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THEME:

Phytochemical Study and Biological Activities of *Solanum elaeagnifolium cav* Leaf Extracts

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ABSTRACT

Phytochemical Study and Biological Activities of *Solanum elaeagnifolium cav* Leaf Extracts

Solanum elaeagnifolium is one of the invasive plants in Algeria; studies on its chemical composition and its biological activities are rare in Algeria. The current work aims to investigate the chemical components in *Solanum elaeagnifolium* leaf extracts. This study has shown antioxidant activity, photoprotection activity, and antibacterial activity. The objective of this research is to evaluate this plant and assess its biological activities as well as its total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC). In addition to sun protection factor (SPF) and infrared, The Folin-Ciocalteu method and the aluminium-trichloride ($AlCl_3$) method were used to determine TPC and TFC in hydro-ethanol (HEE) and soxhlet (ethyl cetate, butanol, and chloroformic phases) leaf extracts. Two assays were performed to determine the antioxidant activity: the DPPH test (radical 2,2'-diphenyl-1-picrylhydrazyl) and the TAC tests. Disc diffusion and minimal inhibitory concentration were used to test antibacterial activity against six pathogenic bacteria. The hydro-ethanol extract (99.59 ± 1.94 mg EAG/g) has a greater polyphenol concentration than the ethyl acetat extract (86.74±1.91 mg EAG/g), butanol extract (81.51±1.01 mg EAG/g), and chloroform (38.48±0.72 mg EAG/g). Also, the flavonoid content of the hydro-ethanol extract (25.87± 0.556 mg EQ/g) is greater than that of the butanol extract of soxhlt (18.71 ± 0.699 mg EQ/g) than the chloroformic phase (16.25±0.42 mg EQ/g), and the lowest value is the AcoEt phase (12.76±0.44 mg EQ/g). The DPPH values were IC50 = 0.443 ± 0.0028 mg/mL for hydro-ethanolic extract and (1.439 ± 0.0341 mg/mL) and (1.751 ± 0.064 mg/mL) for ethyl acetate and butanol, respectively. The overall antioxidant activity of the ethanol extract was found to be considerable. In terms of antibacterial activity, the extract is almost weak against some bacteria (*Escherichia coli, Pseudomonas aeruginosa, and Klebsila pneumonia*). *S. elaeagnifolium* leaf extracts contain good SPF values compared with the positive control (sunscreen cream).

Keywords: *Solanum elaeagnifolium*; phenols; flavonoids; antimicrobial activity; antioxidant activity; SPF; infrared.

RESUME

Étude phytochimique et activités biologiques des extraits de feuilles de *Solanum elaeagnifolium cav*

Solanum elaeagnifolium est l'une des plantes invasives en Algérie, les études sur sa composition chimique et ses activités biologiques sont rare en Algérie. Le travail actuel vise à étudier les composants chimiques des extraits de feuilles de *Solanum elaeagnifolium*. A montré d'une activité antioxydante acceptable, une protection contre les rayons solaires et une activité antibactérienne. L'objectif de cette recherche est d'évaluer les activités biologiques de ce plant ainsi que sa teneur en phénols totales (TPC), son contenu total en flavonoïdes (TFC). En plus du facteur de protection solaire (SPF) et de l'infrarouge pour l'extrait éthanoïque, La méthode Folin-Ciocalteu et la méthode trichlorure d'aluminium ($AlCl_3$) ont été utilisées pour déterminer le TPC et le TFC dans les extraits hydro-éthanolique (HEE) des feuilles de *solanum elaeagnifolium* ainsi que les phases obtenues à l'aide du soxhlet (éthylacétique, buthanoliques et chloroformiques). Deux essais ont été effectués pour déterminer l'activité antioxydante : le test DPPH (radical 2,2'-diphényl-1-picrylhydrazyl) et le test TAC. La diffusion de disques et la concentration minimale inhibitrice (CMI) ont été utilisées pour tester l'activité antibactérienne contre six bactéries pathogènes. L'extrait d'hydro-éthanolique (99,59 ± 1,94 mg EAG/g) a une concentration de polyphénols plus élevée que l'extraction d'acétate d'éthyle (86,74 ±1,91 mg EAC/g), l'extrémité de butanol (81,51 ± 1,01 mg EAGE / g) et le chloroforme (38,48 ± 0,72 mg EAP / g). En outre, la teneur en flavonoïdes de l'extrait d'hydroéthanol (25,87 ± 0,556 mg EQ/g) est plus élevée que celle de l'extrait de butanol de soxhlt (18,71 ± 0.699 mg Eq/g), que dans la phase chloroforme (16,25 ±0,42 mg EQU/g). Les valeurs du DPPH étaient IC50 = 0,443 ± 0,0028 mg/mL pour l'extrait hydroéthanolique et (1,439 ± 0.0341 mg/ml) et (1,751 ±0,064 mg/ mL) pour l'acétate d'éthyle et le butanol, respectivement. L'activité antioxydante globale de l'extrait d'éthanol a été considérable. En termes d'activité antibactérienne, l'extrait présent presque une faible antibactérienne activité faible contre certaines bactéries (*Escherichia coli, Pseudomonas aeruginosa, and Klebsila pneumonia*). Les extraits de feuilles de *S. elaeagnifolium* ont a donnés des bonnes resultats du SPF comparativement à celui du control (crème solaire).

Mots-clés: *Solanum elaeagnifolium*; polyphénols; flavonoïdes; activité antimicrobienne; action antioxydante; SPF; infrarouge.

ملخص

دراسة الفيتوكيميائية والنشاطات البيولوجية لمستخلصات أوراق نبات *Solanum elaeagnifolium cav*

السجوة الزيتية هي واحدة من النباتات الغازية في الجزائر ومن النادر في الجزائر إجراء الدراسات عن تركيبتها الكيميائية وأنشطتها البيولوجية .ويهدف العمل الحالي إلى استقصاء المكونات الكيميائية في مستخلصات اوراق السجوة الزيتية .أظهرت نشاطا مضادا للأكسدة ،ونشاطا للحماية الضوئية ونشاطا مضادا للبكتيريا.والهدف من هذا البحث هو تقييم هذه النبتةوتقييم انشطتها البيولوجية وكذلك إجمالي محتواها الفينولي ومجموع المحتوى الفلافونويدي ومجموع القدرة على مكافحة الأكسدة. وبالإضافة إلى عامل الحماية من الشمس والأشعة تحت الحمراء ،استخدمت طريقة Folin و كلوريد الألومنيوم لتحديد مركبات الفينول و الفلافونويدي في مستخلص الماء والكحول ومستخلصات soxhlet ايثيل اسيتات، كلوروفورم،بيتانول).اجري اختباران لتحديد نشاط مضاد الأكسدة :اختبار DPPH و, ومجموع القدرة على مكافحة الأكسدة وبينما استخدم الانتشار القرصي الحد الأدنى من التراكيز لاختبار نشاط البكتيريا ضد ٦بكتبريات مسببة للمرض .ولدى مستخلص مستخلص الماء والكحول (99.59 ± 1.94 mg EAG/g) اكبرتركيز من البوليفينول مقارنة بتركيز مستخلص ايثيل اسيتات (86.74±1.91 mg EAG/g)ومستخلص البيتانول اعطى (81.51±1.01 mg EAG/g)،ومستخلص الكلوروفور .(38.48±0.72 mg EAG/g) كما ان المحتوى الفلافونويدي لمستخلص الايثانول 25.87± 0.556 mg) (EQ/g.وهو اكبر من محتوى مستخلص البيتانول(16.25±0.42 mg EQ/g)اما بالنسبة لمحتوى الفلافونويد لمستخلص كل من الكلوروفورم وايثيل اسيتات اعطيا النتائج التالية بالترتيب (12.76±0.44 mg :(18.71 ± 0.699 mg EQ/g) (EQ/g وكانت قيم IC50 ل DPPH 0.443 ± 0.0028 mg/mL لمستخلص الايثانول و 0.0341 ± 1.439) (mg/mL.لمستخلص ايثيل اسيتات والبيتانول (1.751 ± 0.064 mg/mL) وتبين أن النشاط العام لمضاد الأكسدة في مستخلص الايثانول مرتفع.وفيما يتعلق بالنشاط المضاد للبكتيريا ،فان الاستخلاص يكاد يكون ضئيلا في بعض البكتيريا ((*Escherichia coli, Pseudomonas aeruginosa, and Klebsila pneumonia*)) وتحتوي مستخلصات اوراق السجوة الزيتية على قيم جيدة من SPF مقارنة ب SPFكريمة واقي الشمس التي استعملت كمراقب إيجابي .

الكلمات المفتاحية: السجوه الزيتية ،الفينول ،الفلافونويد ،نشاط ضد البكتيريا ،نشاط ضد الأكسدة ,SPF, نشاط ضد أشعة الشمس والأشعة تحت الحمراء.

ABBREVIATIONS LIST

Abs: Absorbance
AC_2O: Acetic Anhydride
AcoEt: Ethyl Acétate
$AlCl_3$: Aluminium Chloride
ATCC: American Type Culture Collection
BuOt: Butanol
CH_3COOK: Potassium Acetate
$CHCl_3$: Chloforme
DMSO: Dimethyl Sulfoxide
DPPH: 2, 2-Diphenyl-1-picrylhydrazyl
E.O: Essential Oil
EtOH: Ethanol
$FeCl_3$: Ferric Chloride
FTIR: Fourier Transforms Infrared
H_2SO_4: Sulfuric Acid
HCL: Hydrochloric Acid
$HgCl_2$: Mercury Chloride
I_2: Iodine
IC50: Half maximal Inhibitory Concentration
IMC: Minimal Inhibitor Concentration
KI: Potassium Iodide
KPR: *Klebsiella Pneumonia* Reference
KP: *Klebsiella pneumoniae*
MH: Muller Hinton agar
MHG: Müeller–Hinton gelose medium
Na_2HPO_4: sodium phosphate dibasic
NaCl: Sodium Chloride
NaOH: Sodium Hydroxide
NB: Nutrient broth
NH_4OH: Ammonium Hydroxide
PA: *Pseudomonas aeruginosa*
PAR: *Pseudomonas aeruginosa* Reference
RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species
SPF: Sun Protection Factor
TAC: The total antioxidant capacity
TFC: Total flavonoid content
TPC: Total phenolic content
Y%: Yield
ZOI: inhibition zone

INTRODUCTION

Introduction

Humanity has utilised a range of local plants for food and medicine since the beginning of time, treating and healing a wide range of illnesses **(Addoun and Boumediou, 2017)**. This is because plants are easily obtained, widely accessible, and has low levels of toxicity **(Eddouks et *al*., 2017)**.

Growing scientific study has shown a link between phytotherapeutic benefits of plants and physiologically active chemicals produced by secondary metabolites **(Kralova et *al*., 2021)**. For that understanding the molecular components of medicinal plants and obtaining the correct scientific knowledge are essential for their appropriate use. The therapeutic qualities of plants are derived from the chemicals they contain **(Khare et *al*., 2021)**.

Algeria boasts a diversified flora, including over 4,000 species of vascular plants, making it a true phylogenetic reservoir **(Hamel, 2018)**.

S. elaeagnifolium, a member of the Solanaceae family, is a widespread plant found in America and expanding into the Maghreb and Spain. With almost 2000 species, the Solanum genus is the richest in the Solanaceae family. Commonly consumed foods include *Solanum nigrum, Solanum elaeagnifolium*, potato (*Solanum tuberosum*), aubergine (*Solanum melongena*), tomato (*Solanum lycopersicum*), and potato (*Solanum tuberosum*) The plant's rich content of phenols, alkaloids, saponins, terpenes, flavonoids, coumarins, and carotenoids has been linked to health benefits **(Bouslamti et *al*., 2022)**.

S. elaeagnifolium despite being considered a bad plant, is known for its therapeutic properties due to its phytochemicals. Its diverse soil textures make it a polyvalent plant with anticancer, chemiopreventives, insecticides, mollusciciques, analgésiques, and antioxydantes properties **(Xavier et *al*., 2022; Bouslamti et *al*., 2023)**.

According to **DeepaShree et *al*, (2012)** to motivate the application of therapeutic substances from medicinal plants, it is necessary to investigate their composition and action. FTIR is commonly used to identify the functional groups found in plant compounds.

In this context, the objective of this work is to focus on the phytochemical study, the evaluation of the biological activities (antioxidant, antibacterial, and photo-protection) of *Solanum elaeagnifolium* different extracts, the determination of total phenolic and total flavonoid content.

This study consists of two parts:

- The first section is devoted to a bibliographic synthesis, which includes three parts. The first part covers the presentation of the plant (*Solanum elaeagnifolium*, Family: Solanaceae), followed by secondary metabolites and biological activities.
- The second section pertains to the experimental portion and is divided into two chapters, the first covers materials and procedures, and the second compiles all of the findings and discussions.

Chapter I:

Literature Review

Solanum elaeagnifolium

1-The Solanaceae family

A family of dicotyledonous plants in the Solanales order, the Solanaceae family is one of the larger ones. Along with its diverse chemical composition, it is well-known for its assortment of reproductive structures **(Gebhardt, 2016)**.

This plant family, which includes herbaceous plants, trees, shrubs, and lianas, is found primarily in tropical regions of both hemispheres, particularly in South and Tropical America.With nearly 147 genera and around 2930 species, many of these plants are toxic due to steroid terpene and alkaloids **(Judd et *al*., 2002)**.

Despite their toxicity, Solanaceae plants are extensively utilized in pharmacy, with certain species like tobacco (*Nicotiana*), belladonna (*Atropa*), and thorn apple (*Datura*) being potent narcotics **(Benkou, 2014)**.

Humans value the Solanaceae family of plants highly, primarily for their food (*Solanum tuberosum L., potatoes; Solanum lycopersicum L., tomatoes; Solanum melongena L., aubergine; Capsicum annuum L., pepper*); as ornamental plants (*Petunia Juss, Schizanthus Ruiz & Pav, Salpiglossis Ruiz & Pav, Browallia L., Brugmansia Pers*.); and for their toxic, medicinal, or psychoactive properties (*Nicotiana tabacum L., tobacco; Atropa belladonna L., belladone; Mandragora offcinarum l., mandragore; Hyoscyamus niger L., jusquiame; Datura stramonium L.*), spinal pulp **(Gebhardt, 2016)**.

1-1 *Solanum* Genus

With seven subgenres and over 1500 species identified, *Solanum* is one of the most abundant genera of vascular plants. It is found in every place where plants grow, but is most prevalent in the Neotropical zone. Important food species can be found there **(Djouadi, 2012)**.

1-2 *Solanum elaeagnifolium*

Solanum elaeagnifolium is a very invasive weed that has spread rapidly around the world, infesting both irrigated and rainy agricultural systems **(Karmezi et *al*., 2022; Krigas et *al*., 2023)**. *S. elaeagnifolium* is a highly adaptive plant that can withstand drought, saline soil conditions, low annual rainfall (250–600 mm), and high summer temperatures (20–34°C) **(Karmezi et *al*., 2022)**.

S.elaeagnifolium is native to the southwest and northern Mexico. As a result of human activity, it has been spread to several nations, including Greece, Morocco, India, Tunisia, Egypt, and Australia **(Roberts and Florentine, 2022)**.

1-3 Botanical description of *Solanum elaeagnifolium*

One terrifying annual crop in the Solanaceae family is *Solanum elaeagnifolium*. This herbaceous plant can grow up to 60 centimetres in length, depending on the biotope. Its morphological variety is really wide. Buds that produce rhizomes ensure that the roots can descend as far as five meters **(Chafik et *al*., 2013).**

The plant's stems are branching at the top and aligned, with woody tips at the base. This plant seems grey because of the silver-white tint of its leaves. While the upper leaves are smaller, measuring between 1 and 2 cm wide and 6 to 10 cm long, the lower leaves can reach a length of 15 cm. The leaves are lanceolate to oblong in form, with alternating, wavy edges and a rounded or truncated base (**Benmechernene and Hamadi, 2022).** A 0.5- to 2-cm-long petiole carries the leaves. The inflorescence is in a terminal cyme with one to five flowers. The flowers are roughly 3.5 cm wide and are often purple, blue, or occasionally white. Partially welded Five Petal Corollas Welded: Five Sepal Calices There is yellow anthrax in the androecium. They are hermaphrodite blooms. Carried by a 0.5–1 cm-long peduncle. The plant has smooth, spherical, 1.5 cm-diameter berries that are green when young and turn golden, orange, and lustrous when ripe **(Zhang et *al*., 2012)**.

