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THEME

Valorization of secondary metabolites products of some plants belong to *Chenopodiaceae* growing in the Algerian desert

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To My Father & My Mother

... To All My Family

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Summary

This study investigated six medicinal plants belonging to the *Chenopodiaceae* family that grow in the Algerian Sahara, including: *Anabasis oropediorum* Maire, *Bassia muricata* L, *Halocnemum strobilaceum* Pall, *Salsola tetragona* Del, *Salsola foetida* Del, and *Suaeda fruticosa* L. In order to evaluate the secondary metabolites of these plants in the pharmaceutical field.

These six plants were valued by determining the nutritional value of the aerial part of each plant, and applying quantitative and qualitative analyses, including RP-HPLC analysis of methanolic extracts (70%) of the aerial part. The biological activity was evaluated *in vitro* by antioxidant, antibacterial, antidiabetic, anti-inflammatory, and sun protection factor (SPF). In addition to testing the cytotoxicity of the extracts *H. strobilaceum* and *S. fruticose*.

The chemical analysis of the nutritional value showed that the biomass of the aerial part or the six plants is characterized by a high mineral content, as all of them mainly contain calcium. It also contains an important water content, indicating their ability to grow in desert conditions. The plants also contained carbohydrates, lipids, and proteins, with distinct values for each plant.

The phytochemical screening revealed that the methanolic extracts of the aerial part of the studied plants qualitatively contain most of the secondary metabolites; alkaloids, coumarins, mucilage, phenols, saponins, and terpenoids. The FT-IR analysis showed the presence of phenolic, aromatic, aliphatic amine, organosulfur compounds, and alkanes.

According to the quantitative estimation, it is clear that all plant extracts are rich in saponins and phenolic compounds, as *B. muricata* extract had the highest content of saponins (22.94±0.10 µg DE/mg), and *S. foetida* extract had the highest content of total phenols and flavonoids; 67.05±0.23 µg GAE/mg and 35.51±0.92 µg QE/mg, respectively. It also reported that all extracts contain many sections of flavonoid compounds; anthocyanins, flavonols, flavanones, hydrolyzable tannins, and condensed tannins with different values. The extracts of *B. muricata* and *S. foetida* were also distinguished by the presence of flavanols, without the rest of the extracts. RP-HPLC analysis revealed rutin as a common compound in all extracts at varying concentrations.

Several experiments were conducted on methanolic extracts to study their biological activity. Where it was observed that the methanolic extract of *B. muricata* recorded greater activity than the rest of the extracts. The inhibitory concentration of 50% of $DPPH^{\bullet}$ free radicals was about 9.86±0.33 µg/mL, while the percentage of lysis of red blood cells was 40% when hemolysis assay at a concentration of 500 µg/mL. In the *β*-Carotene/linoleic acid, reducing power, and phosphomolybdenum assay, the methanolic extract of *S. foetida* made the best results.

In terms of studying the activity of methanolic extracts in inhibiting bacterial growth, they demonstrated a medium level of antibacterial activity. The results of the three anti-diabetic assays suggest that these plants may be effective as hypoglycemic agents. Whereas, *S. foetida* extract was the best sunscreen with 21.33±0.03. Through the anti-inflammation assay, *B. murticata* extract had the highest inhibition of denaturation of inflammatory protein by 62.88 % at 500 µg/ml. The extracts of *H. strobilaceum* and *S. fruticosa* had weak cytotoxic activity as the percentage of dead cells (Huh-7 and HepG2 cells) did not exceed 30% at the concentration of 100 µg/mL.

Key words: Valorization, secondary metabolites, *Chenopodiaceae*, Algerian desert.

Summary in Arabic (مُلخص)

تثمين نواتج الأيض الثانوية لبعض النباتات التابعة لعائلة *Chenopodiaceae* النامية في الصحراء الجزائرية

أجريت هذه الدراسة على ستة نباتات طبيّة من الصّحراء الجزائريّة تنتمي للعائلة الرّمرامية (*Chenopodiaceae*) وهي: *Anabasis oropediorum* Maire, *Bassia muricata* L, *Halocnemum strobilaceum* Pall, *Salsola tetragona* Del, *Salsola foetida* Del, و *Suaeda fruticosa* L. وذلك قصد تثمين نواتج أيضها الثانوي في المجال الصيدلاني.

تم تثمين هذه النباتات الستة من خلال، تحديد القيمة الغذائية للجزء الهوائي لكل نبات، وتطبيق الإجراءات القياسية والنوعية بما فيها تحليل RP-HPLC للمستخلصات الميثانولية (70%) للجزء الهوائي. كما تم تقييم الفعالية البيولوجية في المختبر عن طريق مضاد الأكسدة، ومضاد البكتيريا، ومضادات السكرى، ومضادات الإلتهابات، وتم تحديد عامل الحماية من أشعة الشمس. بالاضافة الى اختبار السمية الخلوية للمستخلصين *H.strobilaceum* و *S.fruticose*.

أظهرت التحاليل الكيميائية للقيمة الغذائية أن الكتلة الحيوية للجزء الهوائي للنباتات الستة تتميز بمحتوى معدني عالٍ، مُميز بوفرة عنصر الكالسيوم. كما تحتوي على منسوب رطوبة مهم يدلّ عن قدرتها على تحمّل ظروف نموها الصحراوية القاسية. كما تحتوي على الكربوهيدرات واللبيدات والبروتينات، حيث لكل نوع نباتي قيم مميزة له.

أسفر الفحص الفيتوكيميائي على أن المستخلصات الميثانولية للجزء الهوائي لنباتات المدروسة، نوعيًّا تحتوي على أغلب نواتج الأيض الثانوي؛ قلويدات، وكومارين، وصمغ، وفينولات، وصابونين، وتربينات. وأسفر تحليل FT-IR على وجود كل من مركبات فينولية، وعطرية، وألفاتية ومركبات ذات طبيعة كبريتية وألكانات. أما كميا، فقد أفاد التقدير الكمي أن جميع المستخلصات النباتية غنية بالصابونين والمركبات الفينولات؛ حيث كان لمستخلص *B.muricata* أعلى محتوى من الصابونين (22.94±0.10 µg DE/mg)، ومستخلص *S.foetida* كان له أعلى محتوى من الفينولات الكلية والفلافنويدات؛ 67.05±0.23 µg GAE/mg و 35.51±0.92 µg QE/mg على التوالي. وأفاد أيضا أن جميع المسخلصات تحتوي على العديد من أقسام المركبات الفلافنويدية؛ الأنتوسيانين، والفلافونول، والفلافانونات، والتانينات القابلة للإذابة، والتانينات المكثفة بقيم مختلفة. كما تميز مستخلص *B.muricata* و *S.foetida* بوجود مركبات الفلافانول دون باقي المستخلصات. وأسفر تحليل RP-HPLC عن وجود الروتين كمركبًا مشتركا في جميع المستخلصات بكميات متفاوتة.

أجريت عدة تجارب على المستخلصات الميثانولية لدراسة الفعالية البيولوجية؛ حيث لوحظ أن المستخلص الميثانولي لـ *B.muricata* سجل أكبر نشاطية مضادة للأكسدة، فقد بلغ التركيز المثبط لـ50% من الجذور الحرة •DPPH حوالي 9.63 µg/mL، بينما بلغت نسبة تحلل كريات الدم الحمراء 40% عند اختبار Anti-hemolysis. أما في اختبار β-Carotene-linoleic acid و Reducing power و Phosphomolybdenum فقد سجل المستخلص الميثانولي لـ *S.foetida* أفضل النتائج. أما عن دراسة نشاط المستخلصات الميثانولية في تثبيط نمو البكتيريا، فقد كان لجميع المستخلصات نشاطية مضادة للبكتيريا متوسطة. وأعطت الفحوصات الثلاثة للنّشاط المضاد للسكري تنبؤا جيّدا بإمكانية استخدام هذه النباتات كعلاج لخفض مستوى الغلكوز في الدم. و كان مستخلص *S.foetida* أفضل عامل حماية من الشمس 21.33±0.03. ومن خلال اختبار مضادات الالتهاب كان لمستخلص *B.murticata* أعلى تثبيط لتمسخ البروتين الالتهابي بنسبة 62.88% عند التركيز 500 µg/ml. وكان للمستخلصين *H.strobilaceum* و *S.fruticose* نشاط سمية خلوية ضعيف تجاه الخلايا Huh-7 و HepG2، حيث لم تتجاوز النسبة المئوية 30٪ للخلايا الميتة عند التركيز 100 µg/mL.

كلمات المفتاحية: تثمين، نواتج الأيض الثانوية، *Chenopodiaceae*، الصحراء الجزائرية.

Summary in French (Résumé)

Valorisation des métabolites secondaires produits de certaines plantes appartenant aux *Chénopodiacées* poussant dans le désert algérien

Cette étude a été menée sur six plantes médicinales du désert algérien appartenant à la famille des *Chénopodiacées* qui sont : *Anabasis oropediorum* Maire, *Basia muricata* L, *Halocnemum strobilaceum* Pall, *Salsola tetragona* Del, *Salsola foetida* Del, et *Suaeda fruticosa* L. Afin d'évaluer le métabolite secondaire des plantes dans le domaine pharmaceutique et semi-pharmaceutique.

Ces six plantes ont été valorisées en déterminant la valeur nutritionnelle de la partie aérienne de chaque plante, et en appliquant des analyses quantitatives et qualitatives, dont une analyse RP-HPLC des extraits méthanoliques (70%) de la partie aérienne. L'activité biologique a été évaluée *in vitro* par antioxydant, antibactérien, antidiabétique, anti-inflammatoire et facteur de protection solaire (SPF). En plus de tester la cytotoxicité des extraits *H. strobilaceum* et *S. fruticose*.

Les analyses chimiques de la valeur nutritionnelle a montré que la biomasse des parties aériennes des six plantes se caractérise par une forte teneur en minéraux, car toutes contiennent principalement du calcium. Elles contiennent également une importante teneur en eau qui indique sa capacité à résister aux conditions de croissance désertiques. Surtout *S. fruticosa*, dont la teneur en humidité dépassait 80 %. Elles contiennnent également des glucides, des lipides et des protéines, où chaque type de légume a des valeurs distinctes.

Le criblage phytochimique a révélé que les extraits méthanoliques de la partie aérienne des plantes étudiées contiennent qualitativement la plupart des métabolites secondaires; alcaloïdes, coumarines, gommes, phénols, saponines, et terpènes. L'analyse FT-IR a révélé la présence de composés phénoliques, aromatiques, d'amines aliphatiques, de composés organosoufrés, et d'alcanes.

Sur le plan quantitatif, le dosage quantitatif a indiqué que tous les extraits de plantes sont riches en saponines et en composés phénoliques, car l'extrait de *B. muricata* avait la teneur la plus élevée en saponines (22.94±0.10 µg DE/mg) et l'extrait de *S. foetida* avait la plus haute teneur en phénols et flavonoïdes totaux ; 67.05±0.23 µg GAE/mg et 35.51±0.92 µg QE/mg, respectivement. Il a également signalé que tous les extraits contiennent de nombreuses sections de composés flavonoïdes; anthocyanins, flavonols, flavanones, tanins hydrolysables et tanins condensés avec des valeurs différentes. Les extraits de *B. muricata* et *S. foetida* se distinguaient également par la présence de flavanols, sans le reste des extraits. L'analyse RP-HPLC a révélé que la rutine était un composé commun dans tous les extraits à des concentrations variables.

Plusieurs expériences ont été menées sur des extraits méthanoliques pour étudier leur activité biologique. Où il a été observé que l'extrait méthanolique de *B. muricata* an enregistré une plus grande activité que le reste des extraits. La concentration inhibitrice de 50% des radicaux libres $DPPH^{\bullet}$ était d'environ 9.63 ± 0.36 µg/mL, tandis que le taux de lyse des globules rouges était de 40 % lorsque l'hémolyse était testée à une concentration de 500 µg/mL. Dans le test acide *β*-carotène-linoléique, pouvoir réducteur, et phosphomolybdène, l'extrait méthanolique de *S. foetida* a donné les meilleurs résultats.

Quant à l'étude de l'activité des extraits méthanoliques dans l'inhibition de la croissance des bactéries, ils avaient une activité antibactérienne moyenne. Les trois tests d'activité anti-diabétique ont également donné une bonne prédiction de la possibilité d'utiliser ces plantes comme traitement pour abaisser le taux de glucose dans le sang. Alors que l'extrait de *S. foetida* était le meilleur écran solaire avec 21.33 ± 0.03. Grâce au test anti-inflammatoire, l'extrait de *B. murticata* présentait la plus forte inhibition de la dénaturation de la protéine inflammatoire de 62.88 à 500 µg/mL. Les extraits de *H. strobilaceum* et *S. fruticosa* ont une faible activité cytotoxique puisque le pourcentage de cellules mortes (cellules Huh-7 et HepG2) ne dépasse pas 30% à la concentration de 100 µg/mL.

Mots clés : Valorisation, métabolites secondaires, *Chénopodiacées*, Désert algérien.

Nomenclature

***Symbol*:**

°C Degree Celsius is unit of temperature

µg Micrograms

µL Microliter

Al Aluminum

Al_2O_3 Aluminum oxide

C Carbon

Ca Calcium

CaO Calcium oxide

Cl Chlorine

Fe^{2+} Iron

Fe_2O_3 Iron (III) oxide

Fe_2O_3 Iron oxide

K Potassium

L Liter

mg Milligrams

MgO Magnesium oxide

mL Milliliter

Na Sodium

Na_2O Sodium oxide

O Oxygen

S Sulfur

Si Silicon

SiO_2 Silicon dioxide

Zn Zinc

Abbreviations:

A549 Adenocarcinomic human alveolar basal epithelial

Abs Absorbance

APG Angiosperm phylogeny group

ATCC American Type Culture Collection

Caco-2 Colon cancer

CAM Crassulacean acid metabolism

Chl Chlorophyll

CIP Collection of the Institute Pasteur

CNV Central nervous system

DE Equivalent to diosgenin

DPPH$^{\bullet}$ 1,1-diphenyl-2- picrylhydrazyl

DW Dry weight

DU-145 Human prostate cancer cell line

GC Gas Chromatography

EC_{50} The effective concentration

EPH Epiphany

FW Fresh weight

GC/MS Gas Chromatography / Mass Spectrometry

HPLC High performance liquid chromatography

GC Gas Chromatography

HT-29 Human colon cancer

IC_{50} The half maximal inhibitory concentration

MCF-7 Michigan cancer foundation-7

MDA-MB-231 Triple-negative breast cancer cell line

PC3 Plotter configuration version 3

PCA Principal components analysis

RP-HPLC Reversed-phase high performance liquid chromatography

SPF Sun protected factor

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INTRODUCTION

Introduction

Due to the geographical location and the vast area, Algeria is characterized by climatic diversity favorable for the growth and development of medicinal and aromatic herbs. Mediterranean climate in the north (collectively known as the hill), semi-arid climate in the high plateaus (high plains) and arid across the desert (Azzi *et al.*, 2012). The Algerian Sahara represents more than 80% of the total area and 20% of the African desert. It also constitutes a resource for desert wild herbs, as it includes more than 500 species of higher plants that are still in common use by the population (Ozenda, 2004).

Plants have been used in medicine and have been a source of many medicines from the earliest times to the present. The medicinal properties of plants are due to the presence of active ingredients that make them a major source of bioactive molecules, known as secondary metabolites (Krishnaprabu, 2020). Plants produce secondary metabolites in order to protect against exposure to external attacks or to attract pollinators, but humans have been able to exploit them and use most of them in the treatment of various human diseases. Among the most important of these biologically active compounds are alkaloids, saponins, and phenolic substances (Tabasum *et al.*, 2016). These components can be extracted and used in the preparation of useful medicines. Through scientific studies the biological and therapeutic importance of plant-derived bioactive compounds traditionally used to treat many human ailments is increasingly being verified (Noor *et al.*, 2022), but there are still countless medicinal plants and beneficial herbs awaiting evaluation, exploitation, and determination of their effective therapeutic application.

The efforts of recent studies seek to reach chemicals from natural sources that have a logical, proven, medical effect that are used as alternatives to medicines and engineered products. The secondary metabolites of plants were the first destination for such research. Where these products are used after their discovery in general in the management of many diseases and disorders. It has produced during the past ages so far, many medicines either in their natural form or within the pharmacological modification melds through the exploitation of the metabolites of medicinal plants. In general, these plants have lower efficacy as bioactive compounds compared to pharmaceutical drugs, but because they are taken regularly and in large quantities as part of the diet, they may have a significant physiological effect in the long term (Ksouri *et al.*, 2012a). The secondary metabolites of the plant are also of nutritional importance due to their extensive use as flavoring agents, food preservatives, fibers, and oils. Additionally, they can be used as glues, waxes, perfumes, herbicides, and insecticides (Tabasum *et al.*, 2016).

Introduction

Desert plants are among the most important sources of secondary metabolites. This is because, in their arid desert habitats, they face severe environmental stresses such as erratic rainfall, high temperatures, and high salinity (El-Keblawy *et al.*, 2016). These arid conditions cause oxidative stress and increase the production of free radicals. Thus, these plants are forced to produce antioxidant products, such as phenolic compounds and flavonoids as strategies to develop supplements for survival and adaptation during different stages of their life cycle (Ghanem *et al.*, 2021). Halophytes, for instance, are naturally salt-tolerant plants that contain medicinal/nutritive properties and may be useful as new sources of functional compounds in nutritional foods, such as vitamins, polyunsaturated fatty acids, flavonoids, and tannins (Ksouri *et al.*, 2012a). Several studies have shown that drought is a factor in increasing the number of secondary metabolites in the tissues of various plant species (Liu *et al.*, 2011).

Mostly, phytochemical researchs depends on selecting plant species for study based on their traditional use, or the selection is done randomly. However, in this study, we focused on a group of plants that have both traditional use and belong to the same botanical family, in order to embody a possible relationship between the botanical families and biological activity and also with phytochemical content. Since this work aims to valorize the botanical resources of the Algerian Sahara, we chose the *Chenopodiaceae* family which is the most widespread in the region and has interesting traditional uses. The *Chenopodiaceae* family is one of the most prominent types of desert vegetation and most of its species are halophytic and have the ability to adapt to different climatic conditions (Dehghani *et al.*, 2021). Although many phytochemical studies have been conducted on the plants of this family at different regions of the world, they have not received sufficient phytochemical and biological evaluation from local research, despite the availability of many of them in the Algerian Sahara.

This work aims to extract the secondary metabolic products of some species of the *Chenopodiaceae* family growing in Algerian desert and evaluate them through the study of biological activity, in order to exploit these natural products in various fields such as food, pharmaceutical industries, or cosmetics. In the current study, six medicinal desert species belonging to the family *Chenopodiaceae* were highlighted, namely, *Anabasis oropediorum* Maire, *Bassia muricata* L, *Halocnemum strobilaceum* Pall, *Salsola tetragona* Del, *Salsola foetida* Del, and *Suaeda fruticosa* L. Where these plants are valued by characterizing their compounds on the one hand, and on the other hand, determining their biological efficacy, and since these six species have biological medicinal properties and therefore directed to the diet, their nutritional content was valued by conducting an analysis of the proximate compositions

of the plant biomass. Moreover, a set of analyses were performed to determine and characterize their phytochemical content through phytochemical screening, fourier-transform infrared spectroscopy (FT-IR), a set of quantitative assessments, and RP-HPLC analysis. Their biological effect was determined by a study *in vitro* for its antioxidant, anti-bacterial, anti-diabetic and anti-inflammatory activities of methanolic extracts. Also, the cytotoxicity of *Halocnemum strobilaceum* and *Suaeda fruticosa* extracts against Huh-7 and HepG2 cells was determined. As for the theoretical part, defining the concepts of the study variables was addressed; secondary metabolites, *Chenopodiaceae* family, and the studied plants.

CHAPTER I

Secondary metabolites

CHAPTER I

Metabolism is a series of chemical reactions that occur to various nutrients by enzymatic agents for the purpose of obtaining energy or building tissues. These interactions are based on the processes of demolition and construction (Rodriguez, 2022). Where the metabolic pathways can be classified into:

Primary metabolism that includes all metabolic pathways necessary for plant survival, responsible for the synthesis of metabolites directly involved in the growth and development of the organism in general. Such as carbohydrates, amino acids, fatty acids and nucleosides (Chen & Wang, 2017).

Secondary metabolism includes secondary metabolic pathways that are different from but related to primary metabolic pathways. It is responsible for the synthesis of a large number of secondary metabolites (Wink, 2010). This is the topic of this chapter.

1. Definition of secondary metabolites

Secondary metabolites are referred to in the literature as phytochemicals, bioactive compounds, plant xenobiotics, and antinutritional factors (Makkar *et al.*, 2007).

Phytochemicals are active non-nutritive organic compounds. They are low molecular weight molecules with diverse chemical structures. More than 100,000 different substances have been discovered in different plant species, and more are likely to be discovered (Hadacek, 2002; Fang *et al.*, 2019).

The secondary metabolites product in plants are synthesized according to specific metabolic pathways in tissues and/or organs by specific synthetic enzymes, according to the type of compound produced and the type of plant. These products accumulate and are stored in high concentrations in plant organs important for survival and reproduction; it represents one to three percent of the dry weight. In general, the lipophilic substances are deposited in the resin ducts, laticifers, trichomes, oil cells, or in the epidermis, and the hydrophilic compounds are stored in the vacuole. These compounds are transported from the synthesis site to the storage sites by xylem, phloem or, in some cases, via the apoplast (Wink, 2010; Chezem & Clay, 2016).

Secondary metabolites are produced in various plant species; trees, shrubs, herbs, cereals, legumes, vegetables, and fruits accumulate in different concentrations in plant parts; roots, stems, bark, leaves, flowers, fruits, and seeds. It is also produced in other organisms such as; algae, fungi, and lichens (Table 01) (Kathirvel, 2021).

Table 01. Some examples of secondary metabolites found in other organisms.

Organism	Scientific name	Secondary metabolite	Reference
Microalgae	*Muriellopsis sp.*	Lutein	(Del Campo *et al.*, 2001)
	Scenedesmus sp.	Lutein, *β*-carotene	(Schüler *et al.*, 2020)
Macroalgae	*Ulva rigida*	Sterols	(Li *et al.*, 2017)
	Laurencia genus	Sesquiterpenes	(Cikoš *et al.*, 2021)
Fungi	*Cystobasidium minutum*	Phenol,2,4-bis(1,1-dimethylethyl)-; Hexanedioic acid, bis (2-ethylhexyl) ester, and Butylated Hydroxytoluene	(Harikrishnan *et al.*, 2021)
	Grammothele fuligo		
	Rigidoporus vinctus		
Lichens	*Stereocaulon caespitosum*	Atranorin	(Jeon *et al.*, 2019)

2. Classification of secondary metabolites

There is no established, agreed-upon order for the classification of secondary metabolites. But according to their chemical structure, plant secondary metabolites can be divided into several class, the most important of which are: phenolic compounds, alkaloids, and terpenes. These classes are also classified into several subclasses according to their chemical composition as shown in Table 02.

2.1. Phenolic compounds

Phenolic compounds are compounds that are specific to the plant kingdom, as they are produced in all parts of the plant from the root to the fruit. Includes more than 8000 identified natural compounds. The main structural elements of phenolic compounds; it is the presence of at least one aromatic ring consisting of six carbons, directly related to at least one free hydroxyl group (OH) (A phenolic ring is shown in Figure 01 "A") and participating in another function: ether, esterification, or glycoside (heterocyclic sugars) (Siddiqui & Prasad, 2016). The main groups of phenolic compounds include flavonoids, phenolic acids, tannins, stilbenes, and lignans (Table 02).

Table 02. Classification of secondary metabolites according to their chemical structure, with an example of each subclass, its plant source, and its biological effect.

Class		Subclasses		Example (compound)	Plant	Effect	References
Phenolic compounds	**Simple phenols**	Phenolic acids	Hydroxycinnamic acid derivatives	Caffeic acid	*Malus domestica*	Antioxidants	(Nisar, 2022)
			Hydroxybenzoic acid derivatives	Gallic acid	*Ceratonia siliqua*		
		Flavonoids	Anthocyanidins	Malvidin	*Vaccinium corymbosum*		
			Flavanols	Catechin	*Camellia sinensis*		
			Flavonols	Quercetin	*Allium cepa*		
			Flavones	Apigenin	*Apium graveolens*		
			Flavonones	Naringenin	*Citrus fruits*		
			Isoflavones	Genistein	*Glycine max*		
		Phenolic alcohol		Oleuropein	*Olea europaea*	Anticancer	(Nediani *et al*., 2019)
	Complex phenols (Tannins)	Condensed tannins		Procyanidin-B2	*Vitis vinifera*	Antioxidants	(Kocabey *et al*., 2016)
		Hydrolyzable tannins	Ellagitannins	Punicalin	*Terminalia catappa*	Anti-inflammatory	(Lin *et al*., 1999)
			Gallotannins	Tannic acid	*Caesalpinia spinosa*	Antioxidants	(Cabezas *et al*., 2015)
	Stilbenes			Pinosylvine	Vine	Antioxidants	(Gabaston, 2018)
	Lignans			Matairesinol			

Alkaloids	**Nonheterocyclic alkaloids**	Tropane alkaloids		Colchicine	*Colchicum autumnale*	Cytotoxicity	(Qiu *et al*., 2014)
		Phenyl ethyl amine alkaloids		Mescaline	*Lophophora williamsii*	Asthma	
		Modified diterpene		Taxol	*Taxus baccata*	Anticancer	(Malik *et al*., 2011)
	Heterocyclic alkaloids	Mon-nuclear alkaloids	Pyridine	Lobeline	*Lobelia inflata*	Asthma	(Qiu *et al*., 2014)
			Pyrrol	Murrayanine	*Murraya koenigii*	Anti-inflammatory	(Mahapatra *et al*., 2018)
			Pyrrolidine	Nicotine	*Nicotiana tabacum*	CNS stimulants	(Cano, 2022)
			Piperidine	Coniine	*Conium maculatum*	Neurotoxin	
			Imidazol	Pilocarpine	*Pilocarpus jaborandi*	Parasympatholytic	
		Poly-nuclear alkaloids	Indole	Ajmalicine	*Rauwolfia serpentina*	/	(Qiu *et al*., 2014)
			Isoquinolin	Morphine	*Papaver somniferum*	Anesthetic	(Cano, 2022)
			Purine	Caffeine	*Coffea arabiga*	CNS stimulants	
			Quinazoline	Sparteine	*Sarothamnus scoparius*	Neurotoxin	(Qiu *et al*., 2014)
			Quinoline	Papaverine	*Papaver somniferum*	Antispasmodic	(Cano, 2022)
			Tropane	Atropine	*Atropa belladonna*	Anticholinergic	
Terpenes	**Monoterpenes (C_{10})**	Volatile oils		Pinene	Pine trees	Antiviral	(Cox-Georgian *et al*., 2019)
	Sesquiterpenes (C_{15})			/	*Helianthus annuus*	Antibacterial	
	Diterpenes (C_{20})			Phytol, gibberellin	/	/	
	Triterpenes (C_{30})	Saponins		Steroid saponin		Detoxifcation	
				Triterpene saponin			
		Sterols	Tocopherols	*β*-Tocopherols	*Allium cepa*	Antioxidants	(Aiboudi *et al*., 2016)
			Phytosterols	*β*-sitosterol		Anti-inflammatory	
	Tetraterpenes (C_{40})		Carotenoids	*β*-carotene	/	Important for vision	(Cox-Georgian *et al*., 2019)
			Xanthophylls	Lutein	*Muriellopsis sp.*	Antioxidants	(Del Campo *et al*., 2001)
	Polyterpenes			Resin	*Tree spruce*	Source of energy	(Demko & Machava, 2022)

Flavonoids are one of the most important classes of simple phenolic compounds (Table 02). This section includes more than 4000 identified compounds, most of which are the compounds responsible for the color in most parts of the plant. Its chemical structure consists of two aromatic rings and one heterocyclic ring (Figure 01 "B"). Flavonoids are of interest to researchers because of their large number and variety of biological activities (Kumar & Pandey, 2013).

