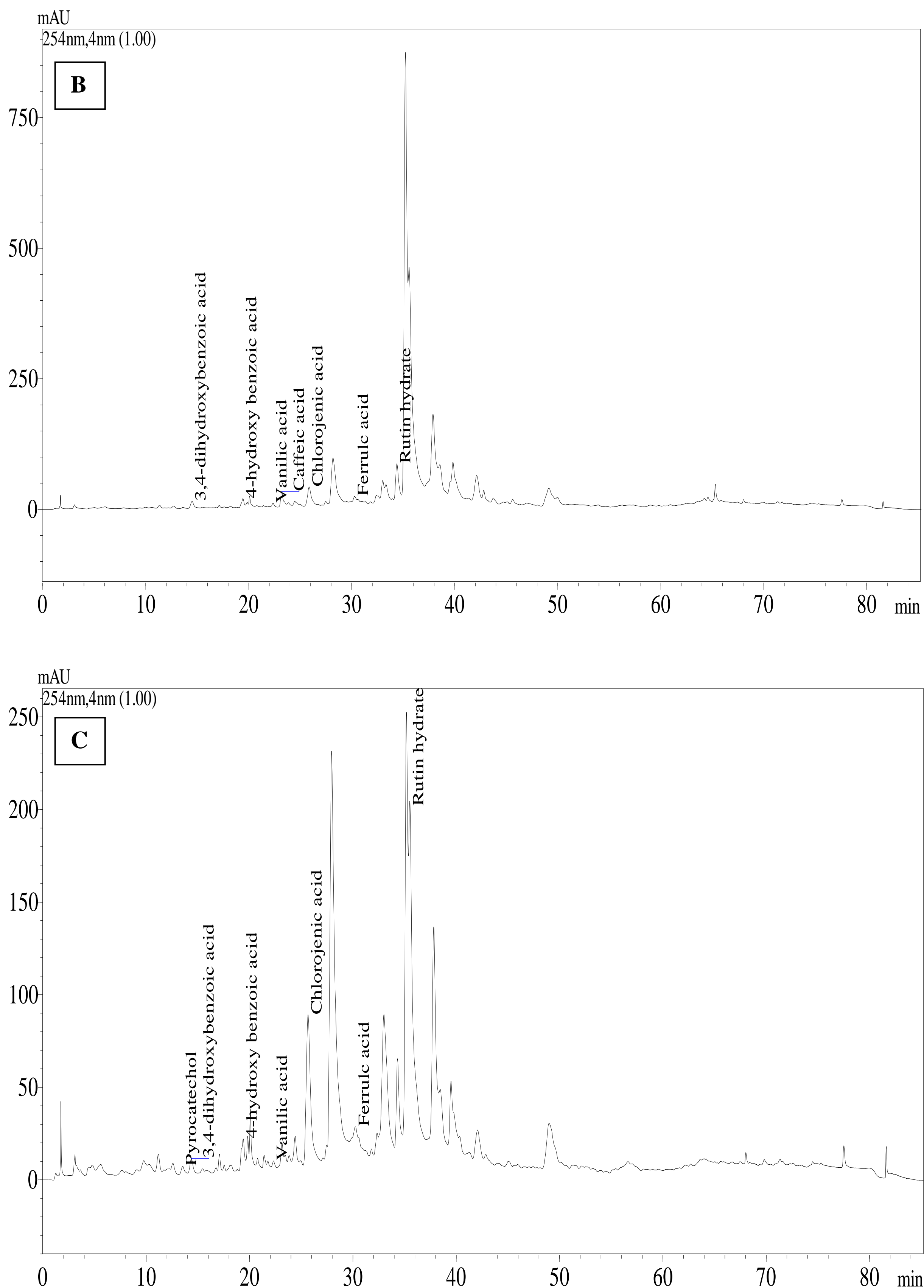
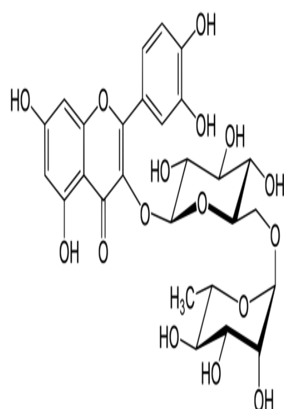


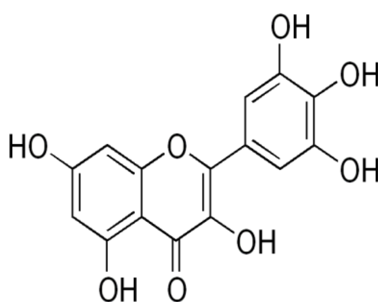
Figure 17. HPLC chromatograms of the total polyphenols of **(A)** ME, **(B)** EA, **(C)** Bu of *B.incrassatum* at 254nm.

Several research on chemical composition of essential oils from *Bunium* genus have been performed **(Sharafati Chaleshtori *et al*., 2018)** and on *B.incrassatum* **(El kolli *et al*., 2017; Boussetla *et al*., 2011),** however, there is still lack in data in phytochemical study of their extracts. Therefore, the present reserach may provide more information for the genus.

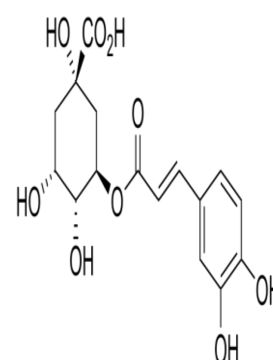
The appearance of chlorogenic acid in relatively high quantities in the plant kingdom and species belonging to the Apiaceae family was described mainly in literature **(Generalic Mekinic *et al*., 2016; Upadhyay *et al*., 2013**). Previous studies reported a number of phenolic compounds from other *Bunium* species, including phenolic acids (caffeic acid, *ρ*-coumaric acid) and flavonoids (kaempferol) have been identified in methanolic extract of *B.persicum* **(Sharifi-far *et al*., 2010). Souilah *et al*. (2021)** showed the presence of seven flavonoids and eight phenolic acids were founed in ME extract of *B.crassifolium*, in opposite toMeOH extract(70%) eight flavonoids and eight phenolic acids were detected, in which *p*-coumaric protocatechic, malic, quinic, fumaric, chlorogenic, sinapic and tr-ferulic acids and *p*-hydroxybenzoic were detected in large amounts. Gallic and caffeic acid were founded in large quantities in ME extract. **Zengin *et al*. (2019)** showed the presence of some phenolic compounds in *B.brachyactis, B.pinnatifolium, B.microcarpum*, and *B.sayai* including chlorogenic acid, rutin quinic acid, isoquercitrin, and apigenin.



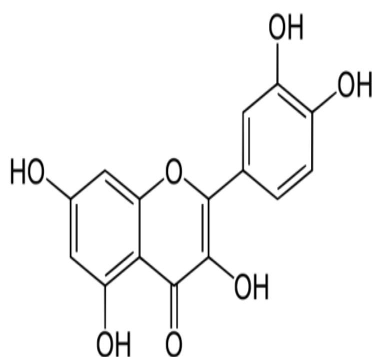
Rutine



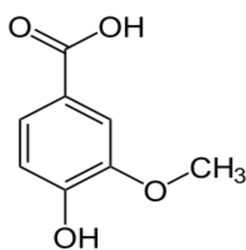
Myricetin



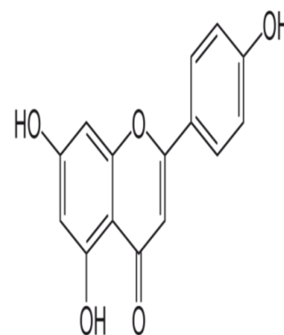
Chlorogenic acid



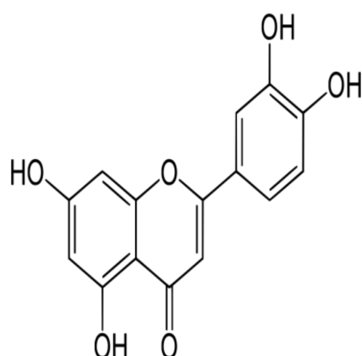
Quercetin



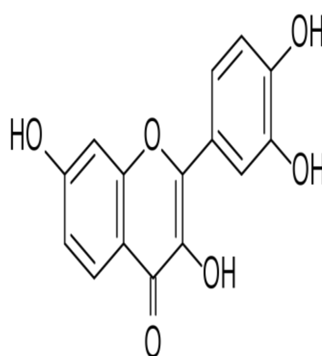
Vanilic acid



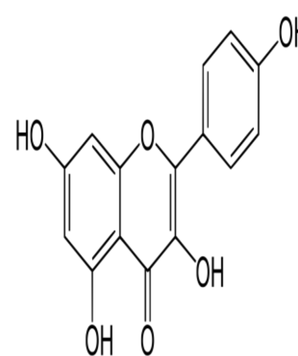
Apigenin



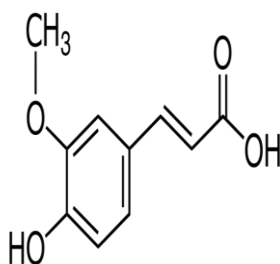
Luteolin



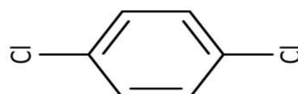
Fisetin



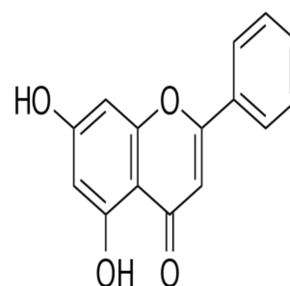
Kaepmferol



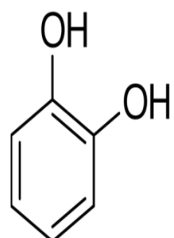
Ferrulic acid



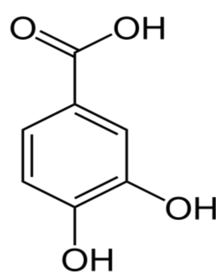
1,4diclorobenzene



Chyrsin



Pyrocatechol



Protocatechic acid

Figure 18. Chemical structures of some compounds identified from *B.incrassatum* extracts.

II. Biological investigations

II.1. Exploration of antioxidant capacities

Antioxidants were explored by using DPPH radical scavenging, ABTS cation radical scavenging, β-carotene-linoleic acid, cupric reducing antioxidant capacity (CUPRAC) and metal chelation in which α-Tocopherol, BHA, BHT, Quercetin and EDTA were used as antioxydant standards, which are well known compounds with strong antioxidant properties. The results can be expressed as percentage of the antiradical activity or using the parameter $IC_{50}/A_{0.50}$.

II.1.1. Free radical scavenging assay by DPPH

The DPPH test aims to measure the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picryl hydrazil (DPPH) formed in solution by donating of a hydrogen atom or an electron **(Tepe *et al.*, 2006).** DPPH gives a strong absorption band at 517 nm in visible spectroscopy. The stable free radical DPPH has been widely used to test the free radical-scavenging capacity of various antioxidants. If the extracts can scavenge the DPPH free radical, the initial purple solution will change to a yellow color due to the formation of diphenyl picryl hydrazine **(Pavithra and Vadivukkarasi, 2015).**

In the DPPH assay, the radical scavenging ability of the extracts and the positive controls (BHT and α- Tocopherol) was analyzed in triplicates. DPPH scavenging activities of plant extracts and standard antioxidants are given in (Figure 19). As can be seen, the DPPH radical scavenging activity varied from 58.82 to 84.25µg/mL. The highest antioxidant activity was related to EA extracts. At 100 µg/mL, the highest radical scavenging activity was obtained with the EA fraction of *B.incrassatum* (84.25± 0.04 µg/mL), followed by its Bu fraction (83.32 ±0.13µg/mL). EA fractions of all studied plants showed much higher radical scavenging activity than BHT (73.91±0.11µg/mL). However, none of the extracts and fractions exhibited stronger DPPH radical scavenging activity than α-Tocopherol (93.77±0.07 µg/mL).

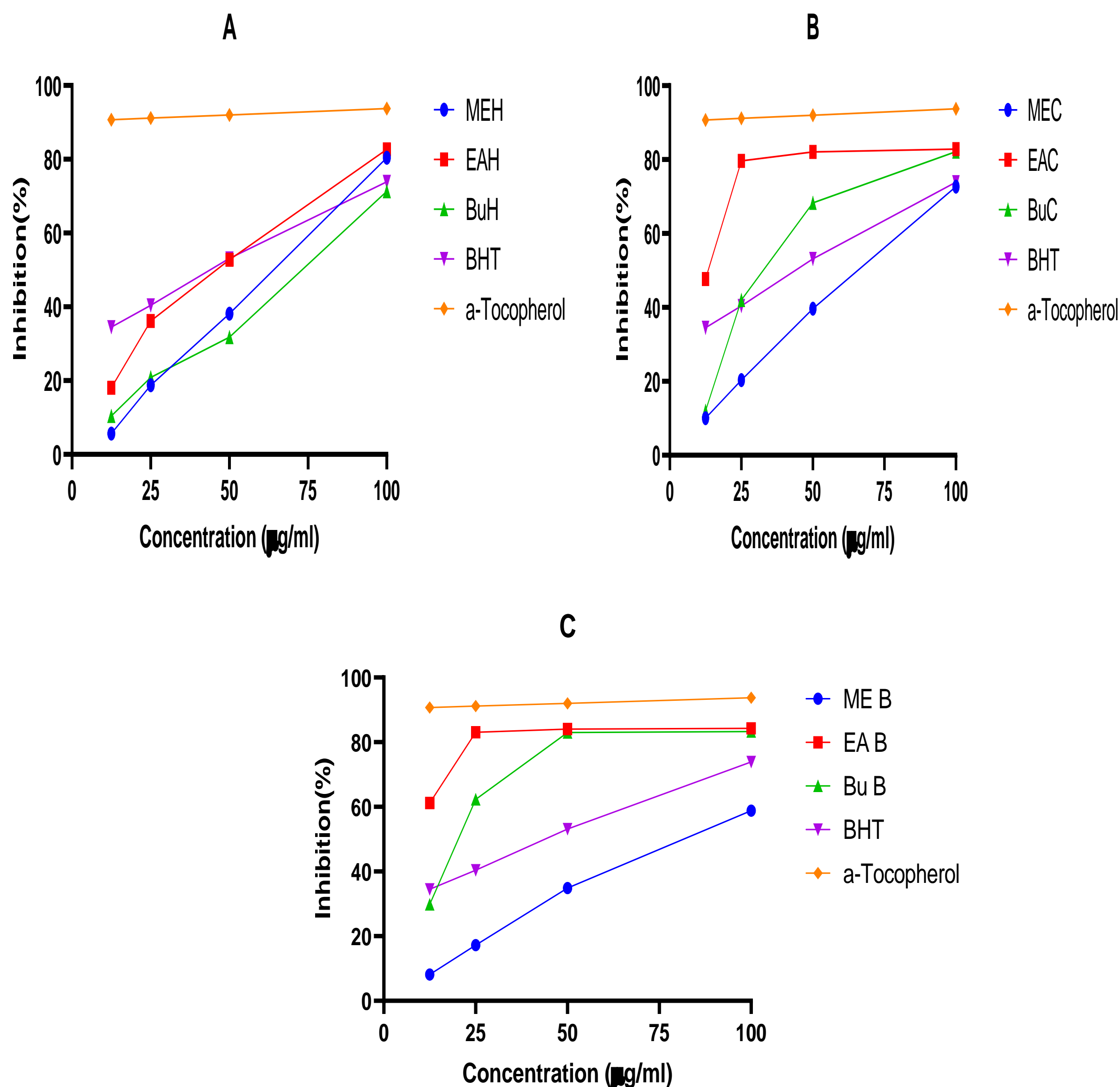


Figure 19. DPPH free radical scavenging activity of different plant extracts. **(A):** *S.hoggariensis*, (**B**)**:** *S.coronopifolius*; **(C):** *B.incrassatum,* **ME:** methanolic extract, **EA:** ethyl acetate extract, **Bu**: *n*-butanol extract.

The antioxidant activities obtained by the DPPH method for extracts are presented as IC_{50} in Figure 20. The analysis of variance revealed significant differences between various extract. The ethyl acetate fraction of *B.incrassatum* showed excellent activity with better IC_{50} values of 1.53±0.39µg/ml than α-Tocopherol (IC_{50}: 12.26±0.07 µg/ml) and BHT (IC_{50}: 45.37±0.47 µg/ml), followed by EA fraction of *S.coronopifolius* (IC_{50}: 4.30±0.34 µg/mL), then it Bu extract (IC_{50}: 29.83±4.71 µg/mL). Bu extract of *S.hoggariensis* (IC_{50}: 88.56±3.75

µg/mL) had the lowest free radical scavenging activity. Both EA and Bu extracts of the three exhibited good antioxidant activity than the crude extracts.

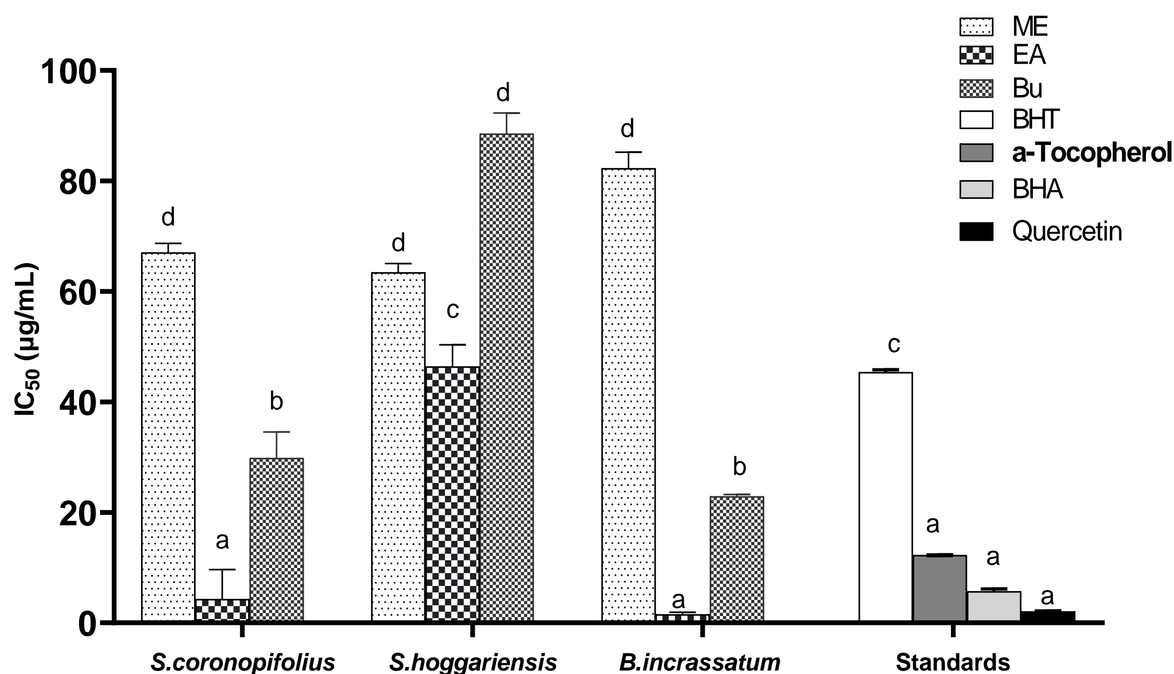


Figure 20. The IC_{50} values in the DPPH radical scavenging activity assay of the extracts. BHT, BHA, α-Tocopherol and quercetin were used as references antioxidants. Each value is expressed as a mean ±S.D (n=3). Bars with superscripts with different letters in the histogram were significantly ($p < 0.05$) different from each other.

II.1.2. $ABTS^{+\cdot}$ radical cation decolorization assay

In the ABTS assay, also known as Trolox equivalent antioxidant capacity (TEAC) assay, the green–blue stable radical cationic chromophore,2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$) is produced by oxidation, and has absorption maxima at 414, 645, 734, and 815 nm **(Prior *et al.*, 2005)**. The $ABTS^{+}$ radical-scavenging assay was measured according to the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS to produce the radical cation, in the presence or absence of antioxidants **(Re *et al.*, 1999).** With the improving technology for determination of the blue ABTS the reaction between ABTS and potassium persulfate. Measurements are preferably made at 734 nm, because of possible interferences from other components in coffee at the other maxima **(Prior *et al.*, 2005).**

As can seen in Figure 21, the ABTS radical scavenging activity of extracts ranked in the same way as the values for DPPH radical scavenging activity, with EA fraction as the highest inhibition. BHT and BHA (94.87±0.42%, 94.20± 0.42%, respectively), showed a significantly higher value than any of the other extracts witch demonstrated relatively strong ABTS radical scavenging activity. The analysis of variance revealed significant differences between various extract. The highest ABTS radical scavenging activity (%) was noticed at 100µg/mL for EA extract of *B.incrassatum* followed by EA extract of *S.coronopifolius* with a percentage of 90.41±0.01% and 91.21±0.07%, respectively.