Figure 1: *Solanum elaeagnifolium* **(Krigas et *al*., 2023)**

1-4 Scientific Classification

The species *Solanum elaeagnifolium* was classified in 1795 by the Spanish botanist Antonio José Cavanilles **(Tataridas et *al*., 2023).**

Table 1: Classification of *Solanum elaeagnifolium Cav*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta

Subphylum	Angiospermae
Class	Dicotyledonae
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*Solanum elaeagnifolium Cav*
EPPO Code	SOLEL

1-5 Identity

According to **Sastry et *al*, (2019)** this plant has different names.

Preferred Scientific Name

Solanum elaeagnifolium Cav

Preferred Common Name

Silverleaf nightshade

Other Scientific Name

- ✓ Solanum dealbatum lindl;
- ✓ Solanum flavidum torr;
- ✓ Solanum leprosum Ortega;
- ✓ Solanum obtusifolium willd**.**

Table 2: International denomination of *solanum elaeagnifolium*

Region	Nom	Causes	Reference
Greece	Lernaean Hydra	Its vigorous regeneration following herbicide treatment.	**Krigas et *al.* (2021)**
Africa	Satansbos, or Satan's apple, or Silver leaf bitter apple	/	**(Wilson et *al.*, 2013)**
America and other countries	Bull nettle, Tomatillo, Meloncillo, trompillo, and white horse nettle	The stem has several spines	**(Kwong et *al.*, 2006)**
Algerian	Echouka	which means thorn	**(Adjim and Kazi Tani 2018)**
South Korea	Eun-bit-kka-ma-jung	The combination of its silvery colouring and a common plant	**(Hong et *al.* 2014)**

		found in the country	
French	Yellow nightshade(morelle jaune, Yellow Morella)	/	**(Utah and Rico, 2007)**
Marocain	Chouka safra Chouika	/	

1-6 Growth and Development

S. elaeagnifolium is a perennial plant that grows best in the summer and has a vast root system. The roots' capacity for extremely deep and horizontal growth can produce a plant with a six-foot stem. The seedlings emerge from the established plants in late March or early April as the earth warms. Plants bloom from spring until late summer. Around June is when wall fruits, which are available from summer to autumn, become available. Plants die throughout the winter and reappear from their roots in the spring **(Stanton et *al*., 2009; M'sadak et *al*., 2015).**

1-7 Effects and Biological Activities of *S. elaeagnifolium*

The silver nightshade is a weed that has invaded this area like an alien plant. It is one of the most hazardous plants in the Mediterranean region due to its detrimental impact on crops and the environment. SOLEL generally causes considerable qualitative and quantitative damage to all crops whose biological cycles overlap fully or partially with their own. It interferes directly through allelopathy and competition and indirectly through acting as an intermediate host for cultural enemies. For certain livestock, it is poisonous. In another way, natural ecosystems are severely unbalanced by its incursion **(Ben-Ghabrit et *al*., 2016).** This plant's seeds sprout early in the spring and spread swiftly because of the reserves kept in its fully formed roots. The yellow mollusk has a competitive edge over many other edible species because of this trait **(Boukhobza, 2014)**.

The high concentrations of flavonoids, alkaloids, terpenes, saponins, phenols, carotenoids, and coumarins found in the *Solanum* species are thought to have positive effects on both human and animal health. Certain species have demonstrated anti-tumor, antidepressant, anti-inflammatory, antihypertensive, antioxidant, hypolipidaemic, hypoglycemic, anti-obesogenic, hepatoprotective, and anti-diabetic properties **(Bouslamti et *al*., 2023)**.

Secondary Metabolites

Secondary metabolites

Secondary metabolites molecules are complex organic compounds that are synthesized and accumulated in small amounts by autotrophic plants**.** Plants produce a large number of secondary metabolites, often with extraordinary structural diversity **(Amara et *al*., 2016)**.

The production of secondary metabolites can be stimulated by changes in climatic, edaphic, and ecological conditions or by interactions between plants and animals. The concentration of these latter varied according to the species, age, season, and portion of the plant. These substances can be present in vacuoles (flavonoids, alkaloids), as well as in unique creatures such as chromoplasts (carotenoids) and chloroplasts (containing chlorophyll) **(Aneb, 2017)**.

1-1 Phenolic compounds

A large family of chemical compounds called polyphenols is present in the kingdom of plants **(Polyphenols in Plants, 2014)**.

The naturally occurring secondary metabolites of higher plants are termed polyphenols, and they have important applications in both industry and medicine.

In the literature, there have been reports of about 8,000 different varieties. These polyphenols are primarily made up of single or double aromatic rings that are joined by one or more hydroxyl groups (OH) **(Prabhu et *al*., 2021)**.

They are involved in defence reactions against a variety of biotic or abiotic stresses (pathogens, UV radiation, etc.) and contribute to the organoleptic quality of plant-based food (colour, aroma, bitterness) **(Collins et *al*., 2024)**.

The various extraction techniques, such as Soxhlet, microwave, heat reflux, ultrasonic, liquid-liquid extraction, supercritical fluid extraction, and ultra-high pressure extractions, can be used to extract the polyphenols from the various origins. Moreover, nuclear magnetic resonance (NMR), reverse phase liquid chromatography (RPLC), high-performance liquid chromatography (HPLC), and other spectroscopic methods can be used to identify (qualitatively or quantitatively) polyphenols **(Prabhu et *al*., 2021)**.

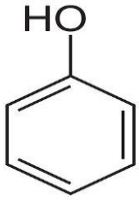


Figure 2: Structure of the phenol nucleus **(Gherhard, 1993)**

1-1-1 Classification of phenolic compounds

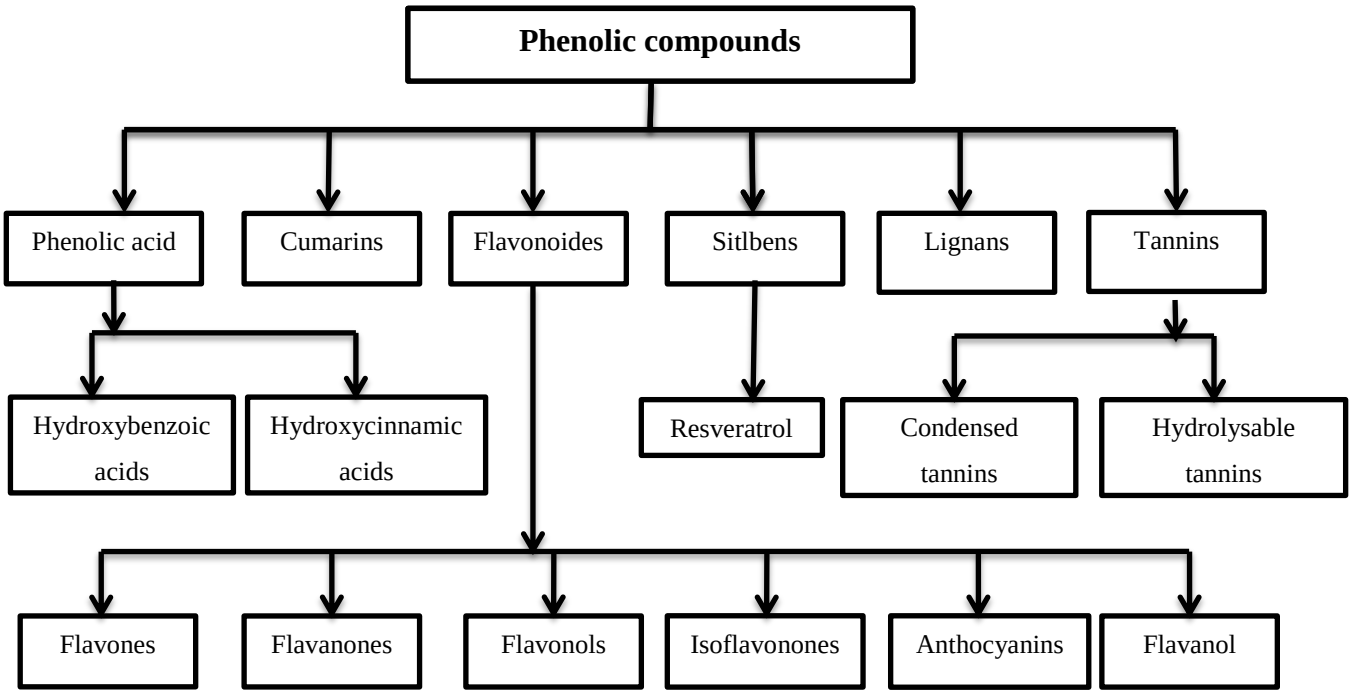


Figure 3: Different classes of polyphenols **(Polyphenols in Plants, 2014)**

Table 3: Main Classes of Polyphenols **(Macheix et *al* ., 2005)**

Carbon skeleton	Class	Examples
C6	Simple phenols	Catechol
C6-C1	Hydroxybenzoic acids	Salicylic acid, gallic acid
C6-C3	Hydroxycinnamic acids	Caffeic acid,ferulic acid
	Coumarins	Scopolin,Esculetin
C6-C3-C6	Flavonoids	
	Flavonols	Kaempferol,quercetin
	Anthocyanins	Cyanidin,pelargonidin
	Flavanols	Catechin,epicatechin

	Flavanones	Narigenin
	Isoflavones	Daidzein
C6-C2-C6	Steblenes	Resveratrol
(C6-C3)2	Lignans	/
(C6-C3)n	Lignins	/
(C6-C3-C6)n	Tannins	Pinoresinol

1-1-2 Phenolic Acid

Any organic compound with one phenyl hydroxyl and one carboxyl function, at least one, is referred as a phenolic acid **(Singla et *al*., 2019)**:

- The base formula of **hydroxybenzoic acids** is type C6–C1, and they are obtained from benzoic acid, which is produced by hydroxylating benzoic acid. Protocatecic acid, vanillic acid, gallic acid, ellagic acid, syringic acid, salicylic acid, and gentisic acid are the hydroxybenzoic acids that are frequently found in plants. Olive products also include certain hydroxybenzoic acids, which have anti-inflammatory, cardio protective, and antioxidant properties **(Călinoiu and Vodnar, 2018)**.
- The aromatic acid class (C6–C3) that is produced from cinnamic acid is represented by **hydroxycinnamic acid**. Common examples of hydroxycinnamic acids are caffeineic acid, ferulic acid, coumaric acid, sinapinic acid and cinnamic acid **(Prabhu et *al*., 2021).**

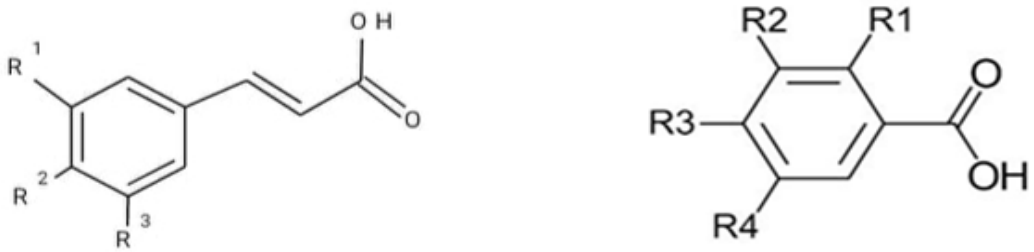


Figure 4: Structure of Phenolic acids **(Călinoiu and Vodnar, 2018)**

1-1-3 Flavonoids

Flavonoids are polyphenol chemicals made up of two aromatic cycles joined by three carbon bridges or 15 carbon atoms, producing a C6-C3-C6 structure. Among all the phenolic chemicals, they are the most prevalent. As secondary metabolites, they play a variety of functions in plants, including pigmentation, disease resistance, UV defence, and stimulation of nitrogen-fixing nodules **(Stringlis et *al*., 2019).**

The fundamental "2-phenylchrome" skeleton of these bioactive compounds is made up of two C6 cycles (A and B) joined by a C3 chain, which has the ability to develop into a heterocyclic (cycle C).They produce colours that range from golden yellow to light yellow **(Harborne, 2013)**.

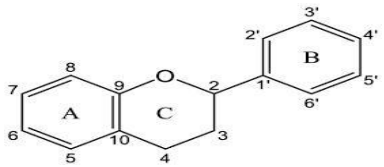
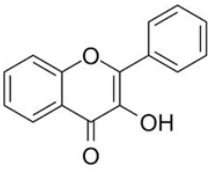
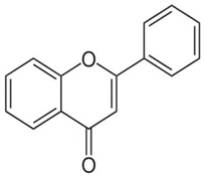
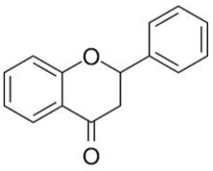
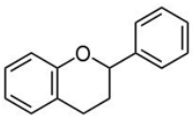
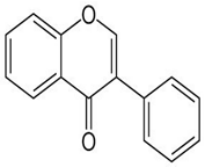
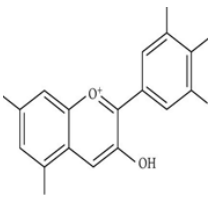


Figure 5: Structure of flavonoids **(Yao et *al*., 2004)**

Table 4: Different classes of flavonoids

Class	Structure	Character	Role	Reference
Flavonols		The flavonol is obtained by substituting a hydroxyl group for the preceding skeleton at position 3. These substances comprise the flavonoid class that is most sensitive.	Have potential clinical applications for blood coating prevention, gingivitis therapy, cardiovascular disease prevention, and cell regeneration.	**(Afonso et *al*., 2007)**
Flavones		Characterized by a double bond in the flavonoid skeleton between C-2 and C-3.	Have opposite effects on cardiovascular disease, while citrus fruits with nobiletin can prevent cancer and inflammation	**(Hostetler et *al* ., 2017)**

Flavanones		The molecule is referred to as a flavanone if it has a carbonyl group at position 4 of the flavone. Typified by the double bond between C2 and C3 not existing. Due to its high reactivity, the structure experiences reactions such as hydroxylation, glycosylation, and homethylation.	Are well-known for their flavor and health advantages. Flavanones include eriodictyol, hesperidin, and naringenin.	**(Akroum, 2011)**
Flavanes		The primary subclass of flavanes is flavan-3-ols. These structures include polymeric ones like anthocyanidins and basic monomers like catechins.	/	**(Akroum, 2011)**
Isoflavones		Are identified by the bonding cycle B-C3. This substance resembles the steroid hormone "estradiol," which prevents ovulation, in terms of structure.	/	**(Mullen et *al.*, 2007)**
Anthocyanid-ins		Are distinguished by the existence of an oxygen atom that is cycle-charged	antioxidant properties, potentially lowering blood pressure, oxidative stress markers, and cholesterol levels	**(Kovinich et *al.*, 2015)**

1-1-4 Tannins

Tannins are high-molecular-mass phenolic compounds found in plants, with molecular weights ranging from 500 to over 3,000 Da and up to 20,000 Da. The molecular makeup of tannins is extremely varied. Both free and bound forms of tannins have been discovered **(Atuhaire et *al*., 2022)**.

A- Classes of tannins

In higher plants, two groups' hydrolysable tannins and non-hydrolysable tannins are often differentiated based on structural variation **(Fiorucci, 2006)**.

A-1 Hydrolysable tannins

Gallic acid esters that attach to glucose molecules are known as hydrolysable tannins. More precisely, several molecules of Gallic acid are bound by glucose **(Macheix et *al*., 2005)**.

A-2 Condensed tannins:

Condensed tannins are flavone-3-polymers or oligomers that are made from catechin or any of its several isomers. They can only be degraded by powerful attacks since they are resistant to hydrolysis **(Sarni-Manchado and Cheynier, 2006)**.