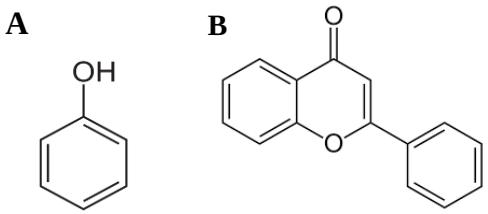

Figure 01. A, The chemical structure of a phenolic ring. **B**, General chemical structure of flavonoids.

Phenolic acids are one of the most common secondary metabolites found in foods. They are divided into two categories: hydroxycinnamic acid derivatives and hydroxybenzoic acid derivatives (Figure 02). The content of hydroxybenzoic acid in edible plants is generally low (Taofiq *et al.*, 2017).

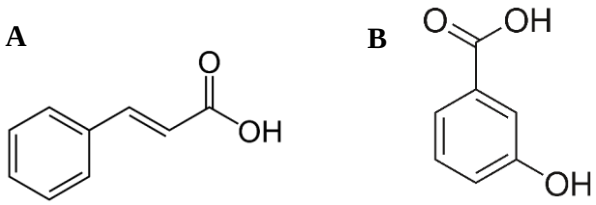

Figure 02. General chemical structure of; **A**, Hydroxycinnamic acid derivatives. **B**, Hydroxybenzoic acid derivatives.

Tannins are water-soluble phenolic compounds, molecular weight from 500 to >20000, prevalent in fruits, vegetables, legumes, and in some grains, located in the vacuole. They cause a pungent taste to plants, and have a biological importance that distinguishes them from others, which is the tanning of leather. The chemical structure of tannins is formed from condensing units of flavonoids (condensed tannins) (Figure 03 "A"), or esterifying D-glycose with gallic acid or its derivatives or ellagic acid, thus forming hydrolyzable tannins (Figure 03 "B, C") (Hall, 2008).

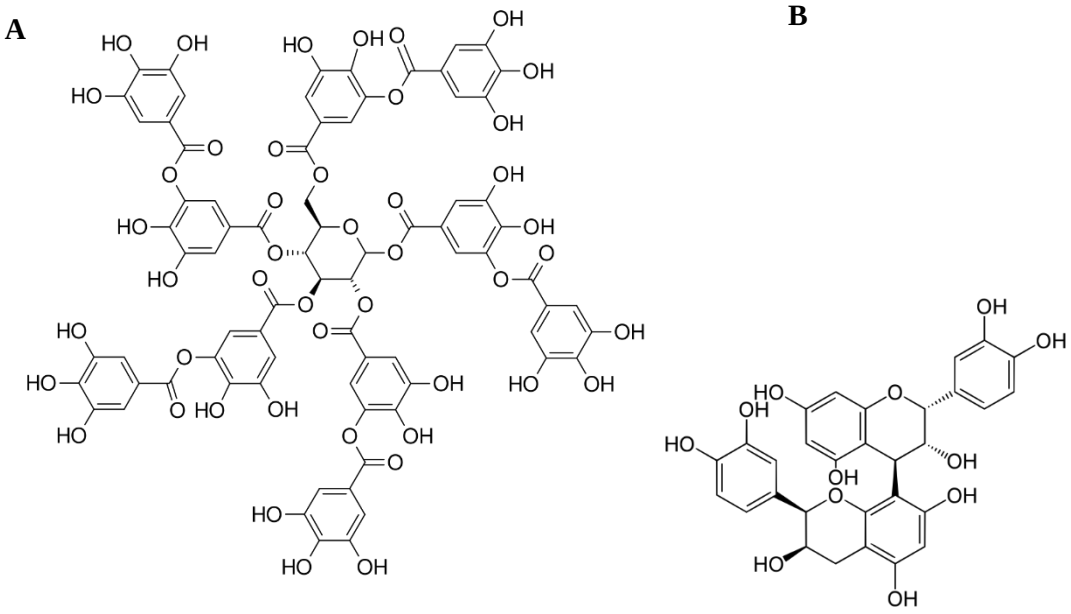

Figure 03. The chemical structure of; **A**, Tannic acid (hydrolyzable tannins). **B**, Procyanidin-B2 complex (condensed tannins).

2.2. Alkaloids

Alkaloids are basic nitrogenous organic compounds that contain in their structural makeup one or more nitrogen atoms. Produced mainly by vascular plants in most of the plant organs, depending on the type of plant. Its presence was also reported in some animals such as especially in millipedes, toads, salamanders, frogs, fish and mammals. Through scientific studies, more than 12,000 alkaloid compounds have been identified. Alkaloids are defensive substances that have the activity of deterring herbivores. It has also been proven to be toxic to insects, bacteria and fungi (Seneca, 2007; Siddiqui & Prasad, 2016). Some alkaloids are colored pigments, such as betalains pigment in some plants of the *Chenopodiaceae* family, and as attractants to pollinators such as in cactus flowers (Cseke *et al.*, 2006b). As for its biological function, it is represented as neuropharmaceutical, cancer chemopreventive, Inhibitors of microorganisms, anti-fungal infection, and antioxidant (Siddiqui & Prasad, 2016).

2.3. Terpenes

Terpenes are compounds derived from a mixture of two or more isoprene units (Figure 04). Terpenes are known as the smallest organic molecules that contain a large number of substances (more than 30,000 terpenoids), the most important of which are essential oils, carotenoids, rubber, and some plant hormones such as gibberellin and abscisic acid (Hanson, 2003; Reuben *et al.*, 2016).

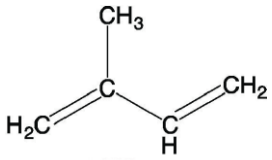

Figure 04. Isoprene unit.

2.4. Saponins

Saponins (Triterpenes) are widely distributed in a large number of plant species and in some marine organisms. The chemical structure of saponin is formed by the bonding of a glycone (sugar) moiety with a steroid or with a terpenoid (triterpene), and from it the saponins are divided into three important classes: steroid saponin (or steroidal) and triterpene saponin (Figure 05). Saponins have foam-forming properties so they are exploited in soap and detergent industry. A number of saponins mainly isolated from medicinal plants have shown pharmacological properties related to cancer inhibition, hormone stimulation, and anti-diabetic activities by modulating blood lipids, improving insulin level, glucose tolerance, and/or lowering blood glucose level (Qi *et al.*, 2010; Ofir, 2020).

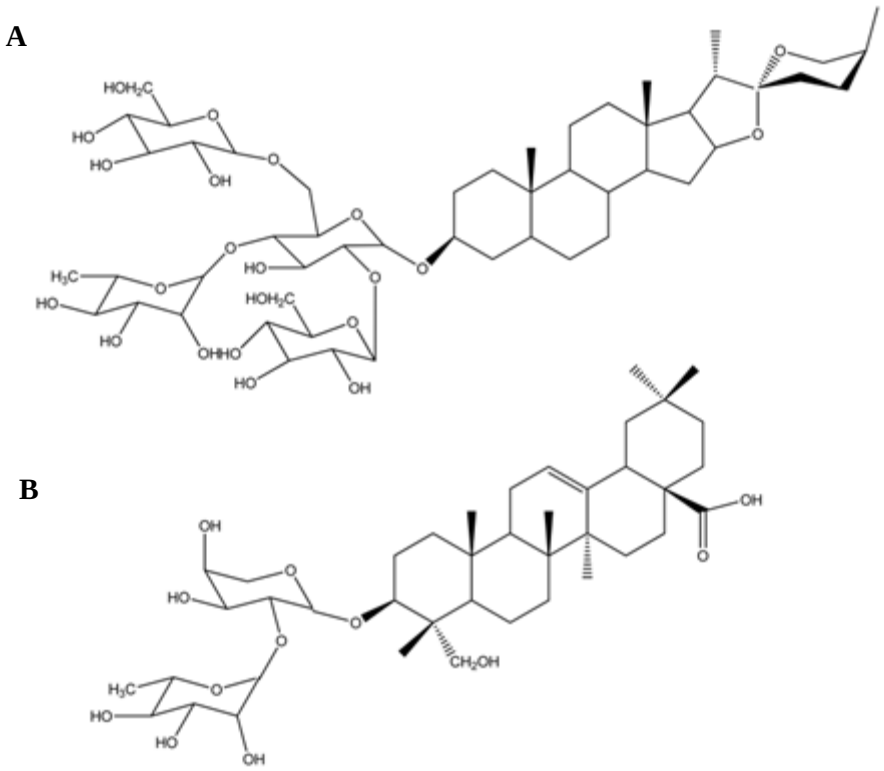

Figure 05. The chemical structure of; **A**, steroidal. **B**, triterpene saponin.

3. Biosynthesis of secondary metabolites

The biosynthetic shikimate pathway is known as the central pathway of plant metabolism that controls the metabolic flow. From carbohydrate metabolism to phenylpropanoid pathway (Hamberger *et al.*, 2006). According to the shikimic pathway, phenolic acids are synthesized by removing an amine group from the amino acids L-phenylalanine and L-tryptophan, in addition to a series of oxidation reactions and adding methyl groups, and through the condensation of phenolic acids, hydrolyzable tannins, lignin and lignans are built (Borrelli & Trono, 2016). According to the shikimic and malonate pathways, flavonoids are synthesized, since the chalcone complex is the main mediator in the synthesis of flavonoids, which results from the association of three malonyl-CoA molecules with a *p*-coumaroyl-CoA molecule, and the condensation of flavonoids results in condensed tannins (Cheng *et al.*, 2012). As for the biosynthesis of alkaloids, several methods have been proposed, where there are types of alkaloid compounds that are synthesized from polyacetate (Aliphatic amino acids) and there are other types that are synthesized from amino acids such as phenylalanine, tryptophane, ornithine, tyrosine, and lysine (Borrelli & Trono, 2016). Terpenes are synthesized from the mevalonate compound with a series of isoprene unit additives. Depending on the number of units added, the terpenes classes is formed. As shown in the diagram (Figure 06) (Mosquera *et al.*, 2021).

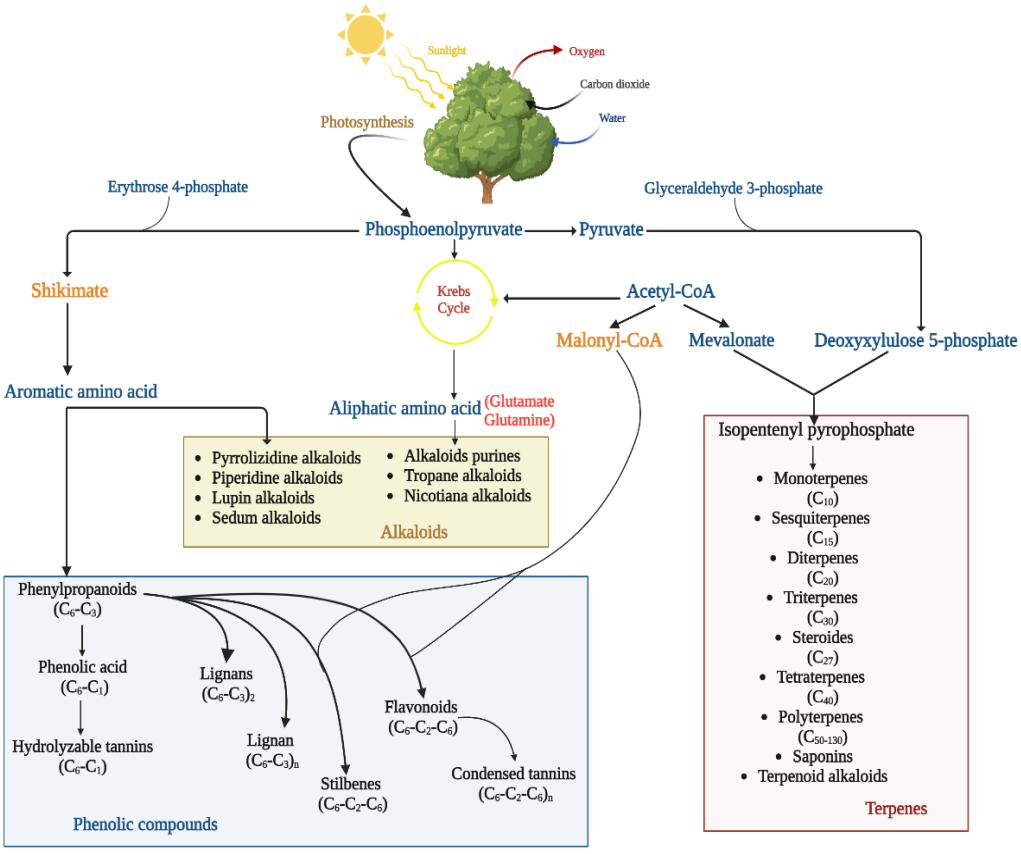

Figure 06. Schematic representation of the biosynthesis of secondary metabolites.

4. Phytochemicals extraction

The first step in the extraction of natural plant products is to identify the appropriate plants of interest and determine the period and habitat of their collection, then collect, dry and preserve them according to the appropriate preservation conditions (light, temperature, humidity ...). Cell rupture is also one of the most important steps in preparing the sample for extraction, as grinding is one of the most important methods used. For example, Taxanes (diterpenes) are extracted by grinding plant tissues in an organic solvent in a mortar (Cseke *et al.*, 2006a). However, there are cases of extraction that do not require grinding, as is the case when extracting volatile oils by steam distillation, where the raw or dried plant material is used without grinding (Runha *et al.*, 2001).

The plant compounds are extracted after preparing the sample using one of the modern techniques or the traditional method. The traditional method is the use of water or buffer, either hot or cold. This technique is also used in food preparation and in the popular use of medicinal plants. As for the methods of modern extraction techniques, they are represented in; using organic solvents, supercritical fluid extraction, microwave extraction, pulse electric field, ultrasound extraction and solid phase extraction *etc* (Cseke *et al.*, 2006a; Hamuel, 2012; Coskun, 2016).

In general, plant extraction is an experimental process where different solvents are used under different conditions such as extraction time and temperature. The choice of extraction technology depends on the physical and chemical properties of the compounds to be extracted. Sometimes further fractionation of the extracted active compounds is performed on the basis of their acidity, polarity or molecular size. Chromatography techniques are among the most important biophysical techniques for the separation and purification of plant components mainly, and include four types: column chromatography, thin layer chromatography (TLC), paper chromatography, gas chromatography, ion exchange chromatography, gel permeability chromatography, and high-pressure liquid chromatography (Cseke *et al.*, 2006a; Hamuel, 2012; Coskun, 2016; Ingle *et al.*, 2017).

5. Detection and analysis methods for phytochemicals

To explore new biomolecules for use in the pharmaceutical, food and agricultural industries, researchers resort to analysing plant extracts in one or different ways of quantitative and/or qualitative analytical techniques, which are:

- Phytochemical screening, a group of scientific tests that help detect the components of plant extracts.
- Fourier-transform infrared spectroscopy (FT-IR).
- Chromatography techniques as mentioned previously, it is a technique in which molecules are separated, and through which it is possible to obtain quantitative and/or qualitative data according to the species.
- Nuclear Magnetic Resonance Spectroscopy (NMR).
- Mass spectrometry (MS) (Okoli & Turay, 2009; Ingle *et al.*, 2017).

6. Biological importance of secondary metabolites

6.1. For a plant

Secondary metabolites are not essential for plant growth and development but help plant survival by:

Secondary metabolites act as buffers that plants use to defend against external attacks. They act as antibiotics against microbial invasion (bacteria and fungi), phytoanticipins and nematodes, and against pathogens (such as viruses, aphids, and nematodes). Many of them, especially volatile terpenes, also have the activity of allelochemicals against competitive herbs and plants (Demain & Fang, 2000).

Secondary metabolic products perform a dual function between the plant and its environment, as they act as deterrents to herbivores, as phenols have a deterrent effect on birds by affecting the intestinal bacteria, including their ability to digest. On the other hand, it attracts pollinators, fruit-spreading animals and symbiotic microbes. It also helps in general protection from UV rays (Nisar, 2022).

Phytochemical compounds act as growth hormones and differentiation effects (such as gibberellins) and signals to regulate metabolic pathways, aid in the germination process by cleansing the environment of competitive microbes during germination, and aid in the ovulation process by stimulating the formation of spores and protecting them from consumption by amoebae. Phenolic compounds, especially phenolic acids, work in the plant's response to several types of stress, and help heal damaged areas (Demain & Fang, 2000; Nisar, 2022).

6.2. For the human

Most of the secondary metabolites of plants are biologically active compounds that are used in the treatment of many disorders and diseases (shown in Table 02), the most prominent example of this, they are used to combat the oxidative damage caused by reactive oxygen species that cause the emergence of many human diseases such as diabetes, cancer, inflammatory conditions, and heart disease (Ferreira *et al.*, 2020). Humans also use many of these compounds for cooking and nutrition (colors, aromas, spices, flavors, and dietary enhancements), soap making, perfumes, and cosmetics (Wink, 2010), and seeks to value them in the field of agriculture by exploiting them in the manufacture of insecticides and herbicides (Shi *et al.*, 2020; Ngegba *et al.*, 2022). The researchers' efforts also seek to produce a hydrocarbon fuel and polymeric materials (paints, coatings, and adhesives) that is safe for the environment by using vegetable oils (Wang *et al.*, 2012; Lligadas *et al.*, 2013).

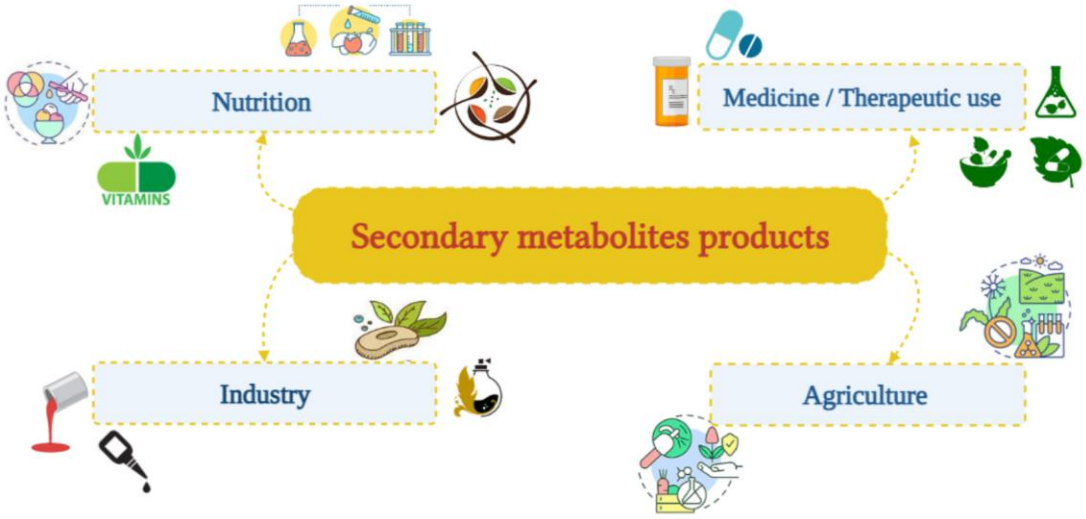

Figure 07. Fields of use of secondary metabolism products produced by plants.

CHAPTER II

Bibliography revision about Chenopodiaceae family

The *Chenopodiaceae* family consists of over 1420 known species of flowering plants in over 102 genera; within the order *Caryophyllales*. It is a sister group of the family *Amaranthaceae* (Borsch *et al.*, 2001). Algeria has 22 genera (For endemic species) and 45 species (Eight endemic species) of them. Specially; 17 genera and 31 species in the Algerian Sahara, it has two endemic species, one in the south of Oran (*Fredolia aretioides*), the other in the middle and west of the Sahara (*Nucularia*) (Quézel & Santa, 1962; Ozenda, 2004).

1. Geographic distribution

The family is widely distributed in temperate and subtropical saline habitats throughout the world. Especially around the Mediterranean, the Red Sea and the Caspian Sea. In the plains of central and eastern Asia, in the deserts of Asia and Australia, the coasts and salt deserts of central western north America and Argentina. It also requires species that grow in strong soil near homes and on the side of roads (Plantes Botanique, 2006).

Figure 08. A map showing the most important concentrations of *Chenopodiaceae* species in the world.

2. Botanical description

In the *Chenopodiaceae* family many species are similar to each other, even from one genus to another; and sometimes some species exhibit an astonishing polymorphism, which makes the appearance of the plant vary among plants of the same species, even from one branch to another, depending on the stage of growth and the season (Ozenda, 2004).

Mostly *Chenopodiaceae* species, succulent (*Suaeda*), halophyte (*Halocnemum*), nitrophily (*Chenopodium*) or cultivated plants (beets and spinach) (Ozenda, 2004).

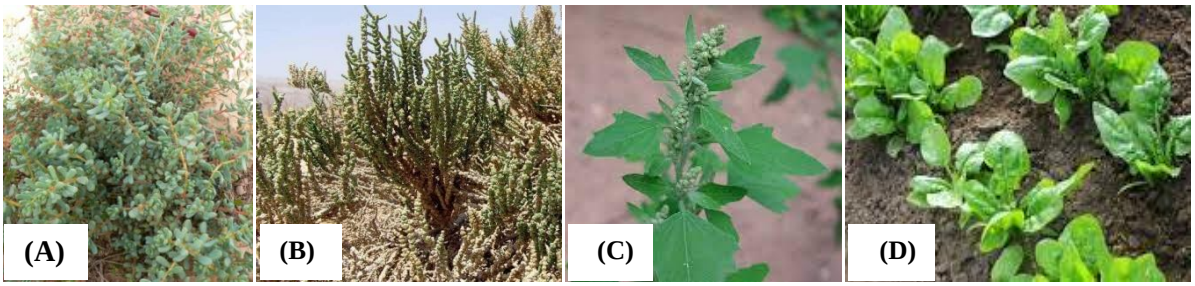

Figure 09. Various botanical varieties of *Chenopodiaceae*; **A**, *Suaeda vermiculata* (Succulent). **B**, *Halocnemum strobilaceum* (Halophyte). **C**, *Chenopodium album* (Nitrophily). **D**, *Spinacia oleracea* (Cultivated).

Plant species belonging to the *Chenopodiaceae* family are distinguished by the following characteristics:

2.1. Vegetation character

Most species in the *Chenopodiaceae* are annual or perennial herbs, subshrubs, shrubs, climbers, or small tree (*Haloxylon*) (Heklau *et al.*, 2012). The species of *Chenopodiaceae* show atypical secondary growth phenomena, where its stems and roots are equipped with a multiple combium ringe (Kraehmer & Baur, 2013).

The vegetative organs of the chenopods are characterized by:

- **Roots:** fibrous, taprooted or thickened (Heywood *et al.*, 2007).
- **Stems and branches:** succulent and/or jointed (Albers, 2002).
- **Leaves:** Often well-developped or reduced to amost absent (In order to adapt to their environments) (Albers, 2002). it is characterized by succulent (*Suaeda*), thick or waxy. Alternate or sometimes opposite, simple and without stipules and often lobed to three like a “goosefoot”, hence the name of the family which comes from the Greek words "cheén" for goose and "pous" for foot (Wiart, 2006) and sometimes it's dentate (*Chenopodium*) or pinnatifida, usually glabrous,

mealy textured or with glandular (often salt secreting) or glandular hairs (*Salsola*), sometimes spiny (*Cornulaca*) (Heywood *et al.*, 2007) (Figure 10).

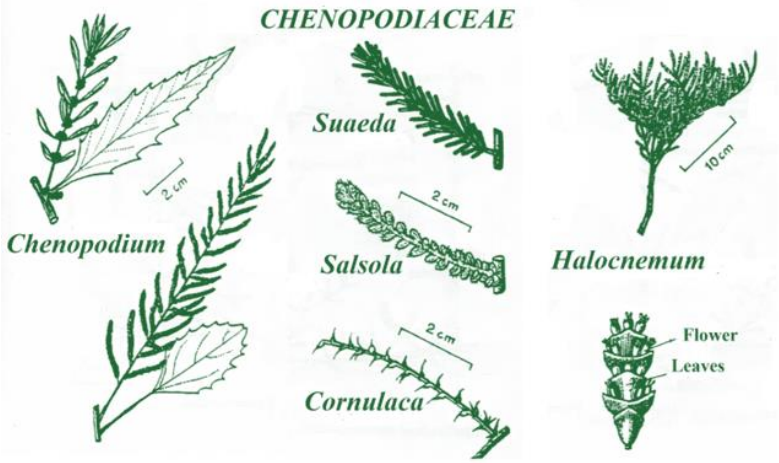

Figure 10. Schematic diagram showing the morphological appearance of the leaves of the most important genera of the *Chenopodiaceae* family.

- **Inflorescence and flowers:** Inflorescence in spikes or pannicles, or solitary flower sometimes, with or without bracts. The flower is very small, often invisible or very small (Figure 11). Unisexual or hermaphrodite, and actinomorphic. The floral periphery consists of 3 to 5 fused sepals (the petals are absent). Stamens five or less. The superior ovary, consisting of one, two or three fused carpels, with one ovary, the basal placentation. According to the following floral formula:

$$S_{(3\text{-}5)}\, A_5\, G_{\underline{(2\text{-}3)}}\, ☿ \text{ or } ♂ \text{ or } ♀ ⊕$$

Where; **S** sepals, **A** Stamen, **G** carpel, ☿ Hermaphrodite, ♂ Male, ♀ Female, ⊕ Symmetrical.

Flowering is generally autumnal by wind, and there is no evidence of self-incompatibility for *Chenopodiaceae* species (Mohlenbrock, 2001; Glimn-Lacy & Kaufman, 2006; Wiart, 2006; Romera *et al.*, 2013; Munro *et al.*, 2014).

Figure 11. Flowering branch of *Anabasis oropediorum* (Maire).

- **Fruits:** is nucule ronde or achene, more rarely a pyxis (Beta). The fruit is usually enclosed in a membranous sheath as the plant *Anabasis articulata* (Forssk.) Moq. (Figure 12).

Figure 12. Fruits of the plant *Anabasis articulata* (Forssk.) Moq.

- **Seeds:** The seed contains narrow cotyledons, the endosperm reduced or absent, abundant or absent (Plantes Botanique, 2006).

2.2. Chromosome number

The basic number of the *Chenopodiaceae* family is x = 9, since most species of the *Chenopodiaceae* family are diploid, including tetraploids, hexaploids, octoploids or decaploids, then the number of chromosomes is (2n=18, 2n=36, 2n=54, 2n=72 or 2n=90) (Behnke & Mabry, 2012; Lomonosova, 2018). with few derived exceptions in *Spinacia* x = 6, *Camphorosma* x = 6 and some species of *Petrosimonia* x = 8 and 7 in *Chenopoduim* (Leung & Marion, 2000; Ghaffari *et al.*, 2015).

2.3. Photosynthesis pathway

The *Chenopodiaceae* includes species depends on both C3 and C4 photosynthesis pathway (Kapralov *et al.*, 2006). Among the Dicotyledon, *Chenopodiaceae* family has the largest number of C4 species; about 550 out of 1420 species (Edwards *et al.*, 2004), and also the greatest diversity in leaf anatomy, include both C4 Kranz and C4 single-cell type species, the difference between the anatomy of C4 leaves lies in:

- The structure and arrangement of the two-layered chlorenchyma adjacent to the vascular bundles.
- By the presence or absence of water storage tissue, hypodermal cells, and sclerenchyma.
- Whether they have continuous or interrupted Kranz tissue (Voznesenskaya *et al.*, 2008).

Also, no species in this family has been reported to exhibit CAM photosynthesis pathway (Freitag & Stichler, 2002).

2.4. Photochemistry

In general, plants of the *Chenopodiaceae* family contain different groups of secondary metabolites of which the most important are alkaloids and coumarins, lipids, essential oils, flavonoids, sterols and steroidal oestrogen-like substances (Singh *et al.*, 2010).

3. Systematics

The *Chenopodiaceae* family is very close to *Amaranthaceae*, with some studies showing a monophyletic between the two, based on the morphology, anatomy and phytochemistry of the two families. (Kadereit *et al.*, 2003; Müller & Borsch, 2005; Heklau *et al.*, 2012; Paul, 2012; Bhargava & Srivastava, 2013). The similarity is embodied in the following characteristics:

- Minute sessile flowers.
- Basal or free-central placentation and anomalous secondary thickening.
- Having curved embryo surrounding the endosperm.
- Presence of betalain pigments.
- Sieve elements with P-type plastids but without a central protein crystalloid.

