الجمهورية الجزائرية الديمقراطية الشعبية
PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
وزارة التعليم العالي والبحث العلمي
MINISTRYOF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

جامعة الإخوة منتوري قسنطينة1-كليةعلوم الطبيعة والحياة

Brothers Mentouri, Constantine1 University - Faculty of Life and Natural Sciences

قسم الكيمياءالحيوية والبيولوجيا الخلوية والجزيئية

Department of Biochemistry, Molecular and Cellular Biology

Thesis submitted for Master Degree in Natural and Life sciences

Field: Applied Biochemistry

Polyphenolic potential and *in vitro* biological screening of antioxidant, antibacterial and antifungal activities of an Algerian wild medicinal plant

Presented by:

Khadidja Yasmine ARIANE and *Zeyneb AISSANI*

On: June 28th 2020

Examination board:

Chairman : Dr. Daffri Amel Daffri (MCB – UFM Constantine 1)

Supervisor : Dr. T. Nardjes MOUAS (MCA – UFM Constantine 1)

Examiner : Dr. Zeyneb Boucherit (MAA– UFM Constantine 1)

2019-2020

ACKNOWLEDGMENTS

Without the help of ALLAH, Most Gracious, Most Merciful, no work could have been accomplished. This is why we thank Him for getting us to where we want to be.

In addition, our research work required framing, help, and contributions that we want to thank here, because it was necessary and precious for the progress of this research.

Our first pleasant duty is to sincerely thank our supervisor Mrs. MOUAS Toma Nardjes, Professor assistant in Brothers Mentouri Constantine 1, for her patience, her availability and especially her judicious advices, which helped to fuel our ideas and reflections. We express our gratitude to her, for all the time she has devoted to us, for providing us with the methodological tools essential to the conduct of this research exercise. Our gratitude for her is beyond measure for giving us her trust.

We would like to thank the eminent professors, members of the jury who agreed to give their time to examine, discuss and exchange opinions on our subject, through their rich and valuable experiences.

We would like also to thank administrative staff of Biochemistry Department, and Biochemistry lab (RDC) engineers for their support, please find here our thanks and recognition.

We cannot forget to have a glance at all our teachers from kindergarten whose priceless teachings lead us to this day.

DEDICATION

Thanks to ALLAH who has lightened my path and who granted me the ability to accomplish this work.

I dedicate this dissertation to my family members and my friends who encouraged me throughout the process. A special feeling of gratitude to my parents, the reason of what I become today, thanks for your great support and continuous care.

Yasmine

DEDICATION

I want to dedicate this humble work to those I love, my dear parents, grandparents, brothers, sisters, uncles, aunts, cousins, and friends.

Without forgetting those who have gone and certainly would have been so glad and proud had they been present, I am thinking about my uncles: Mohamed El Hadi and Mourad.

A special wink to my beloved nephew ADAM.

Zeyneb

Table of contents

Part two Experimental part

List of Abbreviations

ABS	Absorbance.
AIDS	Acquired Immune Deficiency Syndrome.
ATCC	American Type Culture Collection.
CAM	Complementary Alternative Medicine.
DE	Dry Extract.
DMAPP	Diméthylallyl-pyrophosphate.
DMSO	Dimethyl sulfoxide.
Fe^{+2}	Ferrous ion.
Fe^{+3}	Ferric ion.
g	Gram.
GAE	Gallic acid equivalent.
GN	Gentamicin.
IC50	The half maximal inhibitory concentration.
In silico	In computer simulation.
In vitro	Within the glass, outside of a living.
In vivo	Within a living organism.
IPP	Isopentenyl diphosphate.
mg	Milligram.
ml	Milliliter.
mm	Millimeter.
NA	Nalidixic.
nm	Nanometer.
NSA	Non-starch polysaccharides.
OCH_3	Methoxy group.
OH	Hydroxyl radical.
O^{2-}	Superoxide radical.
pH	Potential of Hydrogen.
QE	Quercetin.
ROO-	Peroxyl radical.

ROS	Reactive oxygen species.
SAR	Structure-Activity Relationships.
TCA	Trichloroacetic acid.
TFC	Total Flavonoid Content.
TPC	Total Phenolic Content.
UV-vis	Ultraviolet-visible.
WHO	World Health Organization.
µg	Microgram.
°C	Degree Celsius.
%	Pourcentage.