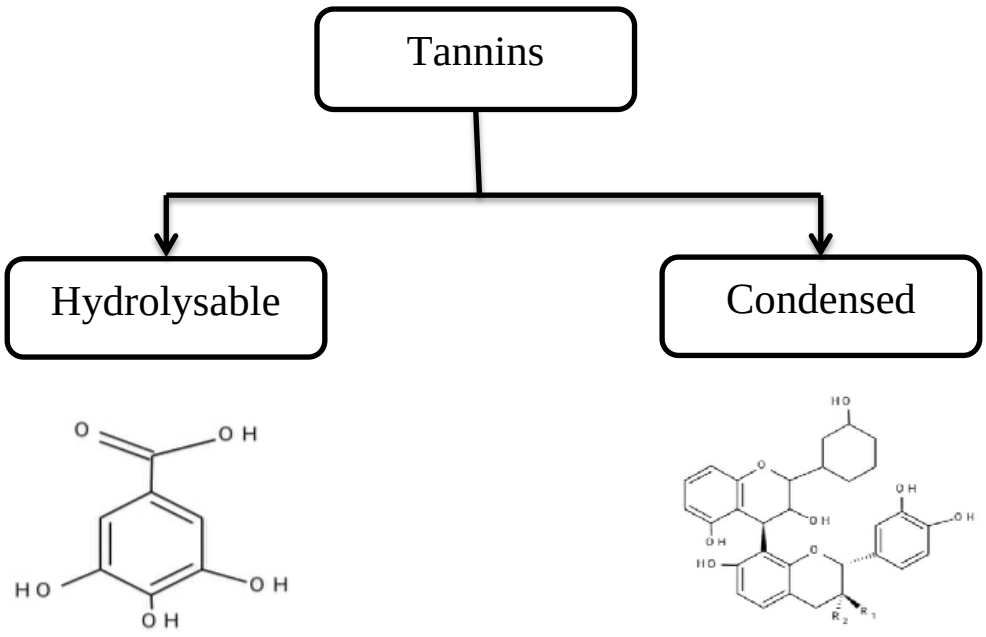


Figure 6: Classes of Tannins **(Ghosh, 2015)**

1-1-5 Cumarins

Secondary metabolites called cumarins are mostly present in higher plants, but they have also been identified in some animal and microbiological species **(Stringlis et *al*., 2019)**. Every component of the plant contains them. They are mainly found in glycosylated form in plant cells, where this glycosylation serves as a kind of storage to prevent these compounds' harmful effects **(Yeh et *al*., 2014)**.

Their characteristic blue fluorescence is caused by their capacity to absorb ultraviolet (UV) light .Recent decades have seen a great deal of research on cumarins, and it has been discovered that these compounds exhibit a wide range of pharmacological actions, including those related to cardiovascular disease, molluscycidal, antiviral (including anti-HIV), anticancer, antidepressant, antioxidant, and anti-inflammatory and anticoagulant agents **(Stringlis et *al*., 2019)**.

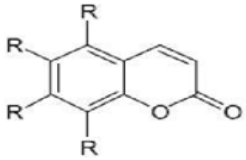


Figure 7: Structure of Cumarins **(Lalande and Parenty, 2003)**

2 Alkaloids

They are nitrogenized organic materials derived from plants that have an alkaline, complicated structure and are found in a variety of plant groups **(Ounis and Boumaza, 2018)**. According to **Gaci et *al*. (2017)**, the majority of alkaloids has a bitter taste and are soluble in alcohol and water. Some of them are extremely dangerous.

2-1 Types of alkaloid

According to **Badiaga (2011)** alkaloids are divided into three groups:

True alkaloids: they comprise the majority of alkaloids, are poisonous, and exhibit a broad range of biological activities. They have a nitrogen atom in a heterocyclic structure and are derived from amino acids.

Proto3-alkaloids: These are amines that are simple and do not have a nitrogen atom incorporated in a heterocyclic. They come from amino acids as well.

Pseudo-alkaloids: these substances do not originate from amino acids but rather possess all the properties of actual alkaloids**.**

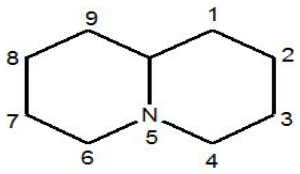


Figure 8: Structure of Quinolizidine type of alkaloids **(Cely-Veloza et *al*., 2023)**

3 Saponosides

Most saponosides are highly polar chemicals that are frequently found in complicated mixes within plants. Additionally, they have a broad range of biological and pharmacological characteristics, such as cytotoxic, immunoadjuvant, immunomodulatory, antitumoral, and cholesterol-lowering abilities **(Boutaghane, 2013)**.

Their physicochemical and biological skills, which are utilized in a variety of conventional and industrial applications (such as soaps, fish poisons, and molluscicides), are reflected in their structural diversity.There is ample evidence about the toxicity of saponins for insects (insecticide activity), parasitic worms (anthelmintic activity), mollusks (molluscicide activity), and their antifungal, antiviral, and antibacterial properties **(Stringlis et *al*., 2019)**.

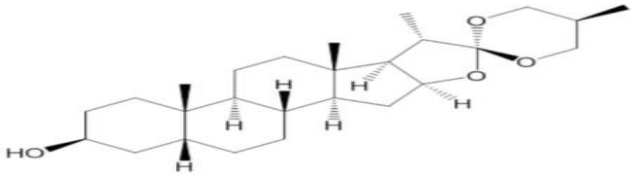


Figure 9: Structure of Saponosides **(Boutaghane, 2013)**.

4 Quinone

Quinone are secondary metabolites isolated mainly from plants and have an aromatic ring (hex cyclically saturated) di-one or di-cetone system. They are usually derived from hydroquinone oxidation. Natural-origin quinines are widespread and include benzoquinones, naphthoquinons, anthraquinones, and polyquinones. Numerous biological activities, like those associated with the redox characteristics of their carbonyl functionalities, have been demonstrated for them, including neurological, antibacterial, antispasmodic, antioxidant, trypanocidal, anti-tumor, and anti-HIV effects **(Eyong, et *al*., 2013)**.

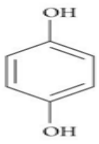


Figure 10: Structure of Free Quinone **(Salazar et *al*., 2015)**

5 Anthraquinones

Over 700 compounds have been identified as anthraquinones, making them the biggest group of molecules. These molecules are found in all parts of plants. Peaches, cabbage, mud, beans, and other foods contain these chemicals. Within the food and pharmaceutical industries, anthraquinones and their derivatives are among the most commonly utilized phytomolecules because of their diverse range of applications. Research has demonstrated that anthraquinones have anti-inflammatory, anti-tumor, antioxidant, diuretic, antifungal, antibacterial, and anti-malarial properties **(Duval et *al*., 2016)**.

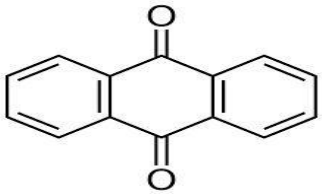


Figure 11: Structure of 9, 10-anthracenedione (Anthraquinone) **(Enin, 2019)**

6 Terpenoids

These molecules are of the many molecules that plants can create, terpenoids (also known as isoprenoids) are the largest and most varied class. Are generally lipophilic compounds made up of one carbon atom per molecule **(Hopkins, 2003)**.

Terpenoids are natural hydrocarbons with cyclic or open-chain structures have the gross formula (C5Hx) n **(Bezzaz, 2013)**.

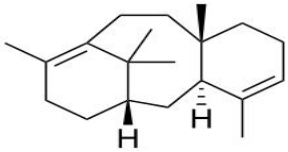


Figure 12: Structure of Terpenoids **(Las Heras et *al*., 2003)**

7 Essential oils

Almost 2,000 plant species from 60 families were found to contain significant amounts of essential oils. Volatile substances known as essences or essential oils can be found in a variety of plant parts, including flowers, leaves, crusts, and roots. These are typically worm repellents, antiseptic, antibacterial, and antifungal agents **(Aribi-Zouioueche & Couic-Marinier, 2021)**.

7-1 Classification of essential oils chemically

Three categories can be used to classify E.Os based on their main components

(Aribi-Zouioueche & Couic-Marinier, 2021):

- Terpene-rich hydrocarbon E.Os (Citron);
- Oxygenated E.O. It is high in esters and alcohols (thyme, girofle, menthe, etc.);
- Sulphurized E.O. (Pellets, Liliaceae).

7-2 Chemical composition of essential oils

The E.Os is mixes of elements that belong to two separate series, each with a distinct bioenergetics origin. They are complicated and remarkably changeable.

- Compounds terpenic ;
- Derivatives of propane phenyl aromatic compounds **(Aribi-Zouioueche & Couic-Marinier, 2021)**.

Biological Activities

1Antioxidant activity

Oxidative stress

Oxidative stress is defined as an imbalance between oxidants and antioxidants in favour of the oxidants. This imbalance is caused either by an exaggerated production of oxidising agents or by an alteration in defence mechanisms **(Sies, 2020)**.

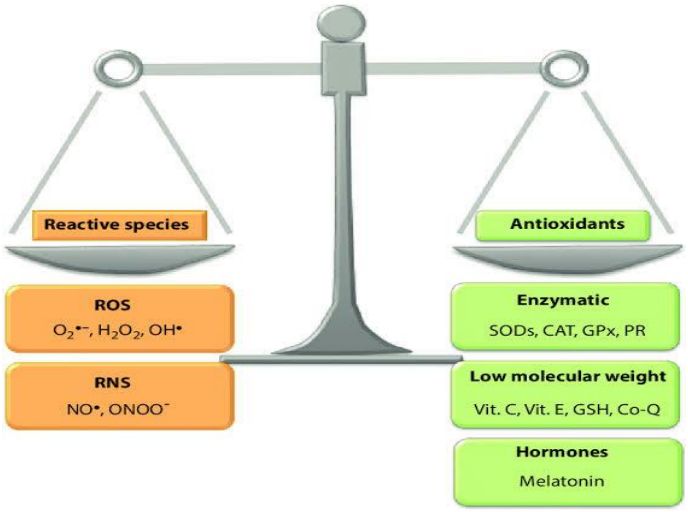


Figure 13 : Balance redox **(Rahman et *al*., 2012)**

1-1 Free radicals

A free radical is defined as any atom or group of atoms having an unpaired (single) electron in their orbital. This electron allows free radicals to react easily with other molecules **(Sies et *al*., 2017)**.

According to **Lobo et *al*. (2010)** free radicals can be produced in a variety of ways. They may be created by normal metabolic processes in the body (endogenous) or from exposure to carcinogens (cancer-causing substances) in the environment (exogenous).

Table 5: Source and origin of free radicals **(Lobo et *al*., 2010)**

Free radicals	Origin
Endogenous	• Mitochondria • Xanthine oxidase • Peroxisomes • Inflammation • Phagocytosis • Arachidonate pathways

	• Exercise • Ischemia/reperfusion injury
Exogenous	• Cigarette smocks • Environnemental polluants • Radiation • Certain drugs, pesticides • Industrial solvants • Ozone

1-1-1 Major types of free radicals

There are several kinds of free radicals, but the most common in humans are reactive oxygen species (**ROS**) and reactive nitrogen species (**RNS**) **(Mohammed et *al*., 2015)**.

Reactive oxygen species (**ROS**) refers to several reactive compounds produced from molecular oxygen. They are available as radical derivatives such as superoxide, hydroxyl radical, and nitric oxide, as well as highly reactive non-radical derivatives such as singlet oxygen, hydrogen peroxide, peroxynitrite, and hypochlorite.

Reactive nitrogen species (**RNS**) refers to a group of reactive molecules containing nitric acid, particularly the nitric oxide radical, which are important components of oxidative bursts and redox status modulation. This spice is also divided into radical spices such as nitric oxide (NO•), nitrogen dioxide (NO_2•), and non-radical spices such as peroxynitrite (ONOO-) and alkyl peroxynitrites (ROONO) **(Mohammed et *al*., 2015)**.

1-1-2 Effect of free radical on body

Each and every cell present in body produces free radical hence prone to free radical attack. Biological compounds available in human changes their property after free radical attack which may alter cellular function of human body and even leads to death of cells or tissue which responsible for generating various diseases. The most susceptible biological compounds' are lipid, protein, DNA etc. **(Qazi and Molvi, 2018)**.

Proteins, lipids, and DNA make up a large part of the human body, so that damage can lead to a vast number of diseases over time. These include:

- Neurodegenerative diseases, such as Parkinson's and Alzheimer's;
- Atherosclerosis or the hardening of the blood vessels;
- High blood pressure, which is also known as hypertension;
- Heart disease;

• Cancer;

• Diabetes.

1-1-3 Role of free radicals in normal physiological processes

Some free radicals are good for health because they maintain cellular homeostasis in the body through participation in various signalling pathways. As mediators and executioners of precancerous and infectious cells, they play an important role in phagocytosis, apoptosis, and detoxification reactions. They also control a wide range of metabolic and cellular processes, such as oxygen sensing, vascular contraction, gene expression, regulation of transcription, signal transduction, etc. **(Qazi and Molvi, 2018)**.

1-2 Antioxidants

Therefore, they are chemicals or systems that can safely interact with free radicals and terminate the chain reaction before critical molecules are damaged. These species have the ability to either decrease or stop the production of free radicals, and in physiological settings, antioxidants have the ability to counteract the effects of reactive oxygen species (**Preiser, 2012)**.

1-1-2 Mechanisms of action of antioxidants

Free radicals are neutralized by antioxidants by the acquisition or provision of electrons, which ends the unpaired radical state. Consequently, reactive radicals can be directly reacted with by antioxidant molecules, which can then destroy them and transform into new free radicals. But compared to previously neutralized radicals, these new radicals are less harmful, less reactive, and have a longer lifespan. Put differently, the antioxidant system functions similarly to a ROS scavenger **(Lobo et *al.*, 2010)**.

1-2-2 Classification of antioxidant

Antioxidants are classified according to their catalytic action type: Enzymatic or Non enzymatic antioxidants.

Enzymatic antioxidants contain specific cofactors and are highly selective to substrate-reactive species. Some examples of enzymatic antioxidants include SOD, CAT, and GPx. Meanwhile, Non enzymatic antioxidants differ from the former in that they do not have a specific substrate and so may neutralise the detrimental effects of both RNS and ROS **(Haida and Hakiman, 2019)**.

According to **Qazi and Molvi, (2018)** the enzymatic antioxidants are divided into primary and secondary antioxidants

- **The primary enzymatic antioxidants** by neutralizing free radicals, the primary enzymatic antioxidants such as glutathione peroxidase, catalase, and superoxide dismutase stop them from forming. Selene's are created by glutathione peroxidase, which lowers peroxides. Up to 6 billion molecules of hydrogen peroxide can be converted into molecular oxygen and water by a single catalase molecule. Hydrogen peroxide is produced from superoxide anions by superoxide dismutase.
- **The secondary enzymatic antioxidants** Secondary enzymatic: antioxidants include glutathione reductase and glucose-6-phosphate dehydrogenase limit the generation of free radicals by producing the reducing compounds glutathione and NADPH, which neutralise free radicals **(Qazi & Molvi, 2018).**

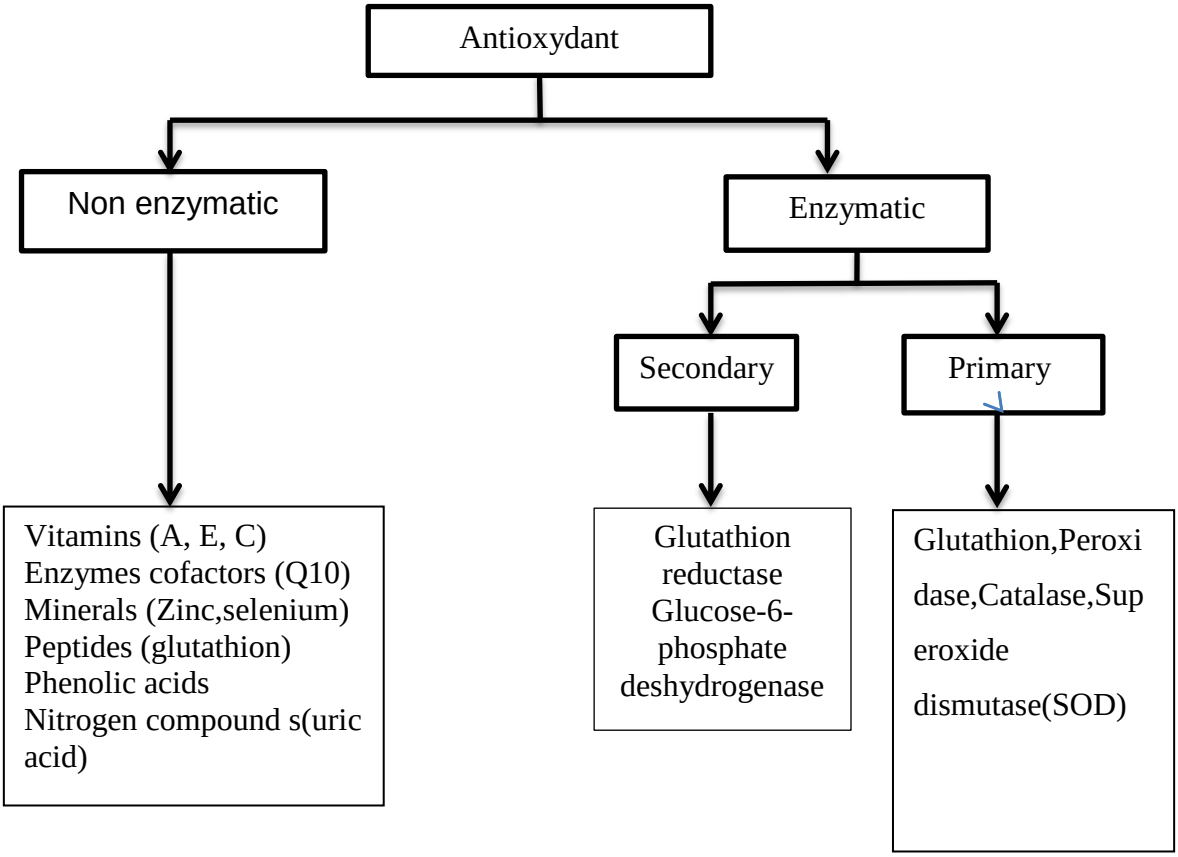


Figure 14: Antioxidants are classified according to their mode of action

1-2-3 Plants with anti-oxidant activity

Many medicinal plants, as well as their purified constituents, have demonstrated antioxidant activity. Examples of these include *Zingiber officinale*, *Terminalia bellerica*, *Solanum nigrum Solanaceae*, *Ocimum sanctum*, *Withania somnifera Solanaceae* and Flavones **(Qazi and Molvi, 2018)**.