Chenopodiaceae differs from the *Amaranthaceae* by the presence of nonscarious perianth and mostly free filaments; whereas in *Amaranthaceae* the perianth is scarious and the filaments are mostly connate below (Paul, 2012).

However, on the basis of data from molecular phylogenies, the APG I system (1998), the APG II system (2003), and the APG III system (2009) classify the chenopods in the family *Amaranthaceae*.

Based on the following studies, the family now includes seven subfamilies (Kadereit *et al.*, 2012; Dehghani *et al.*, 2021):

- ***Betoideae*:** represents a small group, comprises in five genera, and 11–16 species (Iamonico, 2019), distributed in both western Eurasia, and western north America (Hohmann *et al.*, 2006). One of its most popular genera; *Beta* (Kadereit *et al.*, 2006a).
- ***Camphorosmoideae*:** circa 179 species distributed in Australia, Eurasia, north Africa, South Africa, and north America (Kadereit & Freitag, 2011). *Bassia*; it is one of the most famous of its genre (Sukhorukov & Kushunina, 2020).
- ***Chenopodioideae*:** includes about 26 genera and about 170 species. *Chenopodium* of the widest species (Fuentes-Bazan *et al.*, 2012).

- ***Corispermoideae*:** comprises only the three genera (*Corispermum*, *Agriophyllum* and *Anthochlamys*) and about 68 species. distributed in arid regions of Eurasia, central Asia, and north America (Kadereit *et al.*, 2003).
- ***Salicornioideae*:** clade with 13 genera and about 100 species, are found around the world (Dehghani *et al.*, 2021), of the most important genera *Halosarcia* (Kadereit *et al.*, 2006b).
- ***Salsoloideae*:** comprise the largest number of genera within *Chenopodiaceae*, it is split in two tribes, *Caroxyleae* and *Salsoleae*. Tribe *Salsoleae* includes one-third of all known genera currently recognized in the *Chenopodiaceae* family (Akhani *et al.*, 2007), and *Caroxyloneae* has 140 described species, being found in central Asia, Arabia, and northern and southern Africa (Akhani *et al.*, 2007).
- ***Suaedoideae:*** grouped in 2 tribes; *Bienertieae* and *Suaedeae* (Lara *et al.*, 2006). *Suaeda* is the mother of a genus belonging to this subfamily that has a global distribution, concentrated mainly in the coastal strips and central Asia (Plantes Botanique, 2006).

4. Economic importance

4.1. Food

The Chenopodiaceae family includes a few food plants such as beet (*Beta vulgaris*), beet leaf (*B. vulgaris* ssp. *maritima*) used as leafy vegetable, and lambs quarters (genus *Chenopodium*); (Singh, 2019). Quinoa seeds (*Chenopodium quinoa* Willd.) are also an exciting food crop, especially for the people of the Andes region in South America, as it is considered a source of essential amino acids, micronutrients, vitamins, minerals, fibers to health-promoting phytochemicals such as alkylresorcinols, saponins, and phenolic compounds (Agarwal *et al.*, 2022). There are also some wild species, such as *Salicornia herbacea* L, *Suaeda* edulis, and *Salsola soda*, which are considered edible herbs (Costa-Becheleni *et al.*, 2022; Murshid *et al.*, 2022; Noh *et al.*, 2022)

4.2. Forage

Many species, including *Salsola tragus*, *S. baryosoma*, and *Atriplex species* are widely used as livestock feed in different parts of the world (Murshid *et al.*, 2022).

4.3. Medicine

Many chenopods are used traditionally according to different cultures as an antidiabetic agent. In Algeria: *Atriplex halimus* L, *Salsola tetragona*, *Hammada elegans*, *Anabasis articulate* Moq, and in Morocco: *Atriplex halimus* L and *Cucurbita pepo* L., and in Saudi Arabia *Haloxylon*

salicorn leaves are used. Moq. In Japan, *Kochia scoparia* and in India, *Beta vulgaris* is used (Eddouks *et al.*, 2002; El Ghazali *et al.*, 2010; Qi *et al.*, 2010; Makheswari & Sudarsanam, 2012; Lakhdari *et al.*, 2016; Fouad & Lahcen, 2020).

The *Chenopodiaceae* family also includes many species that are used as anti-inflammatory and skin diseases (Benzineb *et al.*, 2019). *Chenopodium ambrosioides* and *Beta vulgaris* used in New York City to treat uterine fibroids and menorrhagia (Ososki *et al.*, 2002). The wild plant *Bassia muricata* is used to treat kidney and rheumatic diseases, and *Cornulaca monacantha* Del is used to treat liver diseases (Shaker *et al.*, 2013; Noh *et al.*, 2022).

4.4. Other uses

The *Chenopodiaceae* family includes saline and alkaline species called Ashnan that are used for washing clothes (Barkoudah & Henderson, 2006). Soda ash is also extracted from many chenopods that grow in salt marshes, coastal areas, and deserts (eg: *Suaeda*, *Salsola*, *Salicornia*) for use in making soap, glass, and porcelain (Tite *et al.*, 2006). Perennial woody species such as *Anabasis articulata* are used as fuelwood (Bidak *et al.*, 2015).

CHAPTER III

Studied plants

CHAPTER III

This chapter is concerned with presenting the plants that were valued during this work, as it includes their classification, morphological, geographical distribution and traditional use, with a list of the most important phytochemical studies related to each plant.

1. *Anabasis oropediorum*

1.1. Classification

Domain: Eukarya

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Dicotyledons

Order: Caryophyllales

Family: *Chenopodiaceae*

Genus: *Anabasis*

Species: *Anabasis oropediorum* (Maire)

Vernacular names: Ajram

1.2. Morphological description

A. oropediorum is a Saharan shrub a dark green color characterized by a thick, sinuous trunk, emitting articulated, almost leafless branches; Single flowers in the axils of each leaf, the fruit is surrounded by five membranous wings (Ozenda, 2004; Gamoun, 2014).

Figure 13. Different parts of *A. oropediorum*: A. Leafy twig; B. Fruiting branch; C. Perianth; D. Seed (Quézel & Santa, 1962).

1.3. Geographic distribution

A. oropediorum is a wild plant common on rocky ridges and sandy areas. Native to Palestine and north Africa (Bouaziz *et al.*, 2009).

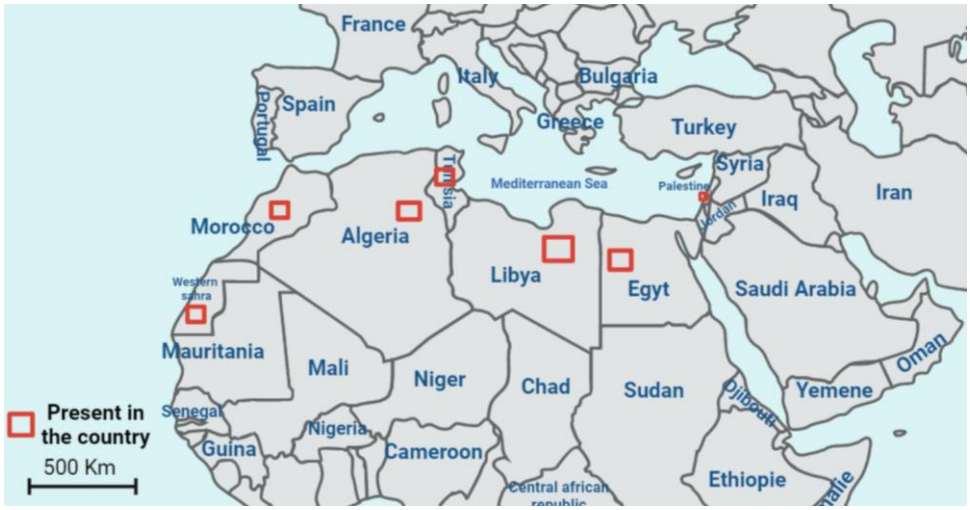

Figure 14. The geographical distribution of *A. oropediorum*.

1.4. The uses

*A. oropediorum*is traditionally used to treat skin diseases, as it is used specifically for eczema (Bouaziz *et al.*, 2009). Its trunks (firewood) are also used to kindle a fire. It is also a wild plant very palatable to livestock (Heneidy & Bidak, 2004).

1.5. Previous studies

No studies have been conducted on the chemical compounds or biological activities of this plant, except for the study of Bouaziz *et al.* (2009) where they determined its antioxidant efficacy and antimicrobial activity of the extract of hexane, methanol, water and ethyl acetate of aerial parts.

2. *Bassia muricata*

2.1. Classification

Genus: *Bassia*

Species: *Bassia muricata* (L.) Asch.

Synonyms: *Salsola muricata L.*, *Kochia muricata* L.

Vernacular name: Ghabitha

2.2. Morphological description

Annual densely branched herb, differ widely in habit, lower branches decumbent. Leaves sessile, linear-narrow elliptic, 10-20 mm long and 1.5-2.0 mm wide, with acute-attenuate tips. Inflorescence spicate, with 1-3 sessile flowers in the axils of leaf like bracts. Fruiting perianth with radiating spines, spines longer than the disc. Seeds orbicular, pale dark brown. Flowers exposed with loose hairs, stigma 1-1.8 mm, hilum/seed ratio less than 0.4. Fruiting: March to Mai (Turki *et al.*, 2006).

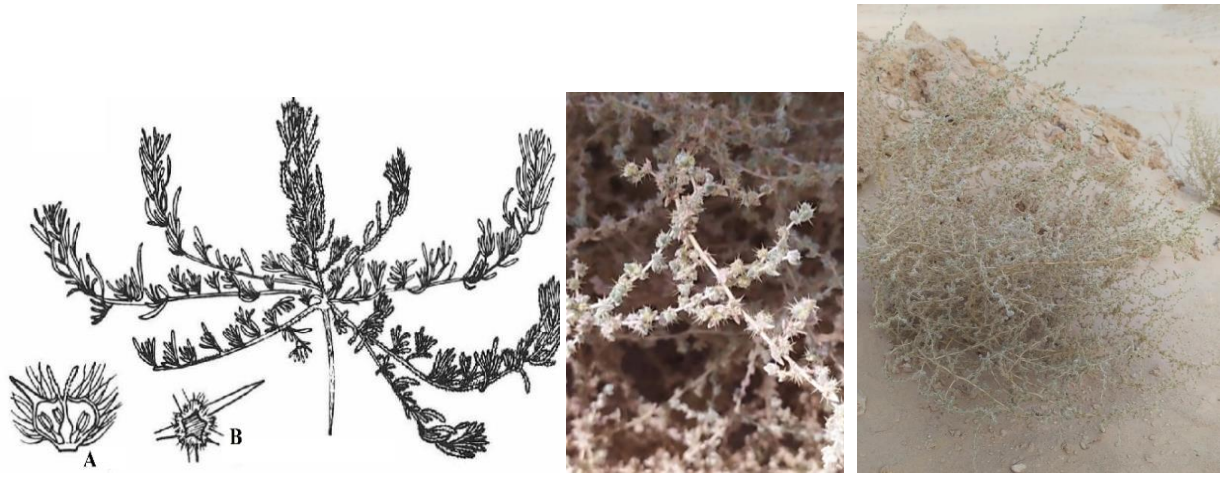

Figure 15. Different parts of *B. muricata*: A. Longitudinal section of a flower; B. Cross section of flower (Quézel & Santa, 1962).

2.3. Geographic distribution

B. muricata is a common species throughout the Sahara and especially in sandy soils and at the margins of desert roads (Turki *et al.*, 2006). It exists in North Africa, subtropical Asia (Saharo-Arabian and Iran) (Abd-ElGawad *et al.*, 2020).

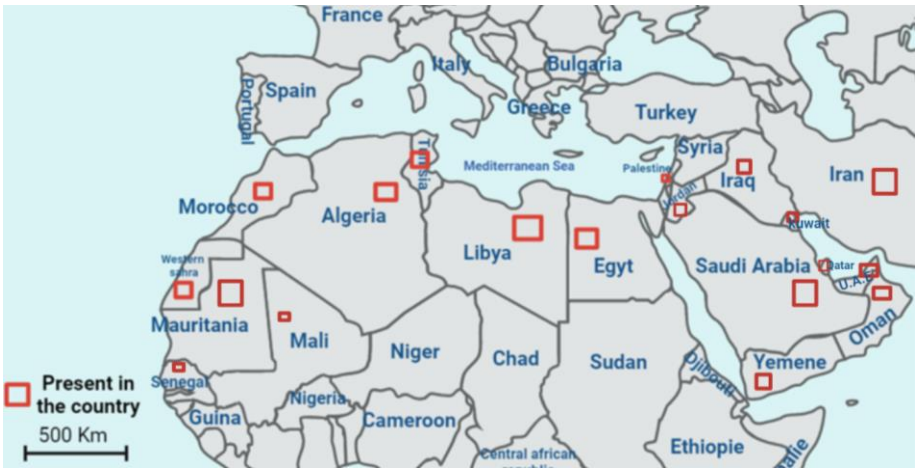

Figure 16. The geographical distribution of *B. muricata.*

2.4. The uses

The leaves and aerial parts of this plant are useful in traditional medicine, where they are used to treat skin diseases, pimples, boils, infected wounds, diarrhea, and skin diseases. It is also used as an anti-diabetic, analgesic and antipyretic plant, and to treat kidney disease, low blood pressure and spasm, and the seed oil is used to treat ulcers (Hammiche & Maiza, 2006; Awad, 2017; Boufous *et al.*, 2017; Mohammedi *et al.*, 2019).

2.5. Previous studies

Through previous biological activity studies, *B. muricata* has been shown to have antioxidant, antimicrobial, insecticidal properties (El-Sayed *et al.*, 1998; Bouaziz *et al.*, 2009; Chemsa *et al.*, 2016). Through chemical isolation reports; Kamel *et al.* (2001) isolated eight chemical compounds, and their structure was determined by MS and NMR spectroscopy: two types of acylated flavonoid glycosides (Quercetin-3-O-(6"-feruloyl)-sophoroside and quercetin-3-O-(6-caffeoyl)-sophoroside) as a new compound, two known flavonoid glycosides (Quercetin-3,7-O-beta-diglucopyranoside and quercetin-3-O-sophoroside), four known triterpenoidal saponins (Chikusetsusaponin IVa, chikusetsusaponin IVa methyl ester, oleanolic acid-3-O-beta-glucopyranoside, and oleanolic acid-3,28-beta-diglucopyranoside).

In other phytochemical study by Shaker *et al.* (2013), they isolated and identified three metabolites which are 3′-methylquercetin, 3,4-dimethoxytoluene, and flavonoid glycoside (3-O-[α-L-arabinopyranosyl-(1→2)-L-α-arabinopyranosyl)]-3′-methylquercetin).

3. *Halocnemum strobilaceum*

3.1. Classification

Genus: *Halocnemum*

Species: *Halocnemum strobilaceum* (Pall.) M. Bieb.

Synonym: *Salicornia strobilacea* Pall. (1771)

Vernacular names: Guerina

3.2. Morphological description

Shrub, annual, pale green, with short root, woody branched stem, 60-25 cm tall; leaves reduced to articulated, short triangular segments (Figure 17). The flowers are small, have three membranous petals, grow from the lateral axillary of the leaves, where they bloom during the

fall season (October-November). The fruit is a brown grape with wings 7-8 mm wide (Ozenda, 2004).

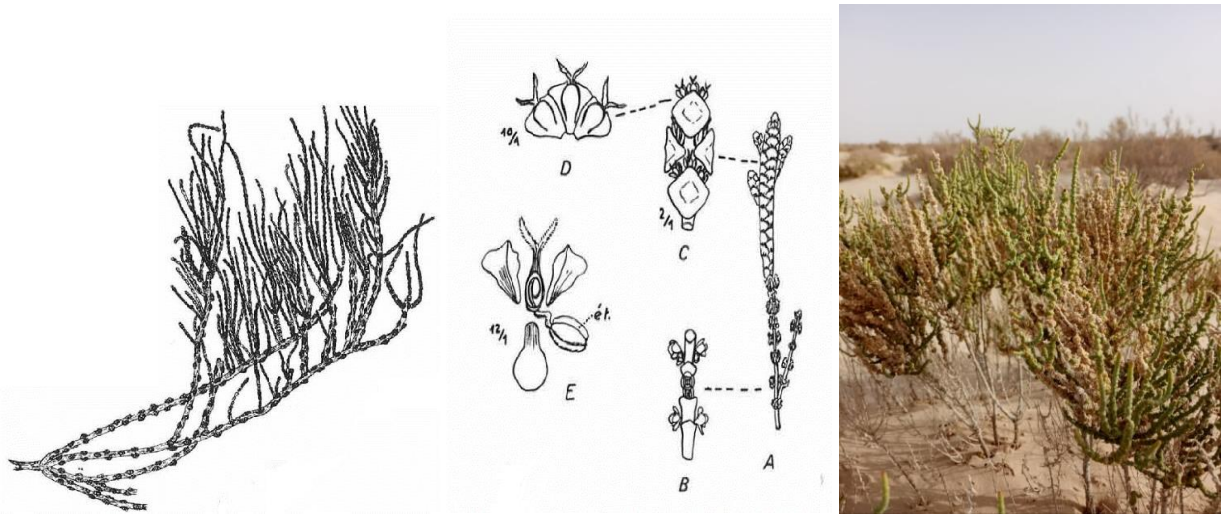

Figure 17. Different parts of *H. strobilaceum*: A. Port; B. Fragment of flowering spike; C. Fragment of flowering spike; D. Floral leaf; E. Flowers (Quézel & Santa, 1962).

3.3. Geographic distribution

H. strobilaceum is a succulent halophytic plant that grows in low lying wet saline soil or beside lakes, in coastal, desert and dryland biomes. It is widespread in the countries of the Mediterranean, Central and Western Asia, the Arabian Peninsula, Iran, Pakistan, Mongolia, Russia, and China (Su *et al.*, 2012).

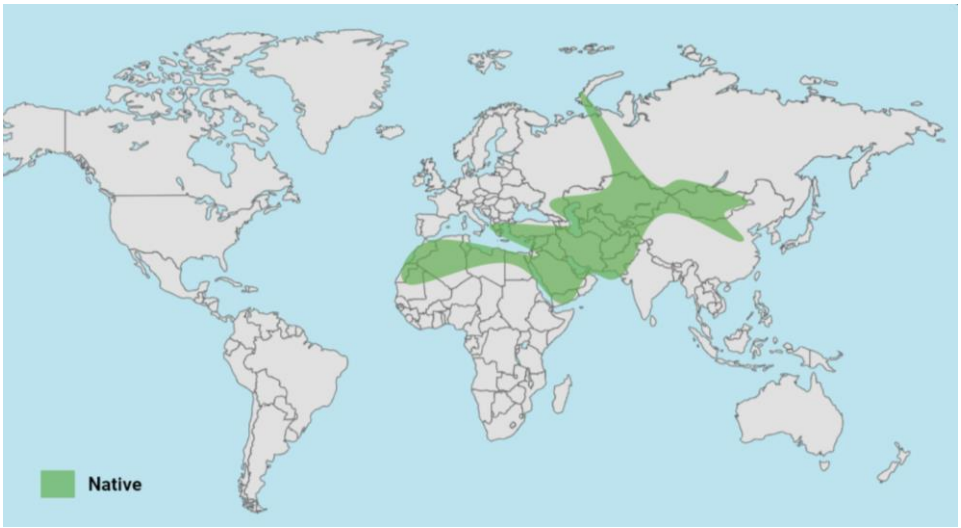

Figure 18. The geographical distribution of *H. strobilaceum*.

3.4. The uses

This salt-grass is one of the most important saline forage pasture plants (Singh, 2005), but it is believed that it causes lung disease in camels when it is overfeeding (Acheuk *et al.*, 2018). In folk medicine, it is used as a stimulant for the digestive system and a treatment for fever and headache (Abdelrazek *et al.*, 2019). It is also used to treat women's problems; pregnancy difficulties and dysmenorrheal (Heneidy *et al.*, 2017).

3.5. Previous studies

Through the foregoing researches, the effectiveness of the ethanol extract of this plant as an insecticide was evaluated, against *Tribolium castaneum* (Coleoptera: *Tenebrionidae*) (Acheuk *et al.*, 2018), and hexane extract of roots and aerial part as an antimicrobial activity and cytotoxicity (Abdelrazek *et al.*, 2019). It has also been proven that this plant has antioxidant, anti-diabetic properties, and anticancer activity against MCF-7, PC3, and A549 cells (El-Manawaty & Gohar, 2018; Mariem & Mari, 2018; Handoussa *et al.*, 2019).

Chemical investigations of *H. strobilaceum* have shown that it contains various phytochemicals, including saponins, flavonoids, anthocyanins, tannins, coumarins, and alkaloids (Acheuk *et al.*, 2018). Specifically, it contains coumarin, 7-hydroxy-3-methylcoumarin, oreoselone, heraclenin (Miftakhova *et al.*, 2001), scopoletin, stigmasterol, α-amyrin, Campesterol, β-sitosterol (Radwan & Shams, 2007), *p*-coumaric acid, rutinhydrat, 3,4-Dimethoxybenzoic acid, O-coumaric acid, rosmarinic acid (Mariem *et al.*, 2018), cromolyn, and astaxanthin (El-Amery *et al.*, 2020). This halophyte also contains mineral elements; sodium, potassium, calcium, magnesium, and vitamins C and E (Maatallah Zaier *et al.*, 2020).

GC/MS analysis of Abdelrazek *et al.* (2019) work resulted in *H. strobilaceum* containing saturated fatty acids (hexadecanoic acid, a methyl ester and 9-octadecenoic acid (Z)-methyl ester), long-chain hydrocarbons (hexadecane, pentadecane, heptadecane, and octadecane), alkyl hydrocarbons (icosan, docosan, tricosan, tetracosan, pentacosan, and heptacosan), and hydrocarbons (dodecane, heptadecane, and octadecane). GC/MS analysis in (Mariem *et al.*, 2018) study revealed the presence of five saturated fatty acids (lauric acid, myristic acid, palmitic acid, stearic acid, and behenic acid), oleic acid, and linoleic acid.

4. *Salsola tetragona*

4.1. Classification

Genus: *Salsola*

Species: *Salsola tetragona* Delile.

Synonym(s) Homotypic: *Caroxylon tetragonum* (Delile) Moq.

Vernacular names: Belbel

4.2. Morphological description

This halophyte is small. Stem well-branched, with four corners branching, erect, well defined. Leaves opposite, sessile, semi-globular, grey. The flowers are solitary and sessile in the leaf axils between two bracteoles (Figure 19). Flowering in November December (Ozenda, 2004; Chehma, 2005).

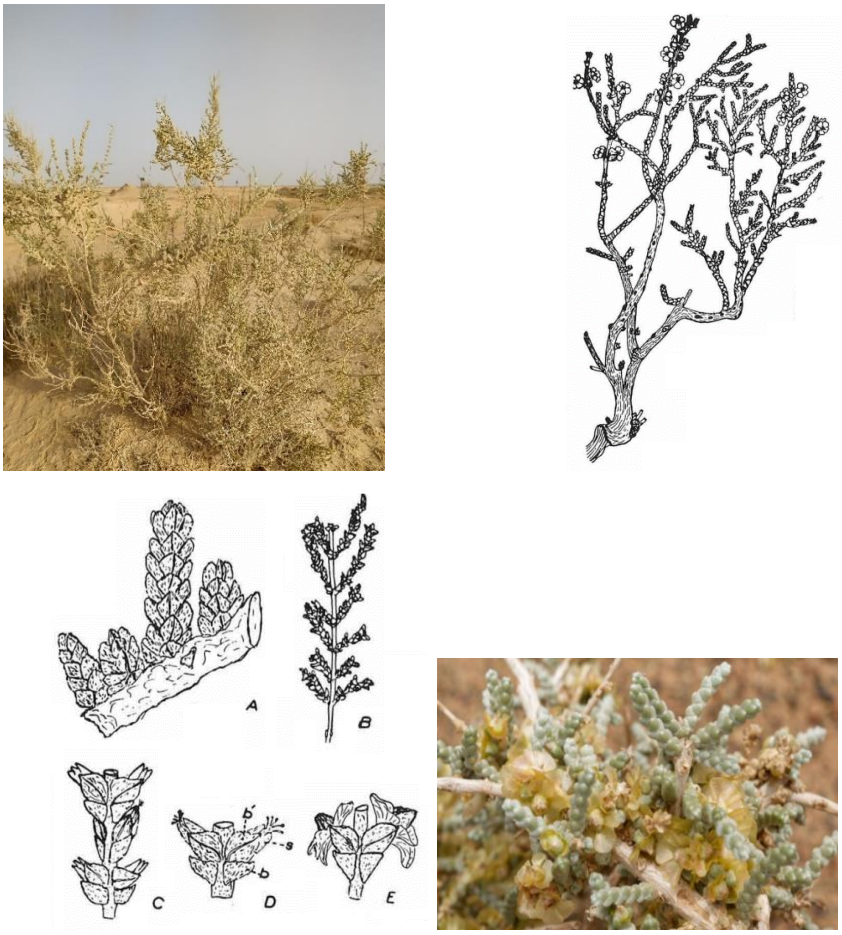

Figure 19. Different parts of *S. tetragona*: A. non-flowering branch; B. part of a flowering branch; C. Three pairs of flowers; D. one pair of flowers (two opposite flowers): each flower has one bract b two bracteoles b' and five sepals s; F. pair of fruits (Quézel & Santa, 1962).

4.3. Geographic distribution

S. tetragona is one of such plant grows in saline habitats, it is found from the desert of Morocco to Palestine and Jordan (Plants of the World Online).

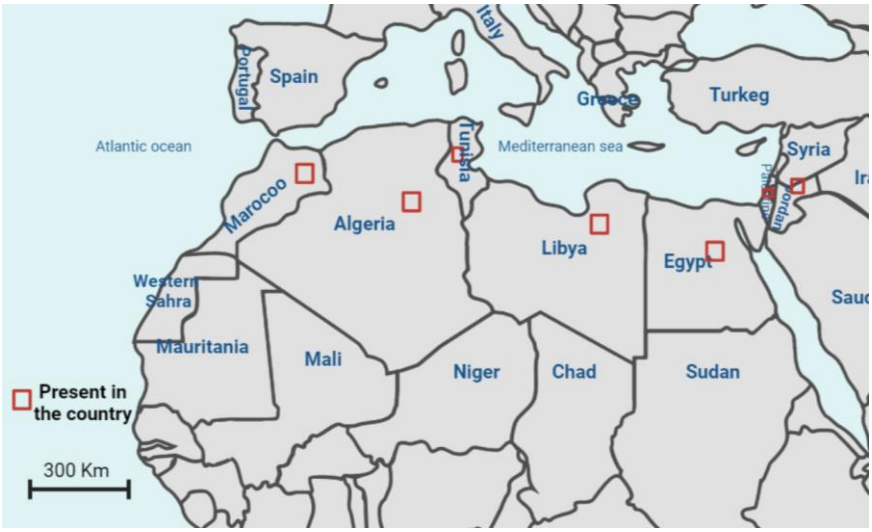

Figure 20. The geographical distribution of *S. tetragona*.

4.4. The uses

S. tetragona is used as an important medicinal plant to treat diabetes and skin wounds or infections. It is used to relieve vomiting, indigestion and fever (Martínez *et al.*, 2010; Ghourri *et al.*, 2013). It is one of the types of plants that are nurtured by animals in the Mediterranean countries (Laudadio *et al.*, 2009).

4.5. Previous studies

According to phytochemical studies, the aerial part of *S. tetragona* possesses five phenolic compounds (phloglucin, vanillic acid, protocatchuic acid, canthoside C, and canthoside D), and five cardenolides; salsotetragonin, uzarigenin, desglucouzarin, 12-dehydroxy-ghalakinoside and calactin. three flavonoids; kaempferol-3-O-β-D-glucopyranoside, quercetin-3-O-β-D-glucopyranosyl-(1→6)-glucopyranoside, and rutin. It also contains two fatty acids; oleic acid and 2,3-dihydroxypropylpalmitate, and it contains Mg, Si, Cl, Cu and Zn (Ghorab *et al.*, 2017; Taia *et al.*, 2018).

5. *Salsola foetida*

5.1. Classification

Genus: *Salsola*

Species: *Salsola foetida* Delile ex Spreng.