List of Figures

List of Tables

Introduction

The field of medicine has long been divided between rationalism and vitalism advocates. While rationalists searched for knowledge based on established laws and principles rather than what is directly perceived, vitalists believed that life is "unique and that it possesses qualities that cannot be defined in simple terms of anatomy and physical make up" (Kawalchik and Hylton, 1998). Whereas the rationalist scientific model dominated (at least in Western nations) for the last couple of centuries, vitalistic concepts of health have increasingly emerged and became well developed in recent centuries. Numerous forms of natural healing –both ancient and new- have arisen, some of which were even challenging conventional medicine. Therefore, Alternative Medicine has become Complementary Alternative Medicine (shortened as CAM), (Pengelly, 2004) i.e.; treatments used instead of standard medical treatments.

Medicinal plants are traditionally used since ancient times to treat common illnesses and more serious diseases. Their actions come from their chemical compounds: primary and secondary metabolites, and particularly from the synergy between the various compounds they contain (Ensa, 2011).

Most of the drugs obtained from natural resources come from plants, most of which date back to ancient times. It is quite clear that nowadays application of pure isolates and crude forms of plants in the treatment of alleged disorders was fore-shadowed in traditional remedies. These forms of traditional healing are now practiced worldwide and have been recognized by the World Health Organization (WHO) as an essential building block for primary health care. Even though economic conditions may play an essential role, they are by no means the ultimate decisive factors in a patient's choice between herbal and modern medicine. The former actually bridges the gap between the availability and the demand for the latter.

Traditional values were significantly considered valid for all people by the industrial world. Some locally used herbal remedies have been found to surpass the efficacy of drugs used clinically in the treatment of the same diseases, as evidenced by the complete cure and remarkable successes achieved in the treatment of some otherwise "medically incurable" diseases (Onayade et al., 1990).

The majority of the world developing countries uses medicinal plants for health purposes and for pain treatment. In the African region, traditional medicine practices have been inherited from generation to generation as oral tradition (Ouelbani *et al.*, 2016). According to the WHO, many African countries in recent years have strengthened training programs for traditional health practitioners to increase their knowledge. In Algeria, several authors have published books on traditional phytotherapy and ethnobotany, however; this country remains poorly explored, even though it has considerable natural resources in different ecosystems and considerable floristic diversity. In this area, old knowledge and therapeutic practices are exclusively preserved (Miara *et al.*, 2019). Nevertheless, it is the role of modern researches to prove the efficiency of such practices.

In this regard, the present work aims at valorizing the medicinal plants of Algeria and possible discoveries of metabolites responsible of therapeutic effect. It falls into three parts:

- The first part is a brief review of phytotherapy, medicinal plants, secondary metabolites and attributed biological activities

- The second one is an experimental study that includes biochemical analysis and *in vitro* biological screening of the antioxidant, antibacterial and antifungal activities of different parts of a selected wild medicinal plant.

- The third one gathers all obtained results and their discussion, with the study of a possible correlation between it.

At last, a general conclusion with the future perspectives and advices were given

Part one

Review of the literature

Chapter I:
Phytotherapy and medicinal plants

I.1. Phytotherapy

Phytotherapy is a field of medicine that uses plants extracts of natural origin either to treat disease or as health-promoting agents. Nowadays, there are several specialties that use plants for medical purposes.

I.1.1. Types of phytotherapy

- **Aromatherapy**: It is a therapy that uses the essences of plants, or essential oils, aromatic substances extracted by distillation. These oils may be harmful and are to be used with precautions and prescribed doses, generally administered percutaneously.
- **Gemotherapy**: it is a therapy that is based on the use of alcoholic and glycerol extracts from the embryonic tissues of various trees and shrubs (buds and emerging shoots), but also from the reproductive parts (seeds) and from newly cultivated tissues (rootlets and root cortex).
- **Herbalism**: it is the oldest method of phytotherapy, it uses the fresh or dried plant, it can use the whole plant or a part of it (flower, fruit, root…etc.). Its preparation is done by a simple method, most often water-based: decoction, infusion, maceration. It can be drunk or inhaled, applied to the skin or added to bath water. Nowadays, it also exists in the form of capsules of dry plant powder, this last preparation mostly preserves the active ingredients which are fragile.
- **Homeopathy**: it uses plants preponderantly, but not in an exclusive way: three quarters of the strains are of plant origin, the rest are of animal and mineral origin. Fresh plants are used in alcoholic maceration. These alcoholates are called mother tinctures. The fundamental principle of homeopathy is based on the postulate that any substance capable of provoking a certain number of symptoms in a healthy individual would be likely to cure a sick patient presenting a similar set of symptoms.
- **Chinese herbal medicine**: it is part of a group called "traditional Chinese medicine" which includes acupuncture and Chinese dietetics. This phytotherapy aims to modify the quantities of different energies or the circuit of these energies in the body.