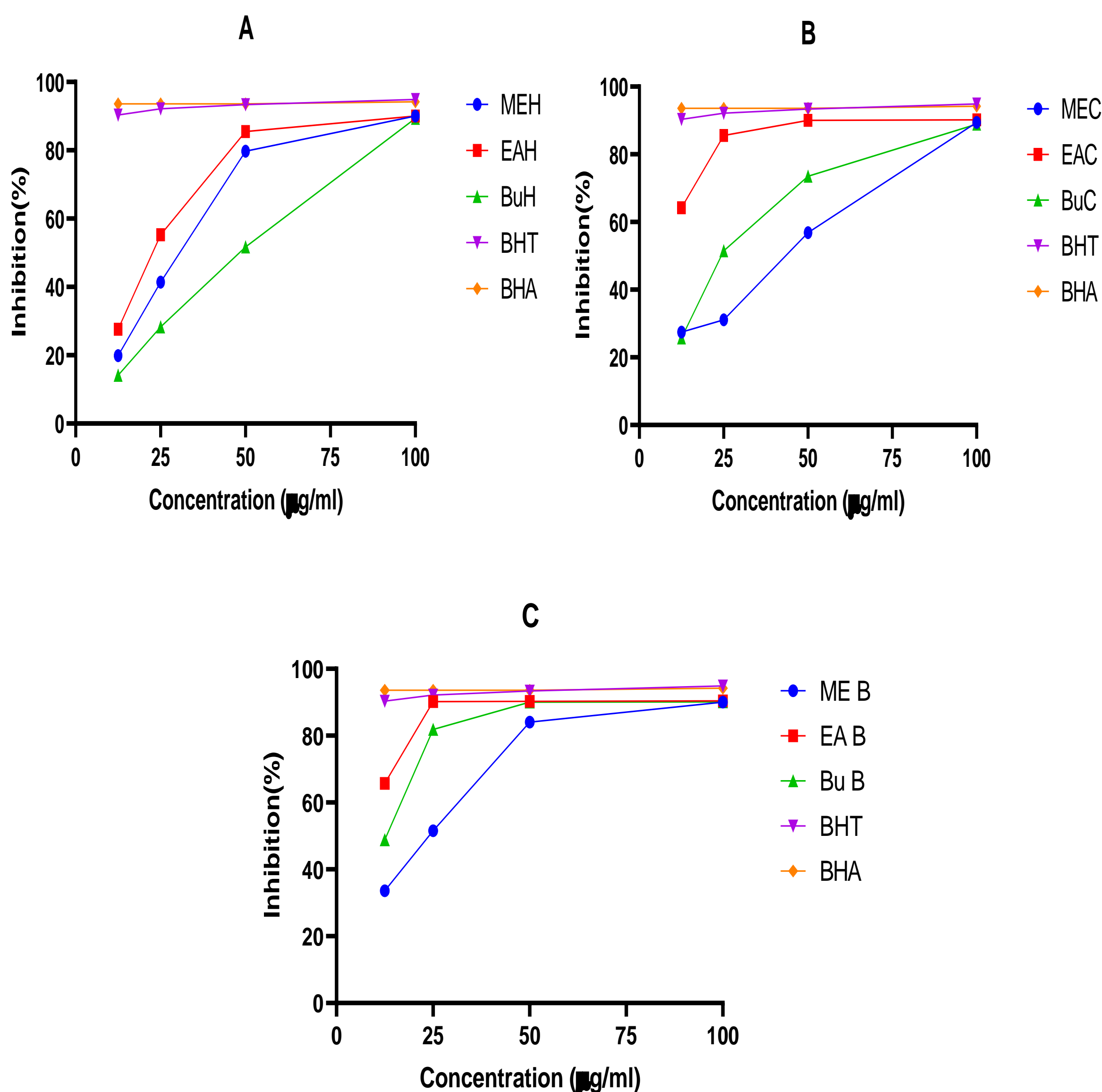


Figure 21. ABTS scavenging activity of different plant extracts. **(A):***S.hoggariensis*, **(B):** *S.coronopifolius*; **(C):** *B.incrassatum.* **ME**: methanolic extract, **EAE:** ethyl acetate extract, **Bu:** *n*-butanol extract.

In the ABTS assay, the EA extract of *B.incrassatum*with an IC_{50}value of 1.28±0.11 µg/mL also had the best activity which could be considered as highly active and close to those of the standard antioxidant compounds BHT (IC_{50}: 4.10±0.06 µg/mL) and α-Tocopherol (IC_{50}: 4.31±0.10 µg/mL), followed by it butanol fraction (IC_{50}: 5.33±1.22 µg/mL) and EA extract of *S.coronopifolius* (IC_{50}:10.62±3.30 µg/mL). Bu extract of *S.hoggariensis* (IC_{50}:50.26±2.16 µg/mL) had the lowest activity (Figure 22).

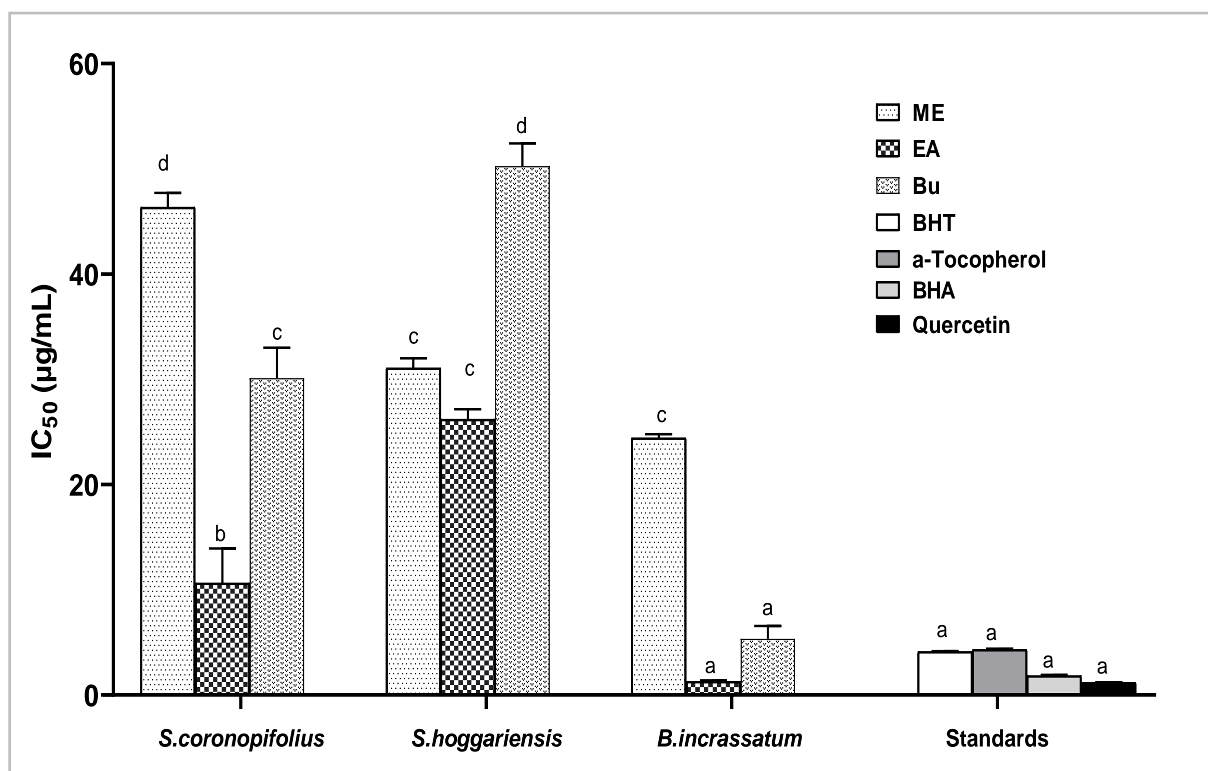


Figure 22. The IC_{50} values in the ABTS radical cation decolorization activity assay of the extracts. BHT, BHA, α-Tocopherol and quercetin were used as a reference antioxidant .Each value is expressed as a mean±S.D (n=3). Bars with superscripts with different letters in the histogram were significantly ($p < 0.05$) different from each other.

II.1.3. β-Carotene bleaching assay

The basis of β-carotene-linoleate assay is discolouration of β-carotene in reaction with linoleic acid free radical formed at elevated temperatures upon removal of hydrogen atom located between two double bonds of linoleic acid. The consequence is the loss of conjugation and accordingly, a decrease in absorbance at 470 nm. Antioxidants can reduce the extent of β-carotene destruction by reacting with the linoleate free radical or any other free radical formed within the system. Thus, by simulation of the oxidation of the membrane lipid components in

the presence of antioxidants, this test gives an insight of the inhibitory effect of extracts on the lipid peroxidation **(Sarikurkcu *et al*., 2009).**

Results above in figure 23 showed that extracts present an inhibition percentage of β-carotene bleaching ranged from 69.52% to 88.26%, The relatively highest antioxidant activity was obtained in EA of *S.hoggariensis* (88.26± 2.13%) followed by EA of *B.incrassatum* (85.39±3.04%) at 100 µg/mL. The results obtained from extracts were all significantly different ($p < 0.05$), compared to BHT and α-Tocopherol as standard (93.60% ±0.16% and 92.10±0.51% respectively).

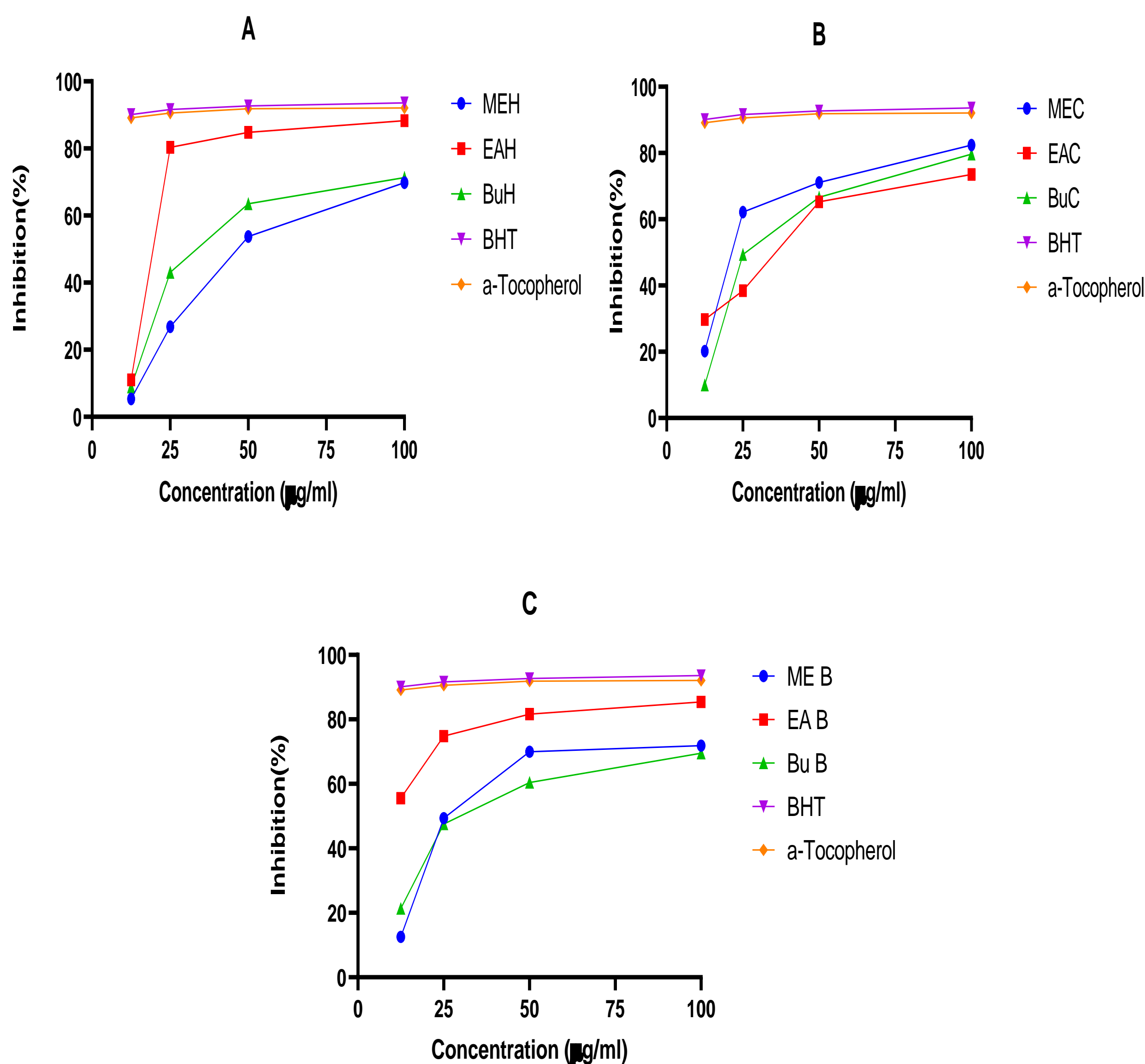


Figure 23. β-carotene-linoleic acid activity of different plant extracts. **(A):** *S.hoggariensis*, **(B):** *S.coronopifolius* ; **(C);** *B.incrassatum*. **ME:** methanolic extract, **EAE:** ethyl acetate extract, **Bu:** *n*-butanol extract.

In EA extract of *B.incrassatum* inhibited the lipid peroxidation in a good manner, it exhibited highest activity with IC_{50} values of 1.94±0.37 µg/mL than that among the extracts ($p<0.05$), followed by Bu and the EA extract of *S.hoggariensis* (IC_{50}: 15.22±4.24 µg/mL, and IC_{50}:18.98 ±1.50 µg/mL, respectively). ME extract of *S. hoggariensis* (IC_{50}: 58.00±1.20 µg/mL) displayed the lowest activity. As described in Figure 24, the analysis of variance revealed significant differences between various extract.

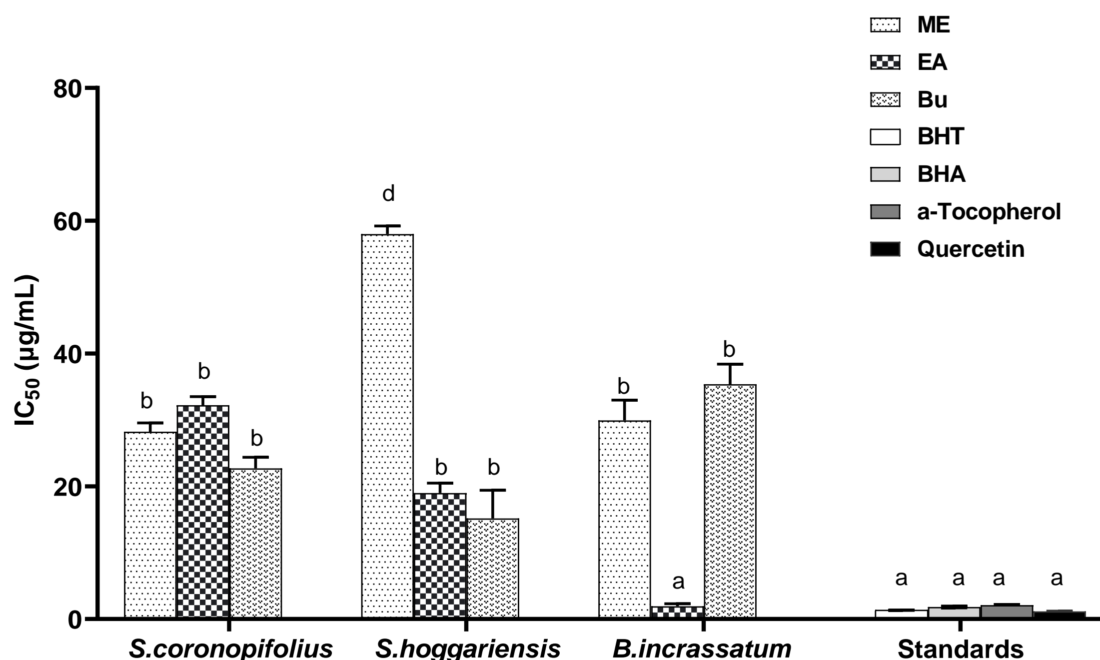


Figure 24. The IC_{50} values in the β-carotene bleaching test antioxidant capacity of the extracts. BHT, BHA, α-Tocopherol and Quercetin were used as a reference antioxidant .Each value is expressed as a mean ±S.D (n=3). Bars with superscripts with different letters in the histogram were significantly ($p < 0.05$) different from each other.

II.1.4. CUPRAC assay

The CUPRAC assay allowed us to measure the total antioxidant potential of the extracts studied plants. This method is based on the reduction of Cu(II) to Cu(I) by antioxidants present in the sample. A chromogenic reagent, neocuproine (2,9-dimethyl-1,10-phenanthroline), forms a complex with Cu(I), which has a maximum absorbance at 450 nm **(Apak *et al.*, 2008).**

Cupric reducing capacity values obtained for extracts (absorbance) increased linearly with the increasing amount of extracts. CUPRAC of the extract were assessed and compared to that of the positive controls BHA and α-Tocopherol. Activity (absorbance) increased

linearly with the increasing amount of extracts. As shown in Figure 25, At 100 µg/mL concentration, EA extracts of *S.coronopifolius* and *B.incrassatum* (2.56 ±0.06 and 2.19±0.01, respectively) exhibit higher reducing power activity than α-Tocopherol (1.85 ± 0.00) and nearly similar the activity of BHT (2.04 ± 0.14) absorbances at 450 nm, followed by its Bu fractions (1.62±0.10 and 1.47±0.02), respectively. However, the crude extracts and fractions of *S.hoggariensis* were less than the positive controls.

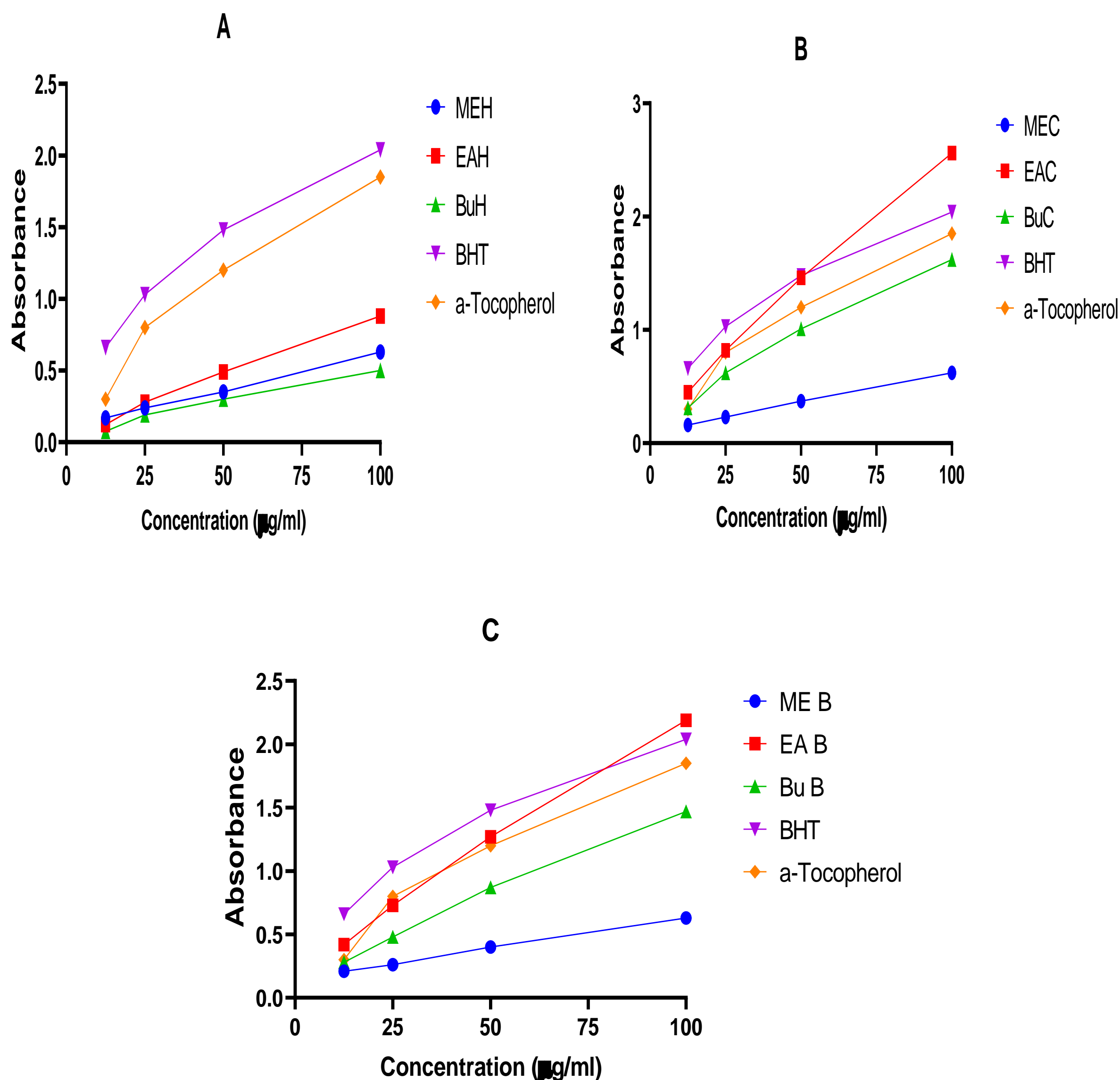


Figure 25. Cupric reducing antioxidant capacity of different plant extracts. **(A):** *S.hoggariensis*, **(B):** *S.coronopifolius* ; **(C):** *B.incrassatum*; **ME:** methanolic extract, **EAE:** ethyl acetate extract, **Bu:** *n*-butanol extract.

EA extract of *S.coronopifolius* ($A_{0.50}$: 10.24±2.45 µg/mL) exhibited the best cupric reducing antioxidant capacity which was close to that of standard α-Tocopherol

($A_{0.50}$:10.20±0.01µg/mL) and low than BHT ($A_{0.50}$: 3.80±0.00 µg/mL) , followed by it Bu extract ($A_{0.50}$: 14.03±4.92 µg/mL) and EA of *B.incrassatum* ($A_{0.50}$: 14.80±0.52 µg/mL). Bu extract of *S.hoggariensis* ($A_{0.50}$: 123.44±4.73 µg/mL) had the lowest activity herein (Figure 26).

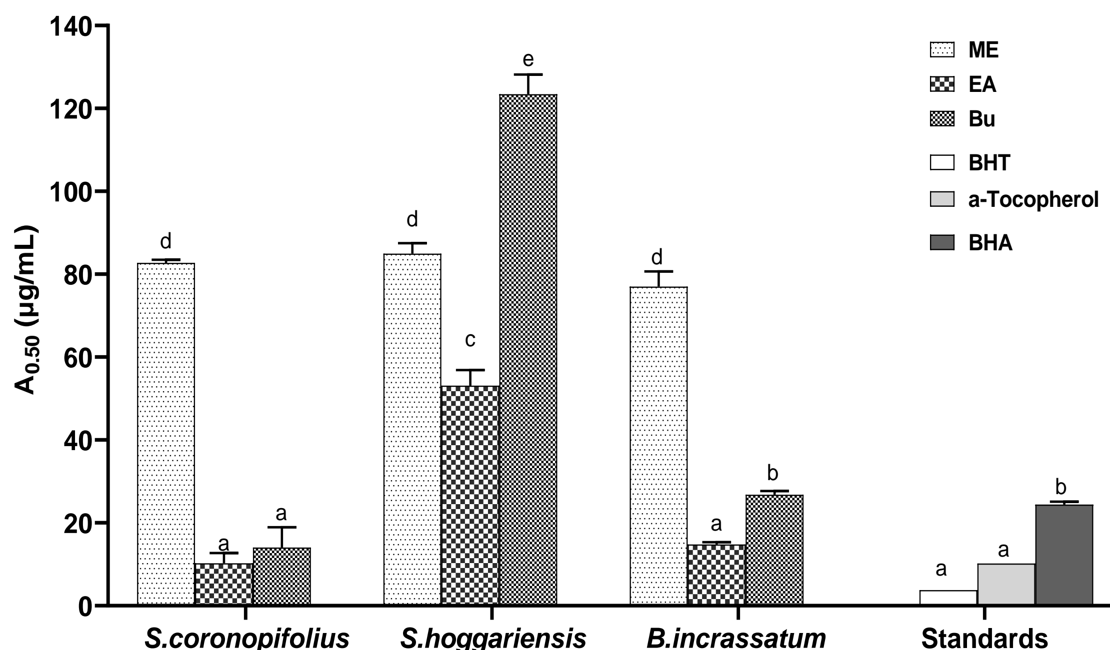


Figure 26. The $A_{0.5}$ values in the cupric reducing antioxidant capacity of the extracts. BHT, BHA and α-Tocopherol were used as a reference antioxidant. Each value is expressed as a mean ±S.D (n=3). Bars with superscripts with different letters in the histogram were significantly ($p < 0.05$) different from each other.