1-3 Methods for evaluating anti-oxidant activity

Methods evaluate anti-oxidant activity based on involvement of free radical, solvent, and feasibility, divided into in-vitro and in vivo methods **(Qazi and Molvi, 2018).**

Table 6: some methods for evaluating antioxidant activity

In-vitro methods	In-vivo methods
➢ DPPH scavenging activity ➢ Hydrogen peroxide scavenging (H_2O_2) assay ➢ Trolox equivalent antioxidant capacity method/ABTS radical cation de-colorization assay ➢ Total radical-trapping antioxidant parameter (TRAP) method ➢ Ferric reducing-antioxidant power (FRAP) assay ➢ Hydroxyl radical scavenging activity ➢ Reducing power method (RP) ➢ Phosphomolybdenum method ➢ Cupric ion reducing antioxidant capacity (CUPRAC) method	➢ Ferric reducing ability of plasma ➢ Reduced glutathione (GSH) estimation ➢ Glutathione peroxidase (GSHPx) estimation ➢ Glutathione-S-transferees (GSt) ➢ Superoxide dismutase (SOD) method ➢ Catalase (CAT) ➢ Glutathione reductase (GR) assay ➢ LDL assay

2-Antibacterial activity

Even though the majority of bacteria are good for the body, numerous pathogen species can cause infectious diseases that are lethal to the host. As is understood, bacterial infections can be effectively treated with antibiotics. Antibiotics are manufactured or natural active substances that either eradicate or stop the growth of microorganisms **(Butler et *al*., 2017)**.

Antibacterials are a type of antibiotics that can be obtained naturally from fungal sources, semi-synthetic members (natural materials that have been chemically altered), or synthetic sources. Natural antibiotics and antibacterial include gentamicin, cephalosporins, cefamycins, and benzylpenicillin.When compared to manufactured antibacterial, natural antibiotics and antibacterial frequently show greater toxicity. The benefit that synthetic antibiotics have over natural antibiotics is that the bacteria are not exposed to the compounds

until they are released, making them even more effective and less dangerous **(Ullah and Ali, 2017)**.

Antibacterial are frequently classed based on their method of action: bacteriostatic (which slows or inhibits the development of germs) and bactericidal (which kills bacteria by attacking the cell wall or cell membrane) **(Uddin et *al.*, 2021)**.

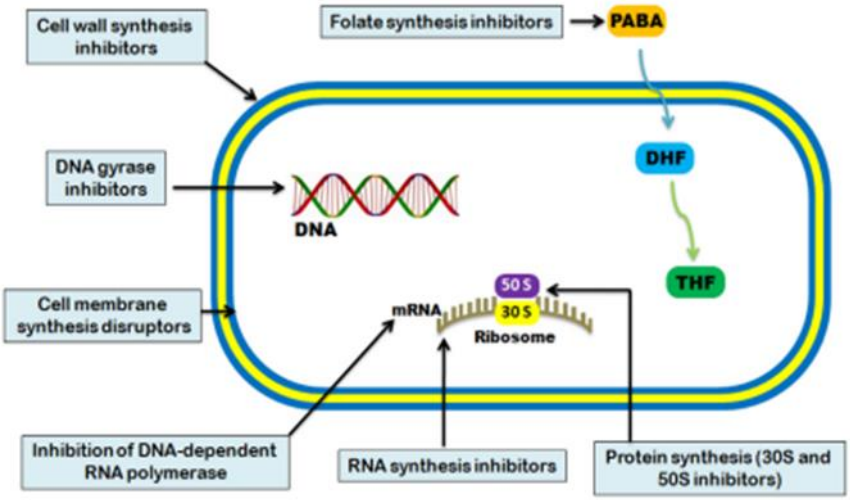


Figure 15: mode of action of antibiotics **(Uddin et *al.*, 2021)**

Certain types of bacteria may develop resistance to certain medications over time. They may mutate in such a way that antibiotics cannot kill them. The term for this is antibiotic resistance. Antibiotic resistance is classified into two types: naturally occurring and acquired. Natural resistance can be inherent (often expressed in organisms) or mediated (genes found in bacteria that are only activated to resistant levels after antibiotic treatment). While Acquired resistance can arise when bacteria receive genetic material by translation, conjugation, transposition, or mutations in their chromosomal DNA **(Uddin et *al.*, 2021)**.

3- Sun protection factor (SPF)

The skin is the body's first line of defence against external stimuli. Skin indicators of ageing are most obvious. Although ageing skin does not pose a hazard to a person's health, it might have a negative impact on their psychological well-being. Much of premature ageing comes as a direct or indirect effect of the skin's contact with the environment **(Mbanga et *al.*, 2014)**.

Each year, approximately one million people are diagnosed with skin cancer. Solar radiation has a detrimental impact on the skin .UV radiation is primarily responsible for skin

damage. UV radiation increases oxidative stress in skin cells, leading to the beginning and progression of cancer **(Hashemi et *al*., 2019)**.

3-1 Solar radiation's detrimental effects

Are mostly induced by the ultraviolet (UV) area of the electromagnetic spectrum, which is classified into three regions: UVA (400 to 320 nm); UVB (320 to 290 nm); and UVC (290 to 200 nm). The atmosphere filters off UVC rays before they reach the planet. The ozone layer does not entirely filter UVB light, which causes sunburn and pyrimidine dimers. UVA radiation penetrates the deeper layers of the epidermis and dermis, causing rapid ageing of the skin and generating free radicals **(Mbanga et *al.*, 2014).**

3-2 The photo-protection of topical sunscreen

The photo-protection of topical sunscreen against UV radiation can be tested in vivo or in vitro, with in vivo determination ideally employing photo-testing on human volunteers, which is time-consuming and expensive .The development of in vitro techniques for evaluating sunscreen photo-protection has led to the adoption of appropriate methods for calculating SPF, a measure of UV protection. These approaches include measuring absorption or transmission through sunscreen product films as well as spectrophotometric measurement of dilute solutions **(Mbanga et *al*., 2014)**.

3-3 The sun protection factor (SPF)

The sun protection factor (SPF) of a sunscreen is a laboratory measure of its efficiency; the greater the SPF, the more protection a sunscreen provides against UV-B (UV radiation that causes sunburn) **(Singh and Sharma, 2016)**.

3-4 The efficacy of a sun protection product

The efficacy of a sunscreen product has been identified as an important public health issue, and it is typically expressed as the sun protection factor (SPF), which is defined as the UV energy required producing a minimal erythema dose (MED) on protected skin divided by the UV energy required to produce a MED on unprotected skin. The minimum erythemal dose (MED) is the shortest time interval or amount of UV light irradiation required to cause a minimal, detectable erythema on exposed skin. The greater the SPF, the more efficient the product is at preventing sunburn **(Mbanga et *al*., 2014)**.

Chapter II:

MATERIALS

&

METHODS

Materials and methods

The aim of this study

This piece of work is divided into three parts:

Phytochemical part:

- Preparation of *Solanum elaeagnifolium* leaf extracts.
- Phytochemical screening.
- Dosage of total polyphenols and flavonoids.

Biological part:

- Evaluation of the antioxidant activity of extracts obtained by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical trapping and phosphomolybdate methods.
- Evaluation of antibacterial activity.
- Determination of sun protection factor (SPF).

Qualitative part:

- Determination of functional group of ethanol extract by Fourier-transform infrared spectroscopy (FTIR).

Presentation of the experimental study area

In February 2024, the experimental study was carried out in pedagogical laboratories of Abass Laghrour University - Khenchela.

1-Materials

Identification and collection of plant material

S. elaeagnifolium leaves were collected in (October 2023), 35° 25′ 55″ north, 7° 08′ 40″, and an altitude of 1 122 m, located in the north of Algeria, in the region of Aures, between the steppe chain and the high plateaus, giving it an agro-pastoral and Saharan forest character. Fresh leaves were air-dried before being ground into powder with an industrial blender and utilised to produce a variety of extracts.

Chemical reagents and equipment

Table 7: Chemical reagents and culture media used

Reagent/solvent/culture media	Manufacturer
Ethanol Hydrolic acid (HCl) Iodine (I_2) Sodium chloride (NaCl)	VWR chemicals

Ammonium molybdate Hxane Butanol Ethyl acetate	
Methanol Acetic anhydride (Ac2O) Sodium carbonate(Na_2CO_3)	FLUKA _HONYWELL
Chloroform Ferric chloride ($FeCl_3$) Folin & ciocalteu's phenol Dimethyl sulfoxide (DMSO) Sodium phosphate dibasic (Na_2HPO_4)	SIGMA-ALDRICH
Fehling reagent A Potassium iodide (KI) Ammonium hydroxide (NH_4OH) Ascorbic acid Gallic acid Aluminium chloride($AlCl_3$) Potassium acetate(CH_3COOK)	BIOCHEM- CHEMOPHARMA
Fehling reagent B	FAROCOCHIM
Muller Hinton agar	INNOVALID
Nutrient broth Nutrient agar	CANDALAB
Sulphuric acid (H_2SO_4)	LOBA CHIMIE
Sodium hydroxide (NaOH) Mercury chloride ($HgCl_2$) 2,2-Diphenyl-1-picrylhydrazyl(DPPH)	SPECILAB
Sunscreen cream	Venus
Naphthalene Quercetin	/

Instruments

Table 8: Instruments and manufacturer

Instrument	Manufacturer
Analytical balance	OHAUS
Vortex Rotary evaporator	DLAB
Spectrophotometer	SHIMADZU
Refrigerator	LIBHERR
Water bath Universal oven to 220 °C with ventilation	MEMMERT
Vacuum pump	KNF LAB
Heating magnetic stirrer	NAHITA
Autoclave	RAYPA
PH Meter	HANNA
UV chamber	VILBER LOURMAT
Heating mantle	WISDELY
Ultrasonic cleaner	BAKU
FTIR spectrometer	PerkinElmer

2-METHODS

2-1 Extraction

The extraction of bioactive compounds was carried out using 3 methods (Maceration, Soxhlet, and Infusion).

Extraction by Maceration

200 g of the powdered leaves were subjected to maceration for 3 replications by using ethanol/distillated water « 7:3 ». The ethanol extract was concentrated using the rota evaporator (40°C).

Extraction by infusion

5 g of air-dried powder of plant material was infused in 20 mL of boiled water for 10 min, and then filtered.

Soxhlet extraction with different solvents (Hexane, Chloroform, Acetate, and Butanol)

Fifty grams of air-dried powder of the plant material were extracted successively using the following solvents: hexane, chloroform, acetate, and butanol, using a Soxhlet

extractor. After complete extraction, the extract of each solvent was concentrated by evaporation using a rotary evaporator (30°– 40°C and 50°C for butanolic extract).

After extraction, the yields of each extract (ethanol and soxhlet extracts) were estimated using the following equation:

Equation 1: Calculation of yield **(K et *al*., 2019)**

$$Y(\%) = \frac{m}{m_0} \times 100$$

Where:

Y(%) :yield of extraction

m :weight of plant extract

m_0: weight of dried powder.

II-2 Phytochemical screening

These tests were only made for ethanol and aqueous extracts. The ethanol extract used was at a concentration of 10 mg/mL.

Test for phenolic compounds

0,1g of EtOH extract and 1 mL of aqueous extract were diluted in 3 mL of methanol before adding 5 drops of 2% $FeCl_3$. The greenish-blue colour appeared due to the presence of phenolic compounds **(Nigussie et *al*. 2021)**.

Test for flavonoids

In a beaker, 10 g of dry powder was immersed in 150 ml of HCl (1%). The beaker was covered with aluminium foil and stirred for 24 hours. Then the mixture was filtered and carried out the following test:

10 ml of the filtrate was alkalized with 10% NH_4OH drop wise. Obtaining a precipitate confirms the presence of flavonoids **(Shalini and Sampathkumar 2012)**.

Alkaloids test

1mL of each extract was taken in test tubes, and a few drops of 1% HCl were added. Then, the content of each tube was divided into two, where the first half was treated with 0.5 mL of Mayer's reagent, while the other half was treated in the same way with Wagner's reagent. The turbidity or white-brown precipitation with these reagents was taken as evidence for the presence of alkaloids **(Shalini& Sampathkumar 2012)**.

Test for steroids and triterpenes

0,5 mL of acetic anhydride was added to the 5 mL of each extract with 0,5 mL of sulfuric acid (H_2SO_4).After incubation for 15 minutes, the colour changing from purple to blue or green in some samples indicates the presence of steroids and triterpenes **(Shalini and Sampathkumar, 2012)**.

Test for terpenoids

5 mL of each extract was mixed with 2 mL of chloroform, and concentrated H_2SO_4 (3 mL) was carefully added to form a layer. A reddish-brown coloration of the face that forms indicates a positive result for the presence of terpenoids **(Shaikh and Patil, 2020)**.

Test for tannins

To estimate tannins, 2 mL of each extract was placed in a test tube, and 0.5 mL of ferric chloride reagent ($FeCl_3$ at 10%) was added. A blue-black precipitate confirmed the presence of Gallic tannins, while a dark green precipitate confirmed the presence of catechins tannins **(Shaikh and Patil, 2020)**.

Test for anthraquinones

5 mL of each extract was mixed with 5 mL of ammonium hydroxide (NH_4OH diluted to 10%). The mixture was subjected to stirring, and the presence of anthraquinones was confirmed by the appearance of a purple colour **(Shaikh and Patil, 2020)**.

Test for reducing compounds

2 mL of each extract was placed in a test tube, and 5 mL of the mixture of equal volumes of Fehling solutions A and B were added and boiled in a water bath for 2 minutes. The presence of a brick-red precipitate indicates the presence of reducing compounds **(Shaikh and Patil, 2020)**.

Test for coumarins (UV- fluorescence)

1 ml of each extract was added to two test tubes. In one of the test tubes, 0.5 ml of 10% NH_4OH was added, while the second tube, which was not treated with NH_4OH, served as a control. Then, a drop of each prepared solution was placed on filter paper, and the appearance of intense fluorescence under ultraviolet light (366 nm) indicated the presence of coumarins **(Ghankoba et *al*., 2024)**.

Test for free quinones

To 5 mL of each extract, a few drops of 10% NaOH were added. The presence of quinones is indicated by a change in colour from yellow, red, or purple **(Allangba et *al*., 2016)**.