Synonyms: *Caroxylon imbricatum* (Forssk.) Moq., *Chenopodium baryosmum* Schult., *Salsola baryosma* (Schult.) Dandy., *S. imbricata* Forssk. subsp. imbricata., *S. vermiculata* var. scopiformis Maire.

Vernacular names: Gudham

5.2. Morphological description

S. foetida is a perennial shrub with thick, woody white stems, 1-4 feet in height. The leaves are spherical, smooth, and have a slightly unpleasant odor (Ozenda, 2004). Flowers are green, barely visible. Spring in the spring period (March-May) (Chehma, 2005).The fruits are very small (2-4 mm) with white membranous wings perianth (Quézel & Santa, 1962).

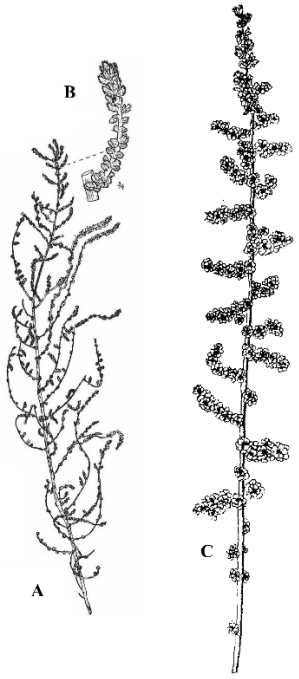

Figure 21. Photograph of *S. foetida*: A. flowering branch; B. enlarged details of a branch; C. fruitful branch (African Plants Database).

5.3. Geographic distribution

This wild halophyte grows in North Africa, the deserts of the Middle East, Iran, Afghanistan, Pakistan, and northwest India (Shehab & Abu-Gharbieh, 2014).

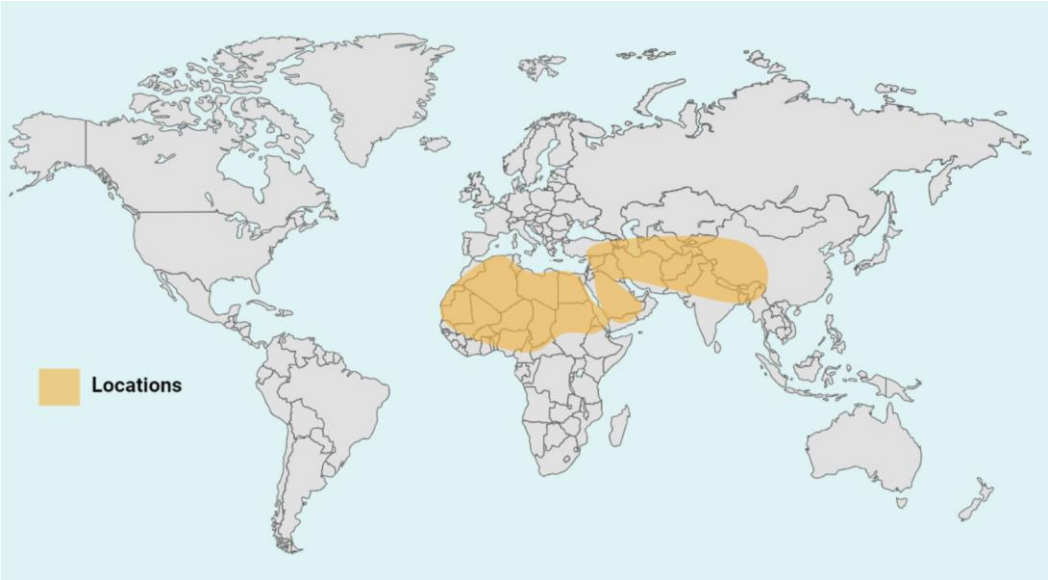

Figure 22. The geographical distribution of *S. foetida*.

5.4. The uses

S. foetida is an edible halophyte, and it is considered one of the most important grazing plants for camels, but it is less consumed by other herbaceous animals. However, it has been proven to be used as an anthelmintic for livestock (Chehma, 2005; Ajaib *et al.*, 2019). It has also been widely used to treat various diseases such as indigestion, diarrhea, dysentery, cold, and asthma. It also relieves skin itching, sinus congestion and is considered as diuretic and female contraceptive (Shehab & Abu-Gharbieh, 2014; Aslam & Janbaz, 2017).

5.5. Previous studies

Scientific research has proven that the extract of the aerial part of *S. foetida* has anti-inflammatory, hepatoprotective, antimicrobial, antispasmodic activities, and helps relieve bronchiectasis (Shehab *et al.*, 2015; Gannoun *et al.*, 2016; Osman *et al.*, 2016; Aslam & Janbaz, 2017). It also possesses antidiabetic activity (Khacheba *et al.*, 2014), tyrosinase inhibitor (Khan *et al.*, 2003), central nervous system stimulant, and antioxidants activity (Khan *et al.*, 2003; Khacheba *et al.*, 2014; Shehab *et al.*, 2015). Additionally, it's *in vivo* antifertility potential has been confirmed (Shehab & Abu-Gharbieh, 2014). But it has no cytotoxic effect (Taha & Alsayed, 2000; Osman *et al.*, 2016).

This plant has also received many scientific studies related to determining its phytochemical content, the most important of which can be described and summarized as follows:

The GC analysis of the study (Gannoun *et al.*, 2016) confirmed that the leaves, stems and roots of *S. foetida* are rich in volatile compounds such as carvone. The researcher (Ajaib *et al.*, 2019) revealed, through chemical detection, the presence of flavonoids, tannins, saponins, alkaloids, anthraquinones, oligosaccharides, and cardiac glycosides. Previous phytochemical analysis of the aerial part of this wild plant also confirmed that it was rich in phenolic compounds (Shehab *et al.*, 2015; Osman *et al.*, 2016; Ajaib *et al.*, 2019), and resulted in the presence of:

Phenolic acids; gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, vanillic acid, ellagic acid, benzoic acid, coumaric acid, cinnamic acid, isovanillic acid, ferulic acid, and *p*-hydroxy benzoic acid.

Flavonoids; catechin, chrysin, quercetin, rosmarinic acid, hesperidin, rutin, quercitrin, naringin, hesperitin, apigenin, scopoletin, bergaptol, daphnoretin, bergaptol 5-O-β-D-glucopyranoside, daphnorin and chrysoeriol 7-O-β-D-glucopyranoside.

Compound isorhamnetin and isorhamnetin glycosides (isorhamnetin-3-O-β-D-glucuronyl (1” → 4”) glucuronide, isorhamnetin-3-O-β-D-diglucuronate dimethyl ester, and isorhamnetin-3-O-β-D-galactopyranoside). In addition to having both; N-trans-Feruloyltyramine and catechol.

6. *Suaeda fruticosa*

6.1. Classification

Genus: *Suaeda*

Species: *Suaeda fruticosa* (L.) Forssk.

Synonym(s) Homotypic: *Chenopodium fruticosum* L., *Chenopodina vera* (Forssk. ex J.F. Gmel.) Moq., *Suaeda* vera (Forssk. ex J.F. Gmel.) Maire & Weiller.

Vernacular names: Suaeda

6.2. Morphological description

S. fruticosa is a dense succulent tree of dark green color with a height of 50 cm. The leg is woody, very branched, puberulent becoming glabrous. Leaves alternate, fleshy. green, more or less linear, rounded in section, 5-30 mm long 0.5-1.5 mm across, glabrous or glabrescent. Flowers small, in three to five flowered axillary clusters arranged in leafy spikes, subtended by a bract and two bracteoles; bract leaflike, longer than flowers; bracteoles shorter than flowers,

membranous. Perianth green, of five more or less equal segments. Stamens five (anthers yellow). Ovary superior. Fruit a achene with membranous (Miller & Morris, 1988).

Figure 23. Different parts of *S. fruticosa*: A. Flowering branch; B. Male flower; C. Etamine; D. Fruit; E. Seed; F. Seed (cut) (Quézel & Santa, 1962).

6.3. Geographic distribution

S. fruticosa is a euhalophytes, it has mechanisms that make it resistant to high salinity levels (Maatallah Zaier *et al.*, 2020). It grows in saline and wet soils, in marshes or palm orchards (Chehma, 2005). This wild plant has a global distribution, as it is found in South America (the San Joaquin Valley south to Mexico), south of Europe and Africa and Asia (Asia Minor, the Arabian Peninsula, Iran, Afghanistan, Pakistan, India) (Paysen, 1980).

Figure 24. The geographical distribution of *S. fruticosa*.

6.4. The use

S. fruticosa is an edible herb (Oueslati *et al.*, 2012a), forage for cattle and wild animals (Singh, 2005), and is used in traditional medicine to treat skin diseases, specifically; herpes, scabies and dermatitis (El-Mokasabi *et al.*, 2018). Hypoglycemic and hypolipidemic activities are known to occur when ingested (Ksouri *et al.*, 2012a).

6.5. Previous studies

Through previous studies, it was confirmed that *S. fruticosa* has a wide spectrum of biological activities, namely: antioxidant, anti-inflammatory, antibacterial, anticancer, hepatoprotective, and hypoglycemic effect *in vivo* (Rashid *et al.*, 2000; Benwahhoud *et al.*, 2001; Oueslati *et al.*, 2012a; Oueslati *et al.*, 2012b; Ullah *et al.*, 2012; Rehman *et al.*, 2013). Its ability to inhibit the enzymes: acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-amylase, and α-glucosidase has also been verified (Saleem *et al.*, 2021). As demonstrated by Oueslati *et al.* (2012b) that it has activity against Detroit 551, Caco-2, HT-29, and A549 cells. By quantitative RP-HPLC analysis for the study of (Qasim *et al.*, 2017) it was proved that *S. fruticosa* contains quercetin, kaempferol, caffeic acid, chlorogenic acid, catechin, and gallic acid.

CHAPTER IV

Materials & Methods

CHAPTER IV

This chapter includes a description of the following procedures:

Materials

- Plant material
- Bacterial strains
- Cells
- Chemicals

Methods

- Valorization of plant biomass.
- Obtaining plant samples and extracts.
- Phytochemical analysis of plant samples.
- Biological efficacy evaluation tests.

1. Materials

1.1. Plant material

The aerial parts of *A. oropediorum*, *B. muricata*, *H. strobilaceum*, *S. tetragona*, *S. foetida*, and *S. fruticosa*, were collected from four different location of the northern border for Algerian Sahara; Ben Ghasha, Debila, Chott Zibahir in Magrane, and Chott Amar in Sidi Amrane (Figure 25 and Table 03), during the flowering period (spring-autumn 2020). These plant species have been identified taxonomically using botanical references; "Flore et végétation du Sahara" (Ozenda, 2004), "Nouvelle flore de l'Algérie et des régions désertiques méridionales" (Quézel & Santa, 1962) and (African Plants Database), and confirmed by Pr. Noureddine SLIMANI (Higher School of Desert Agriculture, El Oued University) and Dr. Youcef HALIS (Centre of Scientific and Technical Research on Arid Regions, Touggourt, Algeria). The collected plant materials were air-dried in darkness at room temperature.

Table 03. Site geography, collection dates, and altitude of studied plants.

N°	Plants	Geographic coordinates	Name of region	Collection dates	Altitude
01	*A. oropediorum*	34° 12′N, 7° 19′E	Ben Ghasha	October	169 m
02	*B. muricata*	33° 31′N, 6° 56′E	Debila	March	56 m
03	*H. strobilaceum*	33° 49′N, 6° 55′E	Chott Zebahir	March	-26 m
04	*S. tetragona*	33° 49′N, 6° 55′E	Chott Zebahir	May	-21 m
05	*S. foetida*	33° 31′N, 6° 56′E	Debila	May	54 m
06	*S. fruticosa*	33° 31′N, 6° 00′E	Chott Amar	October	42 m

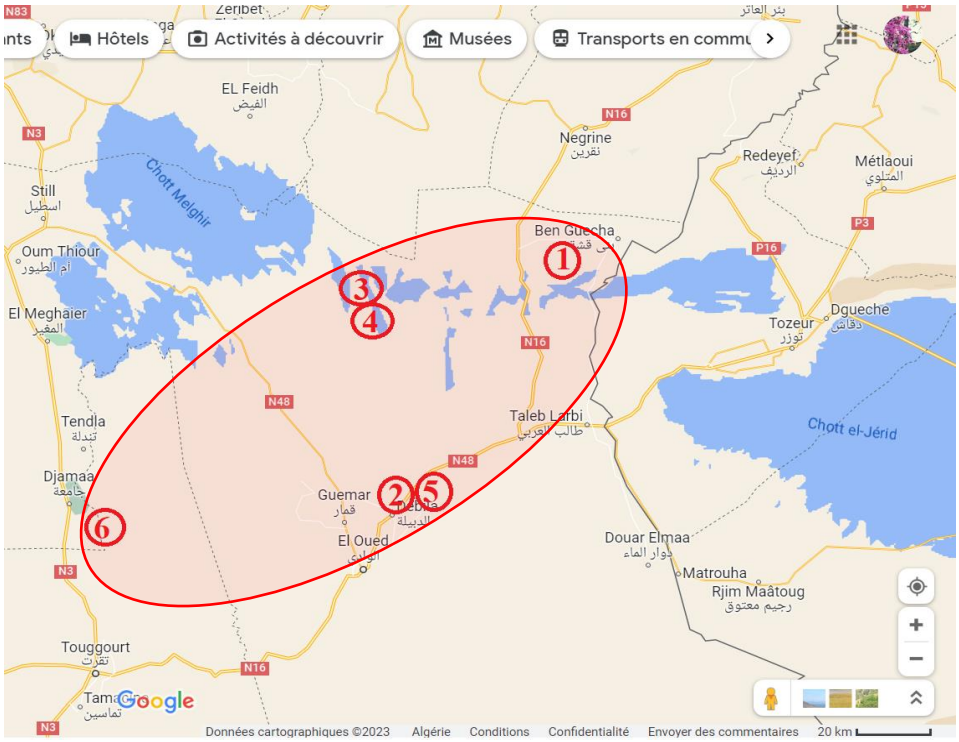

Figure 25. Map showing sample collection locations: (1) *A. oropediorum*; (2) *B. muricata*; (3) *H. strobilaceum*; (4) *S. tetragona*; (5) *S. foetida*; (6) *S. fruticosa* (Google Maps).

1.2. Bacterial strains

In this study, six bacterial strains of pathogens were selected, were:

- Gram positive: *Bacillus subtilis* ATCC 6633, *Listeria innocua* CIP 74915, *Staphylococcus aureus* ATCC 6538.
- Gram negative: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028.

All strains were brought from the Pasteur Institute in Algiers. Bacteria were activated by growing a colony of frozen preservation agar (2°C) in nutrient agar and incubating at 37°C for 18–24 hours.

1.3. Cells

In the cytotoxicity test, two types of cells were used: hepatocellular carcinoma cells (Huh-7) and hepatocellular carcinoma cells (HepG2) which were brought from Nawah Scientific Inc (Mokattam, Cairo, Egypt).

1.4. Chemicals

Aluminium chloride ($AlCl_3$), ferric chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), trichloroacetic acid were obtained from Prolabo (USA). Folin Ciocalteu reagent, Folin-Denis reagent and hydrogen peroxide (H_2O_2) were obtained from Biochem chemopharma Co (France). The rest of the chemicals, reagents and organic solvents were obtained from Sigma-Aldrich (USA). Amoxicillin®, Gentamicin® and Penicillin®, three antibiotics, were utilized. Aspirin® and Metformine® are the medications that are being used.

2. Methods

2.1. Determination of the nutritive value

Given the importance of wild herbs in traditional medicine and in the diet of humans or an animal that consumes them. Consequently, it is required to investigate the nature and properties of these herbs. This analysis aimed to investigate the chemical composition of six herbs of *Chenopodiaceae*. The nutritional value of the dry plant material was determined by determining the following criteria:

2.1.1. Determination of water content

Water content or moisture content means the percentage of water to the solid in the fresh plant matter. It was determined using a drying method (Maatallah Zaier *et al.*, 2020), in which the fresh plant material was dried in an incubator at 60°C. After every 24 hours, the plant material is weighed until the weight is stable. The percentage of water content was calculated according to the equation 01:

$$\text{Water content (\%)} = \frac{W1-W2}{W1-W3} \times 100 \quad \text{(Equation 01)}$$

W_1: Weight of empty container and sample before drying.
W_2: Weight of empty container and sample after drying.
W_3: Weight of empty container.

2.1.2. Determination of photosynthetic pigments content

Chlorophyll a, chlorophyll b, and carotenoids were determined by mixing 0.1 g of fresh plant material in 10 mL of acetone (80%) at 4°C for 24 hours. After centrifugation for 10 minutes at 5000 x g the absorbance of the supernatant was read at wavelengths; 663, 645, and 470 nm using

a spectrophotometer (Nayek *et al.*, 2014). By applying the following mathematical relationships, the content of chlorophyll a, chlorophyll b, and carotenoids:

$$\text{Chl a (mg/mL)} = 12.25 \times Abs_{663} - 2.79 \times Abs_{649} \quad \text{(Equation 02)}$$

$$\text{Chl b (mg/mL)} = 21.5 \times Abs_{649} - 5.1 \times Abs_{663} \quad \text{(Equation 03)}$$

$$\text{Carotenoids (mg/mL)} = (1000 \times Abs_{470} - 1.82 \times \text{Chl a} - 104.96 \times \text{Chl b})/198 \quad \text{(Equation 04)}$$

2.1.3. Estimation of carbohydrates, lipids, and proteins content

Before estimation, the first metabolites were extracted from the samples, was performed according to the method of (Shibko *et al.*, 1967). 0.5 g was soaked in 5 mL of trichloroacetic acid (20%). The mixture was shaken in vortex for five minutes and then separated by centrifugation (3000 rpm/10 minutes). The supernatant was exploited for carbohydrate determination, and the precipitate was added to 2 mL of chloroform/methanol (1/1 V). Then the separation was further centrifuged (3000 rpm/15 minutes). The second supernatant was used to estimate the lipids content, and the precipitate was added to it 5 mL of sodium hydroxide (0.1 N) and this solution was used to estimate the total proteins.

- **Carbohydrate content determination**

The first supernatant was used for determination of carbohydrate content, using the phenol–sulfuric acid method described by Dubois *et al.* (1951). The principle of this method is that the reaction of carbohydrates with concentrated sulfuric acid produces furfural derivatives. This leads to an additional reaction between furfural derivatives and phenol, which leads to the appearance of a color that can be determined by measuring the absorbance. The steps for this method are as follows: 2 mL of an aliquot of the carbohydrate solution was mixed with 1 mL of a 5% aqueous solution of phenol, and then 5 mL of concentrated sulfuric acid was added. The mixture was shaken and incubated for 10 minutes, then the absorbance was measured at 490 nm. The glucose was used to determine the standard curve (Figure 26). The carbohydrate content was expressed as: µg glucose/mg dry matter.

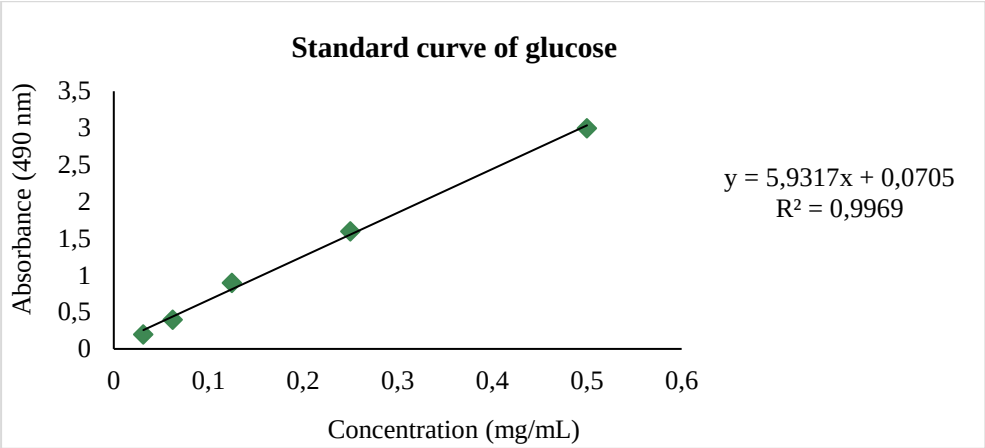

Figure 26. Standard curve of glucose for determination of carbohydrate content in studied plants.

- **Lipid content determination**

The second supernatant was used to estimate the lipid content, using the method of (Goldsworthy *et al.*, 1972). This method is based on the reagent sulfophospho vanillinic and concentrated sulfuric acid. First, the sulfophospho vanillinic reagent was prepared by dissolving 76 g of vanillin in 11 mL of distilled water, then 39 mL of phosphoric acid (85%) was added to the mixture and shaken well to completely dissolve the vanillin. The steps for the determination are as follows: 0.1 mL of an aliquot of lipid solution was mixed with 1 mL of concentrated sulfuric acid, and then the mixture was placed for 10 minutes in a water bath at 100°C. After cooling, a volume of 0.15 mL was taken from the mixture and 1.5 ml of sulfophospho vanillinic reagent was added to it. The mixture was shaken and incubated for 30 minutes, then the absorbance was measured at 530 nm. Soya oil was used to determine the standard curve (Figure 27). The lipid content was expressed as: µg soybean oil/mg dry matter.

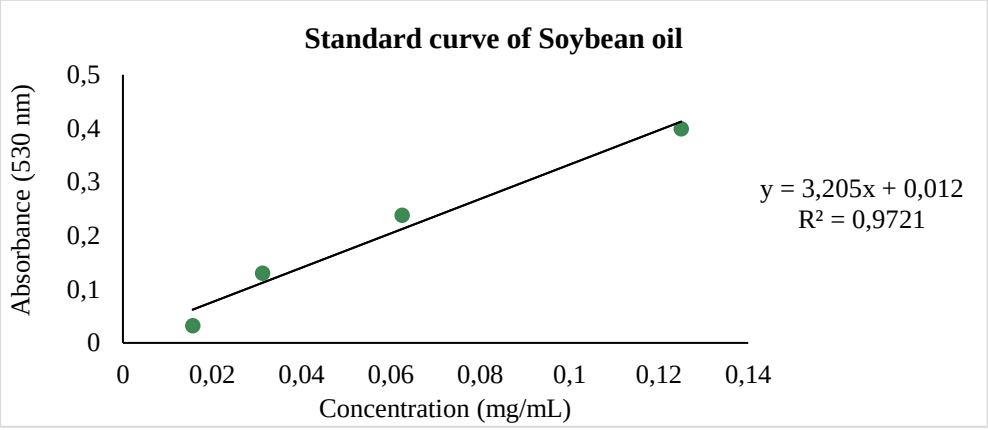

Figure 27. Standard curve of Soybean oil for determination of lipid content in studied plants.

- **Protein content determination**

The protein concentration is determined according to the method of (Bradford, 1976) which uses Coomassie blue (G 250) as a reagent. The amine (-NH_2) groups of proteins react with a reagent based on orthophosphoric acid, ethanol and Coomassie brilliant blue to form a blue complex. The appearance of this color reflects the degree of ionization of the acid medium and the intensity establishes the concentration of proteins in the sample.

Coomassie brilliant blue reagent was prepared by dissolving 100 mg of CBB G-250 in 50 mL of ethanol, then 100 mL of phosphoric acid (85%) was added to the mixture, then distilled water to a volume of one liter was added to the mixture. The protein content of samples was determined by mixing a volume of 0.2 mL of the third sample supernatant with 1.8 mL of Coomassie brilliant blue reagent and then left the mixture for 5 minutes before reading the absorbance at wavelength 546. BSA was used as a standard compound (Figure 27). The protein content was expressed as: µg BSA/mg dry matter.

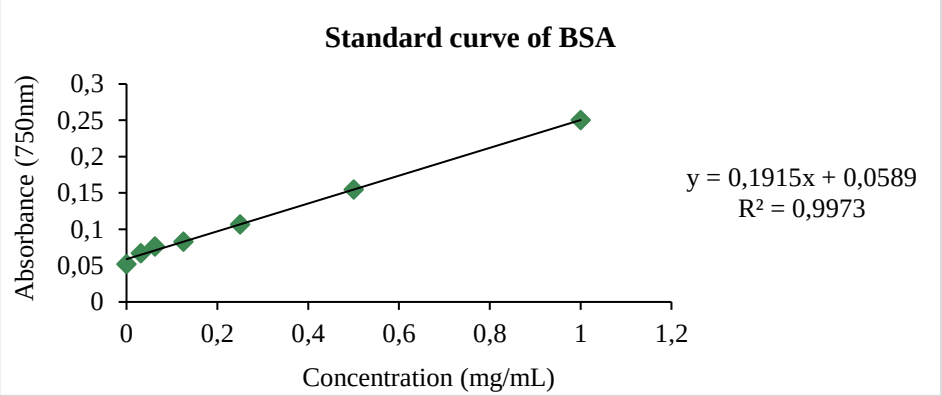

Figure 28. Standard curve of BSA for determination of protein content in studied plants.

2.1.4. Ash estimation

Fresh plant material consists of water, minerals and organic matter. Mineral matter (ash) includes all the minerals and inorganic elements stored in the plant. By burning completely dry plant matter in a muffle furnace at 550°C, raw ash was determined. Exploiting the weights of the samples before and after burning, the ash percentage was calculated using the following formula:

$$\text{Ash content (\%)} = \frac{W1-W2}{W1-W3} \times 100 \quad \text{(Equation 05)}$$

2.1.5. Determination of mineral nutrients by EDX Analysis

Scanning electron microscopy makes it possible to scan part of the surface of the sample using an electron beam with a diameter of a few nanometers. This method allows visualization of morphological features with high magnification and increased depth of field. In addition, an interaction is created between the electron beam and the atomic envelopes of the elements of the material to be analyzed. During scanning, X-ray fluorescence radiation is created which can be recorded by an energy dispersive spectroscopy (EDX) and used for analysis (Djekic & Ćirić, 2022).

The essential chemical elements of dry plant matter were determined by the Phenom Pro Desktop scanning electron microscope (SEM) with an optical magnification range of 20-134×, an electron magnification range of 160-150,000×, maximum digital zoom of 12×, acceleration voltages of 5, 10 and 15 kV, a backscattered electron detector. Backscattered electron detector (BSD) and energy dispersive spectroscopy (EDX), with a nominal resolution of 6 nm or less. The microscope is equipped with a temperature-controlled sample holder (temperature range -25°C to 50°C).

The results of the scanning electron microscope (SEM) analysis attached to the energy dispersive spectroscopy (EDX) analysis are shown as shown in Annexe 01. However, in this study a Table 07 was presented showing the elements and oxides present in each sample.

2.2. Qualitative and quantitative investigations of extracts

2.2.1. Preparation of the extracts

In a beaker, 100 g of dry and finely ground aerial part powder was macerate in 600 mL of solvent (methanol/water: 70/30%) for 24 hours at room temperature. After filtration, the process repeats more than once, collect the extracts and then concentrated using a rotary evaporator. (Buchi Rotavapor R-200) at a maximum temperature of 45°C and freeze at 4°C before use.

The yield of extraction operations was calculated according to the equation 06:

$$\text{Percentage of yield} = \frac{\text{Mass in grams of the evaporated extract}}{\text{Mass in grams of the initial dry plant material}} \times 100 \quad \text{(Equation 06)}$$

2.2.2. Qualitative phytochemical analysis

2.2.2.1 Phytochemical screening

The qualitative phytochemical analysis gives a preliminary idea about the presence of specific class of phytochemical (Kumar *et al.*, 2020). According to standard methods the presence of alkaloids, coumarins, mucilage, phenols, saponins, and terpenoids in the extracts was determined. Any change in the colors or composition of the precipitate has been adopted as an indication of a positive response to these tests.

Dragendorff test: 2 mL of plant extract was mixed with 2 mL of 1% hydrochloric acid, then 2 mL of the mixture was added to 2 mL of Dragendorff reagent (María *et al.*, 2018).

Alkaline reagent test: 1 mL of 10% sodium hydroxide was mixed to 1 mL of the extract. The appearance of the yellow color indicates the presence of coumarin (Zeghib, 2020).

Alcohol test: 1 mL of the extract was mixed with 3 mL of methanol at 60°C. After stirring, the floc precipitate indicates the presence of mucilage (Zeghib, 2020).