II.1.5. Metal-chelating assay

Transition-metal ions have a significant role in the production of oxygen free radicals in organisms. Iron has two distinct oxidation states: ferrous ion (Fe^{2+}) and ferric ion (Fe^{3+}). The ferric ion (Fe^{3+}) is the biologically inactive type of iron. However, depending on the conditions, espacially pH, it could be reduced to the active form Fe^{2+} **(Strlic *et al.*, 2002),** and oxidized again through fenton type reactions with thydroxyl radicals production or could be throught Haber-Weiss reactions with superoxide anions **(Wong and Kitts, 2001)**. Ferrozine can form quantitative complexes with Fe^{2+}. In the presence of chelating substances, the complex formation is supressed and the the complex's red colour disappears. Therefore measuring of the colour reduction allows estimation of the chelating activity of co-existing chelator **(Yamaguchi *et al.*, 2000).** In this test, the interferation of natural compound with the

formation of the ferrozine Fe^{2+} complex, suggested that it has chelating action and catches ferrous ions before ferrozine.

As seen in Figure 27, All extracts except two extracts did not show any remarkable colour changes, although decreases in absorbance readings were recorded. Quercetine is a strong metal chelator, hence, it is used as a standard metal chelator agent in this study. Compared to the results of positive controls, all fractions had moderate ability to chelate metal ion. The highest ferrous ion chelating effect among the samples was shown by ME fraction of *S.hoggariensis*, with the value of 69.26 ±0.21%, followed by the Bu fraction of *B.incrassatum* (45.50± 0.22 %).

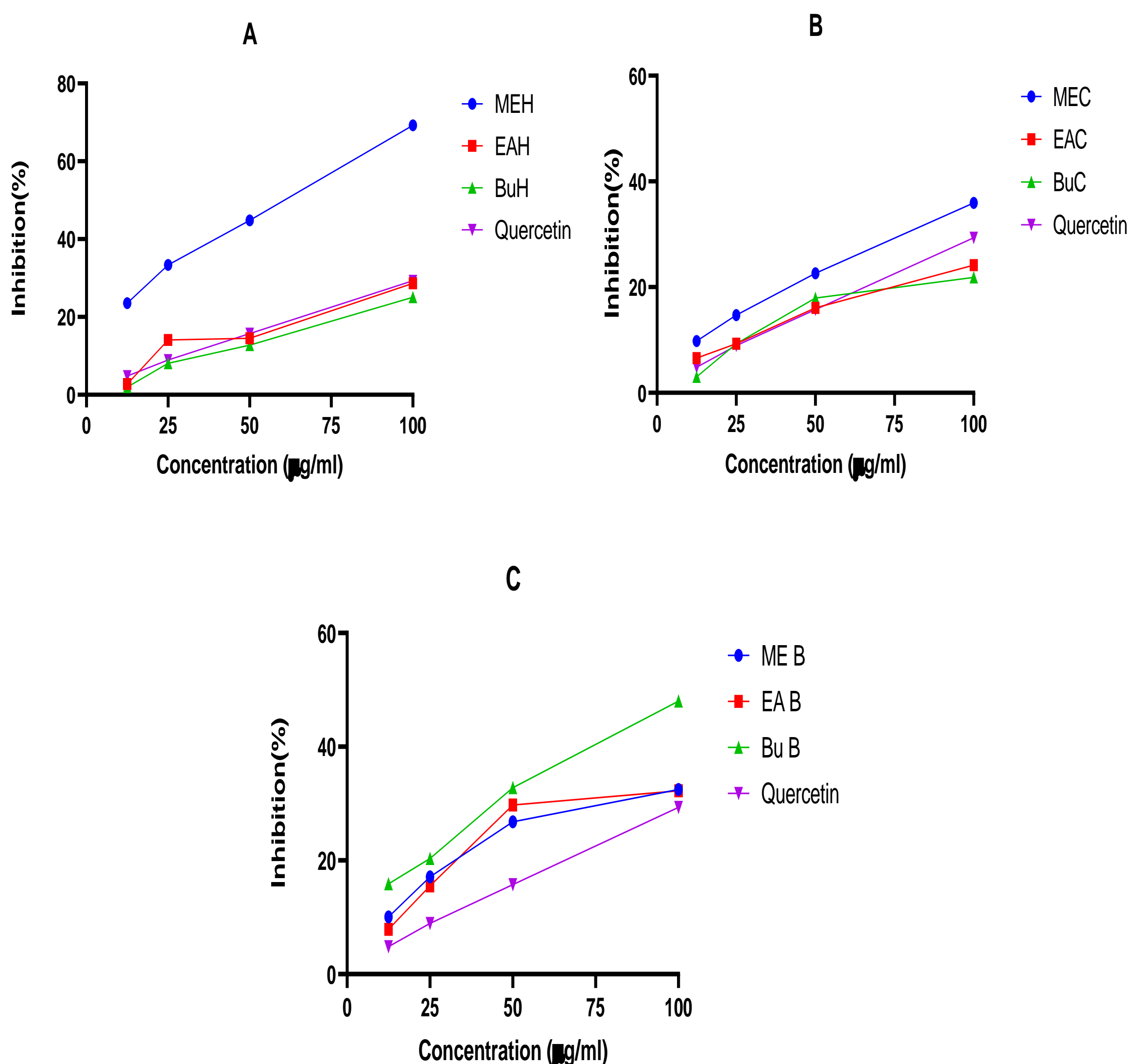


Figure 27. Metal chelating activity of different plant extracts. **(A):** *S.hoggariensis*, **(B):** *S.coronopifolius* ; **(C);** *B.incrassatum.* **ME**: methanolic extract, **EAE:** ethyl acetate extract, **Bu:** *n*-butanol extract.

IC_{50} for all extracts except ME extract of *S.hoggariensis* (52,78±2,21% µg/mL) and Bu of *B.incrassatum* (78.97±1.73 µg/mL) were not identified because it was higher than the area of measurement (IC_{50}> 100µg/mL). The metal chelating activity of the others extracts of plant studied close to each other and exhibited moderate activity compared to standards quercetine (IC_{50}=250µg/mL).

Various methods were used to analyze antioxidant capacity because antioxidant agents with different compositions and contents present different mechanism for their antioxidant capacities. It has earlier been argumented that antioxidants have the ability to act as free radical scavenger ($DPPH^{•}$ and $ABTS^{+•}$ scavenging assays) **(Mishra *et al*., 2012),** as reducing agents (cupric ions) **(Apak *et al*., 2004)** or as hydrogen atom donators (inhibition of linoleic acid oxidation) **(Sánchez-Moreno *et al*., 2002).** The evaluation of antioxidant capacity of some compounds in EOs and extracts has been the subject of a number studies **(Ranilla *et al*., 2010).** However, antioxidant potentials of plant products cannot be carried out accurately by only one method **(Huang *et al*., 2005).** Therefore, we used a variety of antioxidant assays to gain a better inderstanding of the antioxidant potential of plants extracts. the following tests were performed: free radical scavenging activities ($DPPH^{•}$ and $ABTS^{+•}$), inhibition of lipid peroxidation (β-carotene bleaching assay), metal chelating and cupric reducing antioxidant capacity.

To date, no study has reported the antioxidant activity of *S.hoggariensis* extracts. **Ajiboye *et al*. (2018),** stated in their study on *S.biafrae*, which is a part of the same genus, that the crude extract showed low inhibitory abilities against all free radicals in a concentration-dependent manner, with IC_{50} in ABTS radical scavenging ability (78.25g/ml), DPPH radical scavenging potential (92.08g/ml) and Fe^{2+}chelating ability (118.76g/ml). In another study, **Tundis *et al*. (2012)** have also reported the antioxidant activity of the methanol extract of *Senecio stabianus Lacaita* by using DPPH and ABTS methods (IC_{50} values of 66 mg/ml and 72.3 mg/ml, respectively). These findings correspond with those of our study. Moreover, ME extract of *S.chrysanthemoides* demonstrated very low potency in both DPPH and ABTS free radical scavenging assays **(Singh *et al*., 2018).** The presence of flavonoids and phenolics in our extracts such as gallic acid, chlorogenic, caffeic acid, and rutin may reduce cellular oxidative stress **(Adefegha *et al*., 2015).**

Previous studies have investigated and confirmed the antioxidant potential of *S.coronopifolius*. In this study, our extracts exhibited potent antioxidant activities, which can be attributed to their high content in total phenolic and favonoid compounds. Relevantly,

Mohamed. (2015) investigated the antioxidant activity of the root methyl alcohol extract of *S. glaucus* subsp. *coronopifolius* growing in Egypt, using the DPPH method, the extract had a potent activity (IC_{50} =79.57 ± 0.74 µg/ml). Furthermore, **El kahtani *et al.* (2020)** found that the crude and butanol extracts of *S.glaucus* found in Saudi Arabia, displayed strong antioxidant activity through two screening methods DPPH and ABTS, with a value of 35.9 ± 3.5; 47.5 ± 2 % in DPPH and 38.3 ± 4.6; 48.3 ± 4.6% in ABTS at a concentration of 500 µg/mL, respectively .A recent study which reported that ME extract of aerial parts of the same species displayed a strong DPPH scavenging activity (10µg by 69.35%) (**Mohamed *et al.*, 2022**).

In general, according to our finding, EA extracs were the most active fraction. Previous work had found similar good for ethyl acetate and Bu fractions and have documented that this fractions are sources of antioxidant substances **(Tung *et al.*, 2007**). It is important to compare data obtained with other plants under the same genus, significant antioxidant activities were recorded in ethyl acetate fractions : for *S.inaequidens, S. vulgaris*, (inhibition of DPPH equal to 61.60% and 44.57% of inhibition, respectively, at concentration of 0.31 mg/mL **(Conforti *et al.*, 2006),** *S.gibbosus* (IC_{50} of 0.01 mg/mL on DPPH) **(Conforti *et al.*, 2006b**), *S.clivicolus* (IC_{50} of 0.10 mg/mL on DPPH) **(Faraone *et al.*, 2018)** and *S.argunensis* **(Zhou *et al.*, 2008)** and showed higher inhibition than that obtained in present study. It is possible to explain these results with the different methods used to obtain the ethyl acetate fraction. In fact, **Conforti et al. (2006)** extracted the plant materials with methanol; then, the methanolic extract was acidified with 2.50% H_2SO_4 and partitioned with *n*-hexane, dichloromethane, and ethyl acetate. Moreover, in our study the fractionation steps was carried out without acidification.

EA and Bu extracts of *B.incrassatum* extracts demonstrated strong antioxidant than ME extract. Such properties have been previously reported from this plant . In conformity with the present work, The results of **(El Kolli *et al.*, 2017).** ME extract of *B.incrassatum* from aerial parts recorded even higher IC_{50} than the other studies with an IC_{50} of 55.77±3.25 µg/ml. According to our IC_{50} values, it appears that the antioxidant capacity of extracts from aerial parts is higher than that obtained from tubers, Extracts from *B.incrassatum* tubers showed weak reducing activity against DPPH radical with IC_{50} values equal to 21.11 mg/mL, while for aqueous extract no activity was recorded even for the highest dose tested (21 mg/mL)(**Dehimi *et al.*, 2020).** Moreover, crude extract displayed activity on DPPH 1.602± 0.002 mg/mL and ABTS 0.744 ± 0.0001 mg/mL **(Aiouaz and Arezki, 2022).** In other species

and in the study of **Zeignig *et al.* (2019**) the most effective in DPPH and ABTS scavenger was: *B. microcarpum* followed by *B.pinnatifolium*, while the least effective scavenger was *B.sayai*. Similarly, *B.microcarpum* showed the strongest cupric reducing activity, while *B.pinnatifolium* exhibited the highest ferric reducing effect. Again, the weakest cupric and ferric reducer was displayed by *B.sayai*. On the other hand, the least effective antioxidant in the phosphomolybdenum and metal chelating assays was *B. microcarpum*, while the strongest antioxidant among the tested *Bunium* species in the respective assays were *B.pinnatifolium*.

II.1.6. Correlation between TPC and antioxidant activity

The relationship between phenolic content (TPC) in extracts, and antioxidant activities (DPPH, ABTS, β-carotene , CUPRAC and Metal chelating activities) were analyzed by determining the Pearson's correlations between them. Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$ **(Thaipong *et al.*, 2006).** Results showed a significant positive correlation between TPC and all antioxidant activities (Annexe 3).

Table 13. Correlation between TPC contenet and antioxidant activities of the studied extracts

Plant	Extract	DPPH	ABTS	β-carotene	CUPRAC	Metal chelating
S.coronopifolius	**ME**	0.998^{**}	0.993^{**}	0.808^{ns}	0.999^{***}	0.997^{**}
	EA	0.647^{ns}	0.691^{ns}	0.923^{ns}	0.998^{**}	0.992^{**}
	Bu	0.903^{ns}	$0{,}922^{ns}$	0.858^{ns}	$0{,}991^{**}$	0.923^{ns}
S.hoggariensis	**ME**	0.999^{***}	0.904^{ns}	0.937^{ns}	0.998^{**}	0.997^{**}
	EA	0.987^{*}	0.856^{ns}	0.658^{ns}	0.997^{**}	0.946^{ns}
	Bu	0.994^{**}	0.996^{**}	0.846^{ns}	0.988^{*}	0.992^{**}
B.incrassatum	**ME**	0.994^{**}	0.888^{ns}	0.790^{ns}	0.999^{***}	0.940^{ns}
	EA	0.622^{ns}	0.598^{ns}	0.808^{ns}	0.998^{**}	0.887^{ns}
	Bu	0.785^{ns}	0.701^{ns}	0.866^{ns}	0.997^{**}	0.993^{**}

^{ns} **P≥ 0.05** not significant ;

* **P < 0.05** significant;

** **P< 0.01** very significant **;**

*****P< 0.001** extremely significant

A strong positive (r >0.9) and significant correlation (P <0.01) was observed between TPC and CUPRAC assay in all studied extracts. In opposition to the β-carotene finding, all extracts revealed hight positive relation without any significance (p>0.05). High positive

significant correlation ($P<0.01$) was observed between TPC and metal chelating activity except in Bu of *S.coronopifolius*, EA of *S.hoggariensis*, ME and EA of *B.incrassatum* which found to be not significant. ME of *S.coronopifolius* and *S.hoggariensis* was recorded as the most strong ion chelators ($r = 0.997$).

Positive correlations between TPC and DPPH in the present study. ME of *S.hoggariensis* revealed very high significantly activity ($P<0.001$) with a correlation coefficient of $r=0.999$, followed by significant correlation ($P<0.01$) in ME of *S.coronopifolius*, Bu of *S.hoggariensis* and ME of *B.incrassatum* extracts (r= 0.998, 0.994 and 0.994, respectively). EA of *S.coronopifolius*, Bu and EA extracts of *B.incrassatum* (0.647, 0.785 and 0.622) revealed the least positive correlation. ME of *S.coronopifolius* and Bu of *S.hoggariensis* among all studied extracts revealed significant positive correlations ($p<0.05$) between TPC and ABTS scavenging activity. Others extracts showed positive no significant correlation ($p>0.05$).

In general, the significance positive correlations between TPC and CUPRAC test in the present study suggested the hypothesis that an increase in the total phenolic content would increase the antioxidant activity of the extracts, as has been previously reported **(Chirinos *et al.*, 2013)**. Thus, it can be postulated that phenolic compounds contributed to the antioxidant activity extracts in CUPRAC assays. However, the correlation obtained for β-carotene–linoleic acid was not significance. These results suggest that other compounds than phenolics may play a key role in lipid peroxidation in these extracts.

Dudonne *et al.* (2009) proved that there is a significant relationship between total phenolic content and antioxidant activity; they suggested that it is due to the phenolic compounds' major contribution to antioxidant properties of the used plant. In contrast, **(Ghasemi *et al.*, 2009)** reported that the extracts with high total phenolic content showed high radical scavenging activity, but by using linear regression analysis, they found that there is no direct correlation found between antioxidant properties and total phenolic compounds quantity. The difference in the correlations between TPC and antioxidant assays indicates the diversity of the group of phenolic compounds and their different responses to different methods for the determination of the antioxidant activity. A reason for this difference is due to the fact that Folin-Ciocalteau method determined the sum of phenolic compounds, whereas individual phenolic compounds have very different responses on the Folin-Ciocalteau reagent and made different contributions to the antioxidant activity **(Matthaus, 2002).** This is in agreement with the findings by **Goupy *et al.* (1999)** who tested the antioxidant activity of

individual phenolic compounds and found that flavan-3-ols possessed the highest radical scavenging activity while the mechanism of antioxidant activity depended on specific structures e.g. the number of hydroxyl groups and the presence of the -CH=CH-COOH group. For a deeper insight on the effects of the extracts on the methods for the determination of the antioxidant activity, it would be necessary to characterize the individual phenolic compounds.

II.2. Anticholinesterase activity

AD has been describe by a reduction in the levels of the neurotransmitter acetylcholine (ACh), which is hydrolyzed mainly by acetylcholinesterase (AChE) and then by butrylcholinesterase (BuChE) **(Racchi *et al.*, 2004).** Therefore, the use of inhibitors of these enzymes is considered an effective therapy for AD **(Tewari *et al.*, 2018).** Plants are considred as the most important source of new acetylcholinesterase and butyryl-cholinesterase inhibitor drugs which may be used for curing neurodegenerative illnesses like Alzheimer’s **(Mustafa *et al.*, 2018).**

In the current study, the inhibitory activity of plant extracts was determined by Ellman’s method. This method estimates AChE using acetylthiocholine iodide (substrate) and dithiobis nitro benzoic acid. The enzymatic activity was measured by the yellow colour compound produced by thiocholine when it reacts with dithiobis nitrobenzoate ion **(Ellman *et al.*, 1961).** Figure 30 shows the AChE and BChE percent inhibitions of the plant various extracts using galantamine as a positive control. Generally, the extracts exhibited a dose-dependent AChE and BChE percent inhibitions. The inhibitory activities of the extracts on AChE and BChE were reported as potent (>50 %), moderate (30-50 %), inactive or low (>30 %) activity **(Vinutha *et al.*, 2007).** According to this classification and in regarding to results presented in Figure 28, when EA extract of *S.hoggariensis* and *S.coronopifolius* (37.01±1.48 %) and (31.08±1,30%) showed moderate inhibitory activity against AChE, all other extracts showed low inhibitory activity against AChE exept BC, BH, MH and MC wich found to be inactive. Moreover, All extracts were found to be low except MC and MH extract exhibited no inhibition against BChE. Generally, the ethyl acetate extracts displayed a superior action against AChE and BChE enzymes.

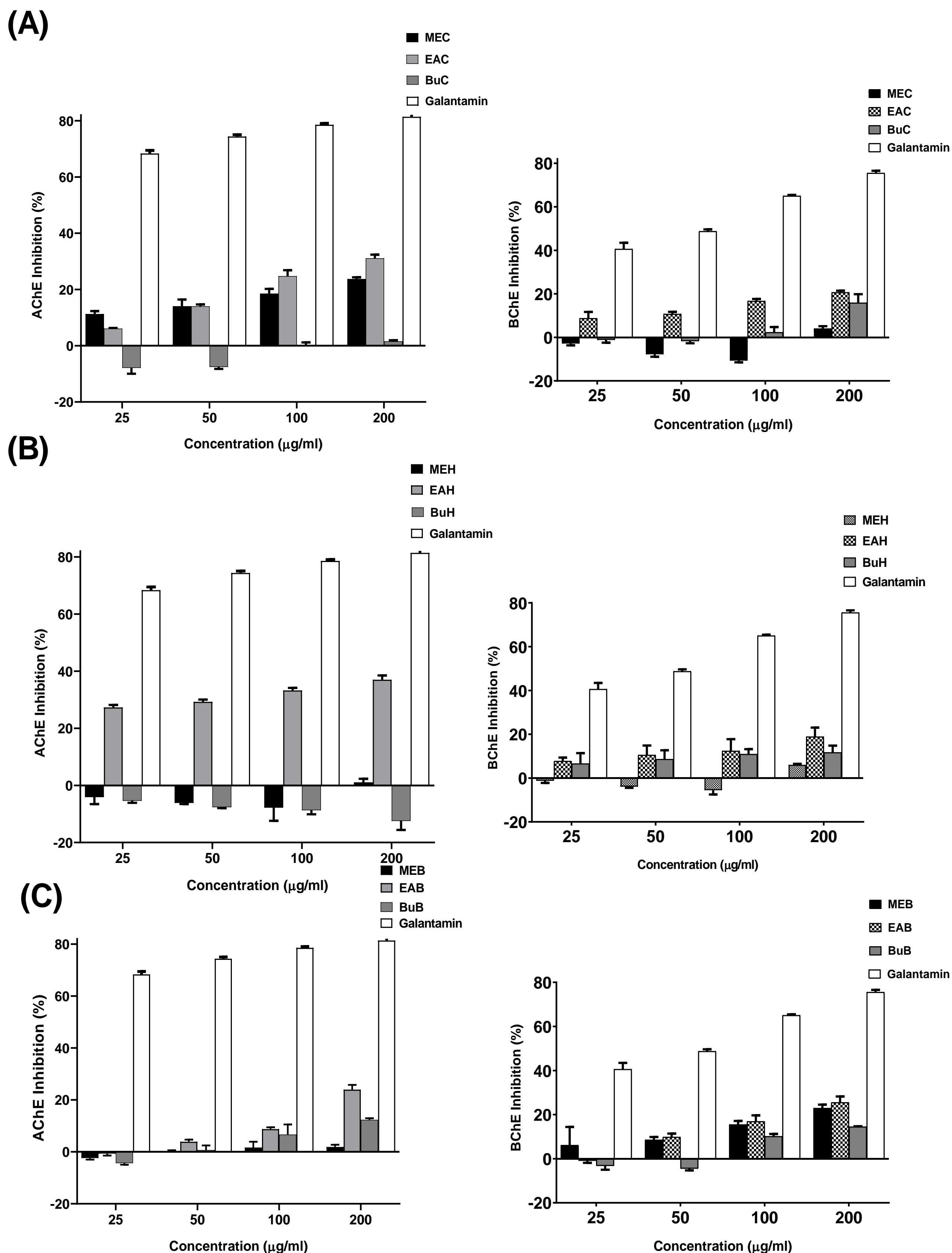


Figure 28. AChE and BChE inhibition by **(A)** *S.coronopifolius*, **(B)** *S.hoggariensis* and **(C)** by *B.incrassatum* extracts. Values represent means ± SD of triplicate readings.