2-3 Determination of total phenolic content (TPC)

The total phenolic content of *Solanum elaeagnifolium* ethanol extract, $CHCl_3$, AcoEt, and BuOt phases was determined using the Folin-Ciocalteau method, with gallic acid, a phenol component used as a reference for the calibration curve **(Habibatni et al., 2016)**.

The principle of polyphenols determination

The polyphenol content was determined using the Folin-Ciocalteu technique. This method use the Folin-Ciocalteu reagent to oxidise the phenolic chemicals in the sample. When phenols are oxidised, a mixture of phosphotungistic acid ($H_2PW_{12}O_{40}$) and phosphomolybidic acid ($H_3PMo_{12}O_{40}$) is reduced to a blend of blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The resulting blue tint has a maximum absorption of approximately between 725 and 760 nm, which is proportional to the rate of phenolic chemicals**.** Total phenolic component content is given in milligrams per litre of equivalent gallic acid **(Boizot and Charpentier, 2006)**.

Protocol

100 µL of each sample solution were mixed with 200 µL Folin-Ciocalteu reagent, 2 mL of H_2O, and 1 mL of sodium carbonate (Na_2CO_3) 15% then vortexed , and the absorbance was measured at 765 nm, after 2 h incubation in the dark at room temperature, Absorbance was measured spectrophotometrically at 765 nm against a blank without extract. The concentration of phenolic compounds in the extract was expressed in milligrams of Gallic acid equivalents per gram of extract (mg GAE/g) using a calibration curve obtained with different concentrations of gallic acid which serve as a positive control **(Habibatni et *al.*, 2016)**.

2-4 Determination of total flavonoid content (TFC)

Principle

The total flavonoid concentration was determined using the colorimetric technique with aluminium chloride. The aluminium chloride ($AlCl_3$) colorimetric method works on the idea that $AlCl_3$ creates acid-stable complexes with flavones and flavonols' C-4 keto groups and either the C-3 or C-5 hydroxyl groups. In addition, it forms acid-labile complexes with the ortho-dihydroxyl groups in the A- or B-rings of flavonoids **(Makuasa and Ningsih, 2020)**.

Protocol

Based on the aluminum chloride colorimetric method, this method consists of adding 250 µL of each extract, 750 µL of ethanol, 50 µL of an $AlCl_3$ solution (10% in methanol), 50

µL of CH_3COOK (1M), and 1.4 mL of distilled water. The mixture is stirred and left at room temperature in the dark for 30 minutes. The absorbance was spectrophotometrically measured at 415 nm.

The flavonoid content was measured using a calibration curve (y = ax + b) created by quercetin at different concentrations (6.25-100 µg/mL) under the same conditions as the samples. The results are reported as microgram equivalents of quercetin per gram of dry extract **(Nguyen et *al.*, 2020)**.

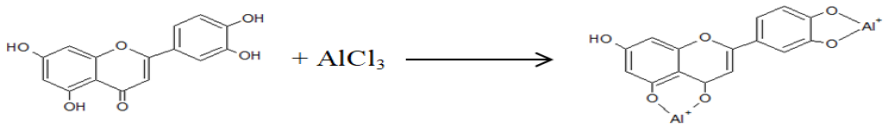


Figure 16: Reaction of flavonoid and aluminium chloride **(Makuasa and Ningsih, 2020)**

2-5 Antioxidant assay

2-5-1-DPPH radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl) and determination of IC50 values

The free radical scavenging activity of *Solanum elaeagnifolium cave* ethanol extract $CHCl_3$, AcoEt, and BuOt phases was estimated by the DPPH method with several modifications.

Principle

The DPPH test is one of the most common methods for evaluating antioxidant activity. This method is based on the reduction of the violet DPPH radical by the antioxidant via a hydrogen atom transfer mechanism to cause a change in colour to stable pale yellow DPPH molecules. The residual violet DPPH radical is measured at 515–520 nm using a UV-Vis spectrophotometer (refer to Figure below). The degree of discoloration indicates the antioxidant compounds or extracts scavenging capacity in terms of hydrogen-donating ability **(Sirivibulkovit et *al.*, 2018)**.

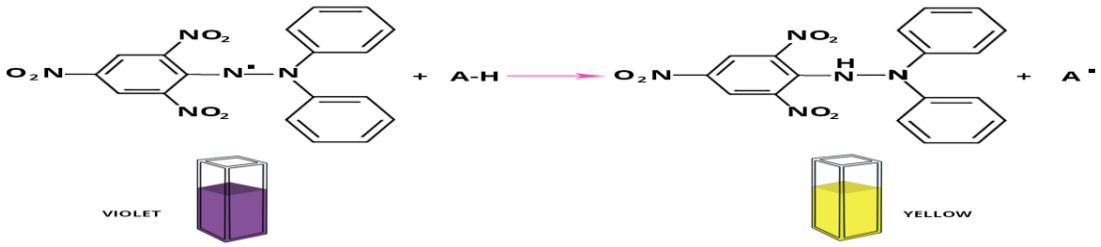


Figure 17: Reaction mechanism of DPPH with antioxidant **(Liang & Kitts, 2014).**

Preparation of DPPH solution

A daily DPPH solution (0.1 mM) was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. The initial absorbance of this solution was measured without sample at 517nm.

Reaction

1 mL of DPPH solution was mixed with 167 µL of each extract in methanol at different concentrations (1.5-0.25 mg/mL). This mixture was vortexed thoroughly and left in the dark at room temperature for 30 min.

The absorbance of the mixtures was measured spectrophotometrically at 517nm.

The same reaction and conditions were used with the ascorbic acid which served as a positive control.

Measurement of DPPH free radical scavenging activity

The following equation was used to calculate the percentage of DPPH radical-scavenging activity of extracts.

Equation 2: Calculation of Radical-Scavenging Activity

$$RSA\ (\%) = \frac{Abs_{ctrl} - Abs_{sample}}{Abs_{ctrl}} \times 100$$

Where:

RSA= radical scavenging activity.

Abs $_{ctrl}$ = the absorbance of the control (DPPH without the sample).

Abs $_{sample}$ = the absorbance of sample (the absorbance of DPPH with sample).

Each experiment was carried out triplicate and the mean of the three values was used to calculate the DPPH scavenging present.

The 50% inhibitory concentration value (IC50) represents the effective concentration of the sample needed to scavenge 50% of the DPPH free radicals. A lower IC50 value

indicates more antioxidant activity, and vice versa. The IC50 values were determined in Microsoft Excel by plotting extract concentration versus inhibition of the DPPH radical **(Sirivibulkovit et *al*., 2018)**.

2-5-2 Phosphomolybdate assay

Principle

The total antioxidant capacity (TAC) of the plant extract is assessed by the phosphomolybdate method. This technique is based on the reduction of Mo (VI) molybdenum present in the form of MoO_{42} molybdate ions to Mo (V) MoO_2^+ molybdene in the presence of the extract to form a green phosphate/Mo (V) complex at acid pH **(Prieto et *al*., 1999)**.

Protocol

An aliquot of 0.2 mL of sample solution was mixed with 2 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were closed and incubated in a boiling water bath at 90 °C for 90 minutes. The absorbance was then measured at a wavelength of 695 nm against a blank (1 mL reagent, 0.1 mL solvent). Total antioxidant activity was expressed in terms of ascorbic acid **(Uchôa et *al*., 2015)**.

2-6 Antibacterial Activity study of seed extracts by Disc diffusion method

Antibacterial activity of the ethanol extract was evaluated by disc diffusion method estimated by **Ponce et *al*, (2003)** with several modifications.

Principle

The ability of plant extracts, fractions, and chemicals to either eliminate or stop the growth of pathogenic microbes has been determined by their antibacterial activity. It provides justification for the selection of chemicals that may be bioactive. The agar-well diffusion approach was used as the basis for the antibacterial screening of the ethanol extract. In this method, the average diameter of the zone of inhibition (ZOI) that the plant extract created for particular pathogenic bacteria was determined in order to estimate the antibacterial activity of the extract **(Ponce et *al*.,2003)**.

Preparation of bacterial strains

In this study, the antibacterial activity of *Solanum elaeagnifolium* was tested against six bacterial strains (three reference and there clinic).

Table 9: strain and gram

Strain	Gram	Reference

Escherichia coli	-	ATCC 25922
Pseudomonas aeruginosa	-	ATCC 27853
Klebsila pneumoniae	-	ATCC 700603

The clinical strains employed were obtained from human samples at the Elhikma (Khenchela) laboratory. These are the strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsila pneumonia.*

Bacterial strains were inoculated on nutrient agar and incubated at 37 °C for 24 h. The inoculum suspension was obtained by taking colonies from 24-h cultures. The colonies were suspended in a sterile 0.9% aqueous solution of NaCl. The density was adjusted to the turbidity of a 0.5 McFarland Standard.

Preparation of stock/ working solution

Two different concentrations of ethanol extract (50 and 100 mg/mL) were prepared in dimethyl sulfoxide (DMSO).

Inoculation of the Mueller Hinton Agar Plates

A sterile cotton swabs were taken out and were dipped into the prepared inoculum. The excess of inoculum was removed by pressing and rotating against the upper inside side wall of the tube above the liquid level and then swabbed carefully all over the plates. The plate was rotated through an angle of 60°C after each swabbing. Finally the swab was passed round the edges of the agar surface.

Then the sterile filter discs were disposed put onto the surface of the inoculated MH agar and, the stock solutions were poured 10µL on the discs with the help of sterilized micropipette. While other discs treated with 10 µL DMSO were used as negative controls.

The inoculated plates were left to dry for few minutes at room temperature with the lid closed. Finally Discs were left for some time till the extract diffuses in them.

All the experiments were done in triplicate. The results are expressed as mean values with standard deviation (±SD) from three experiments.

Incubation and reading of the results

After the incubation of the petri dishes for 18–24 hours at 37°C, the plates were examined for bacterial growth inhibition, which was indicated by a clean zone surrounding the wells which called zone of inhibition (ZOI). The ZOI were measured using a scale, and the mean was recorded for the estimation of the potency of antibacterial substances according to **(Ponce et *al*., 2003).**

Table 10: Classification of strains sensitivity according ZOI **(Ponce et *al*., 2003)**

Strains	Classification
Non sensitive(-) or resistant	diameter less than 8 mm.
Sensitive (+)	Diameter between 9 to 14 mm.
Very sensitive (++)	diameter between 15 to 19 mm
Extremely sensitive (+++)	diameter more than 20 mm

2-7 Photo protective activity of extracts (calculation of SPF protection index)

The determination of the SPF of each diluted extract is based on the measurement of the absorption in the wavelength between 290 and 320 nm (erythematous zone) and is carried out using an UV spectrophotometer **(Mansur et *al*., 1986).**

In order to calculate the value of SPF, **Mansur et *al*., (1986**) proposed the following equation:

Equation 3: Calculation of the value of SPF

$$SPF = CF. \sum_{290}^{320} .EE(\lambda).I(\lambda).Abs(\lambda)$$

With:

(CF=10) is the correction factor (CF=10),

EE (λ) is the erythemogenic effect of radiation on the wave length λ,

I (λ), is the intensity of solar light with the wave length λ

Abs is the spectral photometric absorption value of the sample (diluted solution of the solar product to be tested) at the wave length λ.

The values of EE (λ) × I (λ), are constants determined by **Sayre et *al*. (1979) (Meunier.2008).**

Protocol

Dissolve each extract (BuOt, EtOh, $CHCl_3$, AcOEt) in two milliliters of ethanol. Using a 1 cm quartz cell and white ethanol, the absorption spectra of the solution samples were obtained in the 290 to 320 nm regions. Every 5 nm,

Venus sunscreen used as positive control .Three determinations were performed at each spot, and the Mansur equation was then used **(Dutra et *al*., 2004).**

2-8 Fourier transform infrared spectroscopy (FTIR) analysis

The functional groups present in these chemical constituents of plants are usually identified by FTIR. This helps in structure elucidation with other methods and gained importance to identify medicines in pharmacopoeia of many countries . Initially, FTIR was used to elucidate the structure of isolated compounds. Identification and comparison of biomolecule **(DeepaShree et *al*., 2012).**

Principle

FTIR spectroscopy uses modulated mid-infrared energy to analyze samples. When the binding vibrational energy and the energy of mid-infrared light are equivalent, the bond can absorb this energy. Different bonds vibrate at different energies in a molecule and therefore absorb different wavelengths of IR radiation. The position (frequency) and intensity of these individual absorption bands contribute to the overall spectrum, creating a characteristic fingerprint of the molecule.

This technique consists of irradiating the sample with energy close to that of the vibration and causing a direct transition between two vibrational levels by absorbing the energy of the incident radiation **(Patterson, 1939).** Functional groups are generally recognized by the presence or absence of absorption bands recorded in the infrared spectrum. The absorption of infrared radiation by this material depends on the nature of its constituents and the bonds that bind them

The FTIR spectra were recorded using an FTIR 460 plus PerkinElmer .Naphthalene and sample were combined to form a pellet by exposing to pressure, which was then scanned at room temperature in the 4000 to 400 cm^{-1} spectral region. To increase the signal to noise ratio for each spectrum, 100 interferograms with spectral resolution of $\pm 4cm^{-1}$ were averaged. This study made in physicochemical analyses laboratory of professor **BOUMAZA Abdcharif** University Abass Laghrour- Khenchela department of physic.

Chapter III:

RESULTS

&

DISCUSSIONS

Result and discussion

1-Extraction yield

According to **Zaman et *al*. (2020),** extraction yield assesses both the concentration of secondary metabolites in the plant and the efficacy of the extraction process. Furthermore, extrinsic factors such as age, climate, soil, harvest period, and so on influence the concentration of an active element in the plant **(Banerjee and Bonde, 2011)**.

The values obtained from the calculation of the extraction yield attained for each solvent are displayed in the figure bellow:

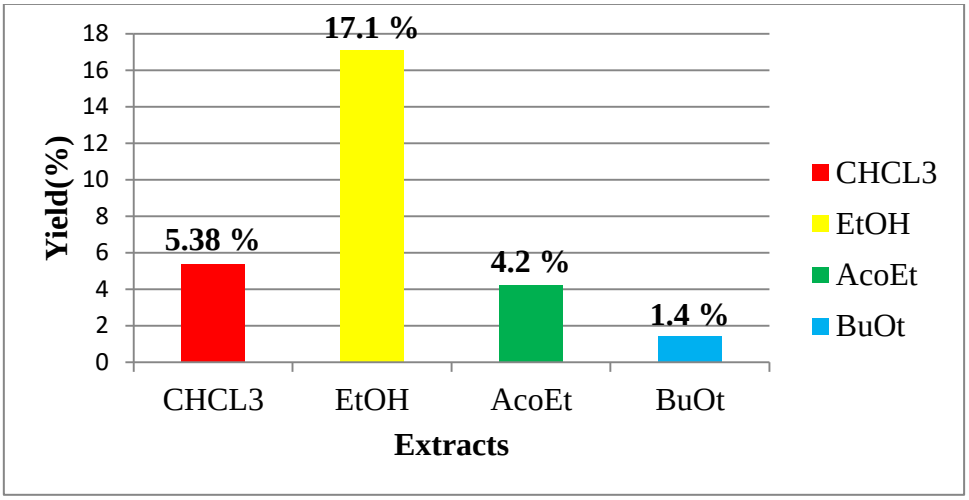


Figure 18: Yield of extraction

The extraction of *S. elaeagnifolium* leaves by maceration with hydro-ethanol yielded 17.1%, but the extract by soxhlet yielded 5.38%, 4.2%, and 1.4% for the extracts of $CHCl_3$, AcoEt and BuOt, respectively.

The variation in the polarity of the solvents employed, which is similarly important in improving the solubility of phytochemical substances, may be the cause of the variation in extract yields. The solvent's polarity effect justifies this outcome **(Guediri et *al*., 2021)**.

Table 11: Previous studies on percentage yield of *Solanum* plants

Plant	**Plant part**	**Recolt region**	**Recolt period**	**Method of extraction**	**Yield %**	**Refrence**
S. elaeagnifolium	Leaves	Moroccan ciity of FEZ	November 2020	Ethanolic Macetration (7-3) for 72 hours and	10.2	**(Bouslamti et *al*., 2022)**

				conserved on 4°C		
		Pithalaipatty village	June 2018	Soxhlet		**(Xavier et *al*.,2022)**
				Ethyl acetat	4.77	
				Ethanol	6.80	
				Acetone	4.94	

So the disparity in yields is obviously attributable to the lack of optimisation of conditions (solvent, environment, plant part, recovery period, etc.).