Ferric chloride ($FeCl_3$) test: a few drops of a ferric chloride (2%) solution were added to 2 mL of the extract. Ferric chloride in the presence of phenolic compounds produces a blue-green or black color (Zeghib, 2020).

Foam test: 2 mL of extract and 2 mL of distilled water are shaken for 15 seconds and left for 15 minutes. A firm foam height of more than one Cm indicates the presence of saponins (Sorescu *et al.*, 2018).

Liebermann-Burchard test: 0.5 mL of the extract was slowly mixed with 2 mL of chloroform and acetic anhydride and drops of sulfuric acid. The appearance of dark green color indicates the presence of terpenoids (Tyagi Phd, 2017).

2.2.2.2 Fourier transform infrared spectroscopy (FT-IR)

FT-IR is a widely used physicochemical analytical technique used in the analysis of herbs and plant extracts. It provides an illustrative picture of the chemical content based on the identification of functional groups of active compounds (Sravan Kumar *et al.*, 2015).

The samples were analyzed by fourier transform infrared spectroscopy of the Agilent Cary 630 type (Agilent technologies). The sample was placed directly on the platform, uniform pressure applied, and the spectra recorded as the average of 8 scans at 16 Cm^{-1} resolution in the region of (400-4000 Cm^{-1}).

2.2.3. Quantitative phytochemical analysis

2.2.3.1. Spectrophotometric quantification

Spectrophotometry is an experimental technique used to measure the concentration of solutes in solutions based on the amount of light absorbed by those solutes using a spectrophotometer. Through this technique, researchers can estimate many classes of active compounds from plant extracts, because the compounds absorb different wavelengths of light with different intensity. In the analysis of each category, it is required to determine the following; a pure special compound (standard compound) of the test class used in the completion of the standard curve that provides the values of changes in absorbance values (amount of light) in terms of the quantitative values of the particular compound. The reaction mixture is capable of desalinating the tested substance in a visible color, and a wavelength suitable for measuring the amount of light is compatible with the visible color of the reaction. In addition to the correct method of blank the device according to the reaction materials (Germer *et al.*, 2014; Xiaohua *et al.*, 2019).

The quantitative expression provided by the spectrophotometric method of analysis is equivalent to the standard component of the standard curve. In this study, nine classes of active compounds were estimated (total saponin, total phenolic, flavonoide, flavonol, flavanol, flavanone, hydroluzable, and condensed tannin) by taking advantage of the standard curves specific to each class.

- **Determination total saponin content**

The total saponin content was determined by the vanillin-sulfuric acid method of Jain and Shrivastava (2017). Diosgenin is used as a reference standard. The same volume was mixed from different concentrations of diosgenin and samples solution with vanillin (8%) and sulfuric acid (72%). The mixture is incubated at 60°C for 10 minutes, then cool in an ice-water bath for 15 minutes. Then the reading is done by a spectrophotometer at 583 nm. The total saponin content is expressed in diosgenin equivalents (µg DE/ mg dry extract) (Figure 28).

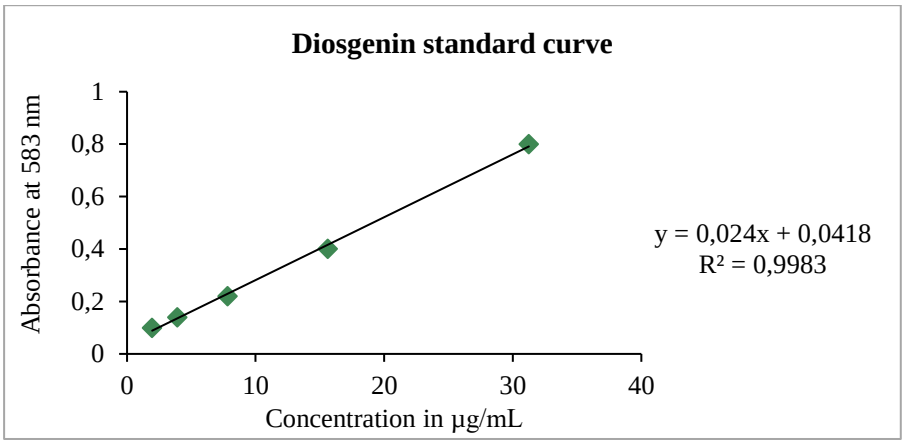

Figure 29. Standard curve of diosgenin for estimating total saponin content.

- **Determination of total phenolic contents**

The total phenolic contents of the crude extracts were determined according to the Folin-Ciocalteu method described by Chekroun-Bechlaghem *et al.* (2019). At a volume of 200 µL of the extract with 1000 µL of Folin-Ciocalteu reagent (10%) were incubated for three minutes. Then 0.8 mL of sodium carbonate solution (7.5%) was added. The tubes were stirred well, after incubation for 30 minutes, the absorbance was measured at 765 nm. The content was expressed as µg gallic acid/mg dry extract.

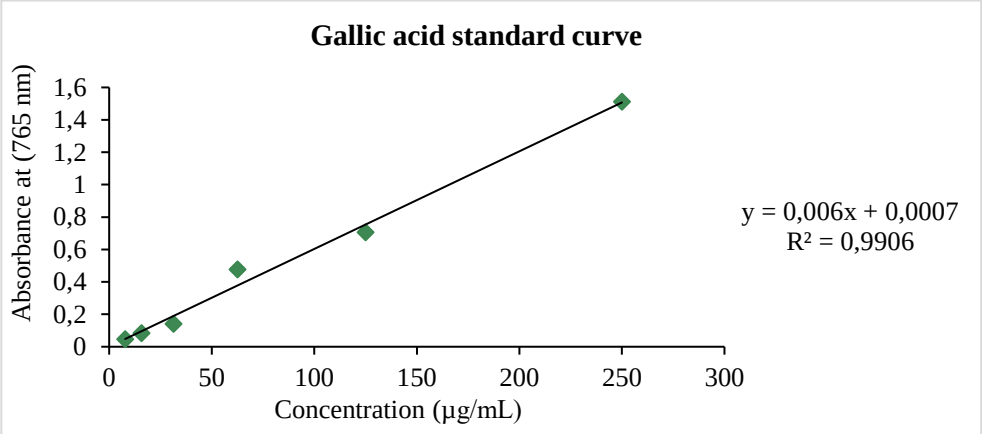

Figure 30. Standard curve of gallic acid for determination of total phenolic content in extracts.

- **Determination of flavonoids contents**

Flavonoids are phenolic compounds that can be chemically determined by the following steps as described by Chekroun-Bechlaghem *et al.* (2019). Quercetin was used as a standard compound to estimate the total flavonoid content (µg QE/mg dry

extract). 250 µL of different concentrations of quercetin and sample were mixed with 255 µL of methanol and subsequently with 100 µL of potassium acetate solution (1 M). Then 100 µL of aluminum nitrate solution (10%) was added to the mixture. The mixture was thoroughly mixed and allowed to stand for 40 min. Absorbance of the mixture was determined at 415 nm.

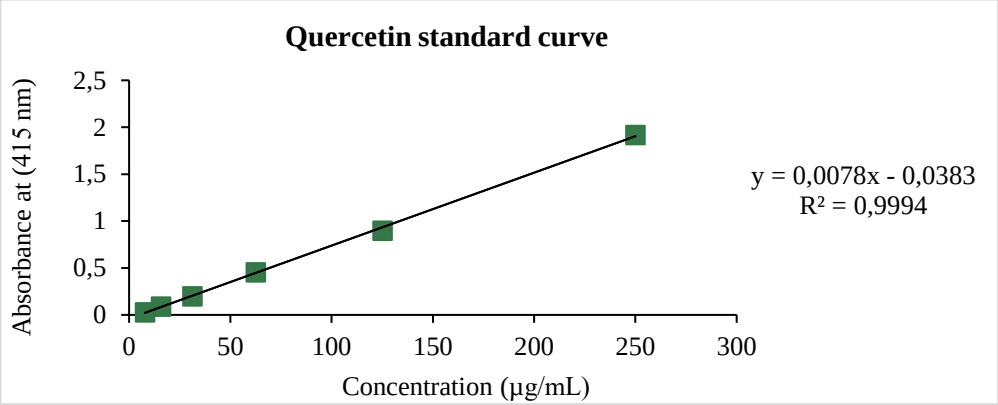

Figure 31. Standard curve of quercetin for estimating flavonoid content.

- **Determination of total anthocyanin content**

Anthocyanin content was determined according to the pH differential method (Lee *et al.*, 2005). Briefly, 400 µL of the extract was mixed with 3600 µL of potassium chloride buffer (0.0025M, pH=1) and sodium acetate buffer (0.4M, pH=4.5) was added each one by one. After incubation for 30 minutes, the absorbance was read at 700 nm and 510 nm. By applying the equation 07, anthocyanin content was expressed in µg equivalent to cyanidin-3-glucoside per milligrams of dry extract.

$$\text{Anthocyanin pigment (µg C-3-GE/mg)} = \frac{\frac{A \times MW \times DF \times 100}{MA}}{100} \quad \text{(Equation 07)}$$

Where the value of "A" is calculated by applying Equation 08:

$$A = (Abs_{510} - Abs_{700})pH_{1.0} - (Abs_{510} - Abs_{700})pH_{4.5} \quad \text{(Equation 08)}$$

Where: **Abs_{510};** absorbance at 510 nm. **Abs_{700};** absorbance at 700 nm **MW;** cyanidin-3-glucoside molecular weight (449.2 g/mol). **DF;** dilution factor. **MA;** molar extinction coefficient of cyanidin-3 glucoside (26.9 l/mol.cm).

- **Determination of total flavonol content**

The content of flavonols was performed as described by Chekroun-Bechlaghem *et al.* (2019). At a volume of 250 µL of each extract and different concentration of

quercetin were mixed with 250 µL aluminium chloride (2 mg/mL) and 1500 µL sodium acetate (50 mg/mL). The absorbance at 440 nm was recorded after two and half hours of incubation. The content of flavonols was expressed as milligrams of quercetin equivalents per gram of dry weight (µg QE/mg).

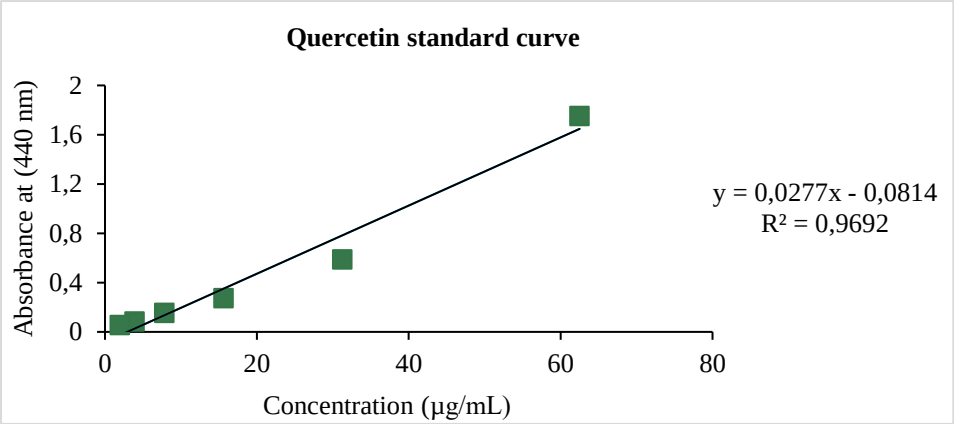

Figure 32. Standard curve of quercetin for determination of flavonol in extracts.

- **Determination of total flavanol content**

The flavanol content was determined using the vanillin and catechin method as a standard, and the results were expressed in (µg CE/mg of dry extract). First the vanillin reagent was prepared by dissolving 0.5 g of vanillin and 200 mL of hydrochloric acid diluted with methanol (4%). Then 1000 µL of each sample was mixed with 5000 µL of vanillin reagent. After incubating for 20 minutes in the dark at room temperature, the absorbance of the mixture was measured at 500 nm. Note: The spectrophotometer was zeroed using a mixture of each sample with hydrochloric acid diluted with methanol (4%) (Yan *et al.*, 2020).

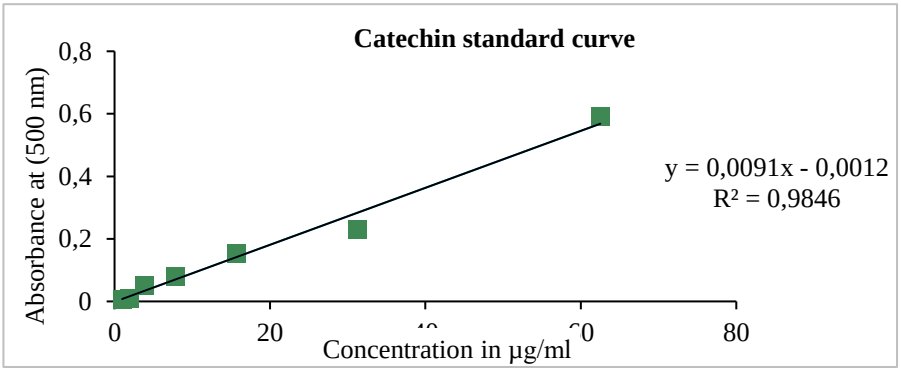

Figure 33. Standard curve of catechin for determination of flavanol in extracts.

- **Determination of total flavanone content**

Flavanone content were evaluated using method described by Oroian *et al.* (2020). First, a dinitrophenylhydrazine solution was prepared by dissolving 1 g of dinitrophenylhydrazine in 2 mL of sulfuric acid (96) and then diluting it to a volume of 100 mL with methanol. Then 2 mL of dinitrophenylhydrazine solution was mixed with 1 mL of extracts or different concentration at 50°C for 50 minutes. After cooling, the mixture was diluted to 10 mL with potassium hydroxide solution (10%) dissolved in methanol. Finally, to 9 mL of methanol, 1 mL of the mixture was added. The absorbance at 486 nm. The amount of total condensed tannins was expressed as microgramme of naringin equivalents per milligram of extract weight (µg NE/mg) from the calibration curve.

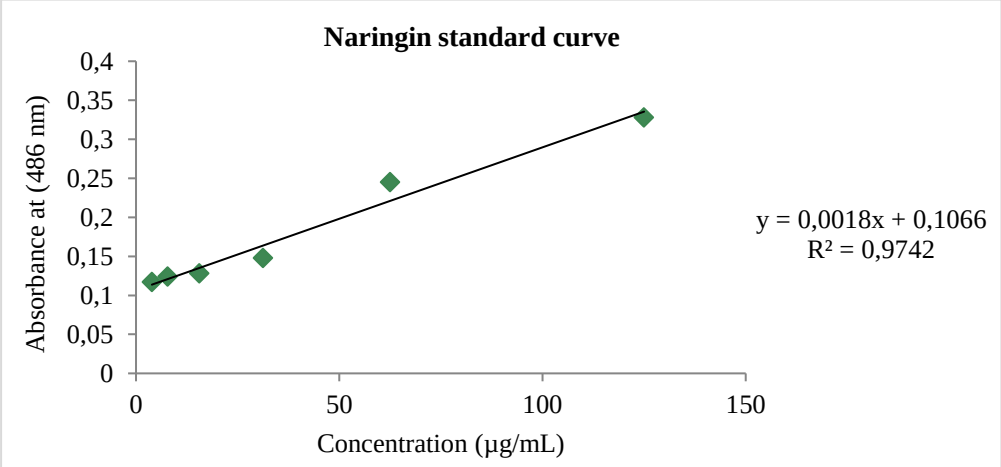

Figure 34. Standard curve of naringin for determination of flavanone in extracts.

- **Determination of hydrolyzable tannins contents**

The determination of hydrolyzable tannin content was performed according to Folin-Denis method (Kousalya & Jayanthy, 2016). A 500 µL aliquot of the extract or different concentration of gallic acid was mixed with 1000 µL of the Folin-Denis reagent and 500 µL of sodium carbonate solution (35%), then distilled water was added to the mixture up to 10 mL. After 30 minutes of incubation in the dark the absorbance was determined at 700 nm. The results were expressed as µg of gallic acid equivalents (CE)/mg sample.

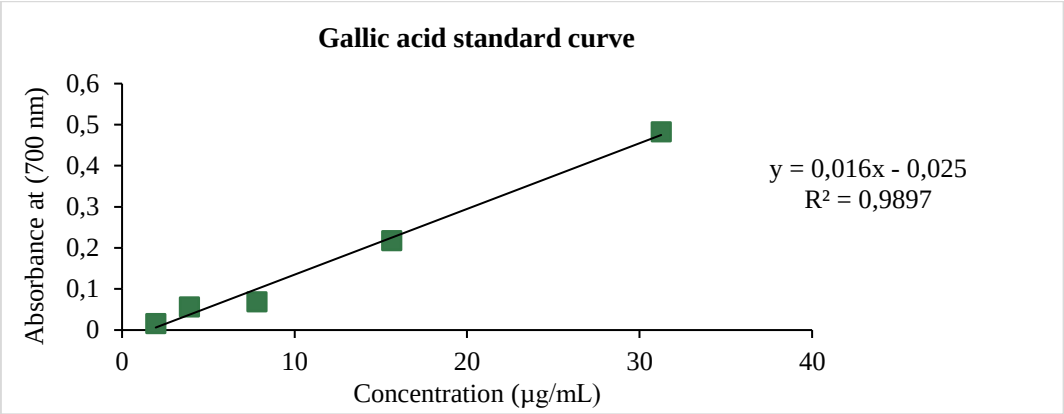

Figure 35. Standard curve of gallic acid for determination of hydrolyzable tannins in extracts.

- **Determination of condensed tannin contents**

The total condensed tannin content was determined by the vanillin hydrochloric acid method (Chekroun-Bechlaghem *et al.*, 2019). 0.5mL of the extracts were mixed with 3mL of vanillin reagent (4%, w/v in methanol) in aluminum-coated test tubes and 1.5mL of HCl (1N) and mixed well. The tubes were incubated at 20°C for 15 min, the absorbance was read at 760 nm. Results were expressed as microgramme of catechin equivalents (CE) per one milligram of extract weight (µg CE/mg).

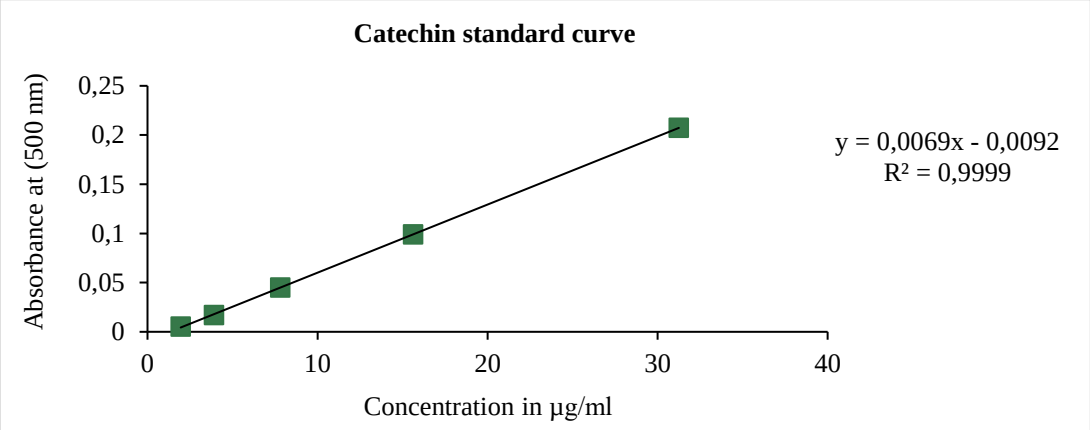

Figure 36. Standard curve of catechin for determination of condensed tannins in extracts.

2.2.3.2.RP-HPLC analysis

Reversed-phase of high-performance liquid chromatography is a technique used to separate, identify and quantify phytochemicals from plant mixtures and relies on pumps to pass a pressurized solvent (mobile phase) containing the components of plant samples through a column filled with a solid absorbent material (stationary phase) (Soto-hernandez *et al.*, 2017). The separation of components of plant extracts

using HPLC depends on the choice of the mobile phase (liquid) and the stationary phase (solid), as well as on the chemical properties of the compounds such as molecular weight, polarity, and charge *etc* (Buszewski & Noga, 2012).

Identification and quantification of phenolic compounds was carried out using a SHIMADZU LC-20 high performance liquid chromatograph, including universal injector (Hamilton®, volume 25 µL), an ultraviolet and visible detector SPD 20A (Shimadzu), analytical chromatographic column C18 (Shim-pack VP-ODS, 5 µm, 4.6 x 250 mm) at a temperature of 25°C, and elution solvents were: acetonitrile (A) and ultrapure water (B). Gallic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, vanillin, rutin, naringin, and quercetin were used as standard compounds.

The compounds were separated by injection of 20 µL volume of the extract solution, filtered with a 0.45 µm organic filter and emptied by sonication. At a flow rate of 1 mL/min, the mobile phase containing the extract solution was passed in the separation column for 50 minutes. At wavelength of 268 nm the separated compounds were detected. How much through the computer the results were recorded as a chromatographic curve (Figure 45). The compounds were identified by comparing their retention time with the retention time of the reference compounds (Table 04) and the content of the specific compounds was estimated by using the equations of the reference compounds (Table 04), the content was expressed as µg/mg dry extract. Separation was carried out using a gradient elution method, starting with 10% acetonitrile acid (A) and increasing over time: 14% (A) at 6 min, 17% (A) at 16 min, 19% (A) at 23 min, 23% (A) at (28-35 min), 40% (A) at 38 minutes, and 10% (A) at 50 minutes.

Table 04. Retention time, and regression analysis of standard compounds phenolic determined by RP-HPLC analysis in 268 nm.

Compounds phenolic	Retention time (min)	Regression analysis
Gallic acid	5.29	y=54681x
Chlorogenic acid	13.392	y=21665x
Vanillic acid	15.531	y=65077x
Caffeic acid	16.277	y=84066x
Vanillin	21.46	y=58930x
p-Coumaric acid	23.817	y=49495x
Rutin	28.37	y=28144x
Naringin	34.788	y=19379x
Quercetin	45.047	y=45378x

y: HPLC peak area. x: Concentration (µg/ml)

2.3. Biological activity tests

2.3.1. Evaluation of antioxidant capacity

2.3.1.1. Free radical-scavenging activity (DPPH• assay)

The ability of the extracts methanolic to scavenge 1,1-diphenyl-2- picrylhydrazyl (DPPH•) radicals were determined by the DPPH• assay described by (Öztürk *et al*., 2007). A volume of 0.6 mL of extract at different concentrations (1-500 µg/mL) was added to 1.2 mL of DPPH• solution (0.1×10^{-3} M) in methanol. After incubation for 30 minutes at room temperature, the absorbance of their action mixture was measured at 517 nm. The percentage of inhibition was calculated by using the following formula:

$$\text{Percentage of DPPH}^{\bullet}\text{ radical inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100 \quad \text{(Equation 09)}$$

Where: $\text{Abs}_{\text{Control}}$; absorbance of the control (containing all reagent except the sample) and $\text{Abs}_{\text{sample}}$; absorbance of the sample.

Antioxidant efficacy was assessed by the free causative inhibition ratio, and IC_{50} values (Concentration of extract necessary to inhibited of DPPH• radical 50%) were calculated. They were determined by exploiting radical inhibition percentage curves in of sample concentration change (Annexe 02).

2.3.1.2. *β*-Carotene-linoleic acid assay

Antioxidant activity to inhibit lipid peroxidation was evaluated using the *β*-carotene bleaching method as described by Chekroun-Bechlaghem *et al*. (2019). The sample was mixed at different concentrations (1-500 µg/mL) with the reaction mixture, which was prepared by mixing 1 mg of *β*-carotene with 1 mL of chloroform, 50 µL of linoleic acid and 400 mg of Tween™ 40, the chloroform was evaporated and the precipitate was dissolved in 200 mL of oxygenated water with rapid magnetic stirring. The absorbance is measured at 470 nm immediately after mixing, and it is called the absorbance at 0 min. Then the mixture was incubated at 50°C for 120 min, then the absorbance was measured and the absorbance was considered at 120 min. To determine the antioxidant activity, the following equation was applied:

$$\text{Percentage of antioxidant activity} = \left(1 - \frac{\text{Abs}_{\text{Control (0 min)}} - \text{Abs}_{\text{Sample (0 min)}}}{\text{Abs}_{\text{Control (120 min)}} - \text{Abs}_{\text{Control (120 min)}}}\right) \times 100$$

(Equation 10)

The effectiveness of lipid peroxidation inhibition was also expressed by the IC_{50} values (Concentrate that provides 50% of effectiveness). They were determined by exploiting antioxidant activity percentage curves in of sample concentration change (Annexe 03). α-Tocopherol was used as a reference compound.

2.3.1.3. Anti-hemolysis assay

The objective of this test is to identify the extent to which plant extracts protect red blood cells from oxidants and free radicals that cause their degeneration, by determining the percentage of red blood cells (RBCs) lysis in the presence of the studied extracts. The efficacy of the extracts has been evaluated, following the protocol described by Chouikh *et al*. (2020). A volume of 40 µL of human erythrocytes (10%) was mixed with 2 mL of different concentrations (500, 250, and 125 µg/mL) of the extract and incubated for five minutes at 37°C. Then, we added 40 µL of hydrogen peroxide (H_2O_2) (30×10^{-3} M), 40 µL of ferric chloride ($FeCl_3$) (80×10^{-3} M) and 40 µL of ascorbic acid solution (50×10^{-3} M) respectively. After one hour of incubation at 37°C, the mixture was centrifuged with 700 rpm for 10 minutes. The absorbance of the supernatant was read at λ= 540 nm. The percentage of hemolysis was determined using the following formula*:*

$$\text{Percentage of hemolysis} = \frac{\text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \times 100 \quad \text{(Equation 11)}$$

2.3.1.4. Reducing power assay

Potassium ferricyanide reacts with Fe^{+3} ions to form a complex with a very high extinction coefficient at 700 nm. Through this assay, the reduction of potassium ferricyanide is monitored by the antioxidants present in the studied extracts (Pisoschi *et al*., 2016). The reducing power of the extract was performed using the potassium ferricyanide method (Muthukrishnan *et al*., 2018). A volume of 0.5 mL of the different concentrations (mg/mL) of the extract was added to 1.25 mL of a phosphate buffer (0.2 M, pH 6.6) mixed with 1.25 mL of potassium ferricyanide (1%). The mixture was incubated in a hot bath at 50°C for 20 minutes, there action is stopped by adding 1.25 mL of trichloroacetic acid (10%), the tubes were then centrifuged at 3000 rpm/10 minutes. 1.25 mL of the supernatant, was mixed with 0.25 mL of ferric chloride ($FeCl_3$) (0.1%) and 1.25 mL of distilled water, and the optical density was read at 700 nm.

The results were compared with the value of EC_{50}, is the value of the effective concentration, which gives an absorption of 0.5 at a wavelength of 700 nm. They were determined by exploiting

the absorption curves at a wavelength of 700 nm in of sample concentration change (Annexe 04).

2.3.1.5. Phosphomolybdenum reducing power assay

The total antioxidant capacity of the plant extracts was expressed as gallic acid equivalent; where the estimation was based upon the method of phosphomolybdate (Muthukrishnan *et al.*, 2018). 0.1 mL of the sample, was mixed with 1 mL of phosphomolybdate solution. Stirred well, then incubated for one hour in a water bath at 95°C. After cooling the tubes, the optical absorption was read at a wavelength of 695 nm using UV–vis spectrophotometer. The phosphomolybdate solution was prepared by mixing the same volume of ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (0.6 M).