Our results go in accordance with **Kaufmann *et al.* (2016)**, they found that methanol extract of *S.scandens* showed no inhibition of AChE activity. In another study reported by **Ajiboye *et al.* (2018)**, the crude extract of *S.biafrae* appeared to be better comparing to our finding with IC_{50} of 347.22µg/ml and 378.79 µg/ml in AChE and BChE inhibitory activity respectively. Contrary, this results and previous report in *S. angulatus* ethyl acetate and butanol extracts, wich showed remarkable potent inhibitory effects with IC_{50} of 6.72 ± 0.10 mg/mL and 8.6±0.08 mg/mL, respectively. These differences may be caused by the collection localities of samples, extraction, purification and the different classes of polyphenols and flavonoids found in the extracts of the studied plant.

To compare our finding with previous reports on same genus *Bunium*, our results were similare to that obtained in *B.crassifolium* **(Souilah *et al.*, 2021).** They found that pure methanol extract demonstrated weak acetylcholinesterase inhibitory activity with IC_{50} value of 352.57±8.72 µg/ml, while hydro-methanolic extract demonstrated very weak activity (IC_{50}: 1,014.05±9.79 µg/ml). In addition, results of butyrylcholinesterase inhibitory activity test showed that extracts demonstrated very weak activity with IC_{50} values of 5,983.20±6.20 and 6,201.67 ± 0.00 µg/ml for hydro-methanolic and pure methanol extracts, respectively. **Zengin *et al.* (2019)** observed that the *B.sayai* and *B.brachyactis* were effective in inhibition of AchE and the most effective BchE was *B.brachyactis*. *B.crassifolium* seems to help in prevention of cognitive decline during aging as it revealed a competitive inhibitory activity of acetylcholinesterase with that of galantamine.

The inhibition of these cholinesterases by EA fractions could be as a result of the important phytochemicals such as caffeine and flavonoids, which have already been characterized in this studies. Extract have shown that the presence of quercetin in conjunction with chlorogenic and caffeic acids, which have been demonstrated to be potent inhibitor of cholinesterases **(Ademosun *et al.*, 2015; Oboh *et al.*, 2013),** could have (but not completely) responsible for this effects. Although, this effect could be considered lower than that of the synthetic inhibitors, such as galantamine but the adverse effects exhibited by these synthetic drugs/inhibitors may not be experience using plants or plant-based inhibitors such as the plants in use (**Ademosun *et al.*, 2016; Chaiyana and Okonogi, 2012).**

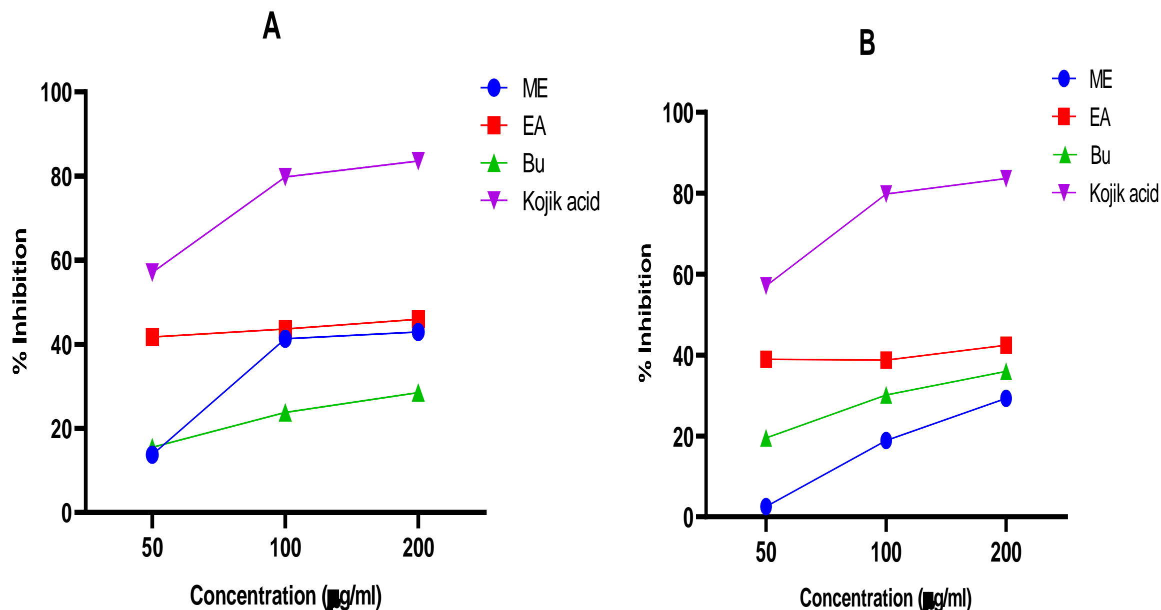
Orhan *et al.* (2007) reported that cholinesterase inhibitory effect of polyphenolic compounds is a function of number and position of their hydroxyl (OH) groups that forms hydrogen bonds with specific amino acids at the enzymes active sites. Therefore, inhibition of AChE and BChE by phenolic extract of *S.biafrae* leaf indicates neuroprotective ability of the

extract which may be attributed to gallic acid, chlorogenic, caffeic acid, rutin, quercetin and kaempferol in the extract **(Oboh *et al.*, 2016).**

II.3. Anti- tyrosianse

Tyrosinase is the key enzyme in melanin synthesis along with in dermatological problems such as spots, age, freckles and melanoma, caused by an excess on melanin accumulation. Therefore, tyrosinase inhibitors have become progressively crucial in the treatment of skin problems **(Haliloglu *et al.*, 2017).** The anti tyrosinase assay is based on tyrosinase inhibition from mushroom.

The look for new natural tyrosinase inhibitors is necessary because of the side effects of synthetic inhibitors currently used. Inhibition on tyrosinase was evaluated to measure the ability of extract and fractions to interrupt this enzyme. The result showed that all studied extracts could inhibit tyrosinase activity (Figure 29). The most pronounced inhibition was observed in the ethyl acetate fractions (*S.hoggariensis* 45.99±1.81, *S.coronopifolius* 42.42±1.39and *B.incrassatum* 36.05) wich may considred as a a weak activity in comparison to the standard (IC_{50} >200µg/mL and 8.02 µg/mL for all extract and kojic acid, respectively).



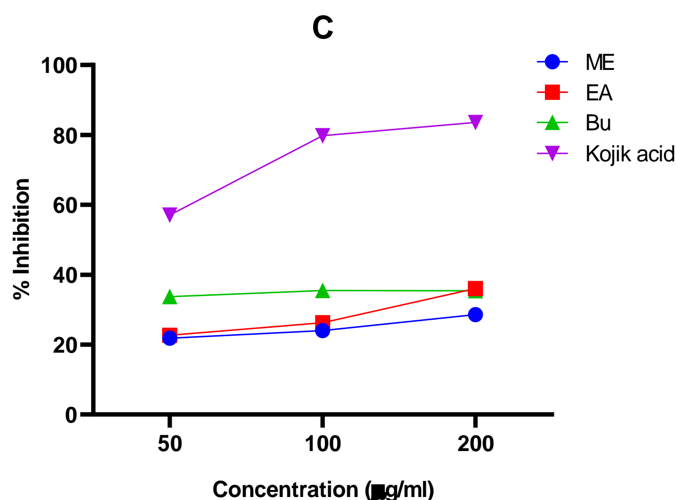


Figure 29. Anti-tyrosinase activity of different plant extracts. **(A):** *S.hoggariensis,* **(B):** *S.coronopifolius* ; **(C):** *Bunium incrassatum* **ME:** methanolic extract, **EAE:** ethyl acetate extract, **Bu:** *n*-butanol extract.

It has been reported that tyrosinase enzyme can be inhibited by aromatic aldehydes and acids, flavonoids and copper chelators **(Xie *et al*., 2003; Souilah *et al*., 2020)** and this is my explains why the ethyl acetate fractions were more potent than the others extract because it was more rich in phenolic acids (e.g. chlorogenic acid) and flavonoids especially, rutin and quercetin according to the obtained HPLC-DAD results. Furthermore, rutin was reported to be a potent antipigment agent due to its tyrosinase inhibitory activity **(Si *et al*., 2012).**

There is no research on antityrosinase activities of *Senecio* species in the literature, to our knowledge, so this is the first review on tyrosinase inhibitors.

In contrast to our results, **Souilah *et al*. (2021)** study revealed that *B.crassifolium* showed a significant tyrosinase inhibitory activity in MeOH extract (IC_{50}: 3.49 ± 3.63 µg/ml) and in pure methanol extract (IC_{50}: 5.72 ± 0.30 µg/ml). Several compounds identified in *B. crassifolium* are known to be strong enzyme inhibitors. In another study, *B.persicum* showed 42% inhibition at 1.14mg/mL for diphenolase activity of mushroom tyrosinase **(Gholamhoseinian and Razmi, 2012).** Cuminaldehyde, kaempferol, caffeic acid and *p*-coumaric acid are found in *B.persicum* **(Sharififar *et al*., 2010)**. They could bind to free enzyme and enzyme-substrate complex and reduced the affinity of substrate for the mushroom tyrosinase and can inhibit mushroom tyrosinase **(Iwai *et al*., 2004; Kim and Uyama, 2005).** The group of **Zengin *et al*. (2019)** showed that the higest activity was exerted by *B.brachyactis*, while the lowest activity was demonstrated by *B.microcaprum*.

II.4. Antimicrobial, antibiofilm and anti quorum sensing activities

II.4.1. Antibacterial activity (minimum inhibitory concentration values)

A common choice of assay used to evaluate the antibacterial actions of plant extracts is using the broth microdilution assay to determine the MIC **(Ncube *et al.*, 2008).** MIC is interpreted as the lowest concentration of an antimicrobial agent that inhibits visible bacterial growth after overnight incubation (**Andrews 2001**). Advantages of broth microdilution method include testing of large samples and with a wider range of concentration compared to other methods such as diffusion techniques. It also has advantages as the method requires small quantities of extract/fraction for testing and this is particularly useful in natural product research due to a common problem of scarcity of fraction or pure compound **(Langfield *et al.*, 2004).**

In this study, the antibacterial activity of the selected plant extracts was examined against five bacteria and one yeast *Candida albicans* ATCC 10239. The results presented in table 14, showed that the plant extracts were active against the tested bacteria, it inhibited the growth of all tested microorganisms with a MIC values between 5 to 10 mg/mL. Among all microorganisms, *E.faecalis* showed the most susceptibility to some tested extracts with MIC of 5 mg/mL for extracts of *S.coronopifolius* and 2.5 mg/mL for the EA extract of *B.incrassatum*.

Table 14. Minimum Inhibitory Concentrations (MIC) of extracts on tested microbial strains mg/mL.

Microorganisms	***S.hoggariensis***		***S.coronopifolius***		***B.incrassatum***	
	EA	**Bu**	**EA**	**Bu**	**EA**	**Bu**
***P.aeruginosa* ATCC 27853**	10	10	10	10	10	10
***E.coli* ATCC 25922**	10	10	10	10	10	10
***S.aureus* ATCC 25923**	**5**	10	**5**	10	**5**	10
***L.monocytogenes*ATCC 7644**	**5**	10	**5**	10	10	**5**
***E.faecalis* ATCC 29212**	10	10	**5**	**5**	**2.5**	10
***C.albicans* ATCC 10239**	**5**	10	10	10	10	10

- : No inhibition the values (MIC) represent means ± SD.

Broadly, our results agree with previous reports, which mention greater activity of EA extracts from roots of *S.coronopifolius* towards *S.aureus*, *K.pneumoniae*, *S. typhimuriu* with MIC values of 3.9, 31.25, 1.95 and 3.9 µg/ml respectively but no activity against *C.albicans* **(Mohamed, 2015).** According to the previously mentioned results on *Senecio* genus **(Tundis *et al.*, 2007)** indicated that EA extract of *S. leucanthemifolius* exhibited a strong activity against *S.aureus* (MIC = 31.25 µg/ml). The Bu extract from *S.delphinifolius* herb showed a weak effect against *E.coli*, with a MIC of 1 mg/mL, but was inactive against *S.aureus* and *P.aeruginosa* (MIC>2 mg/mL) **(Tidjani *et al.*, 2013).** The antibacterial activity of four novel chemical constituents, isolated from the Bu extract of *S.cannabifolius* against different strains had been showed by **Wu *et al.* (2006)**, they were found to have significant antibacterial activities against both Gram positif *S. aureus* and *B. subtilis*, but no activity was recorded against Gram-negative bacterium *E. coli*.

Result of the present study supports the observations of some other researchers who studied the antimicrobial properties of *B.incrassatum*. **El Kolli *et al.* (2017)** assessed the antibacterial activity of EOs wich showed a wide array of antibacterial activity. The application of *B.incrassatum* EO on most bacteria at 10 %, 20 % and 50 % dilutions demonstrated a total resistance. But, concentrations of 100 % gave remarkable inhibition zone diameters with 15 to 20 mm, at concentration of 50 mg/ml. Comparing to different *Bunium* species, in the study of **Amber *et al.*, (2018)** on the anti-microbilal potenty against *S.aureus*, *E.coli* and *K. pneumoniae* by agar well diffusion method. They showed that among alkaloids,flavonoids, and saponins of plant, alkaloids produced significantly high inhibition zones against bacteria.

The minimum inhibitory concentration and minimum bactericidal concentration of phytochemicals and crude methanolic extracts against tested bacterial strains ranged between 12.5-50 mg/ml and 25-50 mg/ml, respectively. Also, it was reported that this plant extracts indicate a significant inhibitory activity against various pathogenic bacteria including *P.aeruginosa*, *S.aureus*, *E.coli*, *H.pylori* and also against pathogenic fungus, *C.albicans* **(Menghani *et al.*, 2011; Atapour *et al.*, 2009).**

In summary, EA fractions were the most effective showing great inhibition against the tested strains. The antibacterial effect shown by these fractions may be due to the chemical composition of the extract, which is rich in flavonoids and phenolic compounds than that of the Bu fractions . In addition, The results indicated that the plant extracts had different degrees of antimicrobial activity against the tested bacteria, particularly in terms of their

abilities to inhibit the growth of Gram positive strains. The differential sensitivity of Gram positive and Gram negative bacteria to plant extracts may be explained by the morphological differences between the microorganisms. The higher resistance of Gram-negative bacteria could be attributed to the differences in their cell membranes, since their outer membrane carries the structural lipopolysaccharide components and renders their surfaces highly hydrophilic **(Smith-Palmer *et al*., 1998).** This constitutes a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da **(Nostro *et al*., 2000).** The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer, which is not an effective permeability barrier and may facilitate the infiltration of hydrophobic compounds **(Burt, 2004).**

II.4.2. Antibiofilm activity

A biofilm represents a structured, organized, and complex group of sessile bacterial cells attached to a surface, which grow and interact as a community **(Høiby *et al*., 2010).** Compared to planktonic cells, biofilms are characterized by significant loss of susceptibility to antibiotics as well as high virulence potential **(Masadeh *et al*., 2013),** Therefore, its formation is considered as a one of the resistance strategies of many pathogens **(De La Fuente-Núñez *et al*., 2012).** Current anti-biofilm research is focused on preventing the development of biofilms through many strategies such as inhibition of adherence, interrupting quorum sensing (QS) or promoting early detachment of cells **(Kaplan, 2005).**

Screening of plants and natural products as possible candidates for anti-biofilm treatment or prevention has been increasing, giving rise to the number of biofilm research **(Musk *et al*, 2006).** As no previous study describes the antibiofilm potential of the selected plant, the current study will provide useful information. In this regard, the effect of studied extracts on the cells attachment and inhibition of biofilm formation within concentration range from MIC – MIC/8 is given in Tables 15. According to established criteria **(Sandasi *et al*., 2008)** the percentage inhibition values ranging between 0 to 100% signify inhibition of biofilm, while enhancement of growth is reflected by values below 0%. Above the 50% inhibition mark the activity is regarded as good, while it is poor if it is between 0 and 49%.

The plant extracts had varying degrees of activity on the prevention of attachment. EA extrac of *B.incrassatum* exhibited the highest percentage of antibiofilm against *S.aureus* ATCC 25923 (72.88±1.83%) followed by EA extract of *S.coronopifolius* with 78.14±0.29 % against *C.albicans* ATCC 10239, at the MIC concentration. EA extract of *B.incrassatum*

showed also a significant inhibition against Gram-negative strains and the inhibition was nearly equal for both *P.aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 with an inhibition percentage of 61.41±2.81 and 60.15±2.23, respectively (Table 15).

In both *Senecio* plants, the antimicrobial activity of EA extracts is related to its flavonoids and phenolic acid contents, which were detected in general and *p*-coumaric acid in particular, as the major compound in these extracts. *P*-coumaric acid is known as a natural antibacterial agent. It has good antimicrobial activity against *E.coli*, but also against other Gram-negative bacteria such as *Salmonella typhimurium* and *Shigella dysenteriae*. This compound can alter the permeability of the cell membrane. It can bind DNA inhibiting cell function by increasing membrane permeability and causing the loss of the barrier function and leakage of cytoplasmic contents demonstrated by the electron micrographs **(Lou *et al*., 2012).** It has been shown that *p*-coumaric acid could bind to the phosphate anion in DNA double helix andintercalate the groove in DNA double helix, which might affect the replication, transcription, and expression. Hence, *p*-coumaric acid has double mechanisms of bactericidal activity: disrupting bacterial cell membranes and binding to bacterial genomic DNA to inhibit cellular functions, ultimately leading to cell death **(Lou *et al*., 2012).** Comparing to the same genus, *Senecio calvus* has a good action against biofilm formation, the presence of lactones, coumarins and flavonoids are related to their action **(Florian-Carrillo, 2015).** It is not surprising that lactones are part of this activity, since QS signals are homoserin-lactones and previous works had demonstrated that halogenated lactones can block biofilm formation **(Galloway *et al*., 2011).** Some other works reported that phenolic compounds and flavonoids also can act as biofilm inhibitors (**Magesh *et al*., 2013; Onsare *et al*., 2015**).

To our knowledge, there is no research on antibiofilm activities of *Senecio* and *Bunium* species in the literature, so this is the first review on antibiofilm activity using this plants.

In general, the tested extracts are capable of inhibiting biofilm formation at MIC and below MIC concentrations thereby making them suitable for preventing biofilm formation of microorganisms. Differences in the action of extracts on *P.aeruginosa* and *E.coli* seems to indicate that the metabolites are acting preferentially against one type of QS signal, since *P.aeruginosa* uses acyl homoserin-lactone (AHL) and *E.coli* uses the AI-2 signal a different molecule, *E.coli* however possesses receptors for AHLs **(Galloway *et al*., 2011).**

Table 15. Antibiofilm activity results of extract of studied extracts

Microorganisms	% inhibition on biofilm formation						
	Planktonic	*S.hoggariensis*		*S.coronopifolius*		*B.incrassatum*	
		EA	Bu	EA	Bu	EA	Bu
P.aeruginosa **ATCC 27853**	**MIC**	59.04 ±1.9	44.37±2.18	40.08±3.62	42.54±2.58	61.41±2.81	24.84±1.80
	MIC/2	38.62±3.4	23.04±1.00	24.55±1.16	33.58±3.72	48.86±2.57	11.20 ±0.93
	MIC/4	28.89 ±1,5	-	-	20.15±3.30	14.24±2.68	-
	MIC/8	-	-	-	-	-	-
E.coli **ATCC 25922**	**MIC**	59.02± 4.7	51.14±6.18	52.16 ±1.7	37.32 ±1.6	60.15±2.23	56.32 ±3.29
	MIC/2	38.62±3.4	32.91±4.33	28.50 ±3.3	21.79± 2.2	33.65±1.91	36.07± 2.68
	MIC/4	37.47± 2.1	23.28±1.00	-	-	26.09±2.82	14.73 ±1.47
	MIC/8	33.86±1.1	-	-	-	7.61± 1.44	4.58± 0.1
S.aureus **ATCC 25923**	**MIC**	49.55±1.00	48.65±2.12	46.40±1.04	40,99±0.21	72.88 ±1.83	70.06 ±1.63
	MIC/2	36.24 1.69	37.09±0.60	39.19±1.4	31,08±2.28	69.82±2.93	39.78± 1.63
	MIC/4	20.82 1.20	-	30.63±0.7	-	41.39 ±3.41	24.89 ±2.81
	MIC/8	13.81 1.00	-	22.82±1	-	27. 94±0.81	-
L.monocytogenes **ATCC 7644**	**MIC**	36.02±3.2	39.10±1.00	18.14±2,58	34.98±2.17	63.70 ±5.47	57.58 ±4.65
	MIC/2	-	-	-	29.87±0.7	36.66 ±3.59	41.74± 1.77
	MIC/4	-	-	-	21.88±1.1	20.62 ±0.85	21.96 ± 1.93
	MIC/8	-	-	-	-	-	-
E.faecalis **ATCC 29212**	**MIC**	58.13±1.00	49,66±1.00	36.54±1.00	37.20±1.00	41.20 ±1.26	71.54± 2.48
	MIC/2	26.24±2.00	-	29.27±1.00	23.99±1.05	21.54 ±1.70	47.20± 1.54
	MIC/4	14.77±2.1	-	12.53±1.3	13.4±0.58	-	32.85 ±1.21
	MIC/8	-	-	-	-	-	7.64 ±0.00
C.albicans **ATCC 10239**	**MIC**	59.56 ±0.4	43.49± 0.5	78.14±0.29	23.26±3,39	75.77 ±1.32	72.75 ±3.86
	MIC/2	41.75±0.42	-	67.34±0.52	-	63.35± 4.97	65.73 ±2.92
	MIC/4	29.47±0.34	-	35.70±2.1	-	45.23 ±3.37	35.42 ±2.40
	MIC/8	-	-	18.9±1.05	-	24.19± 1.74	10.36± 1.72

- : No inhibition the values (MIC) represent means ± SD.

II.4.3. Anti-quorum sensing activity assay

II.4.3.1. Anti-quorum sensing on CV026 and inhibition of violacein pigment production on CV12472

Two assays namely, QS inhibition on *C.violaceum* "CV026", and violacein inhibition on *C.violaceum* "CV12472" were used for the anti-QS activity determination. The MIC values of plant extracts were reported on Tables 16 and 17 respectively. In the both tests, work at MIC and under MIC concentrations eliminates the hypothesis that these activities may be due to the bactericidal effect of tested extracts.

For anti-QS screening, the sub-MICs ranges were selected using agar well diffusion method. *C.violaceum* 026 strain, which is a Gram-negative bacterium, in the presence of C6-AHL on their medium plate, QS- process was mediated, by secreting violacein antioxidant substance (a purple coloured) which protects the bacterial membrane from oxidative stress **(Chong *et al.,* 2018).** On the test plates with a purple lawn coloration produced by activated *C. violaceum* CV026 bacteria, the formation of a cream or yellowish coloured ring around the well was an indication of QS inhibition while a transparent or cloudy ring indicated antimicrobial activity. The QS and antimicrobial activity zone diameters were measured in millimetres and reported on Table 16.