2-Phytochemical Screening

The initial testing used qualitative characterization procedures to identify the different chemical families found in *S. elaeagnifolium* leaves. Certain reagents resulted in colouring or precipitation in these reactions.

The results of phytochemical screening of *Solanum elaeagnifolium* cav are shown in the table below:

Table 12: Phytochemical screening of plant extracts

Test		**Aqueous extract**	**Ethanol extract**
Flavonoids		+	+
Phenolic compounds		+	+
Tannins		+	+
Free Quinone		+	+
Alkaloids	Wagner reagent	-	-
	Mayer reagent	-	-
Redactor compounds		+	+
Terpanoids		+	+
Anthraquinone		-	-
Sterols and triterpenes		-	-
Coumarins		-	-

+: Presence; -: Absent

The phytochemical screening of ethanol and aqueous extracts revealed the presence of flavonoids and phenolic compounds, tannins, free quinone and terpanoids and redactor compounds. While alkaloids, anthraquinone, coumarins, sterol and triterpenes were absent in both extracts.

Bouslamti et *al* (2022) performed a phytochemical screening for ethanol and petroleum ether extracts of *Solanum elaeagnifolium* leaves. The screening's findings demonstrated that tannins, polyphenols, flavonoids, steroids, alkaloids, and saponins were all detected in the ethanol extract.

3-Total phenolic content analysis

One of the most significant groups of secondary metabolites, phenolic compounds is generally present in plants with a variety of structural variations. One of the best electron donors that can ensure the quick conversion of H_2O_2 into H_2O is phenolic compounds. For this reason, they are known as potent antioxidants that break chains **(Feki et *al*., 2014)**.

The total phenolic content of the various extracts of the studied plant leaves was determined using the Folin-Ciocalteu reagent and expressed as milligram gallic acid equivalents (GAE) per gram of plant extract. The total phenolic content of the test fractions was quantified using the Gallic acid standard curve ($y = 0.0029x + 0.1972$; $R^2 = 0.9982$) (Figure19)

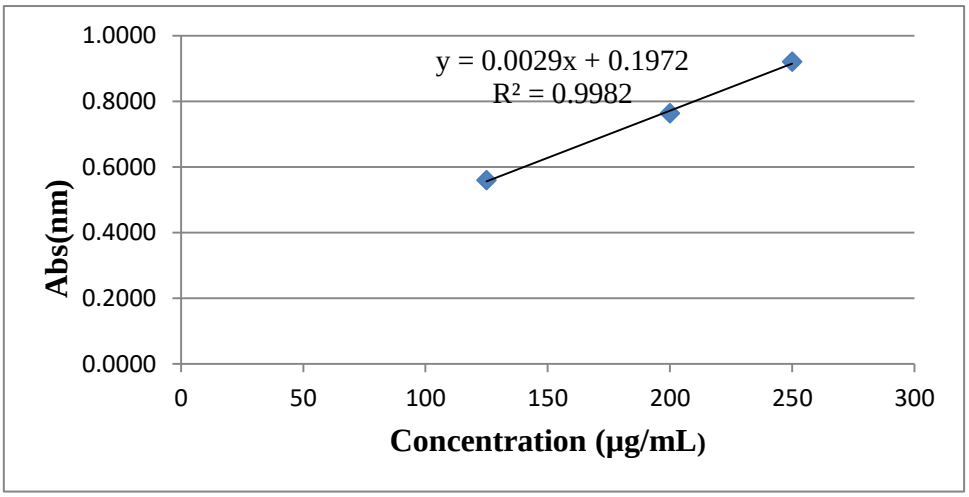


Figure 19: Calibration curve of Gallic acid for total phenolic content determination

The results obtained during the test are presented in the diagram below:

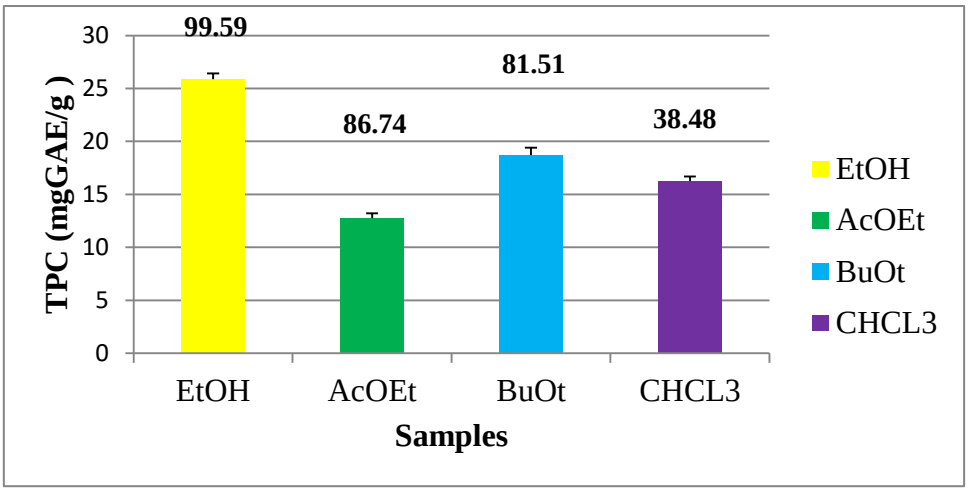


Figure 20: Total phenolic content of *S. elaeagnifolium* leaf extracts.

The current investigation found that the ethanol extract contains a high concentration of polyphenols (99.59 ± 1.94 mg of GA per gramme of dry extract). Compared to other extracts. While the extracts of soxhlet may be arranged according to their total phenolic content as follows: acetate extract (86.74 ± 1.91 mg GAE/g), followed by BuOt with a content of 81.51 ± 1.01 mg GAE/g, and $CHCl_3$ extract (38.482 ± 0.722 mg GAE/g).

Based on their polarity, solubility, and chemical structure, several extraction solvents have had an intriguing impact on the extraction capacity of phenolics. The quick measurement of antioxidant activity might be based on the total phenolic concentration **(Guediri et *al.*, 2021)**.

4-Total flavonoid content analysis

The importance of flavonoids in foods and herbal extracts appears in their protective effects against a spread of diseases associated with ROS through their capacity to transfer free radical electrons, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidation **(Guediri et *al.*, 2021)**.

The total flavonoid content of the various extracts of silver nightshade leaves was determined using the Aluminium Chloride reagent and expressed as milligram quercetin equivalents (QE) per gram of plant extract. The total flavonoid content of the test fractions was quantified using the quercetin standard curve ($y = 0.0059x + 0.1072$; $R^2 = 0.9933$). (Figure21)

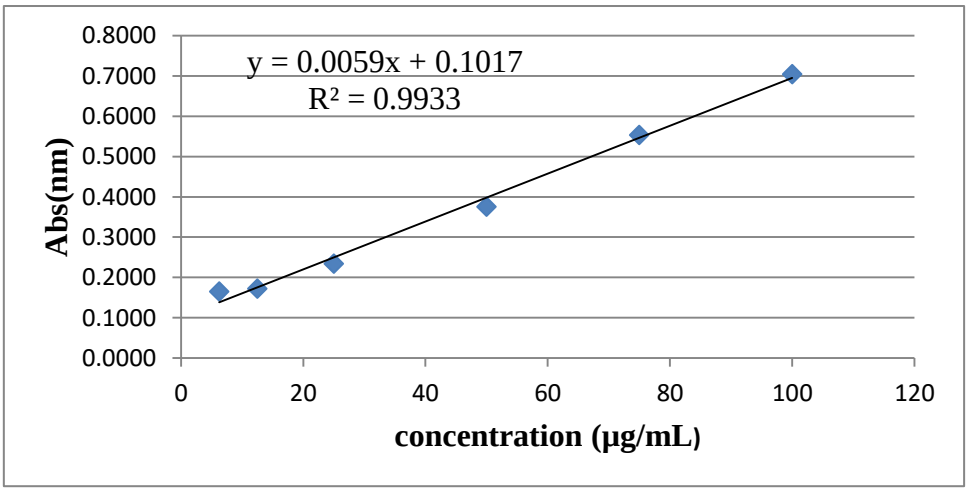


Figure 21: Calibration curve of quercetin for total flavonoid content determination

The results obtained during the test are presented in the diagram below

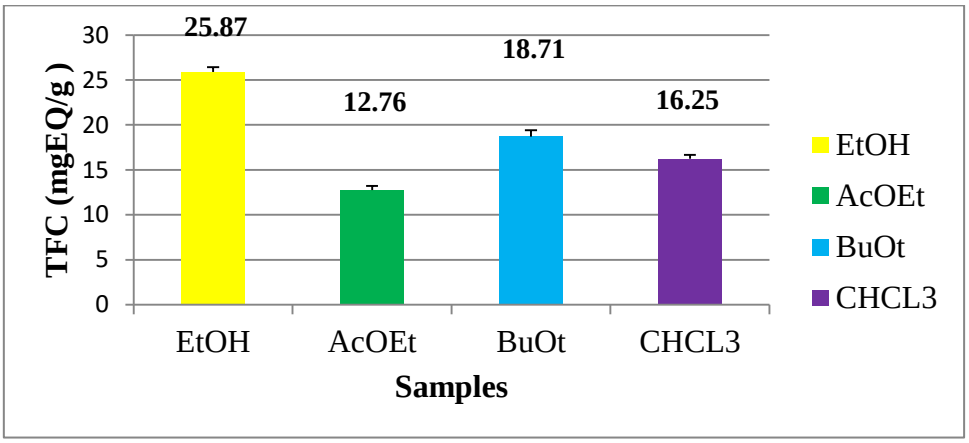


Figure 22: Total flavonoid content of *S. elaeagnifolium* leaf extracts

The current investigation found that the ethanol extract contains a high concentration of flavonoid (25.87 ± 0.556) mg of Q per gram of dry extract. Comparing to other extracts. While the extracts of soxhlet may be arranged according to their total flavonoid content as follows: BuOt extract (18.71 ± 0.699 mg QE/g), followed by $CHCl_3$with a content of (16.25±0.42mg QE/g) and ethyl acetate extract (12.76 ±0.442mgQE/g).

Table 13: Previous studies on TFC and TPC of *Solanum* plants

Plant	Plant part	Solvent	TPC (mg GAE/g)	TFC	Reference
Solanum Nigrum (south of Algeria)	fruit	Ethyl acetate	87.68	18.21	**(Guediri et *al.*, 2021)**
		Butanol	70.65	12.97	
		Chloroform	52.70	24.23	

S. elaeagnifolium (Pithalaipatty village)	seeds	Ethyl acetate	79.04	134.31	**(Xavier et *al*., 2022)**

The amount of phenolic compounds in a plant extract is impacted by the chemical composition of the component, as well as the technique of measurement, standard selection, and the presence of interfering substances **(Naczk and Shahidi, 2006)**.

5-Antioxidant screening analysis

According to multiple studies, medicinal plants are important sources of natural antioxidants, which have emerged as a potential alternative to synthetic antioxidants. We tested the antioxidant activity of the *S. elaeagnifolium* leaf extracts using two methods: the phoosphomylbdemum technique and trapping the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl).

5-1 DPPH free radical trapping test

Based on the obtained values (Abs) of the DPPH assay, the inhibition percentages were calculated using the previously provided formula (equation 1)

The "IC50" index is commonly used to assess a compound's anti-radical capacity, which measures the concentration of the extract necessary to neutralise 50% of the present DPPH.

The anti-radical activity of an extract is inversely proportional to its IC50. Which can be derived using linear regression of inhibition versus antioxidant activity? A lower IC50 value indicates high antioxidant activity.

The IC50 for each extract is shown in the diagram below:

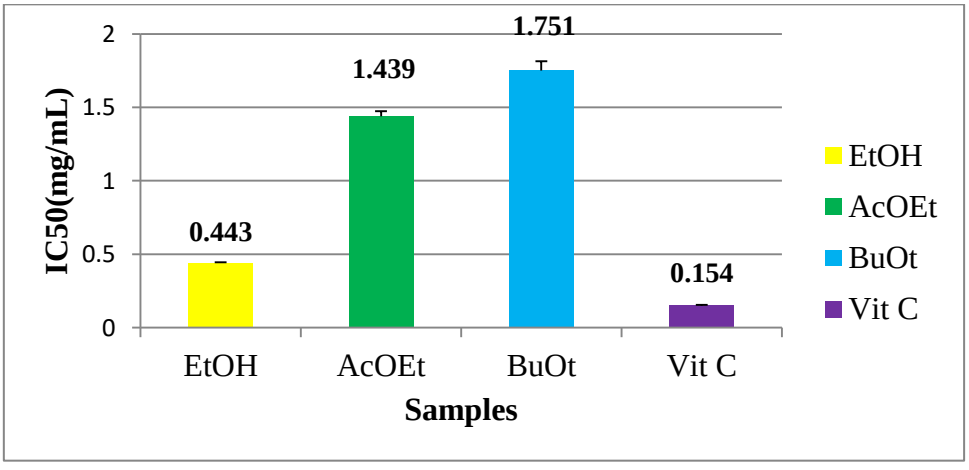


Figure 23: The IC50 of DPPH free radical trapping test

The IC50 for ascorbic acid, employed as a positive control, was 0.154±0.00065 mg/mL.

Overall, it was obvious that the ethanol extract had more scavenging action than the other extracts, particularly the chloroform extract, which had a very high IC50 (>>1.5 mg/mL), indicating that it had extremely low antioxidant activity when compared to ascorbic acid.

All of the extracts tested had lower anti-radical activity than ascorbic acid in terms of DPPH radical trapping.

Flavonoids and phenols are well-known antioxidants. According to several researches, plant extracts with high phenolic component concentrations have relatively high antioxidant activity. Its phenolic hydroxyl groups may account for phenolic compounds' high ability to scavenge radicals **(Feki et *al*., 2014)**.

According to the results of TPC and TFC which was mentioned up it seems that this antioxidant activity is partly due to the presence of phenolic compounds which are the most abundant in dosed molecules (polyphenols and flavonoids).

5-2 Total Antioxidant Capacity (TAC)

The total antioxidant capacity was studied with phosphomolybdate assay of the extract studied is expressed in the number of ascorbic acid equivalents from a calibration curve.

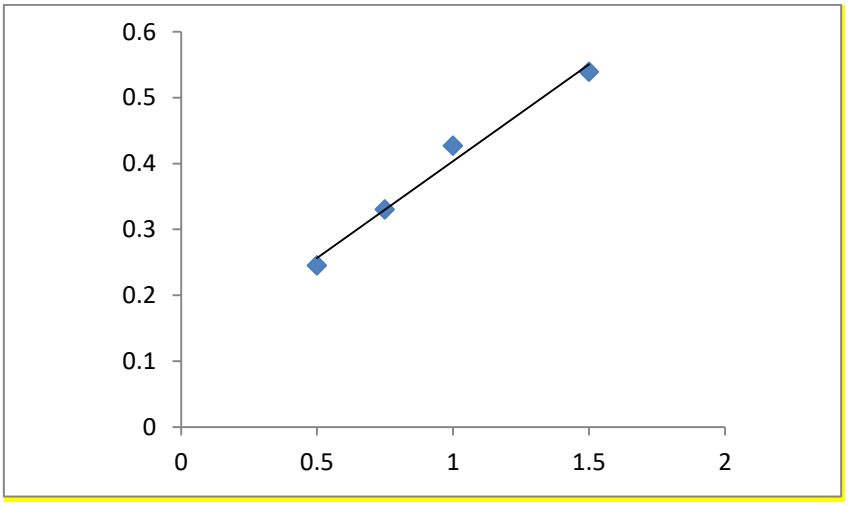


Figure 24: antioxidant activity of the ascorbic acid

This graph shows the correlation between the different concentrations of the EtOH extract and the antioxidant activity by vitamin C equivalent.