- **Pharmaceutical herbal medicine**: it uses products of plant origin obtained by extraction and diluted in solvents such as ethyl alcohol. These extracts are dosed in sufficient quantities in order to have a sustained and rapid action. They are presented in several galenic forms of syrup, drops, suppositories, capsules, lyophilizates, nebulizates (extracts of plants dried by heat) …etc. The concentrations are quite high and the non-toxicity of these drugs is sometimes relative (https://www.larousse.fr/encyclopedie/medical/phytoth%C3%A9rapie/15365).

I.1.2. History of phytotherapy

From a historical perspective, the production of medicines and the pharmacologic treatment of diseases began with the use of herbs. (Volkers Schulz, Varro Eugene Tyler, 2012).

The healing properties of plants were accidently discovered by early mankind in the search for daily food. The observation that animals favored certain plants when they were injured may have helped to guide primitive man in the search of cures for his diseases. (Capasso et al., 2003).

The use of plants as remedies goes back certainly to the great civilizations of the ancient Chinese, Indians, and North Africans who provided written evidence of man's ingenuity in using plants for the treatment of a wide variety of ailments (Phillipson, 2001).

In Middle Ages, medicinal plants began to be catalogued according to their therapeutic action; In the 15th century, several so-called herbals were published containing illustrated information, on medicinal plants. During the 16thand the 17th centuries, medicinal plants continued to play a crucial function in medicine. In the 18th century, Linnaeus made an important contribution to the development of plant science through the introduction of the new system for naming and classifying plants. During the 18th and the 19th century, plants and crude drugs were still being used as powders, extracts or tinctures. However, during this period the isolation and chemical identification of pharmacologically active compounds from crude drugs began, for instance; morphine was isolated in 1803, quinine and caffeine in 1820 …etc. Today, the constituents of the main herbal drugs have been isolated and their structure determined (Capasso et al., 2003).

I.2. Medicinal plants

Also called medicinal herbs are a rich source of a broad range of secondary active metabolites such as flavonoids, phenolic acids, coumarins, terpenoids and sterols that besides giving the plant its specific color, flavor and aroma and protecting it against external dangers, they are responsible of the different effects on human health because of their important role in healing some common diseases such as fever, cold and other medicinal claims that are now supported with sound scientific evidences (Azwanida, 2015), As well as their contribution in the prevention of chronic diseases like cancer, diabetes ...etc, different parts of the plant are used, we mention aerial parts, leaves, fruits and roots as well as stems seeds and blooms.

The study of bioactive molecules in medicinal plants like all other aromatic plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. All stages of extractions, from the pre-extraction and extraction are equally important in the study of medicinal plants. The sample preparation such as grinding and drying affects the efficiency and phytochemical constituents of the final extracts. It can be concluded that, no universal extraction method may be the ideal one and each extraction procedure is unique to a specific plant. Previously developed methods can be used to lead in the selection of suitable methods. However, evaluation and selection of pre-extraction preparation and extraction methods depends on the study objectives, samples, and target compounds (Azwanida, 2015).

As opposed to what is generally believed, several medicinal plants contain toxic ingredients and may cause severe allergic reactions or serious poisoning. But in other cases, these toxic components might be helpful for some curing purposes; We mention for example: The resin podophyllin extracted from the root of the mayapple, podophyllum peltatum, is toxic and is used clinically to remove warts. The major constituent of the resin is the lignan podophyllotoxin which inhibits cell division. Because of its toxic properties it would seem to be not helpful pursuing any medicinal activities even though its effects on cell division would indicate potential use in cancer chemotherapy (Phillipson, 2001).

I.2.1. Phytochemistry of medicinal plants

Herbal medicines are essentially active principles isolated from herbs. Most of them are referred to as secondary metabolites. These plants constituents can be studied using the accumulated skills and knowledge embedded in the natural sciences - especially botany and chemistry or biochemistry. While herbalists value the totality of these constituents at a holistic approach of a herb's action, scientists consider only some specific compounds, which they work on isolating and purifying in order to get active principles, and consider the remaining compounds unworthy.

Knowing each constituent individually is also essential for "developing quality assurance methods, extraction procedures, understanding of pharmacological activity and pharmacokinetics and -most importantly- understanding of potential toxicology and interactions with pharmaceutical drugs" (Pengelly, 2004).

I.2.2. Medicinal Plants of Algeria

Algerian pharmacopoeia is seen as traditional because it has not been written down but has continued to the present by passing from generation to generation among healers and herbalists through oral transmission of knowledge and practice of the medical art., unlike Western pharmacopoeias which have been formalized in forms and codex (Baba AIssa, 1990).