The highest QS inhibition was observed in EA fraction of *S.coronopifolius* at the concentration of 2.5 mg/ml with an inhibition zone of 17.5±0.07mm, followed by the same fractions of *B.incrassatum* then *S.hoggariensis* with an inhibition zone of 21.5±0.07mm and 11±0.42 mm, respectively at 10 mg/ml concentration. While, Bu extracts of the plants extracts showed QS inhibition on CV026 only at MIC values and were inable to inhibit QS at lower sub-MIC concentrations.

Table 16. Anti-quorum sensing activity against *C. violaceum* CV026

Concentration	***S.hoggariensis***		***S.coronopifolius***		***B.incrassatum***	
	EA	**Bu**	**EA**	**Bu**	**EA**	**Bu**
MIC	10	10	2.5	10	10	10
MIC	11±0.42	07.5±0.07	17.6± 0.58	9±0;00	21.5± 0.07	10.5±0.21
MIC/2	7 ±0.49	**-**	13.6± 0.56	**-**	18.5 ±0.84	**-**
MIC/4	**-**	**-**	12 ±0.84	**-**	12±0.84	**-**
MIC/8	**-**	**-**	-	**-**	-	**-**

- : No inhibition.; The values (MIC) represent means ± SD.

C. violaceum CV12472 produces a violet coloration while growing by a quorum sensing (QS) mediated process. This color is due to the presence of violcein and can be observed and quantified easily as a marker-trait that makes this bacterium suitable for quorum sensing activity research **(Kothari *et al*., 2017).** It should be indicated that *C. violaceum* CV12472 produces violacein naturaly during their growth, in opposit to the mutant strain CV026, which can produce violacein only when acylhomoserine lactone hormone is supplied externally **(Tanfu *et al*., 2020).**

On *C.violaceum* CV12472, EA fraction of *S.hoggariensis* was more active (MIC= 5 mg/mL) than *S.coronopifolius* and *B.incrassatum* (MIC=10 mg/mL) meanwhile on *C.violaceum* CV026, EA fraction of *S.coronopifolius* had higher activity (MIC=2.5 mg/mL) than *S.hoggariensis* and *B.incrassatum* (MIC=10 mg/mL).

At MIC concentration, violacein production in CV12472 was totally inhibited by both EA and Bu extracts of *Senecio* plants with 100% inhibition. In addition, only ethyl acetate extract of *S.coronopifolius* showed inhibition (27.71±1.06 %) at lower concentration MIC/16 (Table 17).

Table 17. Violacein production inhibition against *C. violaceum* CV12472.

Concentration	*S.coronopifolius*		*S.hoggarensis*		*B . incrassarum*	
	EA	**Bu**	**EA**	**Bu**	**EA**	**Bu**
MIC	10	10	5	10	10	10
MIC	100±0.00	100±0.07	100±0.018	100±0.00	75.61±0.00	54.98±0.00
MIC/2	77.46±1.00	77.56±1.0	61.73±1.00	83.82±1	23.20±0.35	25.19± 2.0
MIC/4	52.99±1.00	17.81±1.2	28.41±0.59	20.67±0.15	23.18±2.83	/
MIC/8	31.05±0.64	/	10.42±1.04	/	/	/
MIC/16	27.71±1.06	/	/	/	/	/

- : No inhibition. The values (MIC) represent means ± SD.

II.4.3.2. inhibition of swarming motility

Bacterial motility plays a key role in the colonization of surfaces by bacteria and the subsequent formation of resistant communities of bacteria called bioflms. *Pseudomonas aeruginosa* utilizes fagellum-mediated swimming motility to approach a surface, attaches, and then further spreads via the surface through associated motilities such as swarming and twitching **(O'May and Tufenkji 2011).** Flagella motility-dependent swarming is also

regulated by QS. Therefore, a reduction in swarming area compared with the control plate would suggest the presence of anti-QS compounds (**Kazemian *et al*., 2015).** Extracts which shows anti-swarming activity, selectively and distinctly reduced the swarming area of PA01, suggesting that these extracts exept Bu fraction of *Bunium* could inhibit the swarming motility of PA01. As shown in the Table 18, all samples swarming inhibition of EA fractions was greater than Bu extracts.

Table 18. Swarming inhibition against *P. aeruginosa* PA01

Concentration	*S.coronopifolius*		*S.hoggarensis*		*B . incrassarum*	
	EA	**Bu**	**EA**	**Bu**	**EA**	**Bu**
100	26.02±0.21	16.09±0.11	23.97±0.77	13.69±0.19	39.72±0.038	-
75	20.54±0.17	10.95±0.5	13.01±0.38	12.32±0.19	26.98± 0.00	-
50	13.28±0.19	7.53±0.38	8.44± 0.68	-	12.87±0.96	-

* The values means ± SD. Concentration of extract µg/mL

In general, the antimicrobial of plant sources have increasing attention in recent years due to the increase in the incidence of emerging infectious diseases caused by organisms with high resistance rates to standard antimicrobial agents presenting a very challenging and global health burden. Bacteria must not necessarily be killed, but the severity of its infection and resistance can be avoided by reducing its virulence which includes biofilm formation, quorum sensing and swarming.

As no previous studies were performed to investigate the production of violacein or any other QS mediate phenomenon of the three studied plants, this work was undertaken to investigate the anti-quorum-sensing activity by evaluating the effect of the plant extracts on QS-regulated violacein in CV026 and CV 12472 and swarming of PA01. Regarding to our results, the ethyl acetate fraction was found to be the most powerfull inhibitor of violacein inhibition, anti swarming, and anti-quorum sensing activity.It is clear that this activity is totally related to the phenolic compounds of this extract .

In EA fractions of both *Senecio* species , the phenolic compounds *p*-coumaric acid was determine as the major compounds . This later have already been shown to interact with bacterial quorum sensing, sometimes by triggering or by inhibiting the QS-process system **(Rasmussen *et al*., 2005; Bodini *et al*., 2009; Priha *et al*., 2014).**

In EA fractions of *B.incrassatum* rutin and chloroginic acid was determinated as the major compounds . Although chloroginic acid as a QSI, wich has been reported about its inhibition effects on biofilm formation and virulence factors **(Brango-Vanegas *et al*., 2014).** The study of **Wang. (2019)** and others, demonstrate their aabiliies to regulates the quorum sensing in *P.aeruginosa* and *C.violaceum.* In *P.aeruginosa,* chloroginic acid significantly inhibited the formation of biofilm, the ability of swarming, and virulence factors including protease activity, elastase activity, and rhamnolipid production. In *C.violaceum*, it showed similar inhibitory effects on its biofilm formation, swarming motility, chitinolytic activity, and violacein production.

Truchado *et al*. (2012) stated that the phenolic compounds, including rutin, ellagic, and chlorogenic acids were able to reduce the concentration of ALHs on *E.carotovora* and *Y. enterocolitica.*

CONCLUSION

Conclusion

In the present study, antioxidant, anticholinesterse and anti-tyrosinase inhibitory propreties of various extracts of three plant species from arid and semi-arid areas namely: *S.hoggariensis*, *S.coronopifolius* and *B.incrassatum* were determinated with their potential effect on biofilm formation and quorum sensing.

Total phenolics and total flavonoids content were performed according to Folin-Ciocalteu method and to the aluminium nitrate method, respectively. Results obtained in the present study revealed that EAE contained the highest amount of phenols and flavonoids than the others fractions. The highest TPC and TFC was found in EAE of *B.incrassatum* (392.92± 1.50 µg EAG/mg E and 85.06 ±1.57µg/g QE, respectively).

The HPLC analysis revealed the identification of diversified mixture of phenolic components and the major of these compounds were identified as phenolic acids . A total of 16 compounds were detected in ME extract of *B.incrassatum*, while 7 compounds was determinated in its EAE and BuE fractions. It was found that rutin was the major phenolic compounds in ME, EA and Bu extracts of *B.incrassatum* (26.55 mg/g ,16.04 mg/g, 4.45 mg/g, repectively). Nine and eight phenolic compounds were identified in the ME extracts of the two studied *Senecio* ME extract's, in which rutin and Chlorogenic acid were the most dominant in *S.coronopifolius* (10.29mg/g) and *S.hoggariensis* (5.97mg/g), respectively. In the same time, a total of nine and eight components weredetected in ethylacetate extracts of *S.coronopifolius* and *S.hoggariensis*, respectively; wich *p*-coumariq acid was the most abundant compounds with (10.29mg/g) and (10.29mg/g), respectively, meanwhile, the BuE extracts contained low amounts or almost void of target compounds.

Regarding biological activities, it can be noted that extracts exhibited different, but effective degrees of antioxidant activity using various methods. Among all them EAE extracts were shown to possess the best antioxidant activity than BuE and ME extracts in DPPH, ABTS and CUPPRAC tests. The EAE of *B.incrassatum* displayed considerable radical scavenging activity against DPPH (IC_{50}:1.53±0.39µg/mL) and ABTS (IC_{50}: 1.28±0.11 µg/mL) assay except in CUPPRAC test, the EAE of *S.coronopifolius* was the most potent with the best cupric reducing antioxidant capacity (IC_{50}:10.24±2.45µg/mL) close to that of standard α-Tocopherol (IC_{50}: 10.20±0.01 µg/mL) and low than BHT (IC_{50}: 3.80 ± 0.00 µg/mL). Concerning the metal chelating activity, methanol extracts exhibited the highest chelating activity, ME of *S.hoggariensis* (69.26±0.21% µg/mL) showed better activity than

the others tested extracts. Using β-carotene bleaching assay, EAE of *B.incrassatum* exhibited strong inhibition on lipid peroxidation activity with IC_{50} values of 1.94±0.37 µg/mL.

The Results of AChE enzyme inhibition activity clearly indicates that EA of *S.hoggariens* and *S.coronopifolius* extract among all fractions, had moderate inhibition against AChE activity with 37.01±1.48% and 31.07±1.30%, respectively, at 200 µg/mL, when compared to galanthamine (81.41 ±1.03%). Regarding the anti-butyrylcholinesterase test, All extracts were found to below or not active. But, displayed low to moderate tyrosinase inhibitory capacities and EAE fractions were the more potent. the highest tyrosinase enzyme inhibition activity was recorded to EAE of *S.hoggariensis* (45.99±1.81%) in comparison with kojik acid (83.6±0.2%) used as standard, at 200 µg/mL. There is no research on anti-tyrosinase activities of *Senecio* species in the literature, to our knowledge, so this is the first review on tyrosinase inhibitors.

To our knowledge this is the first report of anti-QS and anti-biofilm activities in selected plants. There is a crucial need for developing new therapeutic strategies that can be effective against biofilm-related infections. In general, although extracts, EAE fractions were most powerful than its BuE fractions and had varying degrees of activity on prevention of attachment in biofilms formation. EAE of *B.incrassatum* exhibited the highest percentage of biofilm formation on *S.aureus* ATCC 25923 (72.88±1.83%) at 5 mg/mL. The effect of plant extracts on quorum-sensing (QS) mediated processes such as inhibition of violacein production in *C.violaceum* CV12472 and *C.violaceum* CV026, showed that the studied extracts could not only kill bacteria but also can reduce theirs everity and eliminate their resistance by disrupting QS networks at MIC and sub MIC concentrations. The highest QS activitywasobserved in EAE fraction of *S.coronopifolius* at the concentration of 2.5 mg/ml with an inhibition zone of 17.5±0.07mm. At MIC concentration, excellent inhibition of violacein synthesis in CV12472 was exhibited by the EAE and BuE extracts of both *Senecio* plants with 100% inhibition than extracts of *B.incrassatum*, in which, EAE fraction of *S.hoggariensis* was more active (MIC= 5 mg/mL). At MIC/16 concentration, only EAE of *S.coronopifolius* showed inhibition with 27.71±1.06 %.

Furthermore, The extracts inhibited swarming motility in *P. aeruginosa* PA01, which is a process used by bacteria prior to biofilm formation. The inhibition of swarming motility mostly in flagellated bacteria can reduce the risk of biofilm formation. All extracts exept BuE fraction of *B.incrassatum* could inhibit the swarming motility of PA01. In all samples,

swarming inhibition of EAE fractions was greater than its BuE. The highest anti-swarming activity was recorded to EAE of *B.incrassatum* with 39.72±0.038mm at 100 µg/mL concentration of the extract.

These results are preliminary and it would be interesting to identify, isolate and characterize the active constituents responsible for the strong observed antioxidant and antimicrobial activities in order to determine the exact mechanism. In addition, further studies concerning other biological tests: anti-tumor, toxicity, *in vivo* assays should be carried out.

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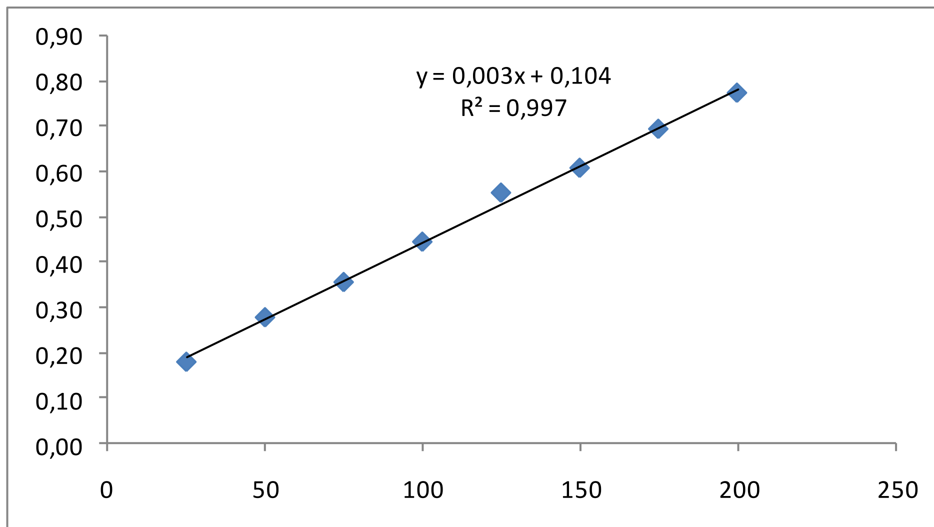
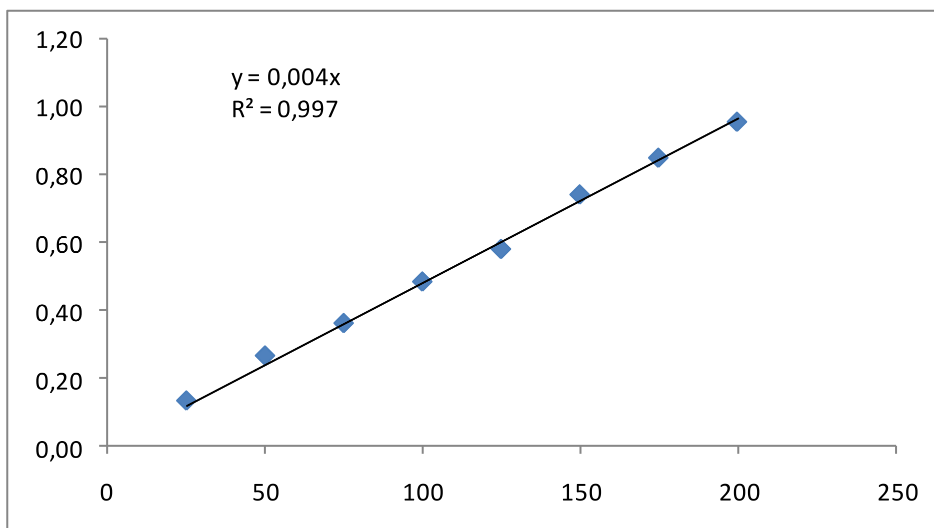
Annexes

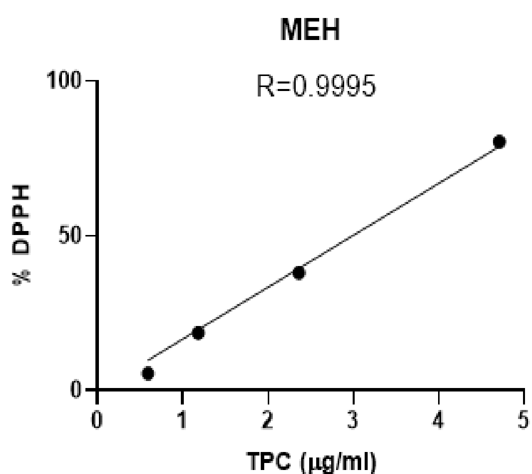
Annexe 1: Standard curve of gallic acid.

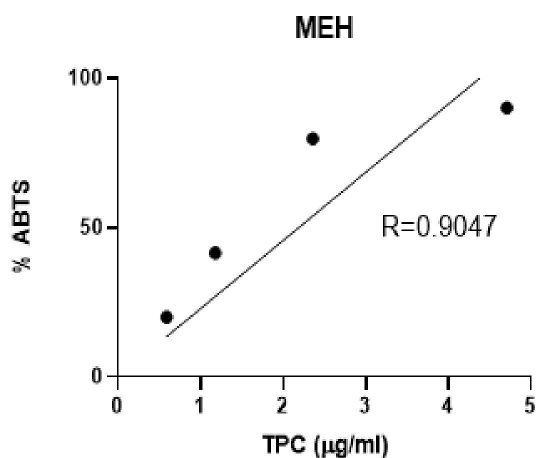


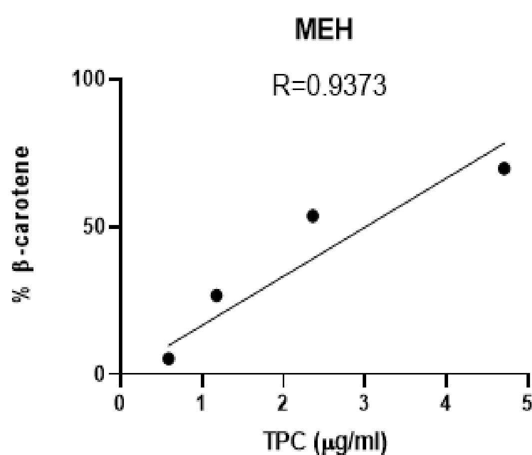
Annexe 2: Standard curve of Quercetine.

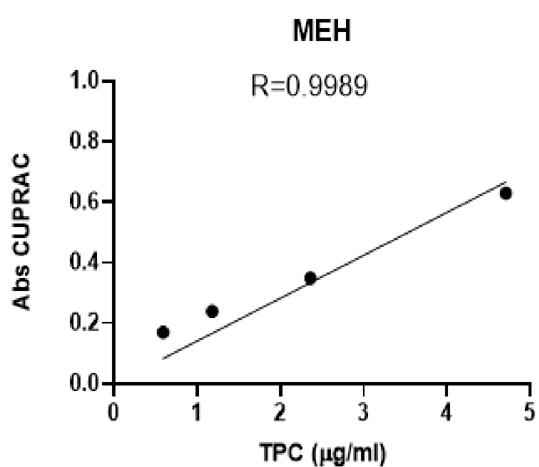


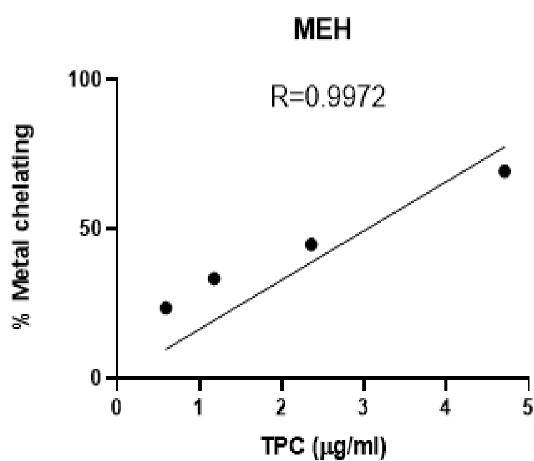
Annexe 3: Correlation between TPC contenet and antioxidant activities of the studied extracts.