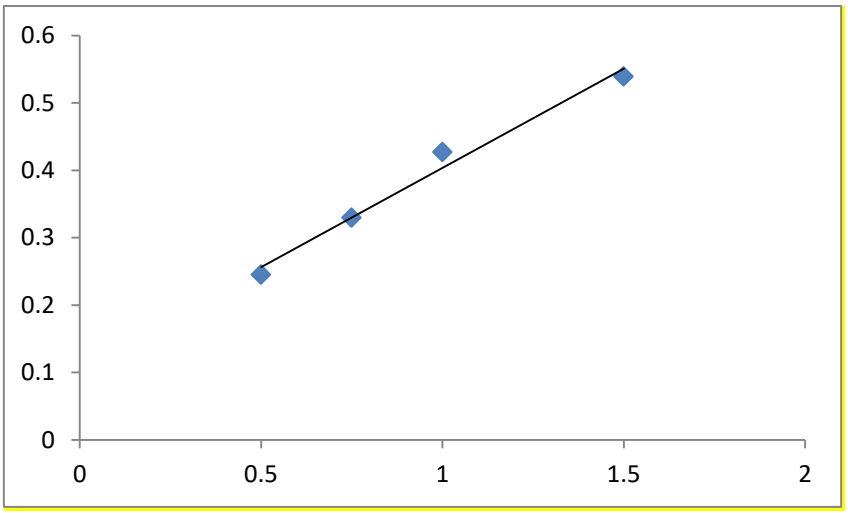


Figure 25: Correlation between different concentrations of EtOH extract and ascorbic acid.

The estimate of TAC is based on the graph. The highest power is in the concentration1.5 mg/mL or TAC=0.176mgEAA/ml.

The variation in total antioxidant capacity has been shown to be a reflection of the extraction solvent's ability to alter the chemical composition of the extract. The results of **Feki et *al.* (2014)** show that the phenolic content of *S. elaeagnifolium* and the antioxidant activity have a high and linear association, suggesting that phenolic compounds may play a significant role in the anti-oxidant action **(Boslamti et *al.*, 2022)**.

6- Antibacterial activity

This study assessed the *S. elaeagnifolium* ethanol extract's antibacterial efficacy against a few harmful bacterial strains. The width of the inhibition zones surrounding the impregnated discs was determined following a 24-hour incubation period at 37 °C. The following table displays the collected findings.

Table 14: Result of Antibacterial activity

Strain	**Inhibition Zone(mm)**		**DMSO**
	C2=50mg/mL	**C1=100mg/mL**	
Pseudomonas Aeruginosa Reference (PAR)	7	8.66±0.57	-
Pseudomonas Aeruginosa(PA)	8	7.66±0.57	-
Klebsiella Pneumoniae(KP)	No activity		-

Klebsiella Pneumonia Reference (KPR)	9.66±1.154	9±1	-
Escherichia Coli (EC)	8.33±0.57	9±1	-
Escherichia Coli reference (EC)	No activity		-

The results reveal that our extract had low activity compared to the concentrations of 50 mg/mL and 100 mg/mL for Gram bacteria, which are as follows: KPR, E. coli, PA, and PAR for ZOI, respectively (9.66±1.154 mg/mL); (8.33±0.57mg/ mL) ;(8±0.00mg/ ml) ;(7± 0.00mg / mL).

According to **Ponce et *al.* (2003),** resistant bacteria that the ZOI<8 as PAR; PA of our results; bacteria sensitive to ZI>9 mm as KPR. In addition, there are bacteria that do not possess activity, such as E. coli and KP.

7- Sun protection factor

The Sun Protection Factor (SPF) or Protection Index (IP) is a measure of the effectiveness of photo protectors against the effects of UVB radiation. This indicates the level of UVB protection provided by the product. This is measured in the laboratory and determined by standardized tests as an example: the diluted solution method of **Mansur et *al.*1986**

The histogram below (Figure 26) represents the sun protection factor (SPF) of the extracts of the different phases obtained from the plant.

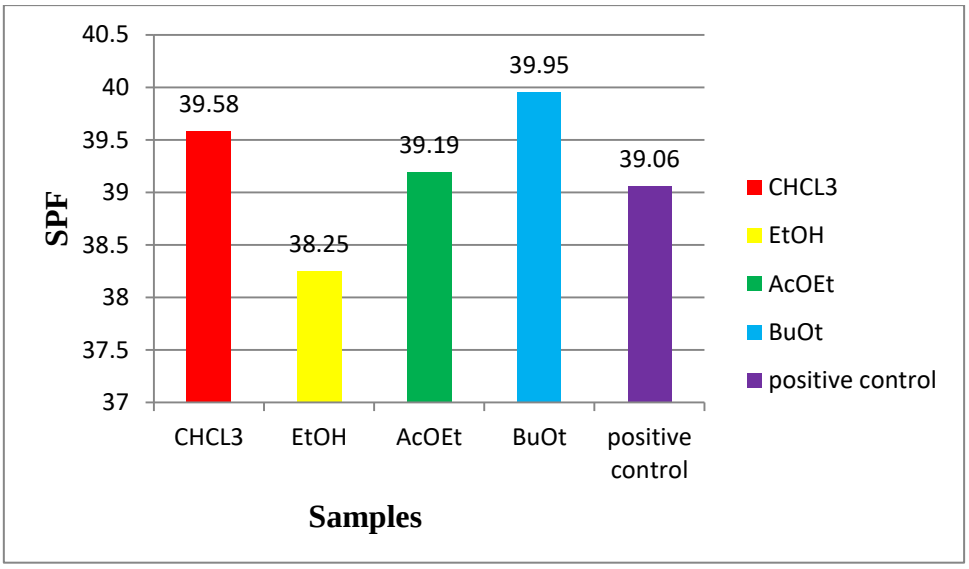


Figure 26: Sun protection factor (SPF) of the extracts of the different phases obtained from the plant.

The results obtained for extracts from our plant are very interesting. The SPF values obtained for a concentration of 2 mg/ml varied from 38.25 to 39.95. The butanol extract, is highly active in photo protection (SPF=39.95), followed by the other extracts (38 < SPF < 39) Despite the SPF values, the photo protective potential of our plant extracts (according to the European Commission) can be classified as follows **Sayre et *al*. (1979)**.

Table 15: Classification of sunscreen products and different *solanum* extracts

SPF measured(Solar products)	**SPF measured(*solanum* extract)**	**Classification**
6-9.9		Low protection
15-19.9		
25-29.9		Medium protection
30-49.9	BuOt 39.95 $CHCl_3$ 39.58 AcOEt 39.19 EtOH 38.25	High protection
50-59.9		
≥ 60		Very high protection

8-6 FTIR results

FTIR analysis for functional groups revealed the presence of a number of distinct functional groups in both samples (dry plant powder and ethanol extract of silver nightshade). (Table 16) shows the frequency range and functional group determined from absorption spectra.

Table 16: FTIR spectra of ethanolic extract of *solanum elagonfluim* leaf **(DeepaShree et *al*., 2012)**

Frequency Range (cm^{-1})	Functional group	Compound class	Reference
3200-3550	O-H stretching	Alcohol	**(DeepaShree et *al*., 2012)**
2500-3300	O-H stretching	Alcohol(intermolecular bonded)	

1566-1650	C=C stretching	Aromatic ring ,cyclic alkene	

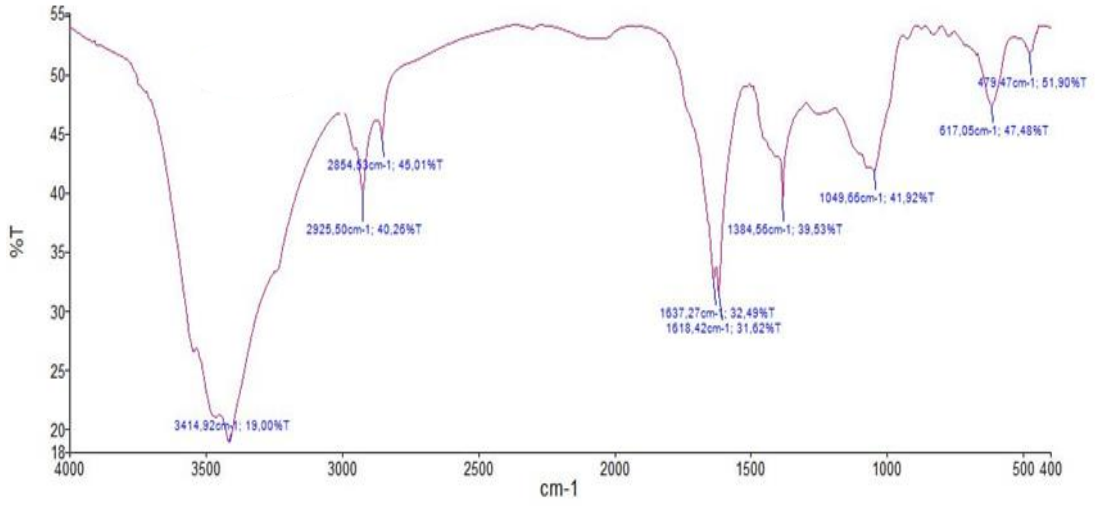


Figure 27: Absorption of spectra of sample before extraction

The absorption spectra of the sample after extraction show O-H stretching, which could be a functional group for alcohol. The presence of this functional group was detected by a prominent absorption band at 3314.92 cm^{-1}. The presence of O-H stretching (alcohol) was confirmed by two additional bonds at 2925.50 and 2854.53 cm^{-1}. This effect could be attributed to the ethanol extract's bioactive components or the alcohol itself.

While the peaks at 1618.42 and 1637.27 were caused by the presence of C=C, which corresponds to an aromatic ring, we can conclude that this extract contains bioactive molecules with an aromatic ring. This idea is backed by the favourable results of SPF, TPC, TFC, TFC, antioxidant activities, and even phytochemical screening, all of which are induced by bioactive substances.

Conclusion

Conclusion

In recent years, herbal medicine has emerged as a significant and increasingly popular area of biomedical research, with researches into medicinal plants taking centre stage alongside chemotherapy. *Solanum elaeagnifolium* is one such plant that has gained interest for its possible therapeutic and preventative effects.To examines the therapeutic potential of the *Solanum elaeagnifolium* plant, we tested several extracts in vitro for antioxidant, antibacterial, and photo-protection properties.

Oxidative stress accelerates the ageing of our bodies and reduces their regeneration potential. Indeed, an overabundance of free radicals will destroy our body's cells. The build-up of these degradations will increase the body's weakening caused by age, and so ageing.

Simultaneously, recurrent oxidative stress contributes to the emergence of a variety of diseases. They include rheumatism as well.

There are hundreds of polyphenols and flavonoids with antioxidant activity that may contribute to the antioxidant mechanisms in humans and animals in general. These substances are good candidates for explaining the health benefits of a fruit and vegetable-rich diet, but there is currently a lack of information on food composition, bioavailability, interactions with other food components and biological effects.

Indeed, the yields obtained from *S. elaeagnifolium* extractions vary considerably depending on the solvent used and the method applied, where the yield obtained by hydro-ethanolic maceration (17.1%) was greater than that obtained by soxhlet extraction.

From the result of this study, it can be concluded that various phytochemicals, phenols, tannins, terpenoids, flavonoids, redactor compounds, free quinons, and anthraquinone were present in both hydro-ethanolic and aqueous extracts of silver nightshade leaves.

For the photo-protection activity, all of the extracts in this investigation gave an outstanding SPF when compared to the sunscreen (Venus), which was employed as a positive control. Antioxidant potential was assessed using the free radical trapping power method (DPPH), which revealed that the ethanol extract of *Solanum elaeagnifolium* had strong antioxidative power when compared to standard ascorbic acid, as well as good total

antioxidant capacity. While the $CHCl_3$ extract had the lowest IC50 value, followed by the butanol extract.

The colorimetric dosage indicated high total polyphenol content and follow-up of flavonoid content with 99.59±1.94 mg EAG/g, 25.87 ±0.556 mg EQ/g respectively of ethanol extract that occupies the first value in both dosages (TPC) and (TFC) comparing with soxhlet phases .

For the infrared analysis the ethanol extract had different functional group such as O-H stretching and C=C stretching.

Numerous experimental perspectives arise from this research. And in the light of these results, it is desirable to complete and deepen this work by:

A study of antioxidant activity by other methods (B-carotene, ABTS, and FRAP) broadens the range of antioxidant and photo-protection activities in vitro and, why not, in vivo? Carry out a comparative study with the other fractions extracted from the same plants. The evaluation of the antimicrobial activity of the extracts against other pathogenic bacteria and fungi and defining their mode of action. Identification of bioactive molecules responsible for the observed antioxidant activities and photo-protection activity by various analytical methods, such as High-performance liquid chromatography (HPLC) coupled with mass spectroscopy or with Nuclear Magnetic Resonance (NMR).

REFERENCES

- Adjim, Z., Tani, C. K. (2018). L'infestation par Solanum elaeagnifolium menace l'Algérie. Revue d'Ecologie, Terre et Vie, 73(4), 569-581.
- Afonso, V., Champy, R., Mitrovic, D., Collin, P., Lomri, A. (2007). Radicaux libres dérivés de l'oxygène et superoxydes dismutases: rôle dans les maladies rhumatismales. Revue du rhumatisme, 74(7), 636-643.
- Allangba, M. R. A., Kadja, A. B., Guessennd, N. K., Mamyrbékova-Békro, J. A., Békro, Y. A. (2016). Bio-guided phytochemical survey, antibacterial and analgesic activities of ethanolic decoctions of a Mimosaceae from Côte d'Ivoire. International Journal of Innovation and Applied Studies, 18(2), 569.
- Allangba, M. R. A., Kadja, A. B., Guessennd, N. K., Mamyrbékova-Békro, J. A., Békro, Y. A. (2016). Bio-guided phytochemical survey, antibacterial and analgesic activities of ethanolic decoctions of a Mimosaceae from Côte d'Ivoire. International Journal of Innovation and Applied Studies, 18(2), 569.
- Amara, N., Melouk, F. Z. (2016). Activité antimicrobienne des extraits des feuilles de la Vigne Sauvage (Vitis vinifera sylvestris). *Algerian Journal of Natural Products*, *4*(3), 358-366.
- Aribi-Zouioueche, L., Couic-Marinier, F. (2021). Huiles essentielles et chiralité moléculaire. *Comptes Rendus. Chimie*, *24*(3), 397-414.
- Atuhaire, J. K., Nabasumba, A. J., Munanura, E. I., Anyama, N. G., Rubayiza, P., Kaggwa,
- Banerjee, S. K., Bonde, C. G. (2011). Total phenolic content and antioxidant activity of extracts of Bridelia retusa Spreng Bark: Impact of dielectric constant and geographical location. J Med Plants Res, 5(5), 817-822.
- Banerjee, S. K., Bonde, C. G. (2011). Total phenolic content and antioxidant activity of extracts of Bridelia retusa Spreng Bark: Impact of dielectric constant and geographical location. J Med Plants Res, 5(5), 817-822.
- Ben-Ghabrit, S. (2016). Historique et caractérisation de l'invasion de la morelle jaune (Solanum elaeagnifolium Cav. SOLEL) au Maroc. Revue Marocaine de Protection des Plantes, (10).
- Benmechernene, R., Hamadi, A. (2022). Contribution à l'étude d'une plante adventice La morelle jaune Solanum elaeagnifolium.