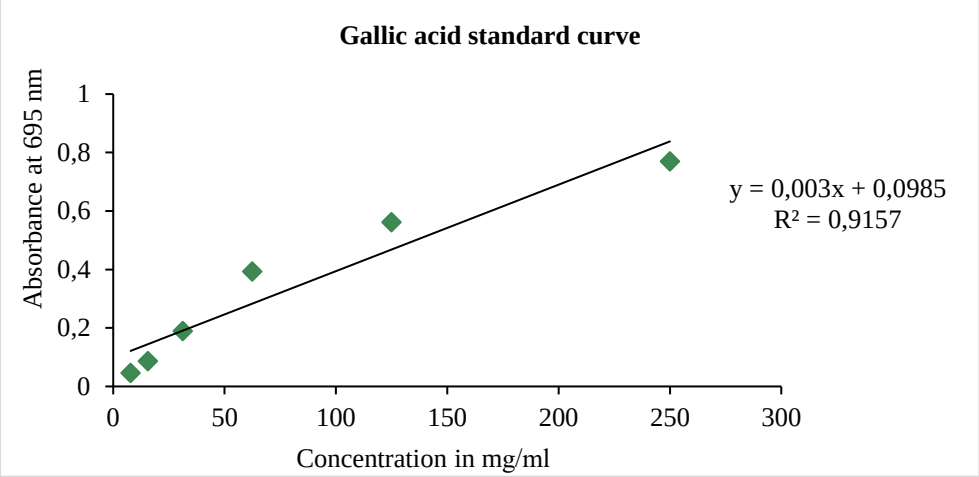

Figure 37. Standard curve of gallic acid for determination of the total antioxidant capacity of the plant extracts.

2.3.2. Evaluation of antibacterial activity

In evaluating the efficacy of antibacterial extracts (*Bacillus subtilis*, *Listeria innocua*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) the sensitivity testing was relied upon and the MICs values were determined as follows.

Preparation of bacterial suspension. The bacterial suspension was prepared from active bacterial cultures, where one or two colonies of the bacterial type were taken and placed in a test tube containing 5 mL of physiological water, it was shaken well until the suspension became homogeneous and turbid. The absorbance of the suspension was read by a spectrophotometer at

a wavelength of 630 nm and used when the absorbance was between 0.08-0.10. Note: The suspension must be used in sowing 15 minutes before its preparation.

2.3.2.1. Antibacterial susceptibility testing (Disk diffusion assay)

The medium (Mueller-Hinton agar) was prepared by adding 20 g of Mueller-Hinton agar powder to 500 mL of distilled water, stirring well, and bringing the mixture to a boil. The mixture was autoclaved at 120°C for 15 minutes and cooled. They were then transferred to sterile Petri dishes. All steps were carried out under sterile conditions.

The sensitivity of six pathogenic bacterial strains to the extracts was tested using the disk diffusion method on solid medium (Mueller-Hinton agar) (Muthukrishnan *et al.*, 2018). The bacterial suspension was sowing on Mueller-Hinton agar (Petri dish), then discs (Whatman paper, 6 mm in diameter, and 0.3 mm height) containing a volume of 10 µL of extracts were diffusion (4, 2, 1, and 0.5 mg). After incubating for 24 hours at a temperature of 37°C, the results were observed by measuring the diameter of the inhibition zone, which indicated that the bacteria were sensitive, moderately sensitive, or resistant to the antibiotics being tested. All steps were performed in sterile conditions. DMSO was used as a negative control as it is the solvent used to dissolve the extracts and prepare different concentrations. Amoxicillin® and Gentamicin® were used as positive controls in the assay.

According to the diameter of the inhibition zone, the antibiotic efficacy was expressed as follows: ≥21mm; strong sensitivity (***), 15-20 mm; moderate sensitivity (**), 10-15 mm; poor sensitivity (*), 10 mm; little or no sensitivity.

2.3.2.2. Determination of minimal inhibitory concentration (MIC)

MICs was determined by testing a sequential dilution of broth using Mueller-Hinton broth (Bbosa *et al.*, 2007). The MIC was defined as the lowest extract concentration that yielded no visible growth. Five concentrations of extracts (100, 50, 25, 12.5, and 6.25 mg/mL) were used, which were prepared by adding 2 mL of Mueller-Hinton broth with 2 mL of solution to the tube that preceded it (the first tube has the highest concentration and Mueller-Hinton broth). Then 0.2 mL of the bacterial suspension was added to each tube, and the solution was slowly shaken. After incubating for 24 hours at 37°C, the results were observed, which is to identify the tubes with a turbid or clear solution. The MIC was determined by the arithmetic relationship:

$$\text{MIC (mg/ml)} = \frac{C_{Clear} + C_{Turbid}}{2} \quad \text{(Equation 12)}$$

C_{Clear}: Concentrate the extract in the first clear tube

C_{Turbid}: Concentrate the extract in the last turbid tube

2.3.3. Evaluation of the antidiabetic activity of extracts *in vitro*

The three assays used are simple *in vitro* tests, which give a simple prediction of the anti-diabetic properties of the studied samples.

2.3.3.1. Glycosylation assay

Through this examination, the effect of plant extracts on the glucose complex is determined *in vitro,* by following the experimental steps of Kebieche (2009), with some modifications. First, the plant extracts and the reference compound (Metformine®) were dissolved in physiological water, then a glucose solution was prepared by dissolving an amount of glucose in a volume of physiological water. The same volume of glucose solution was mixed with the studied substances, in addition to the negative control, it contains physiological water. The mixture was incubated for 15 minutes at 37°C. The dose of glucose that is not related to the studied samples is measured using the glucometer (On Call® Extra). The result is expressed in the values recorded from the device (mg/dL).

2.3.3.2. Non-enzymatic hemoglobin glycosylation assay

The bodies possess enzymatic and other antioxidant mechanisms that reduce the production of reactive oxygen species, which are responsible for causing many degenerative diseases, including diabetes. An increase in glucose in the blood causes it to react with hemoglobin, which can lead to the formation of reactive oxygen species. Through this assay, the efficiency of the extracts in inhibiting the binding of hemoglobin to glycosylation is determined by following the method (Nagarajan *et al*., 2014). First the samples and all test materials were prepared in phosphate buffer (0.01 M, pH = 7.4). Then 1 mL of samples (250, 500, and 1000 µg/mL) was mixed with 1 mL hemoglobin solution (0.06%), 65 µL of Gentamicin® (0.02%), and 1 mL of glucose solution (2%). Then incubated in the dark at 37°C for 72 hours. Finally, the absorbance was read at a wavelength of 520 nm. The percentage inhibition of hemoglobin binding to glycosylation was calculated as follows:

$$\text{Percentage inhibition of hemoglobin binding to glycosylation} = \frac{\text{Abs}_{\text{Samle}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Sample}}} \times 100$$

(Equation 13)

2.3.3.3. Assay of glucose uptake by yeast cells

In this assay the percentage of glucose transport across the cell membrane is studied by the amount of glucose remaining in the medium after a specified time. Hence, this test shows whether the studied samples have a role in promoting glucose uptake by the yeast. *Saccharomyces cerevisiae* is used to glucose uptake test as a model for a cell in the body of an organism. The test steps are as follows: the yeast suspension was prepared by adding an amount of yeast to a volume of physiological water and then it was placed in centrifugation (3000×g, 5 minutes) and the supernatant was obtained. Mixing 2 mL of sample and 2 mL of glucose solution (10 mM) was incubated at 37°C for 10 minutes. Then add to the mixture 200 µL of yeast suspension (10%). The mixture was mixed by Vortex for one minute and then incubated for one hour at 37°C. Then the mixture was placed in a centrifuge for 15 minutes at 3000 rpm. Then the absorbance of the supernatant was measured at 540 nm wavelength using a UV-Vis spectrophotometer (Pitchaipillai & Ponniah, 2016; Liaqat *et al.*, 2021). Determination of the percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Percentage of increase in glucose uptake by yeast cells} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100$$

(Equation 14)

The control is having all reagents except the test sample. Metformine® was used as an antidiabetic drug.

2.3.4. Evaluation of the anti-inflammatory property

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration (Leelaprakash & Dass, 2011). This assay aims to determine the effectiveness of the tested substances in protecting the protein (albumin) from denaturation caused by high temperature. Briefly, 1 mL of serum albumin (5%), 1 mL of different concentrations of the studied samples (125, 250, and 500 µg/mL), and 20 µL of hydrochloric acid (1 N) were mixed. The mixture was then incubated in the incubator at 37°C for 20 minutes and then placed in a water bath at 57 °C for three minutes. After cooling, 2.5 mL of phosphate buffer solution (0.1 M, pH = 6.4) was added. The absorbance was measured at 660 nm (Chouikh *et al.*, 2020). Aspirin® was used as a reference drug. The percentage of protein protection from denaturation was calculated using the following formula:

$$\text{Percentage of protection from denaturation} = (1 - \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}}) \times 100 \text{ (Equation 15)}$$

2.3.5. Determine of sun protected factor (SPF)

The photoprotective activity of the extract was tested by calculating SPF by applying the following equation, after measuring the absorbance of a sample dissolved in ethanol (1 mg/mL) at seven different wavelengths (290-320 nm), with intervals of five nm (Mansur *et al.*, 1986).

$$\text{SPF}_{\text{Spectrophootometric}} = \text{CF} \times \sum_{290}^{320} \times \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{DO}(\lambda) \text{ (Equation 16)}$$

Where: **CF**; Correction factor (10). **EE**; Erythemogenic effect of radiation with wavelength (λ) nm. **I**; Solar intensity spectrum (λ) nm. **DO (λ)**; Spectrophotometric absorbance values at wavelength. The values of **EE(λ)×I(λ)** constant, indicated in the Table 05.

Table 05. Value of erythmogenic effect of radiation with wavelength λ (EE) and spectrophotometric absorbance values at wavelength λ (I)

λ (nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Quercetin and the commercially available sunscreen product Avene were used as the SPF references.

According to the values, the effectiveness of the studied samples was classified into: high sun protection products; SPF≥30 (***), moderate; SPF = 12-30 (**), and minimum SPF; <12 (*).

2.3.6. Cytotoxicity

Cytotoxicity testing was performed by Nawah Scientific, Cairo, Egypt. This assay was performed on only two samples, *H. strobilaceum* and *S. fruticosa*, whose cytotoxicity was tested on two types of hepatocellular carcinoma (Huh-7) and hepatocellular carcinoma (HepG2) cells. The cytotoxic efficacy of the two extracts was evaluated by determining the cell viability percentage.

Cell culture. Cells were maintained in dulbecco's modified eagle medium supplemented with 100 mg/mL of streptomycin, 100 units/mL of Penicillin® and 10% of heat-inactivated fetal bovine serum in humidified, and CO_2 (5% v/v) atmosphere at 37°C.

2.3.6.1. Liver cancer (Huh-7) cell viability

Huh-7 cell viability was assessed by Cell viability was assessed by WST-1 assay using Abcam® kit (ab155902 WST-1 Cell Proliferation Reagent). As described by Sharma *et al*. (2014), 96-well plates were incubated for 24 hours, which contains aliquots of 50 µg/mL cell suspension ($3x10^3$ cells). Then another aliquot of 50 µL media containing the studied samples was added at gradient concentrations (0.01-100 µg/mL) and left for 48 hours, then the cells were treated with 10 µL the cell proliferation reagent (WTS-1). Incubated for one hour. Absorbance was measured at 450 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

2.3.6.2. Hepatocellular carcinoma (HepG2) cell viability

HepG2 cell viability was assessed by SRB assay (Allam *et al*., 2018). Aliquots of 100 µL of cell suspension (5×10^3 cells) were incubated in complete media for 24 hours. Then the cells were treated with another aliquot of 100 µL of media containing the samples at different concentrations. After 72 hours, media was replaced with 150 µL of trichloroacetic acid (10 %) and incubated at 4°C for one hour. Then the trichloroacetic acid solution was removed, and cells were washed five times with distilled water. Aliquots of 70 µL of sulforhodamine B (SRB) (0.4 %) solution were then added and incubated in a dark place at room temperature for 10 minutes. The plates were then washed three times with acetic acid (1 %) and left to air dry. Then 150 µL of TRIS (10 mM) was added. The absorbance was measured at 540 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

Applying the following relationship, the viability percentage is calculated:

$$\text{Percentage of viability of cell} = \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \times 100 \quad \text{(Equation 17)}$$

2.4. Statistical analysis

The results were expressed as the mean ± standard deviation of the mean for 3 independent repetitions of an experiment.

- **Data preprocessing by statistical method**

The data was processed by:

Statistical differences between samples were studied using SPSS Statistic for Windows, version 15.0. using a one-way ANOVA test followed by Duncan's multiple range tests.

Pearson correlation coefficient (r) was used to show correlations and significance levels (*p*-values) between the biological activity and the phytochemical compounds (Quantification values). This analysis was done using SPSS Statistic for Windows, version 15.0. The relationship between the variables was determined based on the following values:

$0.0 < R \leq 0.19$; very low correlation

$0.2 \leq R \leq 0.39$; low correlation

$0.4 \leq R \leq 0.59$; moderate correlation

$0.6 \leq R \leq 0.79$; high correlation

$0.8 \leq R \leq 1.0$; very high correlation

The correlation coefficient can be positive (+R) or negative (-R).

Principal components analysis (PCA) was used to present the determination of the variables with the highest variance, as it was applied to the phytochemical study data (extract yield, quantification values, and the most important biological activities) and nutritional content analysis data, each one separately. This analysis was performed using XLStat (Addinsoft, NY, USA), version 2021.2.2.

CHAPTER V

Results

CHAPTER VI

Discussion

CONCLUSION

Conclusion

Researchers are increasingly interested in applying green chemistry techniques in their research to valorize plant secondary metabolites. Which contribute to sustainable development by identifying substances capable of improving the quality of life with fewer consequences. It also has a role in protecting the environment, achieving biodiversity through caring for vegetation, and at the same time promoting competition between industries towards more environmental, economic and innovative products.

The aim of this study is to highlight six desert medicinal species that belong to the *Chenopodiaceae* family. Their biological effects were determined through an *in vitro* study of their methanolic extracts. Moreover, a series of analyses were performed to determine and characterize their phytochemical content through; phytochemical screening, fourier-transform infrared spectroscopy (FT-IR), a series of quantitative estimates, and RP-HPLC analysis. The nutritional value of these six plants was also determined to assess their potential as food sources.

The desert wild plants, *Anabasis oropediorum* Maire, *Bassia muricata* L, *Halocnemum strobilaceum* Pall, *Salsola tetragona* Del, *Salsola foetida* Del and *Suaeda fruticosa* L, are among the most important plants of the *Chenopodiaceae* family that dominate the vegetation cover in El Oued region of Algeria.

Based on the approximate composition analysis data, these species are rich in nutrients and could serve as a source of food and grazing in desert environments, as the species was distinguished by its high content of carbohydrates, protein and chlorophyll. The wild plant *A. oropediorum* was the most content of these nutrients. It is suggested that these desert species be exploited as food products or additives with sensory properties to foods. They can also be functional foods that have a desirable physiological effect that goes beyond basic nutrition. They could also serve as nutritional supplements or nutraceuticals.

Phytochemical screening and FT-IR analysis revealed that the methanolic extracts of the studied plants contain most of the secondary metabolites. The series of quantitative estimates confirmed that these extracts had a varied phenolic content, which characterized the methanol extract of *B. muricata* and *S. foetida* with the highest content of most phenolic compounds, and most of the studied extracts were characterized by a significant total content of saponins.

RP-HPLC analysis showed the qualitative and quantitative content of the individual phenolic compounds. The analysis showed that the naringin compound was absent from the studied extracts, while all of them contained rutin. *S. fruticosa* extract had the highest quantitative and qualitative

content of phenolic compounds identified in this analysis, whereas *A. oropediorum* extract had the lowest.

Through quantitative and qualitative analysis, it is evident that these plants are important sources of saponins, alkaloids, and phenolic compounds, which could be exploited for medicinal, nutritional, sensory, and cosmetic purposes. It is also proposed to conduct further studies on these plants to isolate, identify, characterize, and elucidate the structure of biologically active compounds, as well as determine their biological activity.

The results of antioxidant activity indicate that the extracts have antioxidant activity. In particular, the methanol extract of *B. muricata* and *S. foetida*. Antibacterial results indicate that all extracts have a growth inhibitory property for each of the tested strains (*Bacillus subtilis*, *Listeria innocua*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*). It is possible to use these plants as an antibacterial agent. The three assays of anti-diabetic activity also gave a good prediction of the possibility of using these plants as a hypoglycemic agent, this encourages further studies needed to evaluate and confirm the origin of the activity. In the results of anti-inflammatory activity, the methanol extracts showed a significant inhibition in protecting the protein from denaturation in different proportions. The methanol extract of *B. murticata* showed higher anti-inflammatory efficacy. By determining the SPF, extracts of *B. murticata* and *S. foetida* can be classified among herbal cosmetics, while the rest of the extracts have poor protection. The cytotoxicity results also indicated that extracts of the two saline plants *H. strobilaceum* and *S. fruticosa* are particularly effective against Huh-7 cells. Statistical analysis; Pearson correlation coefficient and PCA showed that most of the biological activities have a significant correlation with the content of flavonoids, anthocyanins and hydrolyzable tannins.

These results are preliminary, and it would be interesting to test the activity of the high-purity fractions and isolate the responsible molecules that underlie the different activities detected in the different extracts in more efficient ways. In any case, it is important to emphasize that the tests were performed *in vitro*. Thus, it is necessary to confirm these findings by *in vivo* studies to obtain useful information for ultimately therapeutic or nutritional interventions.

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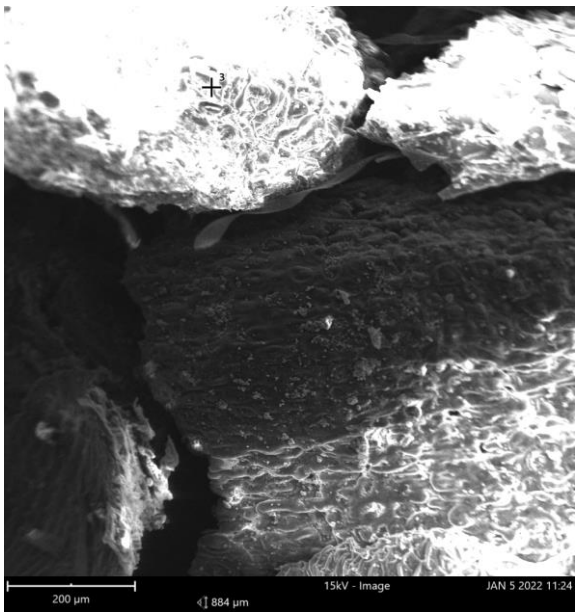
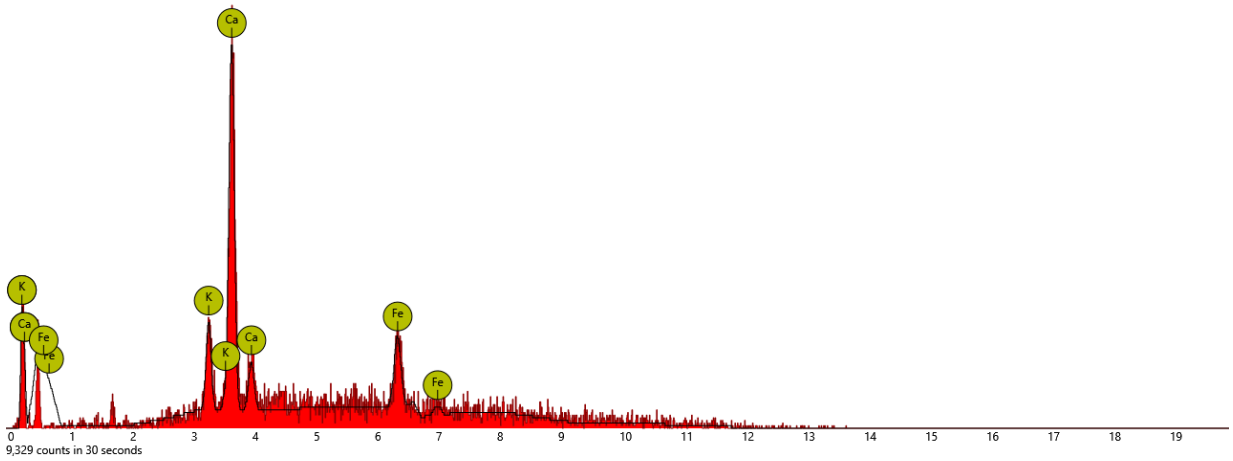
Annexes

Annexe 01.



Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt. Conc.
O	36.31	36.88		
C	59.67	54.66		
Na	0.86	1.39	**Na_2O**	
Ca	59.88	53.89	**CaO**	54.99
Fe	28.94	36.30	**Fe_2O_3**	37.85
K	11.18	9.82		
Si	1.66	3.15	**SiO_2**	9.55
Cl	1.35	3.22		
Mg	1.48	2.54	**MgO**	6.87
Con, concentration. Wt, weight.				

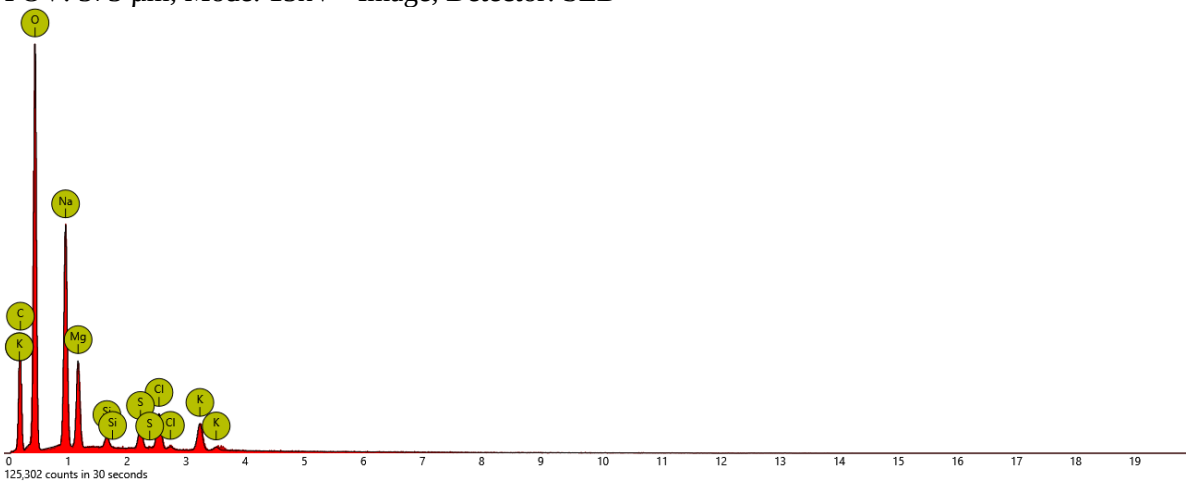
FOV: 884 µm, Mode: 15kV - Image, Detector: SED



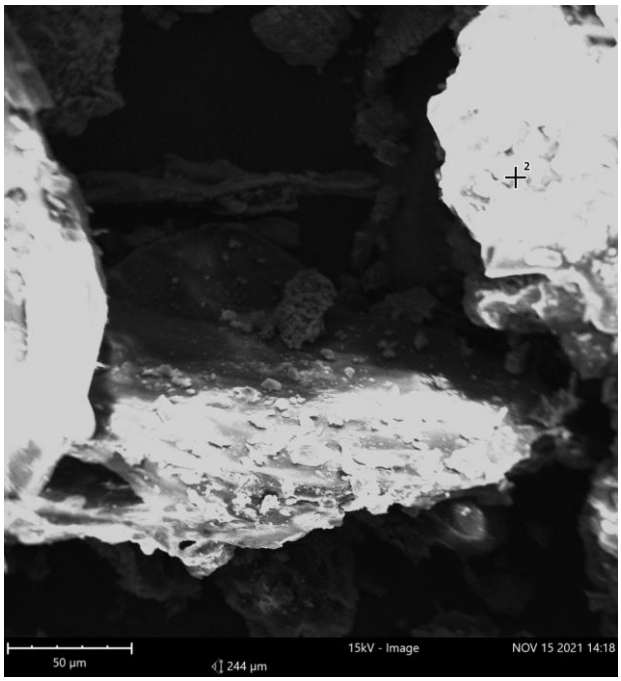
A. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *A. oropediorum.*

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
C	58.45	54.66		
O	34.89	36.88		
Cl	2.88	2.89		
K	1.08	9.06		
Ca	0.45	34.6	**CaO**	6.18
S	0.51	1.47		
Na	0.69	6.72	**Na_2O**	14.28
Mg	0.54	3.44	**MgO**	9.95
Si	0.45	0.88	**SiO_2**	4.50
Con, concentration. Wt, weight.				

FOV: 373 µm, Mode: 15kV - Image, Detector: SED

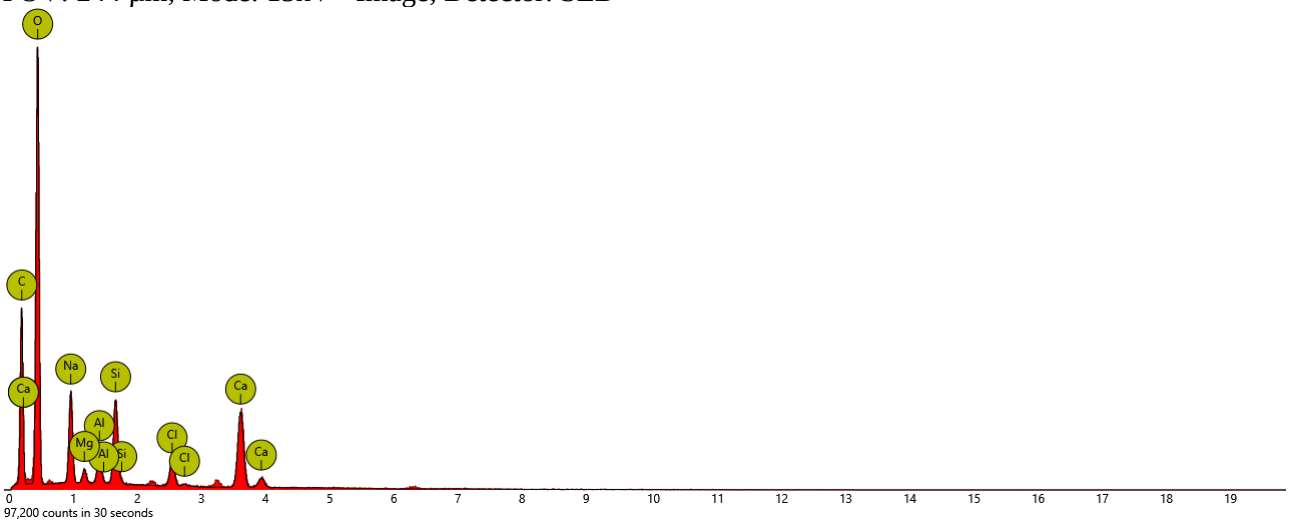


B. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *B. muricata*



FOV: 244 µm, Mode: 15kV - Image, Detector: SED

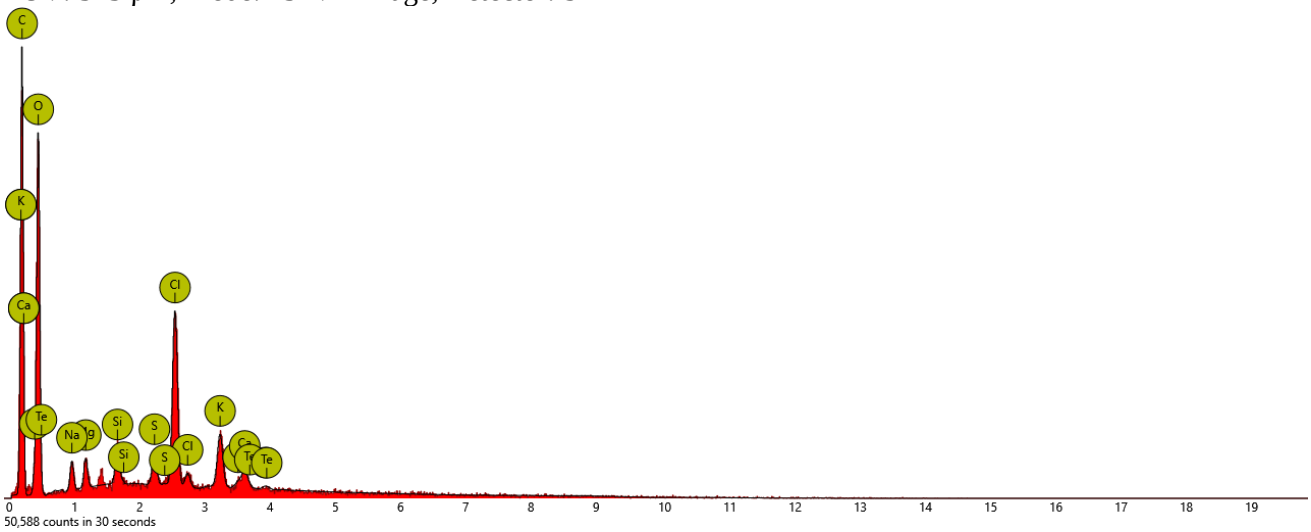
Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	50.47	43.05		
C	38.96	23.06		
Ca	2.91	3.23	CaO	6.61
Na	3.66	7.51	Na_2O	21.3
Si	1.89	1.11	SiO_2	2.1
Al	0.97	0.41	Al_2O_3	1.14
Cl	0.69	6.15		
Mg	0.45	0.17	MgO	3.08
Con, concentration. Wt, weight.				



C. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *H. strobilaceum.*

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
C	58.45	47.18		
O	34.89	37.52		
Cl	2.88	6.86		
K	1.08	2.85		
Ca	0.45	1.22	**CaO**	2.64
S	0.51	1.09		
Na	0.69	1.06	**Na_2O**	2.21
Mg	0.54	0.89	**MgO**	2.26
Si	0.45	0.85	**SiO_2**	2.81
Con, concentration. Wt, weight.				

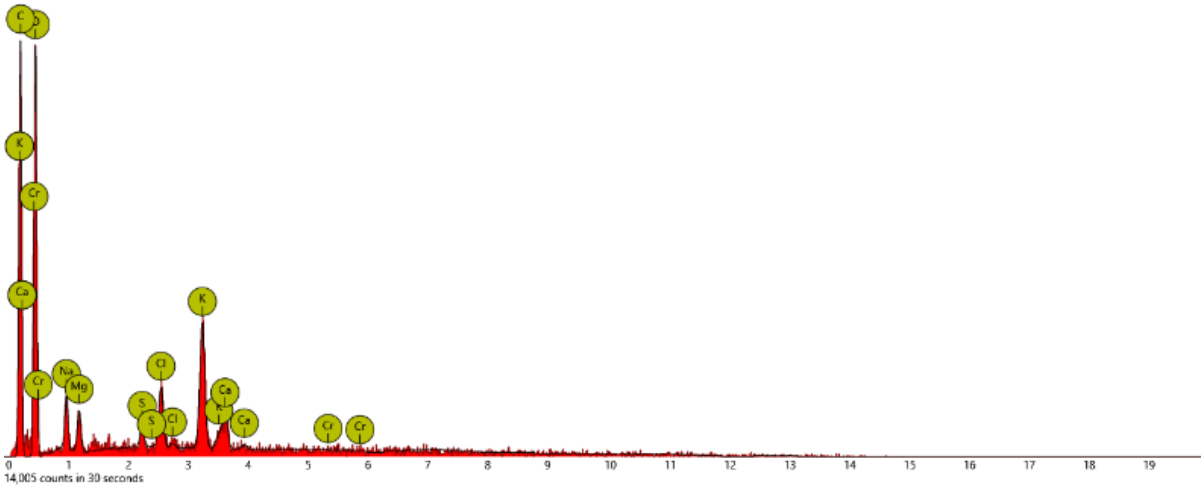
FOV: 373 µm, Mode: 15kV - Image, Detector: SED



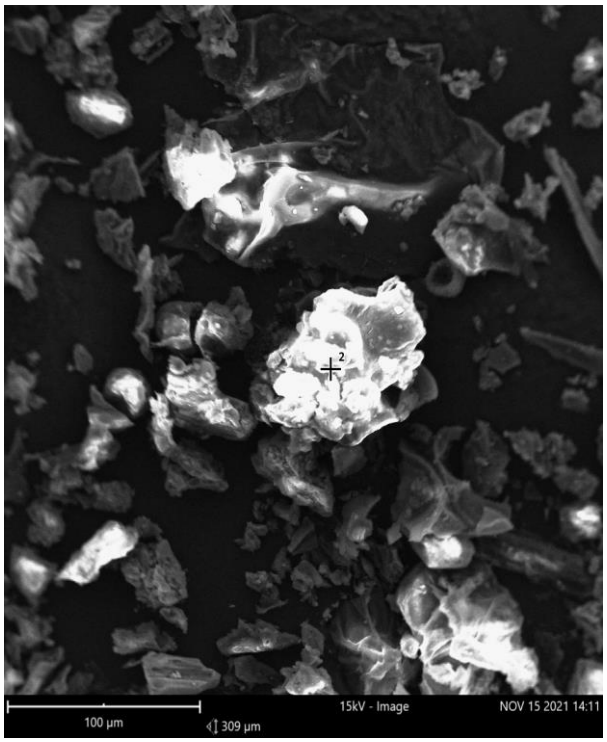
D. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *S. tetragona*.

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	41.35	43.27		
C	51.08	40.13		
K	2.84	7.26		
Cl	1.08	2.50		
Ca	0.94	2.47	**CaO**	5.71
Na	1.47	2.22	**Na_2O**	4.93
Mg	0.88	1.41	**MgO**	3.85
S	0.36	0.75	**SO_3**	3.08

FOV: 309 µm, Mode: 15kV - Image, Detector: SED

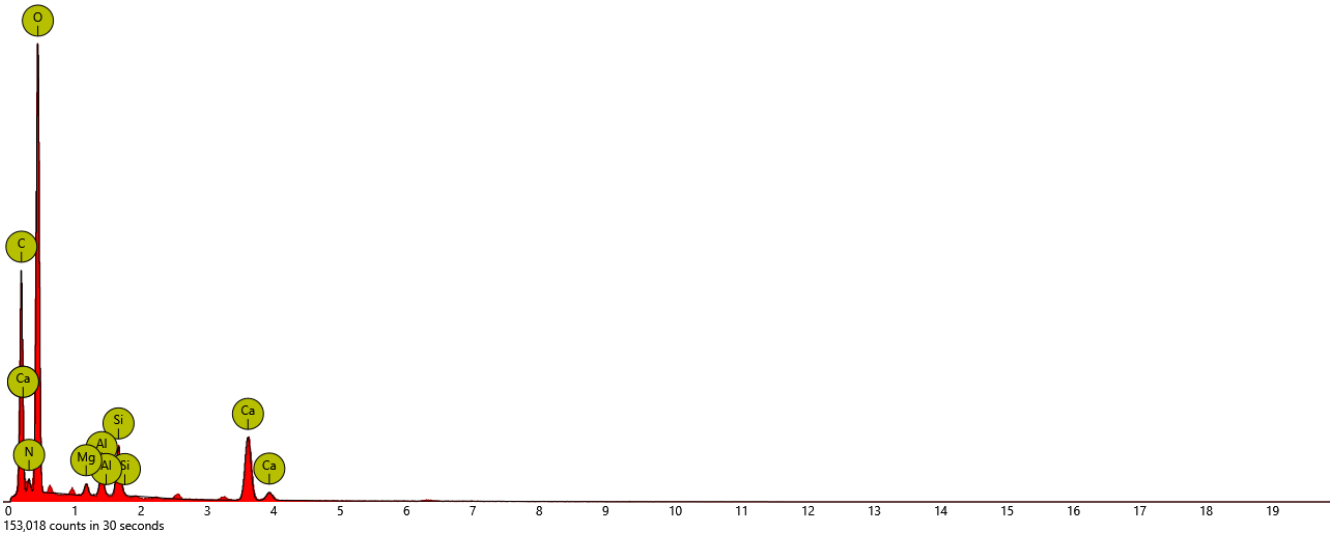


E. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *S.foetida.*



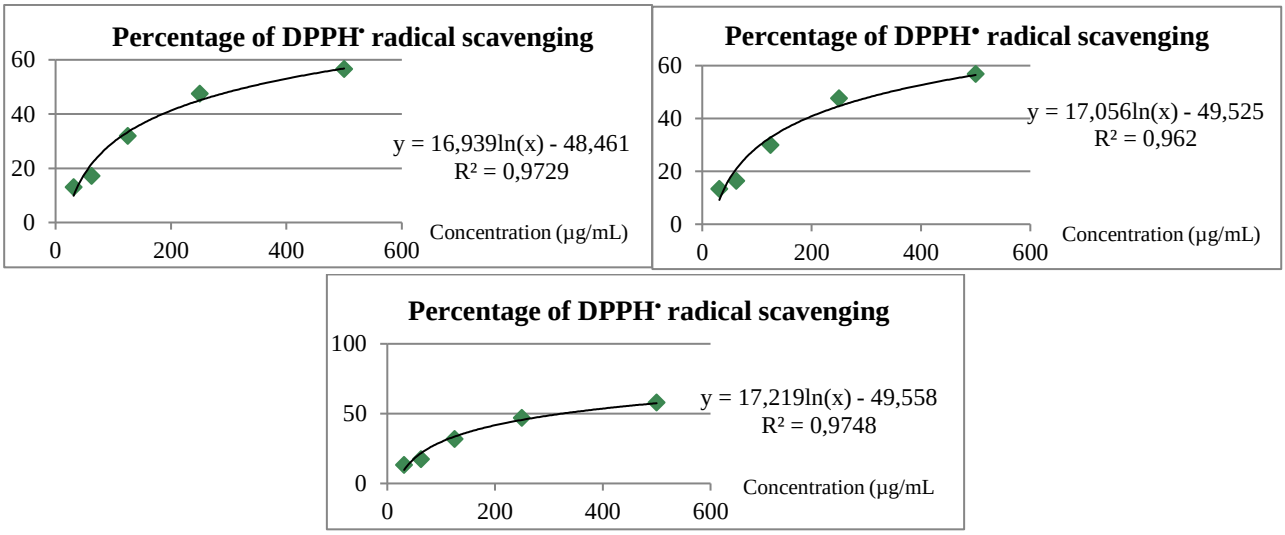
Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol	Stoich. wt Conc.
O	53.96	25.05		
C	35.24	49.55		
Ca	2.42	1.59	**CaO**	4.46
Na	6.27	1.59	**Na_2O**	1.57
Si	1.06	0.49	**SiO_2**	2.1
Al	0.68	0.3	**Al_2O_3**	1.14
Mg	0.36	0.75	**MgO**	3.53
S	0.28	0.25		
Cl	1.01	6.32		
Con, concentration. Wt, weight.				

FOV: 309 µm, Mode: 15kV - Image, Detector: SED

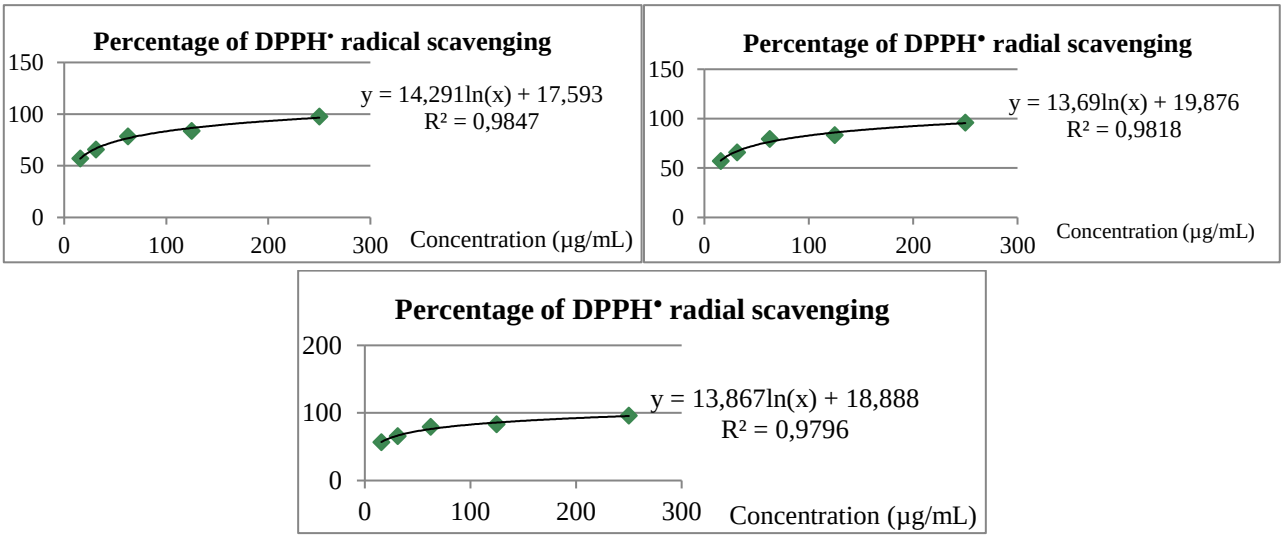


F. Results of a scanning electron microscope (SEM) attached to energy dispersive spectroscopy (EDX) analysis of the dry matter of *S. fruticosa*.

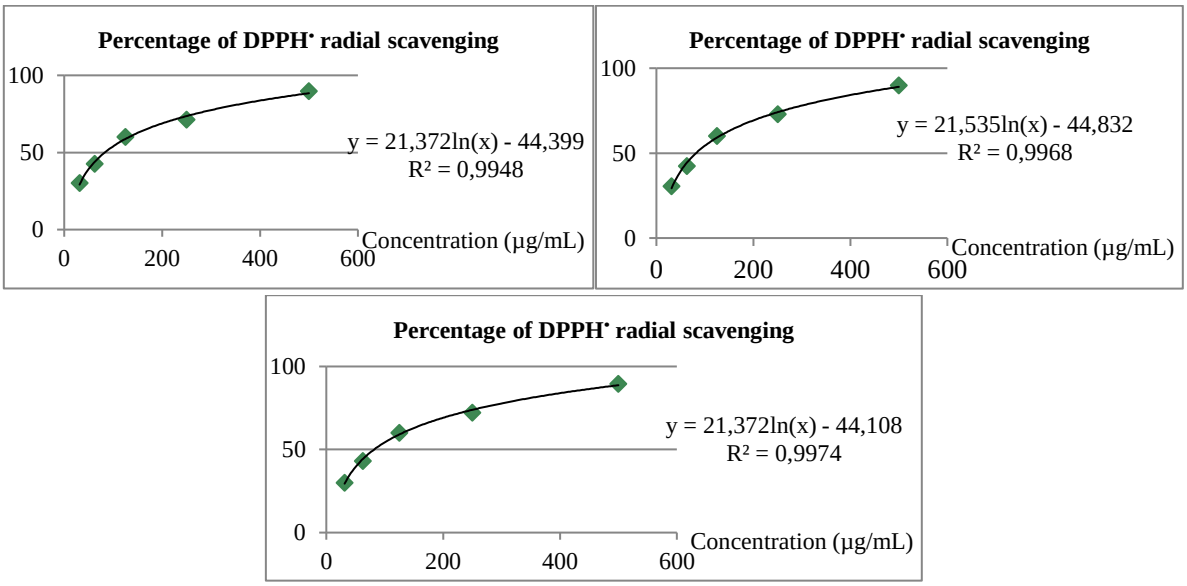
Annexe 02.



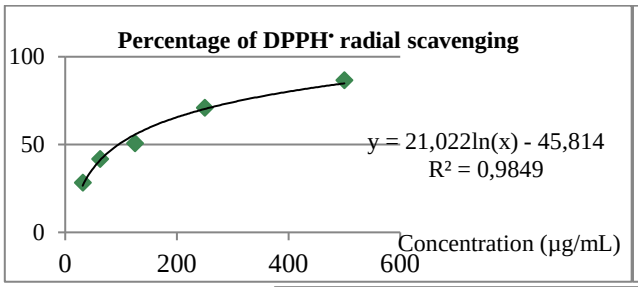
A. DPPH• radical scavenging activity of the different concentration of *A. oropediorum.*

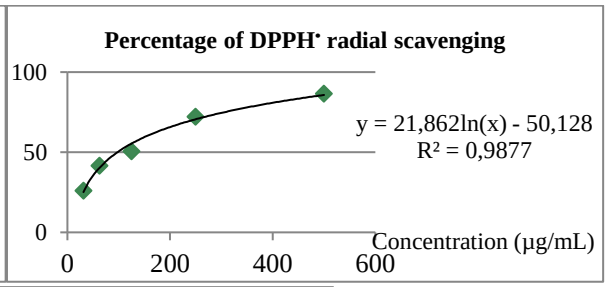


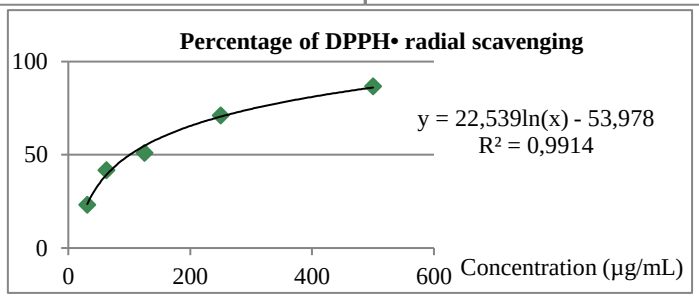
B. DPPH• radical scavenging activity of the different concentration of *B. muricata*.



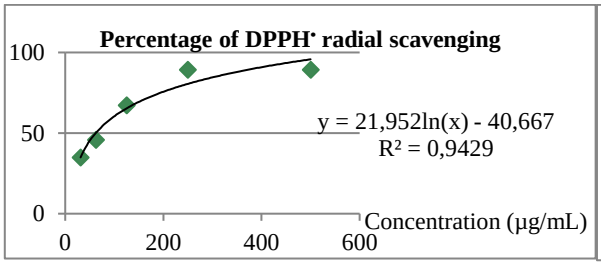
C. DPPH• radical scavenging activity of the different concentration of *H. strobilaceum*

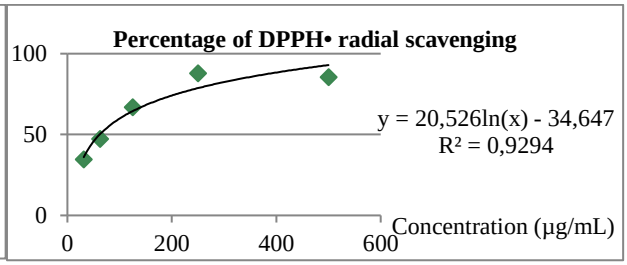


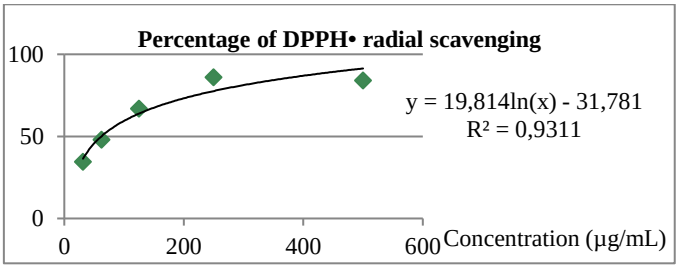




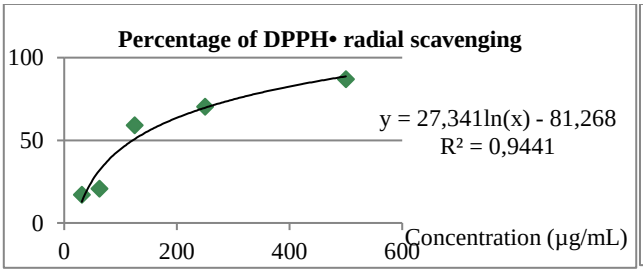
D. DPPH• radical scavenging activity of the different concentration of *S. tetragona*.

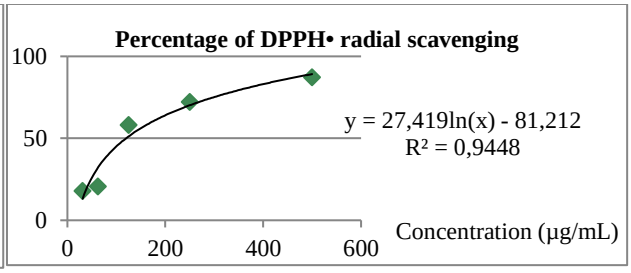


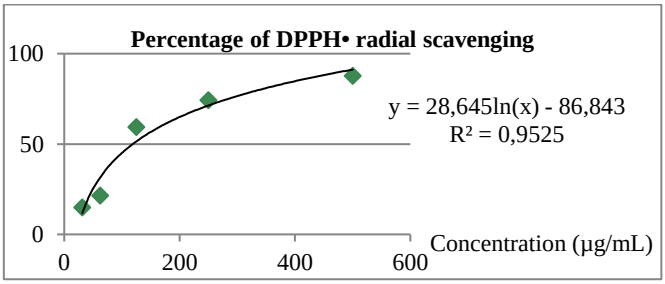




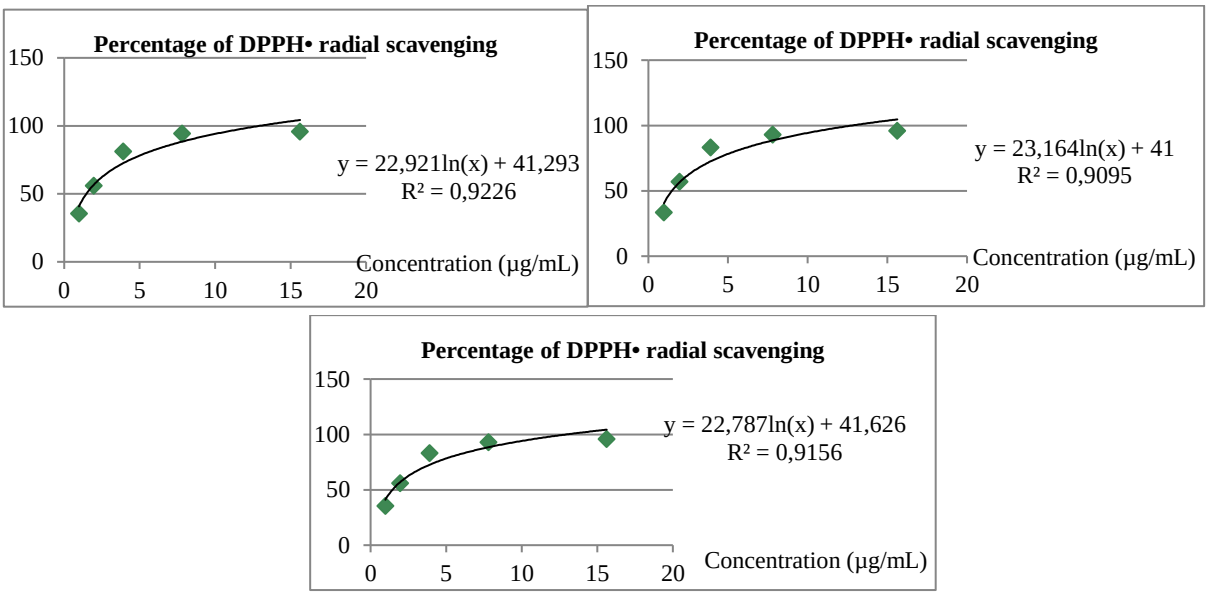
E. DPPH• radial scavenging activity of the different concentration of *S. foetida*.





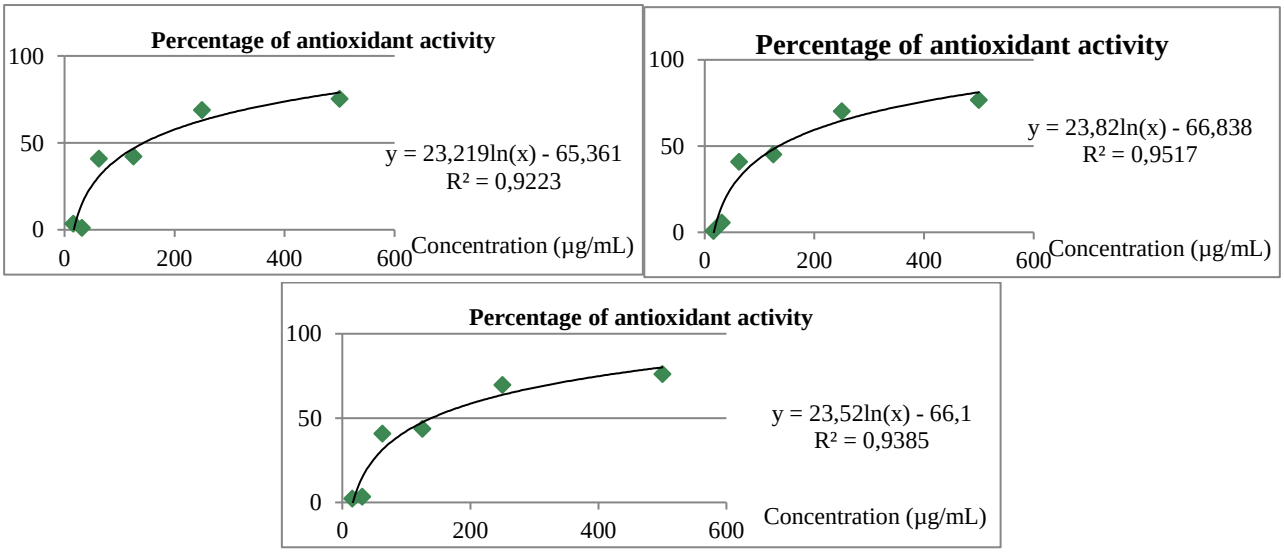


F. DPPH• radical scavenging activity of the different concentration of *S. fruticosa*.

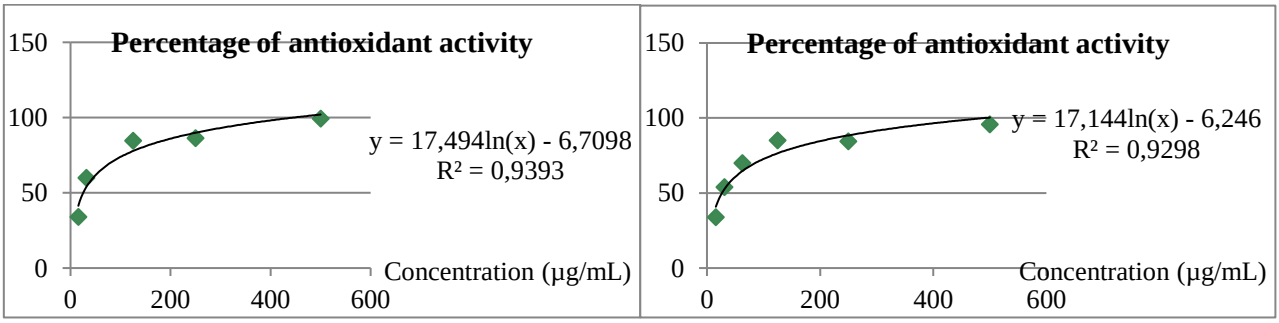


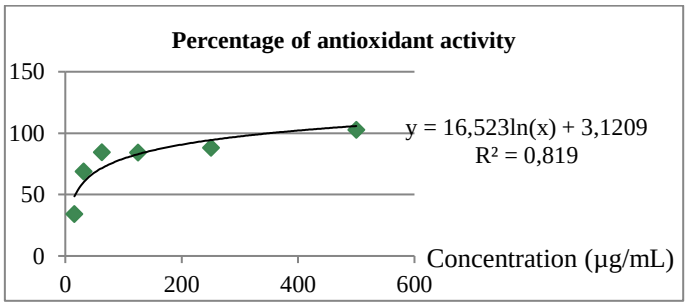
G. DPPH• radical scavenging activity of the different concentration of ascorbic acid.

Annexe 03.