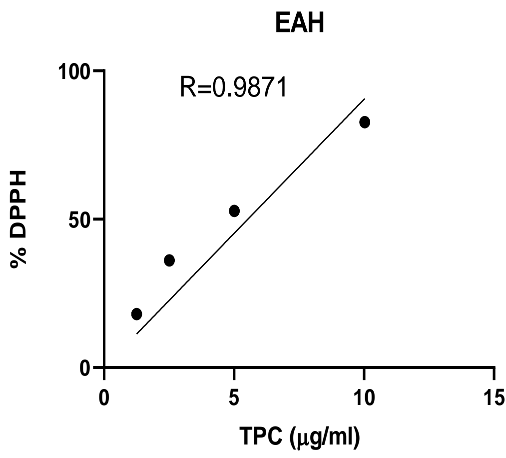











EAH
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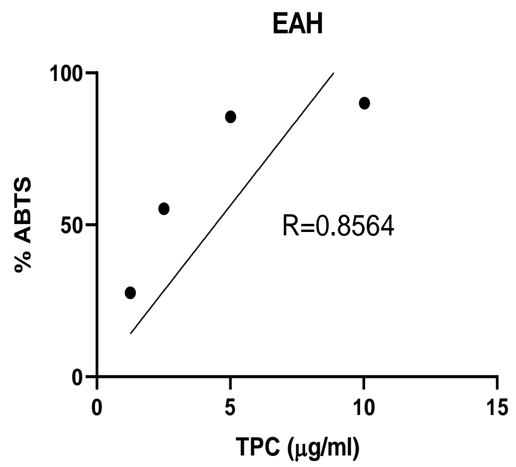
% DPPH
TPC (µg/ml)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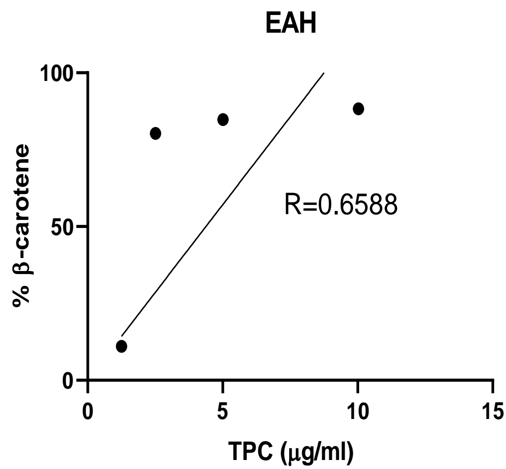
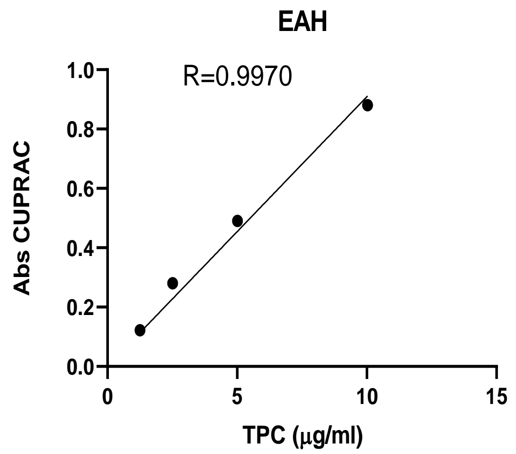
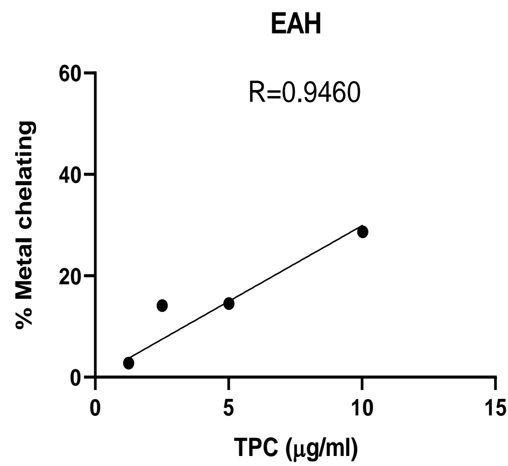
R=0.8564
% ABTS
TPC (µg/ml)


EAH
R=0.6588
% β-carotene
TPC (µg/ml)


EAH
R=0.9970
Abs CUPRAC
TPC (µg/ml)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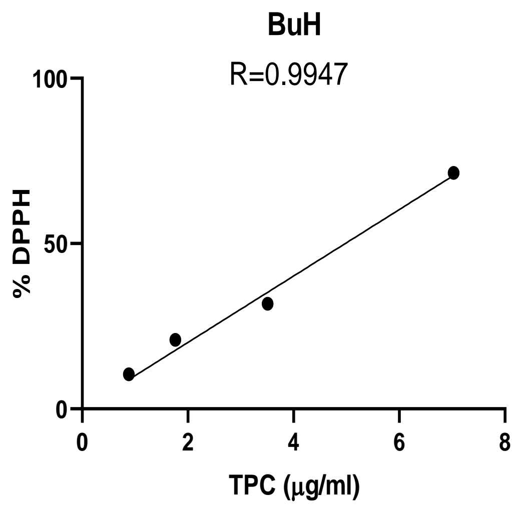
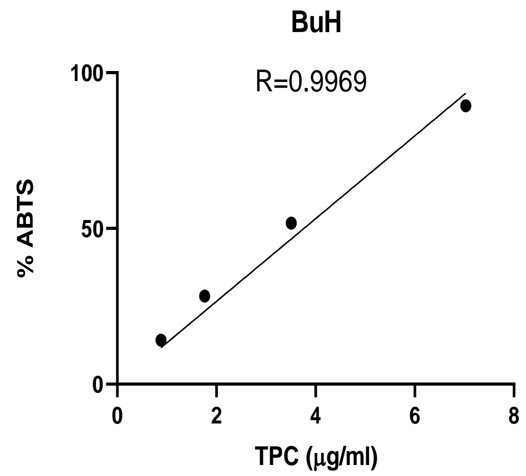
R=0.9460
% Metal chelating
TPC (µg/ml)


BuH
R=0.9947
100
50
0
% DPPH
0
2
4
6
8
TPC (µg/ml)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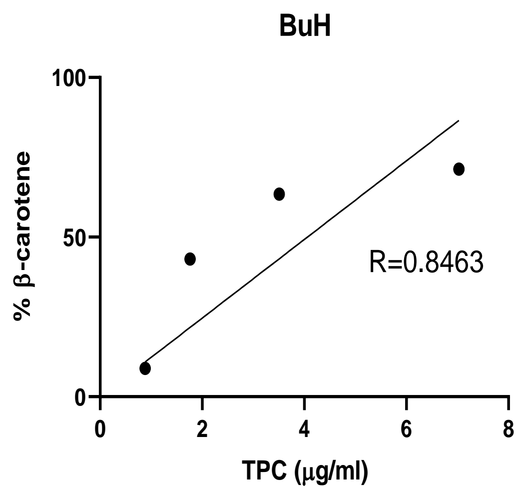
100
50
0
% ABTS
0
2
4
6
8
TPC (µg/ml)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0
% β-carotene
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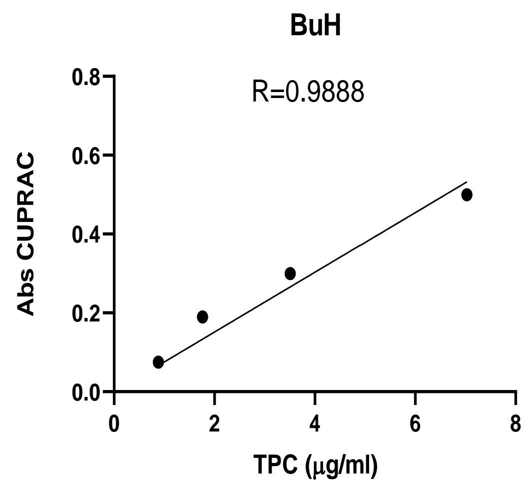
2
4
6
8
TPC (µg/ml)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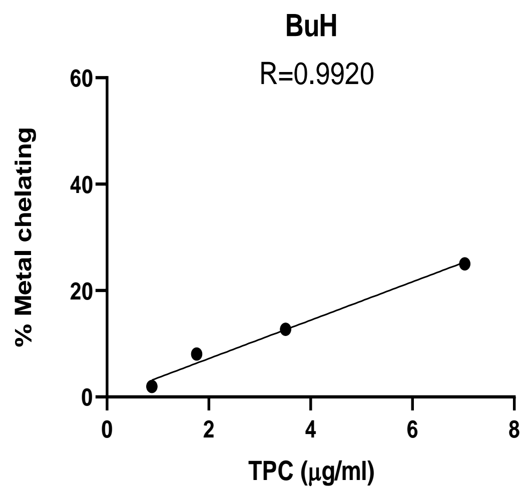
0.6
0.4
0.2
0.0
Abs CUPRAC
0
2
4
6
8
TPC (µg/ml)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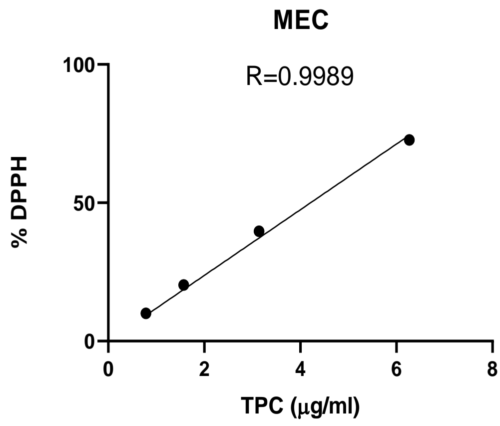
R=0.9920
60
40
20
0
% Metal chelating
0
2
4
6
8
TPC (µg/ml)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% DPPH
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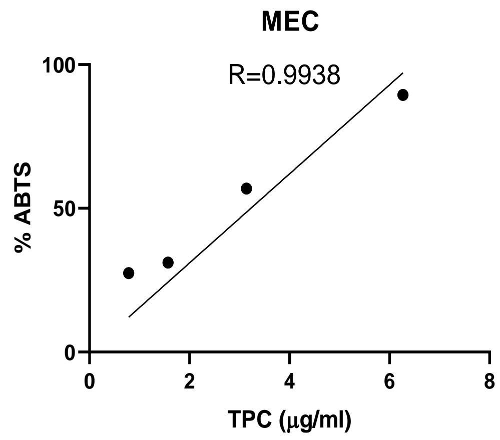
50
0
0
2
4
6
8
TPC (µg/ml)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% ABTS
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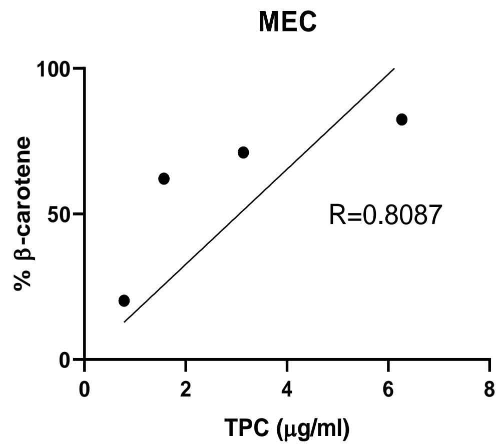
50
0
0
2
4
6
8
TPC (µg/ml)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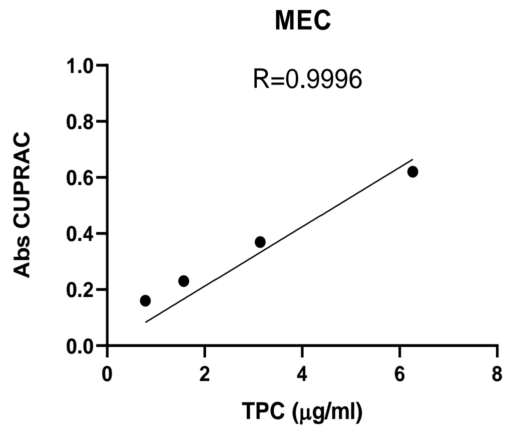
0
0
2
4
6
8
TPC (µg/ml)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Abs CUPRAC
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0.8
0.6
0.4
0.2
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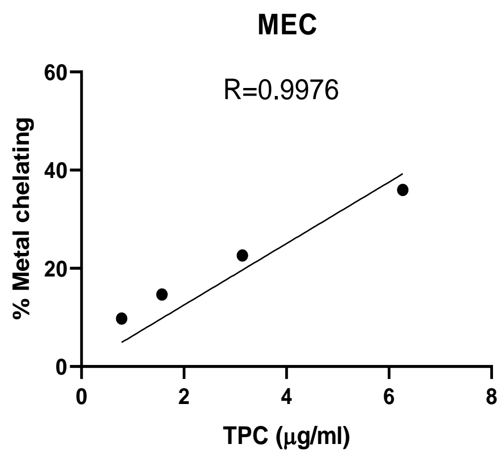
0
2
4
6
8
TPC (µg/ml)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% Metal chelating
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40
20
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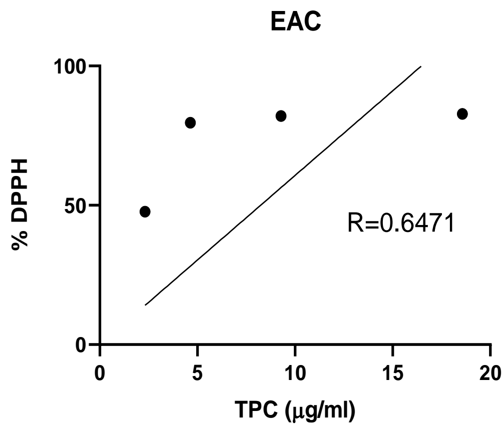
0
2
4
6
8
TPC (µg/ml)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% DPPH
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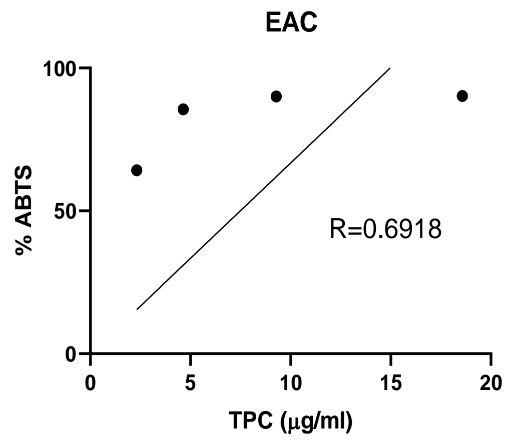
0
5
10
15
20
TPC (µg/ml)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% ABTS
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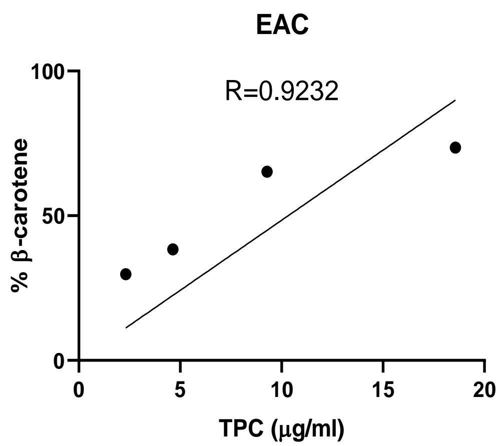
0
5
10
15
20
TPC (µg/ml)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50
0
% β-carotene
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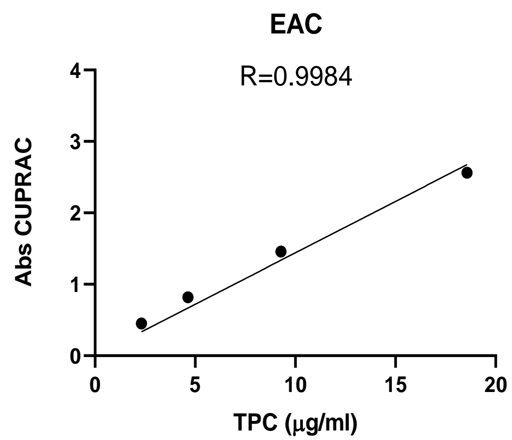
0
5
10
15
20
TPC (µg/ml)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3
2
1
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Abs CUPRAC
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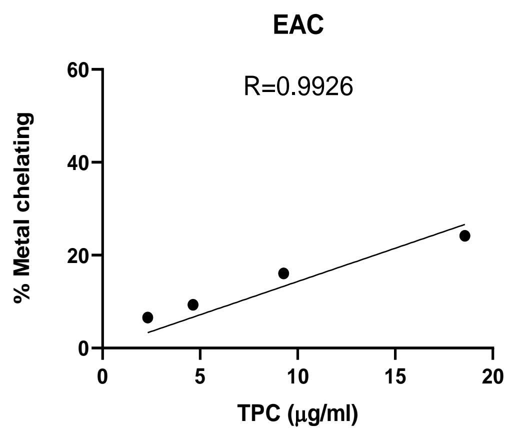
0
5
10
15
20
TPC (µg/ml)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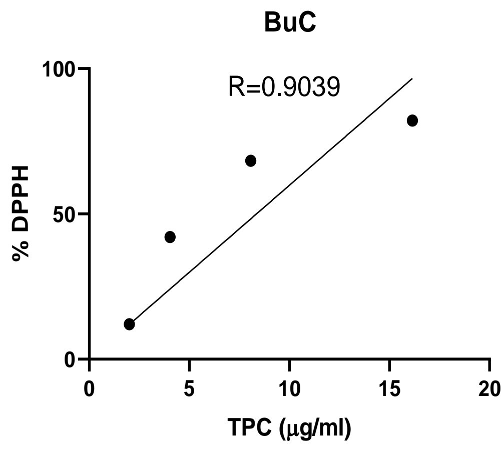
40
20
0
% Metal chelating
R=0.9926
0
5
10
15
20
TPC (µg/ml)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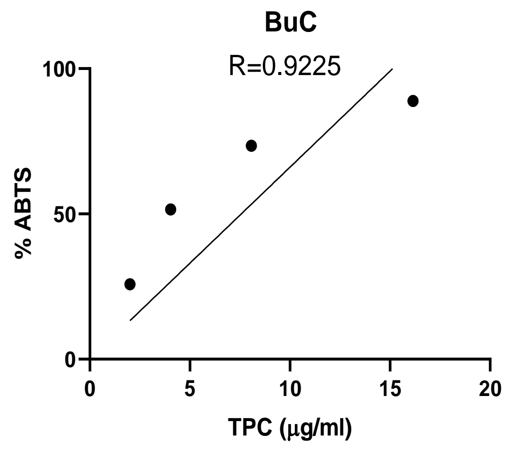
% DPPH
100
50
0
0
5
10
15
20
TPC (µg/ml)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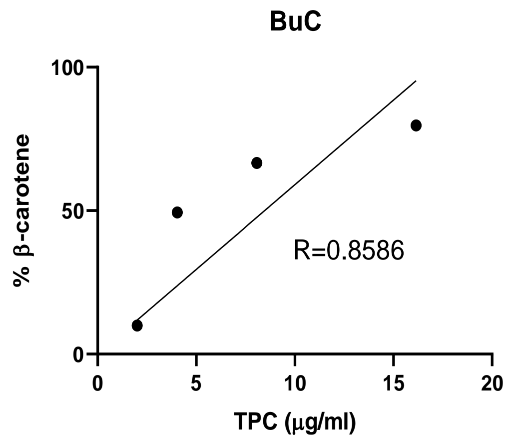
50
0
0
5
10
15
20
TPC (µg/ml)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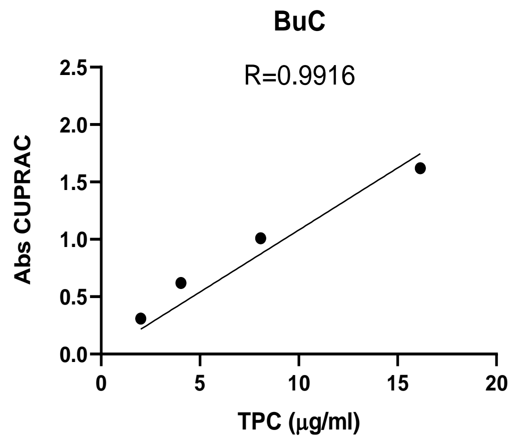
50
0
0
5
10
15
20
TPC (µg/ml)


BuC
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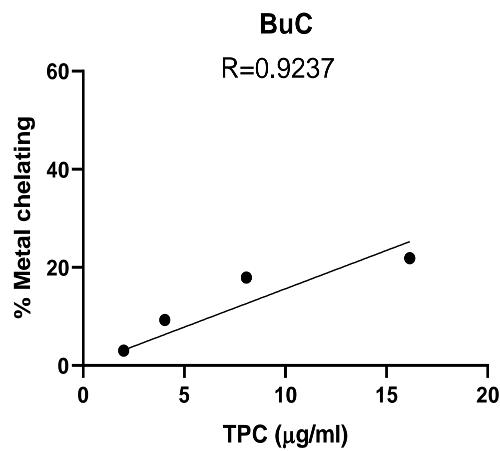
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
TPC (µg/ml)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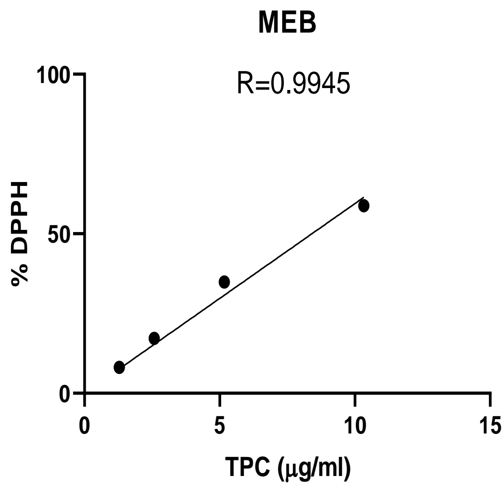
40
20
0
0
5
10
15
20
TPC (µg/ml)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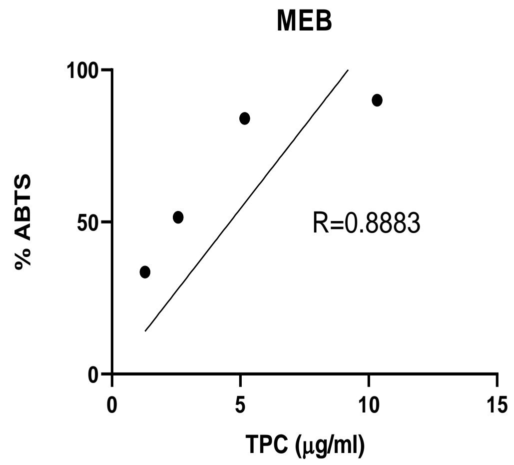
50
0
% DPPH
0
5
10
15
TPC (µg/ml)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50
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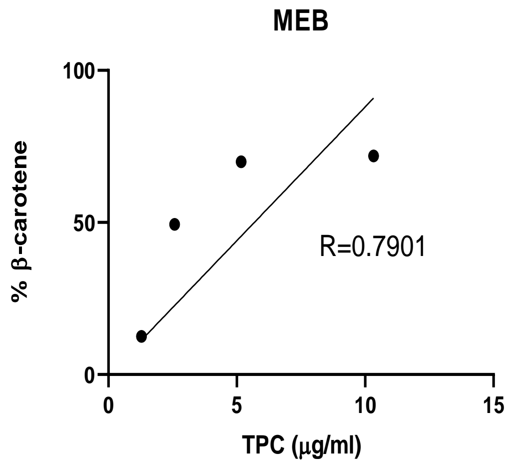
% ABTS
R=0.8883
0
5
10
15
TPC (µg/ml)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% β-carotene
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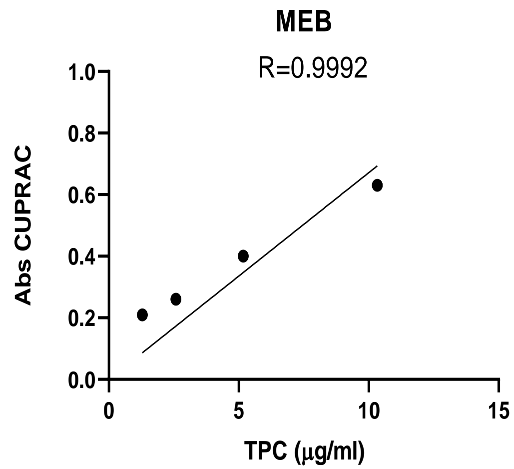
5
10
15
TPC (µg/ml)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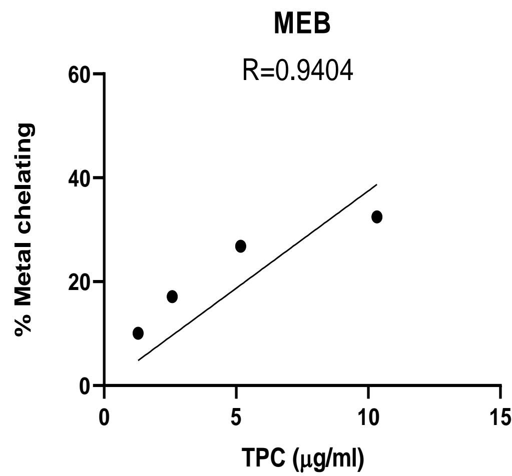
1.0
0.8
0.6
0.4
0.2
0.0
Abs CUPRAC
0
5
10
15
TPC (µg/ml)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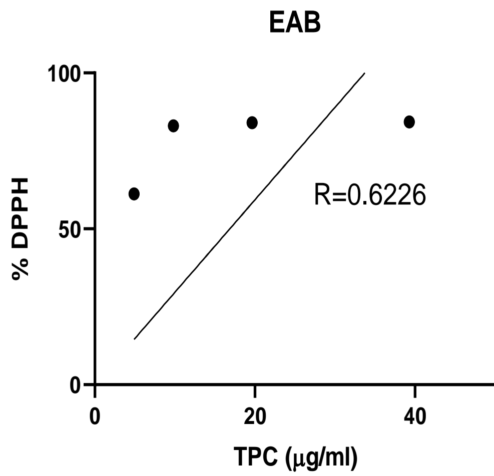
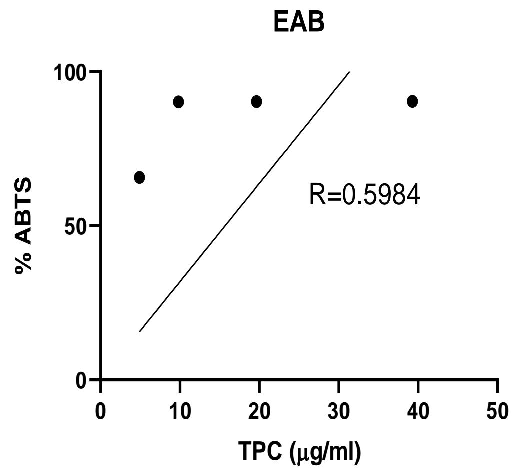
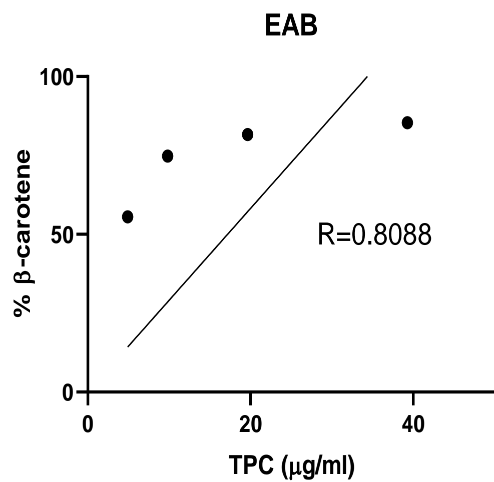
40
20
0
% Metal chelating
0
5
10
15
TPC (µg/ml)