- Boizot, N., Charpentier, J. P. (2006). Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. Le Cahier des Techniques de l'INRA, In: Numéro spécial, 79-82.
- Bouslamti, M., El Barnossi, A., Kara, M., Alotaibi, B. S., Al Kamaly, O., Assouguem, A., Benjelloun, A. S. (2022). Total polyphenols content, antioxidant and antimicrobial activities of leaves of Solanum elaeagnifolium Cav. from Morocco. Molecules, 27(13), 4322.
- Bouslamti, M., Loukili, E. H., Elrherabi, A., El Moussaoui, A., Chebaibi, M., Bencheikh, N., ... Benjelloun, A. S. (2023). Phenolic Profile, Inhibition of α-Amylase and α-Glucosidase Enzymes, and Antioxidant Properties of Solanum elaeagnifolium Cav. (Solanaceae): In Vitro and In Silico Investigations. Processes, 11(5), 1384.
- Boutaghane, N., Kabouche, Z., Voutquenne, L. (2013). Etude phytochimique et pharmacologique de plantes médicinales Algériennes Genista ulicina Spach (Fabaceae) et Chrysanthemum macrocarpum (Sch. Bip.) Coss. Kralik ex Batt (Asteraceae).
- Butler, M. S., Blaskovich, M. A., Cooper, M. A. (2017). Antibiotics in the clinical pipeline at the end of 2015. The Journal of antibiotics, 70(1), 3-24.
- Călinoiu, L. F., Vodnar, D. C. (2018). Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. Nutrients, 10(11), 1615.
- Cely-Veloza, W., Kato, M. J., Coy-Barrera, E. (2023). Quinolizidine-type alkaloids: chemodiversity, occurrence, and bioactivity. ACS omega, 8(31), 27862-27893.
- Chafik, Z., Bouhache, M., Berrichi, A., Taleb, A. (2013). Etat de l'Infestation par la Morelle Jaune Solanum elaeagnifolium (Cav.) dans la région orientale du Maroc. Revue Marocaine de Protection des Plantes, (4).
- Collins, A., Santhakumar, A. B., Francis, N., Blanchard, C., Chinkwo, K. (2024). Impact of sorghum (Sorghum bicolor L. Moench) phenolic compounds on cancer development pathways. Food Bioscience, 104177.
- Dai, J., Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 15(10), 7313-7352.
- Dutra, E. A., Oliveira, D. A. G. D. C., Kedor-Hackmann, E. R. M., & Santoro, M. I. R. M. (2004). Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Revista Brasileira de Ciências Farmacêuticas, 40, 381-385.

- Duval, J., Pecher, V., Poujol, M., Lesellier, E. (2016). Research advances for the extraction, analysis and uses of anthraquinones: A review. Industrial Crops and Products, 94, 812-833.
- Eddouks, M., Ajebli, M., Hebi, M. (2017). Ethnopharmacological survey of medicinal plants used in Daraa-Tafilalet region (Province of Errachidia), Morocco. *Journal of ethnopharmacology*, *198*, 516-530.
- Esimone, C. O., Attama, A. A., Mundi, K. S., Ibekwe, N. N., Chah, K. F. (2012). Antimicrobial activity of Psidium guajava Linn. Stem extracts against methicillin-resistant Staphylococcus aureus. African journal of biotechnology, 11(89), 15556-15559.
- Esseh, K., Afanyibo, Y. G., Ahama-Esseh, K. Y. S., Idoh, K., Koudouvo, K., Agbonon, A., Gbeassor, M. (2019). Screening phytochimique, étude toxicologique, évaluation des activités antiplasmodiale et antiradicalaire de la tige feuillée de Senna occidentalis Linn (Fabaceae). *European Scientific Journal*, *15*(6), 411
- Eyong, K. O., Kuete, V., Efferth, T. (2013). Quinones and benzophenones from the medicinal plants of Africa. In Medicinal Plant Research in Africa (pp. 351-391). Elsevier.
- Feki, H., Koubaa, I., Damak, M. (2014). Secondary metabolites and antioxidant activity of seed extracts from Solanum elaeagnifolium Cav. Mediterranean Journal of Chemistry, 2(5), 639-647..
- Gebhardt, C. (2016). The historical role of species from the Solanaceae plant family in genetic research. *Theoretical and Applied Genetics*, *129*(12), 2281–2294. Ghankoba, M., Soulama, S., Karama, I., Kiendrebeogo, M. (2024).
- Gherhard, R. (1993). Métabolisme des végétaux. Physiologie
- Gherhard, R. (1993). Métabolisme des végétaux. Physiologie et biochimie. Presses polytechniques et universitaires Romandes, Paris. Coumarin content of the feed of Khaya senegalensis (Desr.) A. Juss. According to its origin in Burkina Faso. International Journal of Biological and Chemical Sciences, 18(1), 303–310
- Ghosh, D. (2015). Tannins from foods to combat diseases. *Int J Pharm Res Rev*, *4*(5), 40-44.
- Guediri, I., Boubekri, C., Smara, O., Lanez, T. (2021). Total phenolic contents and determination of Antioxidant activity by DPPH, FRAP, and cyclic voltammetry of the fruit of Solanum nigrum (black nightshade) growing in the south of Algeria.

- Habibatni, S., Miceli, N., Ginestra, G., Maameri, Z., Bisignano, C., Cacciola, F., ... Miceli, N. (2016). Original Research Article Antioxidant and antibacterial activity of extract and phases from stems of Spartium junceum L. growing in Algeria. *International Journal of phytomedicine*, *8*, 37-46.
- Haida, Z., & Hakiman, M. (2019). A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food Science and amp; Nutrition, 7(5), 1555–1563.
- Hamel, J. D., Paquin, J. F. (2018). Activation of C–F bonds α to C–C multiple bonds. Chemical communications, 54(73), 10224-10239.
- Harborne, J. B., Marby, H., Marby, T. J. (2013). The flavonoids. Springer.
- Hashemi, Z., Ebrahimzadeh, M. A., Khalili, M. (2019). Sun protection factor, total phenol, flavonoid contents and antioxidant activity of medicinal plants from Iran. Tropical Journal of Pharmaceutical Research, 18(7), 1443-1448.
- Hopkins, W. G. (2003). Physiologie végétale. De Boeck Supérieur.
- Hostetler, G. L., Ralston, R. A., Schwartz, S. J. (2017). Flavones: Food sources, bioavailability, metabolism, and bioactivity. Advances in Nutrition, 8(3), 423-435.
- Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. (2002). Botanique systématique: une perspective phylogénétique. De Boeck Supérieur.
- Karmezi, M., Krigas, N., Argyropoulou, M. D. (2022). The Invasion and long naturalization of Solanum elaeagnifolium affects the soil nematode community: Evidence from a comparative study.Agronomy, 12(10), 2346.
- Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of infection and public health, 14(12), 1750-1766.
- Kovinich, N., Kayanja, G., Chanoca, A., Otegui, M. S., Grotewold, E. (2015). Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. Plant Signaling and Behavior, 10(7), e10278
- Kralova, K., Jampilek, J. (2021). Responses of medicinal and aromatic plants to engineered nanoparticles. Applied Sciences, 11(4), 1813.
- Krigas, N., Tsiafouli, M. A., Katsoulis, G., Votsi, N. E., van Kleunen, M. (2021). Investigating the invasion pattern of the alien plant Solanum elaeagnifolium Cav. (silverleaf nightshade): Environmental and human-induced drivers. Plants, 10(4), 805.

- Krigas, N., Votsi, N. E., Samartza, I., Katsoulis, G., Tsiafouli, M. A. (2023). Solanum elaeagnifolium (Solanaceae) invading one in five Natura 2000 protected areas of Greece and one in four habitat types: What is next? Diversity, 15(2), 143.
- Kwong, R., Sagliocco, J. L., Weiss, J., Hunt, T., Morfe, D., Kularatne, D. (2006). Feasibility of biological control of solanaceous weeds of temperate Australia; silverleaf nightshade, Solanum elaeagnifolium Cav. and prairie ground cherry, Physalis viscosa L. North Sydney, Australia: Meat and Livestock Australia, 256-258.
- Lalande, J., Parenty, A. (2003). Synthese d'une coumarine. Actualite Chimique, (3), 27-30.
- Las Heras, B., Rodriguez, B., Bosca, L., Villar, A. M. (2003). Terpenoids: sources, structure elucidation and therapeutic potential in inflammation. Current topics in medicinal chemistry, 3(2), 171-185.
- Liang, N., Kitts, D. D. (2014). Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanisms of Action. Molecules/Molecules Online/Molecules Annual, 19(11), 19180–19208.
- Lingegowda, D. C., Kumar, J. K., Prasad, A. D., Zarei, M., and Gopal, S. (2012). FTIR spectroscopic studies on cleome gynandra-Comparative analysis of functional group before and after extraction. Rom. J. Biophys, 22(3-4), 137-143.
- Lobo, V., Patil, A., Phatak, A., Chandra, N. (2010b). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews/Bioinformatics Trends/Pharmacognosy Review*, *4*(8), 118
- M'Sadak, Y., Saad, I. (2015). Characterization qualitative of SOEL for a valorization best of thebiomass produced. Journal of Fundamental and Applied Sciences, 7(1), 33-48.
- Macheix, J. J., Fleuriet, A., Jay-Allemand, C. (2005). Les composés phénoliques des végétaux: un exemple de métabolites secondaires d'importance économique. PPUR presses polytechniques.
- Makuasa, D. a. A., Ningsih, P. (2020). The Analysis of Total Flavonoid Levels in Young Leaves and Old Soursop Leaves (Annona muricata L.) Using UV-Vis Sepctrofotometry Methods. *Journal of Applied Science, Engineering, Technology, and Education*, *2*(1), 11–17.
- Mansur J.D.S., Breder M.N.R., Mansur M.C.D.A., Determinação do fator de proteção solar por espectrofotometria. Anais Brasileiros de Dermatologia, 1986, 61, 121-124.

- Mansur, M. A., Ong, K. C. G., Paramasivam, P. (1986). Shear strength of fibrous concrete beams without stirrups. *Journal of structural engineering*, *112*(9), 2066-2079.
- Mbanga, L., Mulenga, M., Mpiana, P. T., Bokolo, K., Mumbwa, M., Mvingu, K. (2014). Determination of sun protection factor (SPF) of some body creams and lotions marketed in Kinshasa by ultraviolet spectrophotometry. Int. J. Adv. Res. Chem. Sci, 1(8), 7-13.
- Meunier L., Photoprotection (interne et externe). EMC (Elsevier Masson SAS, Paris), Dermatologie, 2008, 98-944-A-10.
- Mohammed, M. T., Kadhim, S. M., Jassimand, A. N., Abbas, S. I. (2015). Free radicals and human health. International journal of innovation sciences and research, 4(6), 218-223.
- Mullen, W., Marks, S. C., Crozier, A. (2007). Evaluation of Phenolic Compounds in Commercial Fruit Juices and Fruit Drinks. Journal of Agricultural and Food Chemistry, 55(8), 3148–3157
- Naczk, M., Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of pharmaceutical and biomedical analysis*, *41*(5), 1523-1542.
- Nguyen, V. T., Le, V. M., Vo, T. S., Bui, L. M., Anh, H. L. T., Danh, V. T. (2020). Preliminary phytochemical screening and determination of total polyphenols and flavonoids content in the leaves of Houttuynia cordata Thunb. In IOP Conference Series: Materials Science and Engineering (Vol. 736, No. 6, p. 062013). IOP Publishing
- Nigussie, D., Davey, G., Legesse, B. A., Fekadu, A., Makonnen, E. (2021). Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complementary Medicine and Therapies*, *21*(1).
- Ounis, R., Boumaza, D., Karouche, S. (2018). Evaluation of the phenolic content and biological activities of Teucrium polium.
- Polyphenols in Plants. (2014). In Elsevier eBooks
- Ponce, A., Fritz, R., Del Valle, C., Roura, S. (2003). Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *Lebensmittel-Wissenschaft + Technologie/Food Science & Technology*, *36*(7), 679–684.

- Prabhu, S., Molath, A., Choksi, H., Kumar, S., Mehra, R. (2021). Classifications of polyphenols and their potential application in human health and diseases. International Journal of Physiology, Nutrition and Physical Education, 6(1), 293–301.
- Preiser, J. (2012). Oxidative Stress. *JPEN, Journal of Parenteral and Enteral Nutrition/JPEN. Journal of Parenteral and Enteral Nutrition*, *36*(2), 147–154.
- Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Analytical Biochemistry, 269(2), 337–341.
- Qazi, M. A., Molvi, K. I. (2018b). Free Radicals and their Management. *American Journal of Pharmacy and Health Research*, *6*(4), 1–10.
- Roberts, J., Florentine, S. (2022). Biology, distribution and management of the globally invasive weed Solanum elaeagnifolium Cav (silverleaf nightshade): a global review of current and future management challenges. Weed Research, 62(6), 393-403.
- Salazar, R., Vidal, J., Martínez-Cifuentes, M., Araya-Maturana, R., Ramírez-Rodríguez, O. (2015). Electrochemical characterization of hydroquinone derivatives with different substituents in acetonitrile. New Journal of Chemistry, 39(2), 1237-1246.
- Sarni-Manchado, P., Cheynier, V. (Eds.). (2006). Les polyphénols en agroalimentaire. Techniques and documentation.
- Sastry, K. S., Mandal, B., Hammond, J., Scott, S. W., Briddon, R. W., Sastry, K. S., ... Briddon, R. W. (2019). Solanum elaeagnifolium (Silverleaf nightshade). Encyclopedia of Plant Viruses and Viroids, 2250-2251.
- Sayre, R. M., Agin, P. P., LeVee, G. J., Marlowe, E. (1979). A comparison of in vivo and in vitro testing of sunscreening formulas. *Photochemistry and Photobiology*, *29*(3), 559-566.
- Shaikh, J. R., Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603–608.
- Shalini, S., and Sampathkumar, P. (2012). Phytochemical screening and antimicrobial activity of plant extracts for disease management. International Journal of Current Science, 1(1), 209-218.
- Sies, H. (2020c). Oxidative Stress: Concept and Some Practical Aspects. *Antioxidants*, *9*(9), 852

- Sies, H., Berndt, C., Jones, D. P. (2017b). Oxidative Stress. *Annual Review of Biochemistry*, *86*(1), 715–748.
- Singh, M., Sharma, V. (2016). Spectrophotometric determination of Sun Protection Factor and antioxidant potential of an herbal mixture. British Biotecnology Journal, 10, 1-8.
- Sirivibulkovit, K., Nouanthavong, S., Sameenoi, Y. (2018, July). Paper-based DPPH Assay for Antioxidant Activity Analysis. *Analytical Sciences*, *34*(7), 795–800.
- Stanton, R., Heap, J., Carter, R., Wu, H. (2009). Solanum elaeagnifolium Cav. In The Biology of Australian Weeds. Volume 3. (pp. 1-35). RG and FJ Richardson.
- Stringlis, I. A., De Jonge, R., Pieterse, C. M. J. (2019). The Age of Coumarins in Plant–Microbe Interactions. Plant & Cell Physiology/Plant and Cell Physiology, 60(7), 1405–1419.
- Tataridas, A., Moreira, M., Frazão, L., Kanatas, P., Ota, N., Travlos, I. (2023). Biology of Invasive Plants 5. Solanum elaeagnifolium Cav. Invasive Plant Science and Management, 16(3), 139-159.
- Uchôa, A. D., Oliveira, W. F., Pereira, A. P., Silva, A. G., Cordeiro, B. M., Malafaia, C. B., ... Correia, M. T. (2015). Antioxidant activity and phytochemical profile of Spondias tuberosa Arruda leaves extracts. *American Journal of Plant Sciences*, *6*(19), 3038.
- Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B.
- Ullah, H., Ali, S. (2017). Classification of Anti-Bacterial Agents and Their Functions. In InTech eBooks.
- Wilson, J. R., Ivey, P., Manyama, P., Nanni, I. (2013). A new national unit for invasive species detection, assessment and eradication planning. South African Journal of Science, 109(5), 1-13.
- Xavier, T. F., Sabitha, R., Kariyat, R., Rose, A. K. F., Ayyanar, M., Subramaniyan, V., Amalraj, S. (2022). In-vitro and in-silico strategies to assess the unexplored pharmacological lineages of Solanum elaeagnifolium Cav. Leaves.
- Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Datta, N., Singanusong, R., Chen, S. S. (2004). Flavonoids in food and their health benefits. Plant foods for human nutrition, 59, 113-122.

- Yeh, T. F., Lin, C. Y., Chang, S. T. (2014). A potential low-coumarin cinnamon substitute: Cinnamomum osmophloeum leaves. *Journal of agricultural and food chemistry*, *62*(7), 1706-1712.
- Zaman, M. a. K., Azzeme, A. M., Ramli, S. N., Shaharuddin, N. A., Ahmad, S., Abdullah, S. N. A. (2020b). Solvent extraction and its effect on phytochemical yield and antioxidant capacity of woody medicinal plant, Polyalthia bullata. Bioresources, 15(4), 9555–9568.
- Zhang, J., an, M., Wu, H., Liu, D. L., Stanton, R. (2012). Chemical composition of essential oils of four Eucalyptus species and their phytotoxicity on silverleaf nightshade (Solanum elaeagnifolium Cav.) in Australia. Plant Growth Regulation, 68, 231-237.