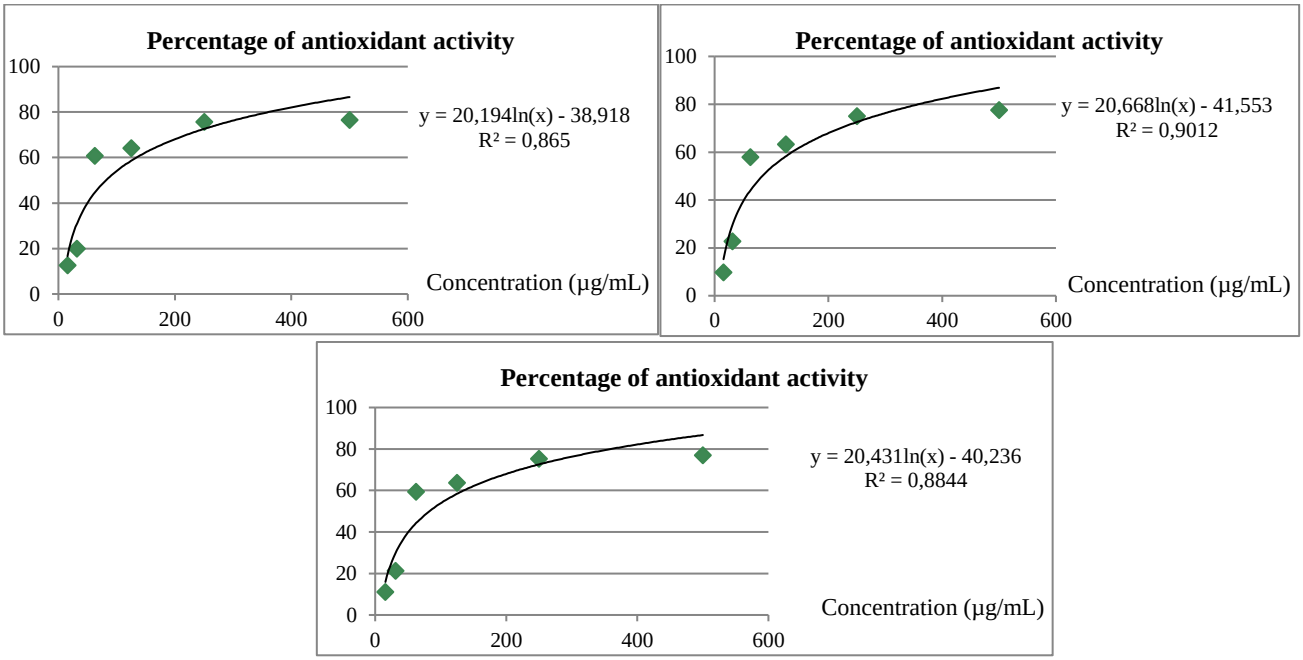


A. Antioxidant activity of *A. oropediorum* extract in the *β*-carotene bleaching assay system.

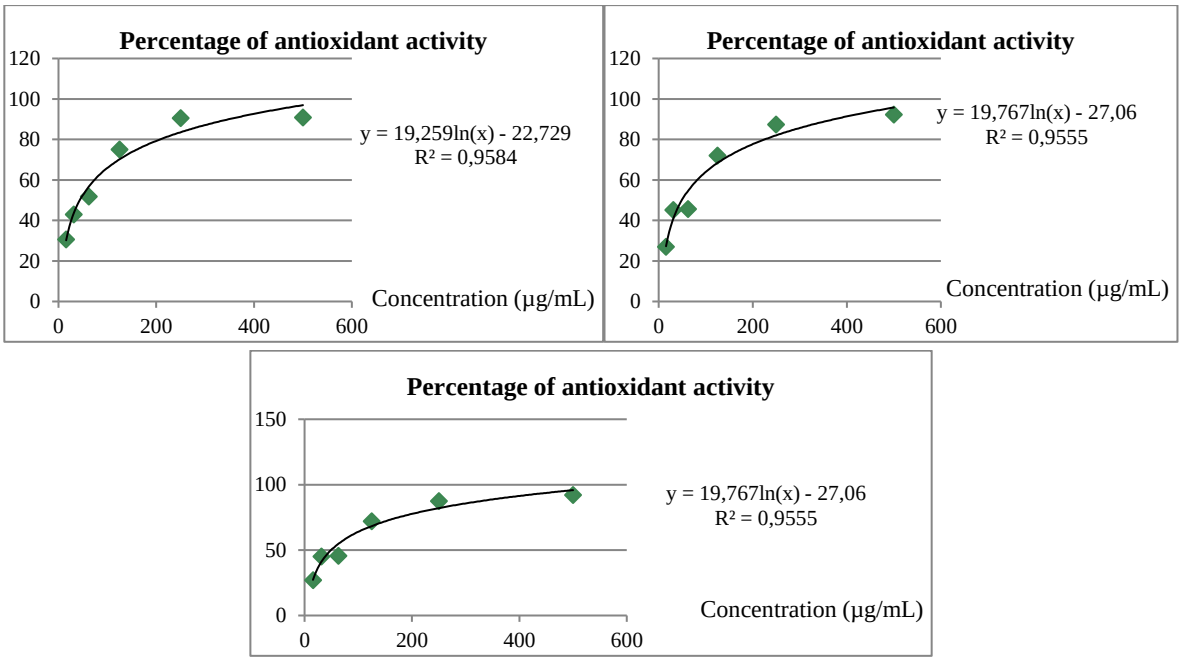




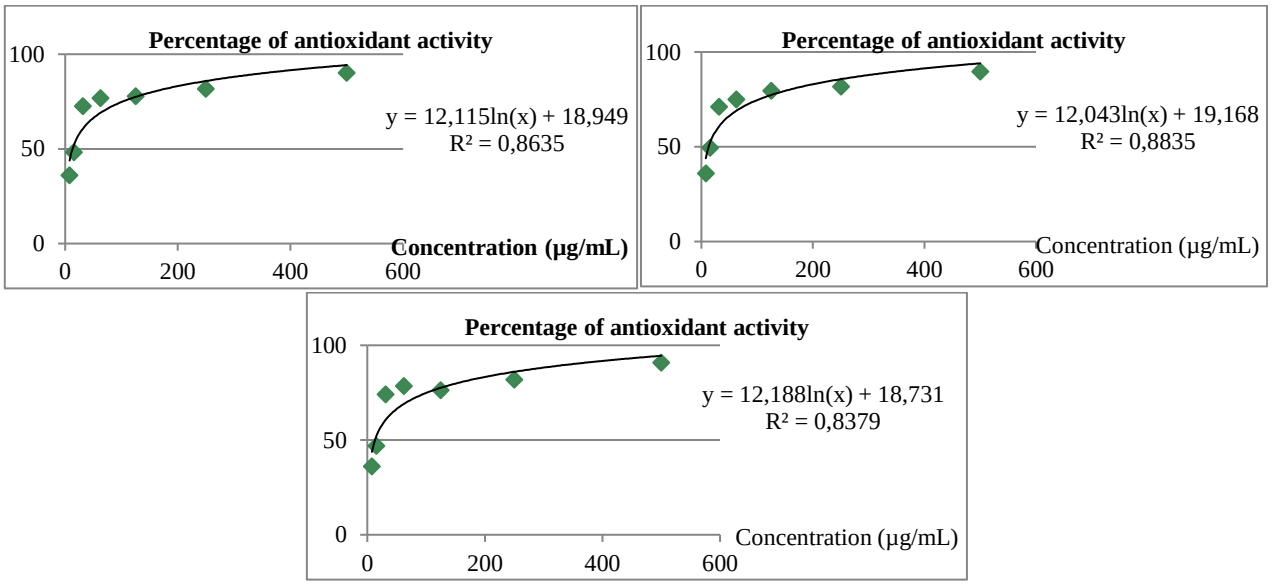
B. Antioxidant activity of *B. muricata* extract in the *β*-carotene bleaching assay system.



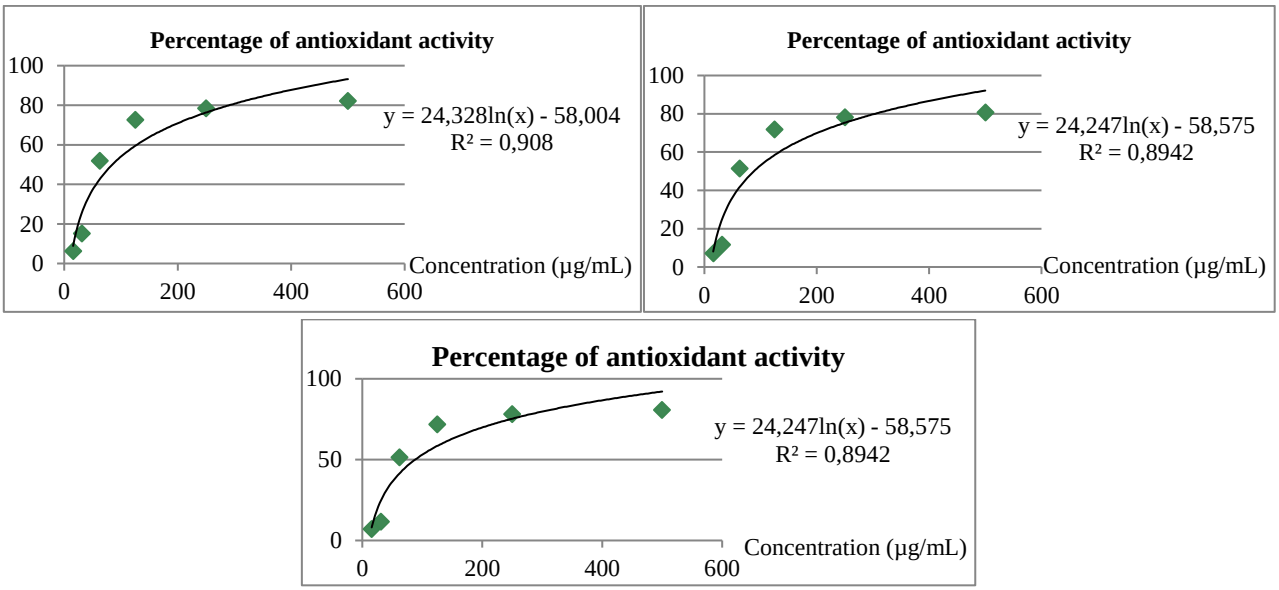
C. Antioxidant activity of *H. strobilaceum* extract in the *β*-carotene bleaching assay system.



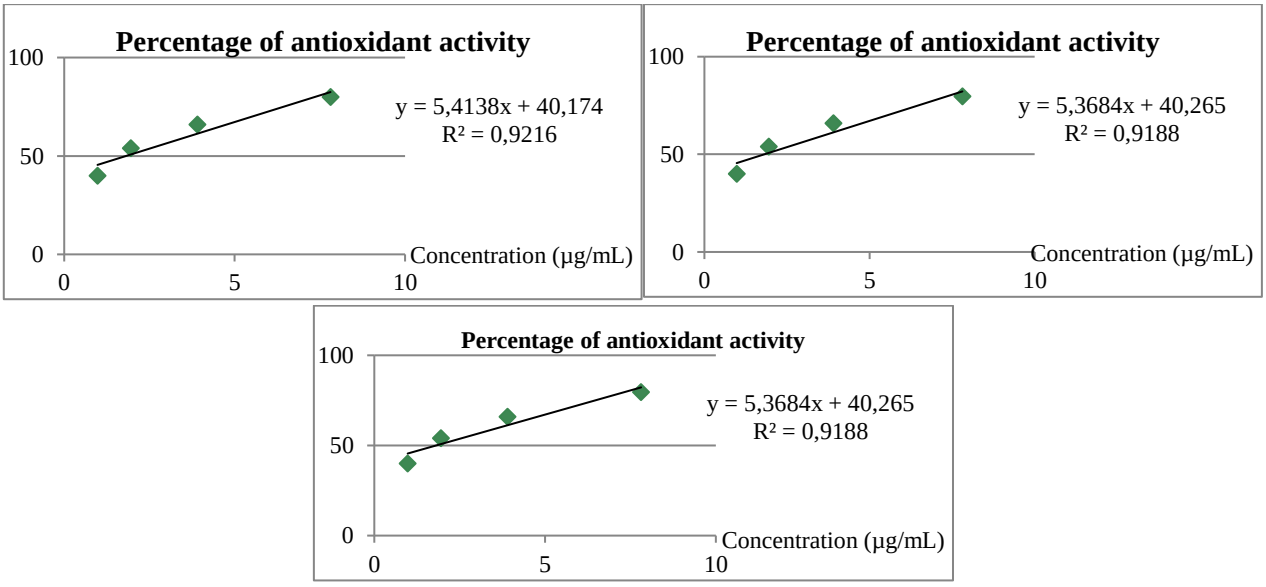
D. Antioxidant activity of *S. tetragona* extract in the *β*-carotene bleaching assay system.



E. Antioxidant activity of *S. foetida* extract in the *β*-carotene bleaching assay system.

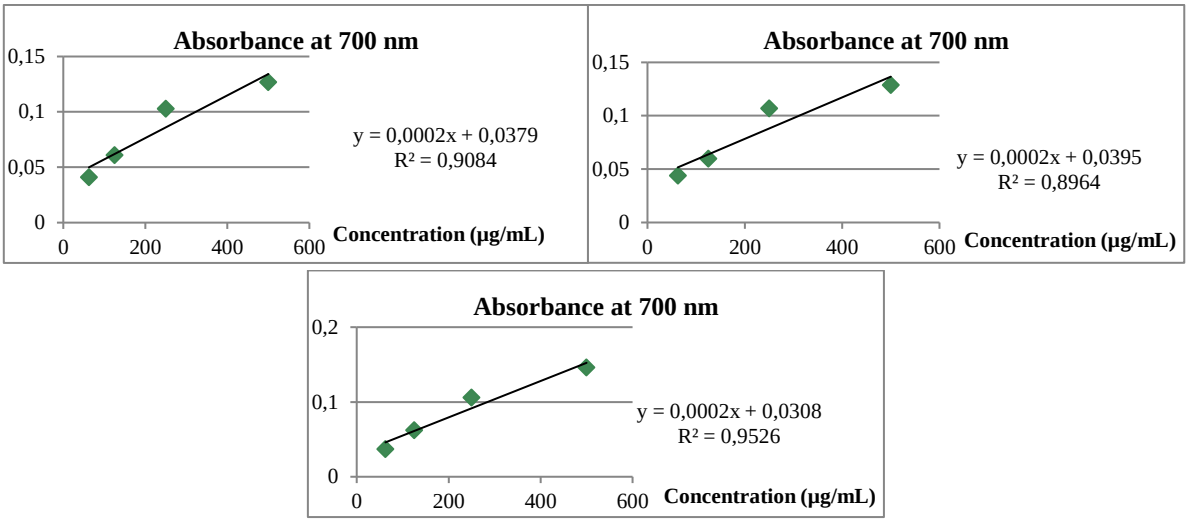


F. Antioxidant activity of *S. fruticosa* extract in the *β*-carotene bleaching assay system.

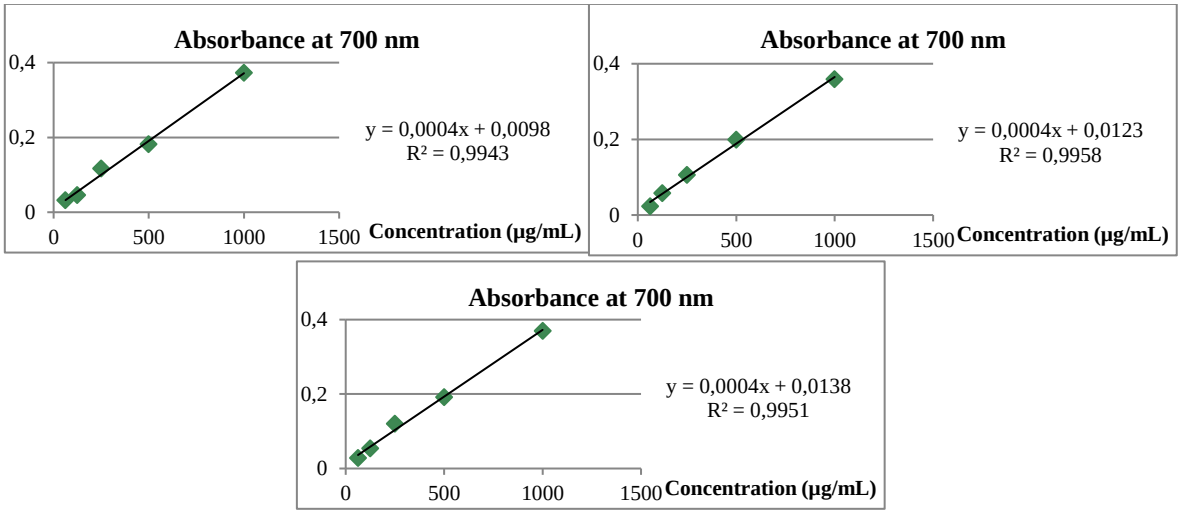


G. Antioxidant activity of α-Tocopherol in the *β*-carotene bleaching assay system.

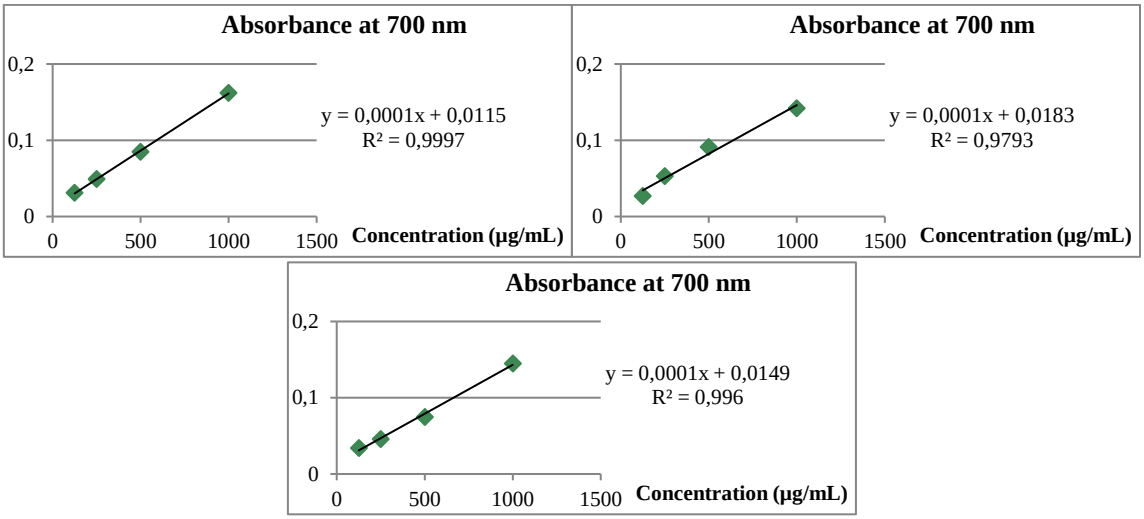
Annexe 04.



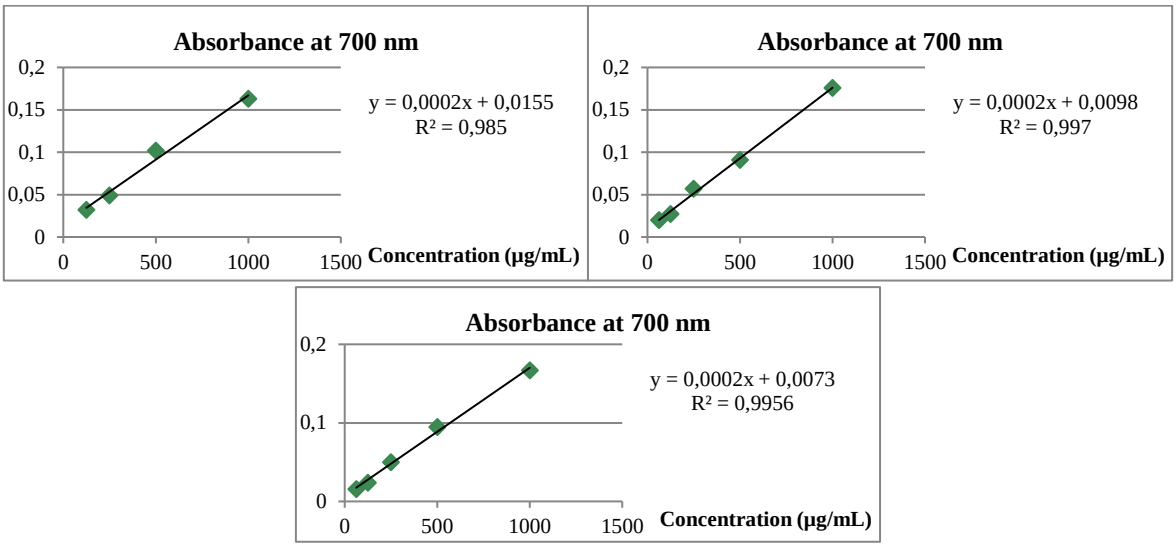
A. Reducing power assay of *A. oropediorum* extract. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).



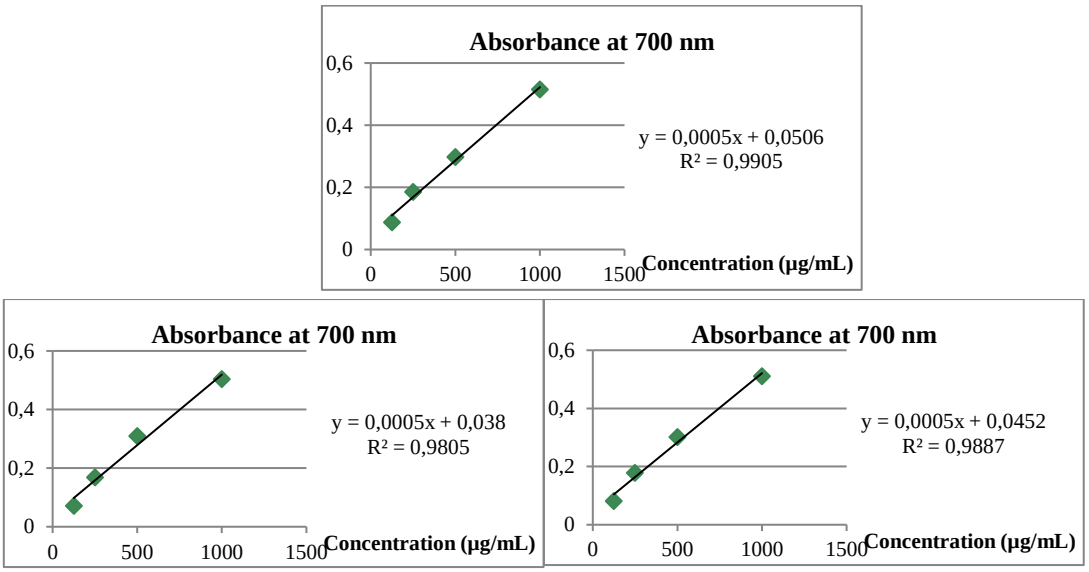
B. Reducing power assay of *B. muricata* extract. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).



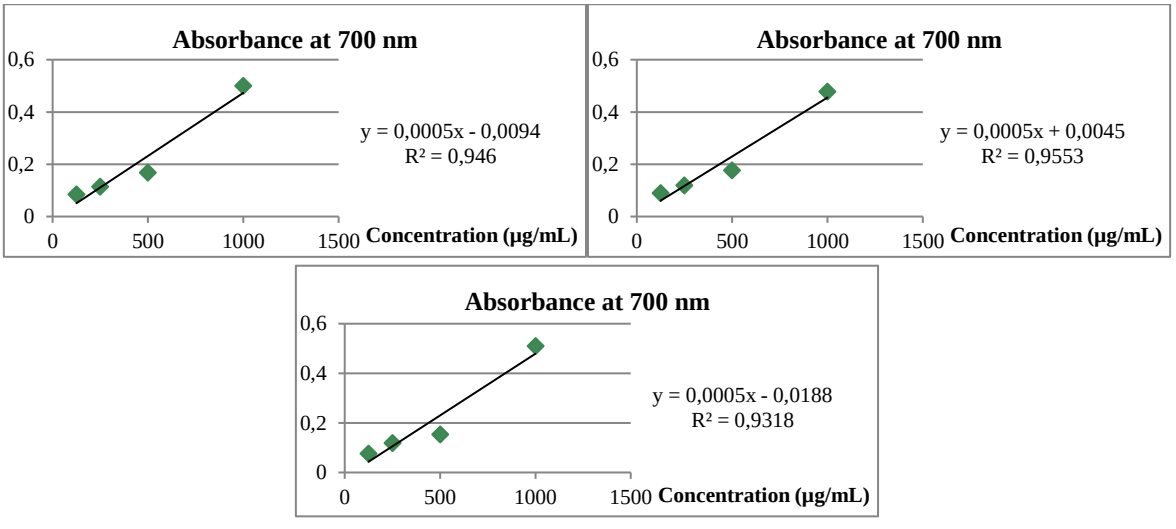
C. Reducing power assay of *H. strobilaceum* extract. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).



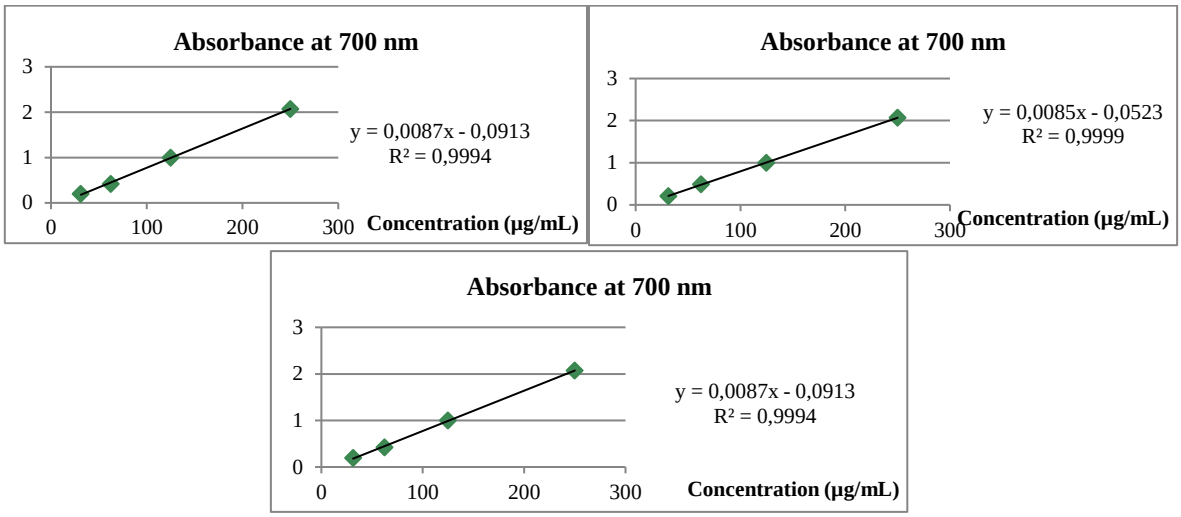
D. Reducing power assay of *S. tetragona* extract. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).



E. Reducing power assay of *S. foetida* extract. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).



F. Reducing power assay of *S. fruticosa* extract. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).



G. Reducing power assay of ascorbic acid. Axis X: concentration (µg/mL), Axis Y: absorbance (at 700 nm).

Annexes 05. « Communications and Publications »

Gheraissa, N., Chemsa, A. E., Elsharkawy, E., & Cherrada, N. (2022). Phenolic compound profile, and evaluation of biological properties of *Bassia muricata* (L.) Asch. aerial part. *International Journal of Secondary Metabolite, Vol. 9, No. 3*, 335-347. https://doi.org/10.21448/ijsm.1080537

A. International Scientific Publications

غـــرايسة، ن و شمـــسة، أ. خ. "الدراسات الفيتوكيميائية والبيولوجية لبعض نباتات العائلة الرمرامية"، ملتقى الدكتوراه الدولي متعدد التخصـــصـــات (IPPM'20) الإصدار الأول، جامعة الشهيد حمه لخضر - الوادى، 23-26 فبراير 2020.

Gheraissa, N., Chemsa, A. E. 'Phytochemical analysis and antioxidant activity of methanolic extract of *Salsola foetida* del. (*Chenopodiaceae* Vent.)', International Conference on the Valorization of Alternative Plants and degraded and Marginal Lands (VPADM, 2022), 11 May 2022.

B. International communications

Gheraissa, N., Chemsa, A. E. 'RP-HPLC analysis of phenolic compounds, antioxidants, and antimicrobial activities from *Salsola foetida* del. (*Chenopodiaceae* Vent.)', First National Seminar on Green Chemistry and Natural Products NGCNP2022, 14 – 15 March, 2022.

Gheraissa, N., Chemsa, A. E., Khirouan, N. E., & ALAMRI., Z. 'Quantification of the phenolic compounds and antioxidant activity of *Suaeda mollis* L. (*Chenopodiaceae* Vent.), 1st National Seminar: Biotechnology, Health and Agro-Environment', 14 – 15 March, 2022.

C. National communications