EAB
% DPPH
R=0.6226
TPC (µg/ml)
100
50
0
0
20
40


EAB
% ABTS
R=0.5984
TPC (µg/ml)
100
50
0
0
10
20
30
40
50


EAB
% β-carotene
R=0.8088
TPC (µg/ml)
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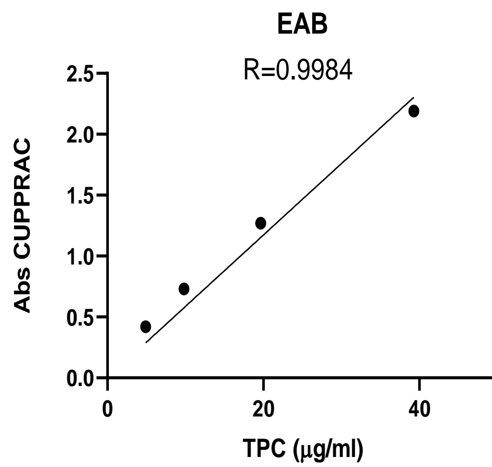
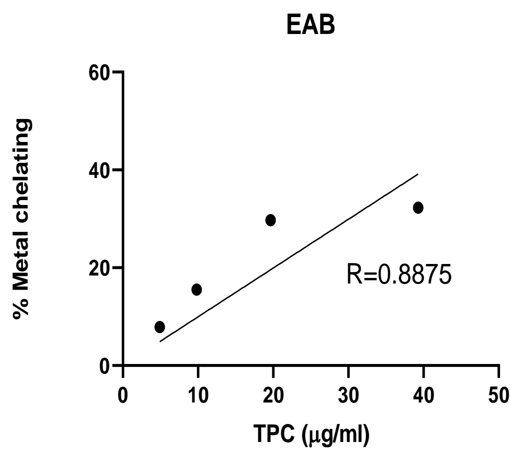
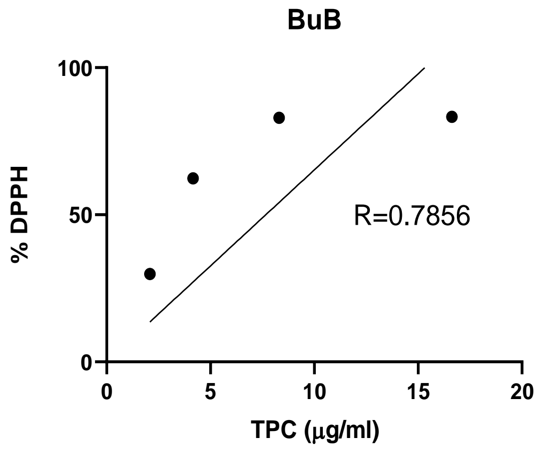
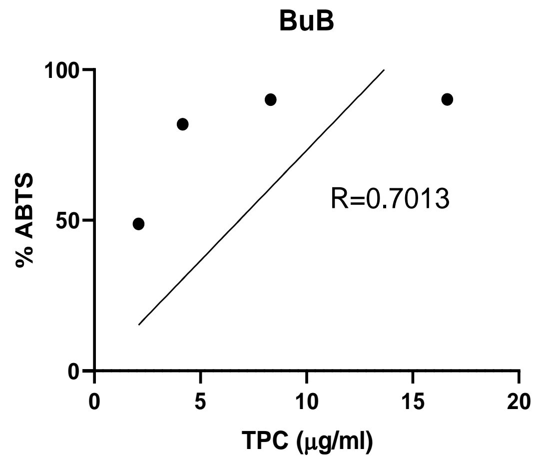
50
0
0
20
40


EAB
R=0.9984
Abs CUPPRAC
TPC (µg/ml)
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40


EAB
% Metal chelating
R=0.8875
TPC (µg/ml)
60
40
20
0
0
10
20
30
40
50


BuB
% DPPH
R=0.7856
100
50
0
0
5
10
15
20
TPC (µg/ml)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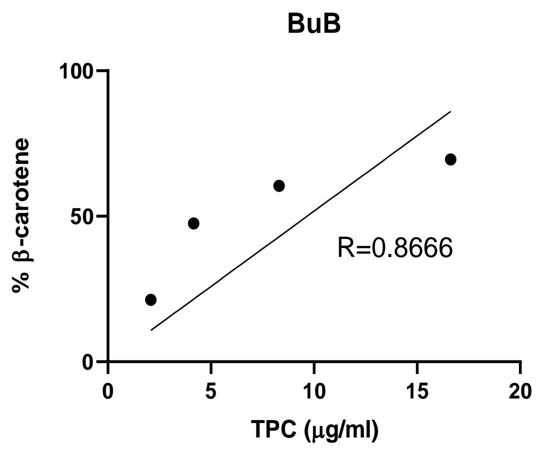
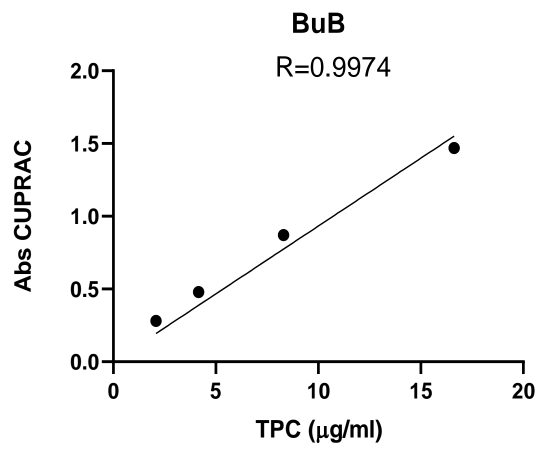
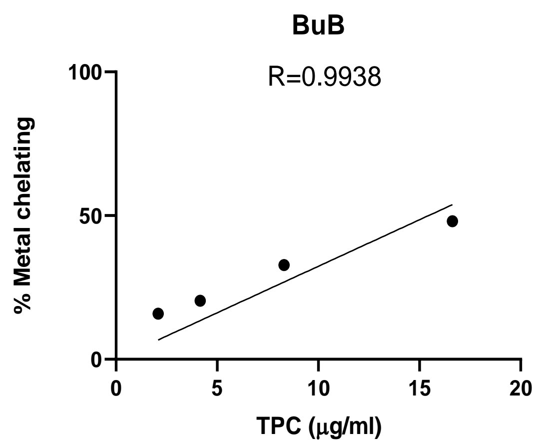
% ABTS
R=0.7013
100
50
0
0
5
10
15
20
TPC (µg/ml)


BuB
% β-carotene
R=0.8666
100
50
0
0
5
10
15
20
TPC (µg/ml)


BuB
R=0.9974
Abs CUPRAC
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
TPC (µg/ml)


BuB
R=0.9938
% Metal chelating
100
50
0
0
5
10
15
20
TPC (µg/ml)

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Assessment of *in vitro* activities and chemical profiling of *Senecio hoggariensis* growing in Algerian Sahara

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Abstract. *Arab Y, Sahin B, Ceylan O, Zellagui A, Olmez OT, Kucukaydin S, Tamfu AN, Ozturk M, Gherraf N. 2022. Assessment of in vitro activities and chemical profiling of* Senecio hoggariensis *growing in Algerian Sahara. Biodiversitas 23: 3498-3506.* The *in vitro* antioxidant, anticholinesterase, tyrosinase inhibitory, antibiofilm, and anti-quorum sensing activities of the ethyl acetate extract of *Senecio hoggariensis,* growing in Algerian Sahara, were studied along with its chemical constituents using HPLC-DAD. The chromatographic analysis unveiled seven phenolic compounds, including p-coumaric acid as a major component. Additionally, the extract showed moderate DPPH radical scavenging activity, compared to known standards. At 200 µg/mL, the extract disclosed equitable acetylcholinesterase (AChE), butyryl-cholinesterase (BChE) and tyrosinase inhibition rates with respective values of 37.01±1.48%, 18.87±4.18% and 45.99±1.81%. Likewise, the extract exhibited a good antibiofilm activity against *Candida albicans* ATCC 10239 biofilm production with an inhibition ratio of 59.56±0.40%, at 50µg/mL. The anti-quorum sensing by QS-regulated violacein pigment production inhibition test was determined using *Chromobacterium violaceum* CV026 and CV12472. The swarming motility inhibition assay was determined using *Pseudomonas aeruginosa* PA01. It is evident from the findings that *Senecio hoggariensis* could be considered potential antioxidant, anti-QS, and antibiofilm compounds. However, the origin of anti-biofilm and anti-quorum sensing activities of the ethyl acetate extract could be revealed by further studies on the mechanism of action of active compounds that can be isolated via activity-guided fractionation.

Keywords: Antibiofilm, anticholinesterase, antioxidant, anti-quorum sensing, anti-tyrosinase, *Senecio hoggariensis*

INTRODUCTION

The genus *Senecio* belonging to the Asteraceae family is widely distributed in tropical and subtropical regions of Africa, America, Asia and Europe, containing more than 1500 species (Tidjani et al. 2013). Most of them are common annual weeds, while some are cactiform and succulent perennials. Their flowers are arranged in clusters at the top of the plants with different colors like white, yellow, red, or purple (Albayrak et al. 2016). *Senecio* spp. is traditionally used for ornamental purposes and in folkloric medicine to treat wounds. They are reported to have antimicrobial, anti-inflammatory, antiemetic and vasodilator activities (Yang et al. 2011). Besides their importance, certain *Senecio* species have adverse effects against livestock due to their high toxicity attributed to the presence of pyrrolizidine alkaloids and some furano-eremophilanes. These are the most important characteristic constituents of *Senecio* plants responsible for more livestock deaths than any other poisonous plants. Besides pyrrolizidine alkaloids (PAs), a comprehensive review of *Senecio* plants showed that they possess flavonoids, coumarins, phenolic acids, alkanes, terpenoids, and steroids. Moreover, they exhibited potent antimicrobial, antitubercular, anti-inflammatory, antiulcer, cytotoxic, antimitotic, antifeedant, and insecticidal properties (Yang et al. 2011). Apart from eremophilanolide derivatives, PAs and sesquiterpenes seem to be one of the most abundant constituents. Regarding bioactivities, antioxidant and antimicrobial activities seem to be the most predominant (Milad 2014).

The limited chemical and biological studies on *S. hoggariensis* incited us to explore their phenolic compositions and various bioactivities thereof. This species grow in the Saharan mountains in Hoggar, Algeria), Niger (Aïr), Chad (Tibesti), and Egypt (Gebel Elba and Sinaï) (Lebrun 1981). Herein, we report the phenolic constituents of ethyl acetate extract of *S. hoggariensis* for the first time using HPLC-DAD, followed by antibiofilm, anti-quorum sensing, antioxidant, anti-tyrosinase and anticholinesterase activities.

MATERIALS AND METHODS

Chemicals and instrumentation

The optical densities for bioassays were measured by using SpectraMax340PC[384] (Microplate reader by Molecular Devices, Silicon Valley, USA). The phenolic profiling of the sample was done using Shimadzu 20AT series (HPLC-DAD (Shimadzu Corporation, Japan). Quercetin, ethylenediaminetetraacetic acid (EDTA), sodium chloride, ferrous chloride, and copper (II) chloride dihydrate ($CuCl_2.2H_2O$), were acquired from Merck (Darmstadt, Germany). DPPH (1,1-diphenyl-2-picrylhydrazyl), butylated hydroxytoluene (BHT), β-carotene, α-tocopherol, neocuproine, polyoxyethylene sorbitan mono palmitate (Tween-40), ferrene, ABTS (2,2′-azino-bis (3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt), linoleic acid, kojic acid, BChE (butyrylcholinesterase) from horse serum (EC 3.1.1.8, 11.4 U/mg) and AChE (acetylcholinesterase) from electric eel (Type-VI-S, EC 3.1.1.7, 425.84 U/mg), mushroom tyrosinase (EC 232-653-4, 250 KU, ≥1,000 U/mg), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), galantamine, butyryl-thiocholine chloride, acetylthiocholine iodide, L-DOPA (3,4-dihydroxy- D-phenylalanine), the certificated reference compounds used to screen the phenolic ingredients were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Solvents and chemicals were of analytical grade.

Plant collection and extraction

For collection of plant samples, *Senecio hoggariensis* Batt. & Trab. Plants were collected in April during their flowering time from El-Hoggar mountains (South-West Algeria). Dr. Youcef Halice, Technical Research Centre of Touggourt, identified confirmed the plant identity up to species level. It was deposited under voucher specimen number ZA67 at the laboratory of Biomolecules and Plant Breeding, Larbi Ben M'hidi University, Oum El Bouaghi, Algeria.

The aerial parts (100 g) of the plant were air-dried, grinded, and macerated with 80% aqueous methanol at room temperature. After filtration, the liquid phase was evaporated under reduced pressure using a rotary evaporator to obtain a solid residue. The obtained material was further dissolved in water and re-extracted using n-hexane, chloroform and ethyl acetate and successively evaporated to dryness under reduced pressure.

Total phenolics and flavonoids contents

The total phenolic contents (TPC) of the extract were calculated using the Folin-Ciocalteau Reagent (FCR) as well as external calibration with Gallic acid. In brief, 2.5 mL of FCR (diluted 1/10 with distilled water) was mixed with 0.5 mL of each extract diluted solution in methanol. After 5 minutes, 2 mL of sodium carbonate aqueous solution Na_2CO_3 (75 g/L) was added to the mixture and incubated for 30 minutes at 40°C then the absorbance was measured At 760 nm. Using the Gallic acid calibration curve, the results are presented as mg of Gallic acid equivalent (GAE)/g of dry extract. All experiments were carried out in triplicates with averaged results (Suleria et al. 2020). The extract was also analyzed spectroscopically to determine flavonoid content using quercetin (5-20 µg/mL) as standard. One (1 mL) of the extract (1 mg/mL) was added to 1mL of $AlCl_3$ (2%), incubated for 10 min at room temperature. Then, the absorbance was measured at 430 nm, and the results were expressed as (µg QE/mg extract) (Durak and Uçak 2015).

Quantitative analysis of phenolic compounds by HPLC–DAD

The chemical constituents of sample extract were determined by the reverse-phase HPLC-DAD system using a validated method against 27 standards (Tokul-Ölmez et al. 2020). The ODS-3 column (Inertsil, 150 mm × 4.0 mm i.d, 4 µm film thickness) was used to separate the compounds. The column oven temperature was set to 40 °C. Each extract's stock solution (8 mg/mL) was prepared in methanol/water (80/20,v/v).The stock solutions were pre-filtered using a disposable LC disk filter (Agilent 0.45 µm). The mobile phases used are 0.5% acetic acid in water (A) and methanol (B). The gradient elution program was of 40 minutes, as 0-0.01 min (0-20% B); 0.01-2 min (20-60% B); 2-15 min(60-80% B); 15-30 min(100% B); 3-35 min (100-10% B); and 35-40 min (10-0% B). The flow was1.5 mL/min, and a 20 µL sample was introduced. The inherent compounds were detected using a photodiode array detector (PDA) in the range of 230-350 nm with reference to the UV data and retention time of each commercial standard. Each analysis was performed in triplicate.

Antioxidant activity

Free radical-scavenging activity (DPPH assay)

The antiradical activity of ethyl acetate extract was tested by the DPPH free radical assay (Kozłowska et al. 2016). DPPH is a colored radical that has a maximum absorbance at 517 nm, and upon reduction, its absorption decreased. Briefly, 0.1 mM DPPH (160 µL) was mixed with 40µL of the sample solution of various concentrations and incubated for 30 minutes in the dark and the absorbance was measured at the same wavelength. The antioxidant activity of tested extract was compared with the known standards. The DPPH radical scavenging was calculated using the equation given below:

$$DPPH\ Free\ radical\ scavenging\ ativity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

From the inhibitory activity versus concentration graph, the IC_{50} (µg/mL) values were calculated.

Lipid peroxidation inhibitory activity

The lipid peroxidation inhibition activity of the plant extract was estimated using the β-carotene-linoleic acid test (Şahin 2013). β-carotene (0.5 mg) was dissolved in chloroform (1 mL), 25 µL of linoleic acid, and 200 mg of Tween-40 emulsifier were mixed in a flask. The mixture was evaporated under a vacuum to remove the chloroform, and 100 mL of oxygen-saturated water was added by vigorous stirring. The prepared reagent (160 µL) was added

separately into wells containing 40 µL of extracts. The absorbance at zero time of each reaction was recorded at 470 nm using a 96-well microplate reader. Each reaction was incubated at 50ºC for 2 hours. α-tocopherol, BHT, BHA and quercetin were used as standards. The equation below was used to estimate the bleaching rate (R) of β-carotene.

$$R = \frac{ln \frac{a}{b}}{t}$$

Where *ln*: natural logarithm, a is the absorbance at 0 time while b is absorbance after two hours. t is the total time in minutes.

The following equation was used to calculate the percent lipid peroxidation inhibitory activity (AA):

$$AA(inhibition\ \%) = \left(\frac{R_{control} - R_{sample}}{R_{control}}\right) \times 100$$

The graph presenting inhibitory activity against concentration was used to calculate the IC_{50} (µg/mL) value.

Cupric reducing antioxidant capacity (CUPRAC)

The standard CUPRAC method with slight modifications was adopted (Maryam et al. 2016) and the absorbance was recorded using an ELISA reader. The aqueous solution including 50 µL of $CuCl_2.2H_20$ (10 mM), neocuproine (7.5 mM in absolute ethanol), and NH_4Ac buffer (100 mM, pH 7.0) was added to 50 µL of sample extract at various concentrations to make 200 µL of final volume then incubated for one hour at room temperature. The absorbance was recorded at 450 nm. The blank contains the same reactants except for the plant extract. The antioxidant standards were used for comparison. The results were expressed as $A_{0.5}$.

ABTS cation radical scavenging activity

$ABTS^{\bullet+}$ scavenging activity assay presents some advantages over DPPH scavenging test which is not convenient with water insoluble or bulky structures compounds. Therefore, the $ABTS^{\bullet+}$ scavenging activity of the extracts was also verified (Gupta et al. 2016). Briefly, 7 mM of ABTS and 2.45 mM of potassium persulfate were dissolved in water, kept for 16 hours in the dark to provide $ABTS^{\bullet+}$ solution. The tested $ABTS^{\bullet+}$ solution was prepared by diluting it with ethanol to get an absorbance of 0.700±0.025 at 734 nm in a one cm pathway. To each well containing 40 µL of the extract in methanol of various concentrations, 160 µL of diluted $ABTS^{\bullet+}$ solution was added and incubated for ten minutes, then the absorbance was measured at 734 nm. For comparison, quercetin, BHA, BHT, and α-tocopherol were used and each assay was performed in triplicate. The sample's capability to scavenge $ABTS^{\bullet+}$ was calculated using the formula given for the DPPH assay. The results of $ABTS^{\bullet+}$ scavenging activity were presented as IC_{50}.

Metal chelating activity assay

The ferrous ion chelating potential of extract was measured using the following standard procedure with slight modifications (Kozłowska et al. 2016). To each well containing 40 µL of the extract in methanol at various concentrations, 40 µL of $FeCl_2$ (0.2 mM) and 40 µL of ethanol were added. Then 80 µL of ferrene (0.5 mM) was added to initiate the reaction. After incubation at room temperature for 10 minutes, the absorbance was measured at 593 nm. The EDTA was used as a chelating standard. The results are presented as inhibition (%) at 100 µg/mL concentrations.

Anticholinesterase activities

The inhibition of acetylcholinesterase (AChE; 5.32 × 10-3U) and butyrylcholinesterase (BChE; 6.85 × 10-3U) of the extract was tested using Ellman's method (Öztürk et al. 2014). In a 96 well plate, each concentration (25-200 µg/mL) of the sample in ethanol (10 µL) was incubated at 25°C for 15 min with 20 µL of enzyme solution and 150 µL of sodium phosphate buffer (100 mM, pH8). After incubation, Ellman's reagent, DTNB (0.5 mM, 10 µL), and substrates (10 µL) were added to each well to make 200 µL final volume. Then measurement was performed at 412 nm for 10 minutes and galantamine was used as a standard. The percent of both enzymes inhibition was calculated using the following formula.

$$AChE/BChE\ inhibiton\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} x100$$

Where $A_{control}$ is the enzyme inhibitory activity of blank and A_{sample} is the enzyme inhibitory activity of the sample. Each test was conducted in triplicate. The results are presented as inhibition (%) at an extract concentration of 200 µg/mL.

Determination of tyrosinase inhibitory activity

In vitro tyrosinase inhibitory potential of plant extract was assessed using mushroom tyrosinase by following the Hearing method (Benso et al. 2018). The L-Dopa was employed as a tyrosinase substrate. Kojic acid was used as a standard to compare the activity. The tyrosinase inhibition (%) at each sample concentration (µg/mL) was calculated as that used in AChE and BChE assays.

Determination of minimum inhibitory concentrations (MIC)

The lowest concentration of extract showing no visible bacterial growth is called MIC. It was determined by a microtiter broth dilution assay (CLSI 2006) by using four bacterial strains including *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and one yeast *C. albicans* ATCC 10239. The Mueller-Hinton Broth (MHB) was used as a test medium, whereas, inoculum density was 5×105 CFU/mL. Before being read, the cell suspensions (100 µL) were incubated separately under aseptic conditions with extract at various concentrations (6.25, 12.5, 25, 50 and 100 µg/mL) at 37ºC for one day.

Effect of extract on bacterial biofilm formation

The biofilm-forming ability of tested microorganisms was tested using a microplate biofilm assay with extract at 1/1, 1/2, 1/4 and 1/8 minimum inhibitory concentration (Wang et al. 2017). Briefly, the tested microbes as mentioned for MIC were incubated at static in glucose (0.25%) containing sterile Tryptose-Soy Broth (200 µL) at 37°C for 48 hours. After incubation, the wells were drained and washed with water. The crystal violet solution (0.1%) was used to stain the remaining bacteria and washed after 10 minutes with water to eliminate the crystal violet solution. The biofilm formed in each well was suspended with 33% glacial acetic acid (200 µL) and was shaken for 5 minutes. Toa sterile tube, the solution (125 µL) was transferred separately from each well. The volume was completed to 1 mL using sterile distilled water. The absorbance was recorded at 550 nm, and % biofilm Inhibition was calculated using the equation below:

$$biofilm\ inhibition\ (\%) = \left(\frac{OD_{550\ contol} - OD_{550\ sample}}{OD_{550\ control}}\right) \times 100$$

Bioassay for quorum-sensing inhibition (QSI) activity using CV026

Method of Koh and Tham (2011) was used to perform quorum sensing inhibition (QSI) extract activity. The bacterial CV026 culture (100 µL) was transferred to the warm molten Soft Top Agar (5 mL). Then, the exogenous AHL source 20 µL of C6-HSL (100 µg/mL) was gently mixed and overlayed onto the solidified Luria Bertani Agar (LBA) plate. After solidification, the 50 µL extracts (sub-MIC concentrations) were transferred to each well and incubated for three days at 30°C. The QSI was visualized by monitoring a cream or white-colored halo around each well against tested CV026 bacteria. Thus, the inhibition zones could be measured to exhibit antimicrobial activity.

Violacein pigment inhibition assay

QSI potential of *Senecio* plant extract was qualitatively determined against *Chromobacterium violaceum* ATCC 12472 (Packiavathy et al. 2012). The overnight culture (10 µL) of *C. violaceum* with 0.400 optical density at 600 nm was transferred to plates that contains LB media (200 µL). The prepared samples with and without sub-MICs of ethyl acetate extract were incubated for twenty-four hours at 30°C. The absorbance was recorded at 585 nm to assess the reduction in the violacein pigments. The formula presented below was used to determine the violacein percent inhibitory activity.

$$Violacein\ pigment\ inhibition\ (\%) = \left(\frac{OD_{585\ control} - OD_{585\ sample}}{OD_{585\ control}}\right)$$

Swarming motility assay

The effect of the extract on the inhibition of *Pseudomonas aeruginosa* was assessed by following the described procedure (Merritt et al. 2011) with few modifications. The swarming plates were prepared by using D-glucose (0.5%), agar (0.5%), NaCl (0.5%), and peptone (1%). The plates were treated with *Senecio* extract (50, 75, and 100 µg/mL) followed by inoculation with a fresh culture of *P. aeruginosa* PA01. A control medium without extract was also prepared for comparison. All the plates were incubated at 37°C in an upright position for eighteen hours. The plate without the extract was maintained as a control. The bacterial growth and extension were measured as swarm motility.

Statistical analysis

Results were calculated and presented as means value±SD of three measurements. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test using GraphPad Prism software (version 8.0.1); p values <0.05 were regarded as significant.

RESULTS AND DISCUSSION

Total phenolics and flavonoids contents

According to the results shown in table 1, the amounts of total phenolics and flavonoids of ethyl acetate extract of *S. hoggariensis* were found to be 100.27±0.74 µg GAE/mg extract and 80.06±1.14 µg QE/mg extract, respectively. The extraction yield was 0.28% with respect to dry weight.

High-Performance Liquid Chromatography (HPLC) analysis

The investigation of the phenolic compounds was carried out using a HPLC equipped with PDA detector. The compounds were identified by comparing their spectroscopic characteristics and retention times (RT) with reference compounds. Seven compounds were detected among which *p*-coumaric is the predominant (10.85 mg/g extracts) followed by caffeic acid (3.38 mg/g). Other constituents were quercetin (0.93 mg/g), 3,4-dihydroxybenzoic acid (0.87 mg/g), 4-hydroxy benzoic acid (0.68 mg/g), vanillic acid (0.38 mg/g) and 6,7-dihydroxy coumarin (0.32 mg/g) (Table 2).

Antioxidant activity

The antioxidant activity was evaluated using five complimentary tests, namely, β-carotene-linoleic acid, ABTS, DPPH, metal chelating, and CUPRAC assays. As shown in Table 3, the results of β-carotene bleaching test are compared with those of BHT and BHA, α-Tocopherol and quercetin. The analysis of variance revealed significant difference between tested samples and the standards. The inhibition of lipid peroxidation was recorded with IC_{50} values of 18.98±1.50 µg/mL. Since IC_{50} of extract is lower than 50 µg/mL, the extract could be considered with good potential for lipid peroxidation.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of *S.hoggariensis*

Plant extract	Yield %	Polyphenols[a]	Flavonoids[b]
Ethyl acetate extract	0.28	100.27±0.74	80.06±1,14

Note: [a]Total phenolic content (µg GAE/mg extract); [b]Total flavonoids content (µg QE/mg extract); results are expressed as means ± SD (n = 3).

Table 2. Retention time, calibration curves, regression coefficient (R^2), linearity ranges, LODs and recoveries of phenolic standards at 254 nm

Compound	RT [a] (min)	Calibration equation	R^{2} [b]	Linear range (µg/mL)	LOD [c] (µg/mL)	LOQ [c] (µg/mL)	Recovery (%)	RSD [d] within day (n= 7)	RSD between days (n=7)	*S. hoggariensis*
3,4-Dihydroxybenzoic acid	14.10	y=76181x-88801	0.9995	3.13-100	3.42	10.35	102.35±4.21	3.19	1.22	0.78±0.08
4-Hydroxybenzoic acid	19.50	y=111102x+21691	0.9993	1.56-50.0	1.58	4.79	100.82±3.89	4.00	2.41	0.68±0.10
6,7-Dihydroxycoumarin	21.99	y=34377x-32740	0.9940	5.00-50	3.98	12.07	104.11±5.06	4.94	3.72	0.32±0.06
Vanillic acid	22.37	y=74653x-9634.1	0.9998	1.56-100	1.56	4.68	103.58±4.43	5.06	3.88	0.38±0.02
Caffeic acid	22.94	y=67972x-32965	0.9880	3.00-30.0	4.54	13.75	102.67±4.92	4.01	5.87	3.38±0.15
p-Coumaric acid	28.43	y=18300x+6153.3	0.9998	6.25-400	5.46	16.56	101.60±2.36	3.14	0.44	10.85±0.31
Ferulic acid	29.93	y=35737x+12977	0.9999	2.34-300	3.96	11.99	100.99±3.54	3.20	0.51	0.93±0.02

Note: [a] RT: Retention time of the compound in minutes, [b] R2: linearity of the calibration graph, [c] LOD: Limit of Detection in mg/m Land LOQ: Limit of Quantification in mg/mL, [d] RSD: Percentage. The values expressed herein were the mean ± S.E.M. of three parallel measurements

Table 3. Antioxidant activity of ethyl acetate extracts of *S. hoggariensis* by β-Carotene-linoleic acid, DPPH•, ABTS•+, CUPRAC and metal chelating assays*

Extract	β-Carotene linoleic acid assay	DPPH assay	ABTS assay	CUPRAC assay	Metal chelating assay
	IC_{50} (μg /mL) **	IC_{50} (μg /mL) **	IC_{50} (μg /mL) **	$A_{0.5}$ (μg/mL) ***	Inhibition (%) (at 100 μg/mL)
S. hoggariensis	18.98±1.50^{b}	46.40±3.95^{b}	26.19±0.96^{c}	53.10±3.79^{b}	28.66±2.70^{b}
α-Tocopherol	2.10±0.09^{c}	12.26±0.07^{d}	4.31±0.10^{e}	10.20±0.01^{c}	NT
BHT	1.34±0.04^{c}	45.37±0.47^{b}	4.10±0.06^{e}	3.80±0.00^{d}	NT
Quercetin	1.81±0.11^{c}	2.07±0.10^{e}	1.18±0.03^{e}	NT	44.09±0.87^{b}
EDTA	NT	NT	NT	NT	96.50±0.07^{a}

Note: *The values (IC_{50} and $A_{0.50}$ and Inhibition%) presented represent means ± SD, followed by the different script letters within the same column indicates significant difference statistically using Tukey test at p<0.05. **IC_{50} values correspond to the μg/mL concentration of 50% inhibition while ***$A_{0.50}$ values correspond to the μg/mL concentration of 0.500 absorbance. NT: not tested

Furthermore, the extract displayed relatively good DPPH radical scavenging activity with IC_{50} values of IC_{50}: 46.40±3.95 μg/mL. Nonetheless, its scavenging activity was higher than those of α-tocopherol (IC_{50}: 12.26±0.07 μg/mL) and BHT (IC_{50}: 45.37±0.47 μg/mL). In ABTS assay, the studied extract exhibited an IC_{50} of 26.19±0.96μg/mL, higher than those of the standards BHT and α-tocopherol with IC_{50}: 4.10±0.06 μg/mL and IC_{50}: 4.31±0.10 μg/mL, respectively.

In CUPRAC assay the antioxidant activity increased with increasing absorbance. The potential of activity was expressed as $A_{0.5}$ corresponding to an Absorbance of 0.5 which was calculated through the absorbance versus concentration graph. As seen in Table 3, *S. hoggariensis* extract exhibited moderate cupric reducing antioxidant capacity ($A_{0.50}$: 53.10±3.79 μg/mL) than the standard α-tocopherol ($A_{0.50}$: 10.20±0.01 μg/mL).

Regarding metal chelating activity, the extract demonstrated an IC_{50} much higher than 100 μg/mL. *S. hoggariensis* extract exhibited an appreciable metal chelating activity where The percentage inhibition was 28.66 ±2.70% at 100 μg/mL compared with quercetin (44.09±0.87 μg/mL) and EDTA (96.50±0.07 μg/mL) (Table 3).

Anticholinesterase activity

Ethyl acetate extract of *S. hoggariensis* was subjected to acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) inhibitory activity tests. The percentage inhibition increased with the increasing concentration. Various concentrations of extract (25-200 μg/mL) were used revealing relatively moderate activity against both enzymes. The results of the highest concentration at 200 μg/mL were reported in Table 4. The extract showed slight AChE and BChE inhibitory activities with respective values of 37.01±1.48% and 18.87±4.18%. Under the same conditions, galantamine highlighted 81.41±1.03% and 75.54 ±1.05% values against AChE and BChE, respectively.

Tyrosinase inhibition

S. hoggariensis extract had moderate inhibitory activity (45.99±1.81%) against tyrosinase enzyme at 200 μg/mL. Under the same conditions, kojik acid displayed 83.6±0.2% (Table 4).

Antimicrobial and antibiofilm activity

The antimicrobial activity of the extract was determined by the broth microdilution method using 96 well plates. The results obtained after evaluating the antimicrobial activity corresponding to the MIC of the extract are shown in Table 5. In this study, two Gram-positive bacteria, two Gram-negative bacteria, and one yeast were used. The MIC values were found to be in the range of 50 to 100 μg/mL against all microorganisms. *S. aureus* showed the highest susceptibility to tested extract, with a MIC of 50 μg/mL. The extract at the MIC and sub-MIC concentrations inhibited biofilm formation by the test microorganisms in various percentages. The highest antibiofilm activity was observed in *C. albicans* ATCC 10239 with 59.56±0.40%, followed by 49.55±1.00 against *S. aureus* ATCC 25923 biofilm production at the 50 μg/mL concentration (Table 5).

Anti-QS potential

Anti-QS activity assay using *Chromobacterium violaceum* (CV026) and Violacein inhibition assay by *C. violaceum* (CV12472). QS bioassay of extract of the plants using CV026 revealed strong anti-QS activities. The ethyl acetate extract of *S. hoggariensis* showed a QS-inhibition zone diameter of 11.00±0.42 mm at 25 μg/mL as shown in Table 6.

Concerning violacein inhibition assay on *CV12472* it is worthy to note that before carrying out the test, MIC values should be determined and the values alongside violacein inhibition percentages are reported in Table 6. In this qualitative analysis, the extract showed inhibition of QS-mediated violacein production in *C. violaceum* ATCC 12472 in a dose-dependent manner. At MIC concentrations, there was 100% inhibition of violacein pigment by the extract. The inhibition decreased for the extract at MIC/16 concentration.

Swarming inhibition assay

The extract was tested for anti-QS potential on swarming motility due to pyocyanin levels against *P. aeruginosa* PA01. The results presented in Figure 1 revealed that the extract positively intervened in the swarming of PA01. Accordingly, a substantial inhibition in the migration of PA01 was achieved. At the highest tested concentration (100 μg/mL), the swarming inhibition with pyocyanin was 26.02±0.21%.

Table 4. Acetylcholinesterase (AChE), butyrylcholineestrase (BChE), and tyrosinase inhibitory activities of the *S. hoggariensis* extract at 200 µg /mL*

Extracts	Enzyme inhibitory activities (inhibition%)		
	AChE assay	BChE assay	Tyrosinase assay
S. hoggariensis	37.01±1.48[b]	18.87±4.18[b]	45.99±1.81[b]
Galantamine**	81.41±1.03[a]	75.54±1.05[a]	NT
Kojik acid**	NT	NT	83.6±0.2[a]

Note: *The values (Inhibition%) represent means ± SD. Followed by the different scripts within the same column indicates significant difference statistically using Tukey test at p<0.05. **Standards used in the studies. NT: not tested

Table 5. MIC and antibiofilm activity of *S. hoggariensis* extract

Microorganim	*S. hoggariensis*				
	Planktonic	% inhibition on biofilm formation			
	MIC (µg/mL)	MIC	MIC/2	MIC/4	MIC/8
P. aeruginosa ATCC 27853	100	59.04 ±1.9	38.62±3.40	28.89±1.50	-
E. coli ATCC 25922	100	59.02±4.70	38.62±3.40	37.47±2.10	33.86±1.10
S. aureus ATCC 25923	50	49.55±1.00	36.24±1.69	20.82±1.20	13.81±1.00
E. faecalis ATCC 29212	100	58.13±1.00	26.24±2.00	14.77±2.10	-
C. albicans ATCC 10239	50	59.56±0.40	41.75±0.42	29.47±0.34	-

Note: -: No inhibition

Table 6. Screening of ethyl acetate of *S.hoggariensis* for anti-quorum sensing activity against *C. violaceum* CV026, inhibition of violacein production against *C. violaceum* CV12472

Plant extract	MIC against CV026	Anti-quorum sensing activity	MIC against CV12472	Inhibition of violacein production
	25	11.00±0.42[b]	50	100±0.00
	MIC/2	7.00±0.49[b]	MIC/2	61.73±1.00
S. hoggariensis	MIC/4	-	MIC/4	28.41±0.59
	MIC/8	-	MIC/8	10.42±1.04
	MIC/16	-	MIC/16	-

Note: -: No inhibition the values (MIC) represent means ± SD. (QS inhibition zone diameters in mm)*

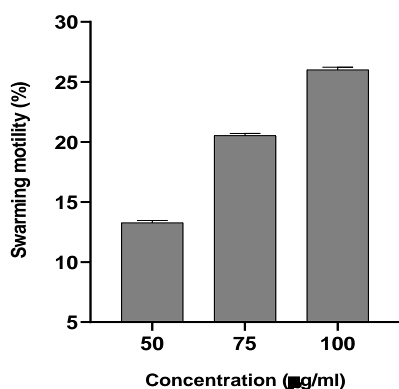

Figure1. Swarming motility inhibition of *S. hoggariensis* extract on *P. aeruginosa* PA01 strain

Discussion

This study was mainly carried out to establish the qualitative and quantitative phenolic profile and to evaluate the antioxidant capacity, anti-tyrosinase, anticholinesterase, antibiofilm and anti-quorum sensing activities of *Senecio hoggariensis* growing in Algerian Sahara.

The Folin–Ciocalteu method and the aluminum chloride colorimetric method for determining total content of phenols and flavonoids, respectively, are quick and accurate assays, giving crucial information regarding phytochemical quantities. However, these approaches do not provide a complete picture of phenolic compound quantification in complicated samples. Therefore, the phenolic profile of *S. hoggariensis* ethyl acetate extract was investigated further using HPLC-DAD and the results were presented in Table 4. Our results revealed the presence of mixture of bioactive phytochemicals from phenolic acids, coumarins to flavonoids. The phenolic composition of *S. hoggariensis* is still limited (Lahlou 2014), which allowed to identify the presence of certain flavonoids like Q 3-glucoside ,I 3 -rutinoside and I 3-monosulphatet in traces.

The ethyl acetate fraction demonstrated a significant potent antioxidant capacity. This is the first contribution of the antioxidant activity of *S. hoggariensis,* therefore, it is important to compare data obtained with other plants under the same genus. Significant antioxidant activities were recorded in ethyl acetate fractions for *S. inaequidens, S. vulgaris,* (inhibition of DPPH equal to 61.60% and 44.57% of inhibition, respectively, at concentration of 0.31 mg/mL

(Conforti et al. 2006a), *S. gibbosus* (IC_{50} of 0.01 mg/mL on DPPH) (Conforti et al. 2006b) and in *S. angulatus* (IC_{50} of 21.85 µg/mL on DPPH) (Bousetla et al. 2021). These findings showed higher inhibition than that obtained in the present study. The diverse procedures utilized to obtain the ethyl acetate fraction can be used to explain these dissimilarities. Conforti et al. (2006b) extracted the plant materials with methanol; then the methanolic extract was acidified with 2.50% H_2SO_4 and partitioned with n-hexane, dichloromethane and ethyl acetate. Conversely, in our study the fractionation steps were carried out without acidification. The antioxidant activity of the extract can be attributed to the content of *p*-coumaric acid and other phenolics such as rutin ellagic acid and quercetin. *P*-coumaric acid is a phenolic acid of the hydroxycinnamic acid family, synthesized from phenylalanine and tyrosine. It has antifungal, antiviral, anti-melanogenic, antioxidant and anti-inflammatory effects and various biological activities. It is also a well-documented antioxidant, known to reduce oxidative stress and have appreciable radical scavenging activity (Yue et al. 2018).

The use of cholinesterase inhibitors is a suitable and effective way to treat neurological illnesses cognitive symptoms (Tamfu et al. 2019). The ability of the plants to inhibit cholinesterase enzymes indicated its potential use in remedying Alzheimer disease. Our results showed that the ethyl acetate extract presented a moderate inhibitory activity against AChE, meanwhile, it was found to be low against BChE enzymes. Tyrosinase is an essential enzyme responsible for the initiation of browning of freshly harvested fruits. It is a copper-containing multifunctional enzyme belonging to the oxidase group that catalyzes the initial two steps of mammalian melanogenesis. Similarly, the hyperpigmentation of human skin is undesirable and is considered comparable to the enzymatic browning of fruits. This has forced researchers to seek novel and more compelling tyrosinase inhibitors for their safe use in functional foods and harmless cosmetics (Chang 2012). The present extract displayed moderate anti-tyrosinase activity in comparison with Kojik acid standard. There is no research on antityrosinase activities of *Senecio* spp. so far in the literature, to our knowledge, this is the first review on tyrosinase inhibitors.

The antimicrobial activity of extract could be related to their flavonoids and phenolic acid contents. In particular *p*-coumaric acid was quantified as a major compound in the *Senecio* sp.. p-coumaric acid is known to have a good antimicrobial activity not only against *E. coli* but also against other Gram-negative bacteria such as *Salmonella typhimurium* and *Shigella dysenteriae*. It can alter the cell membrane permeability and bind DNA, causing increased cell membrane permeability, consequently damaging barrier function due to loss of cytoplasmic contents. The *p*-coumaric acid binds with anionic phosphate of a double helix of bacterial DNA and creates a groove in the helix. This adversely affects the genetic process, including replication, transcription and expression of a bacterium. Due to these facts, the *p*-coumaric acid exhibited a dual mechanism towards bactericidal activity by disrupting bacterial cell membranes and binding the genomic DNA of bacteria to hinder cellular functions, ultimately causing cell death (Lou et al. 2012).

The indiscriminative use of antibiotics to treat bacterial infections is a serious issue causing the development of drug-tolerant strains with persistent and more severe infections, this is due to the formation of highly resistant biofilms by multi-drug resistant strains (Husain and Ahmad 2013). Therefore, QS inhibition can be considered an advanced strategy to control microbial pathogenesis. Thus, the quorum sensing inhibition (QSI) protocols could play a vital role to biomonitor organisms with easily detectable QS responses (Saurav et al. 2017). Herein, the inhibition of QS was detected by the presence of colorless zone (s). Similarly, viable cells around the wells could be differentiated from the growth inhibition zone (antibacterial activity). The swarming migrations of bacterial strains have a significant role in the formation of QS-mediated drugs. The extracts inhibited swarming motility in *P. aeruginosa* PA01. To date, no studies were reported on the effect of *Senecio* spp. on the production of violacein or any other QS-mediated phenomenon. However, certain phenolic compounds amongst other metabolites in these plants could be responsible for the observed anti-QS activity. Naturally occurring phenolic compound *p*-coumaric acid has already been shown to interact with bacterial quorum sensing, sometimes by triggering or by inhibiting the QS-process system (Othman et al. 2019).

In conclusion, the phytochemical analysis and antioxidant, anticholinesterase, tyrosinase inhibitory, anti-QS potential properties of ethyl acetate of *S. hoggariensis* growing in Algeria desert were reported for the first time. Using HPLC-DAD analysis, seven compounds were identified including *p*-coumaric acid as the major constituent. Considering the results obtained, it can be concluded that the plant possessed relatively potent radical scavenging capacity while showing moderate enzyme inhibitory in tyrosinase and acetylcholinesterase inhibitory activities. It can be concluded that *p*-coumaric acid is mainly responsible for critical antioxidant activity. It also possessed high antimicrobial activity and potential inhibition of biofilm formation. The present study also demonstrated the anti-QS properties of *S. hoggariensis* extract against *C. violaceum* CV 026 grown in the presence of C6-HSL. The tested plant extracts inhibited the Violacein synthesis in *C. violaceum* CV 12472 successfully. The said bioactivity of extracts can be mainly attributed to *p*-coumaric acid, which demonstrated anti-QS activity against *C. violaceum*. *Senecio hoggariensis* could be considered for potential antioxidant, anti-QS, and antibiofilm compound. However, the origin of anti-biofilm and anti-quorum sensing activities of the ethyl acetate extract could be revealed by further studies on the mechanism of action of active compounds that can be isolated via activity-guided fractionation.

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