The table below shows some examples of Algerian medicinal plants with the bioactive molecules they contain:

Table 1 Examples of Algerian medicinal plants and their therapeutic effect linked to the active compounds they contain.

Family, plant name	Active compounds	Therapeutic effect	References
➢ *Melissa officinalis.L* الحبق الترنجاني	Essential oil	Antimicrobial activity Antifungal activity Anti-inflammatory activities	(Miraj et al., 2016)
➢ *Zingibar officinalis* الزنجبيل	Gingerols	Inhibition of the induction of several genes involved in inflammatory response	(Ali *et al.*, 2008)
➢ *Olea europaea* الزيتون	Phenols Oleic acid Hydroxytyrosol Tyrosol Oleuropein	Reduce reactive oxygen species (ROS). Antimicrobial activity	(Waterman et al., 2007)
➢ *Pistacia Lentiscus* الضرو	Natural monoterpenes Bioactive triterpenes Gallic acid Ethanolic extract Terpeneol	Antioxidant propriety Inhibitor in human colon cancer Antimicrobial activity	(Ansari and Siddiqui, 2012)

Table 2 Examples of Algerian medicinal plants and their traditional uses and their scientifically proven effects

Family, plant name	Traditional uses	Scientifically proven effects	References
➢ *Olea europaea* الزيتون	Treat gallstones, diarrhea, stomach and intestinal diseases mouth cleanser. Treat respiratory and urinary tract infections. Treat hypertension and induce diuresis Prevent hair loss, oil is applied every night on the scalp then shampooed the next morning. Used as anti-inflammatory and for diabetes.	Efficacy proved in diabetes, cardiovascular disorders, viral and microbial infections.	(Khan *et al.*, 2007)
➢ Melissa officinalis.L الحبق الترنجان	Digestive, carminative, antispasmodic, sedative, analgesic, tonic and diuretic as well as for functional gastrointestinal disorders.	Anti-herpes and anti-HIV effect., antioxidant effects., anti-depression and anti-anxiety and insomnia. Anticancer, antioxidant. Memory and concentration improving, anti-Alzheimer and neuroprotective effects.	(Miraj et al., 2016) (Ali et al., 2008)
➢ *Pistacia lentiscus* الضرو	Used for many treatments such as Eczema, diarrhea, throat infections and as potent antiulcer agent.	Anti-ulcer activities, anti-inflammatory, antimicrobial and antifungal	(Ali-Shtayeh *et al.*, 1998) (Gioxari *et al.*, 2011)
➢ *Zingiber officinale* الزنجبيل	Used to cure a variety of diseases: Nausea, vomiting, asthma, cough, palpitations, inflammation, dyspepsia, loss of appetite, constipation, indigestion and pain.	Antimicrobial activity, Anticancer activity., Antioxidant activity. Antidiabetic activity, nephroprotective activity, hepatoprotective activity. Analgesic activity, anti-inflammatory activity, Immunomodulatory activities.	(Kumar et al., 2011)

I.3. Plants constituents

Medicinal plants contain biochemical substances that belong to two main groups known as primary metabolites and secondary metabolites.

These phytochemicals accumulate in different parts of the plants; roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending on the variety, processing, cooking and growing conditions (Saxena *et al.*, 2013).

Primary metabolites (such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids) cover all processes essential for growth and development whereas secondary metabolites (which include alkaloids, terpenoids, phenolics and essential oils) are indispensable for the survival of the individual in its environment (Hartmann, 2007). Today most scientists accept that many of these compounds (which were once regarded as simple waste products) serve primarily to repel grazing animals or destructive pathogens (Cronquist, 1988). In other terms they protect the plant from adverse factors which threaten its survival in an unfavorable environment besides their important role on human health.

The present study focuses on secondary metabolites;

I.3.1. Secondary metabolites

In all plant parts, a variety of secondary metabolism pathways elicited an array of plant defensive compounds called secondary metabolites, they are found but distributed differently according to their roles. This distribution varies from plant to plant (Yang *et al.*, 2018).

The term "secondary" introduced by A. Kossel in 1891 represents the most valuable phytochemicals of plant secondary metabolism that are derived from primary metabolism and possesses sufficient chemical or structural complexity. The secondary metabolites are present only incidentally and are non-essential for the survival of the organism. Though if they are absent, survival of the organism is impaired to a larger extent (Thirumurugan *et al.*, 2018).

I.3.1.1. Main classes of secondary metabolites

Secondary metabolites include large groups of chemical compounds that have been formerly used as important source of active traditional medicine, such as polyphenols and flavonoids, terpenoids and steroids and alkaloids (Yang *et al.*, 2018).

According to Verpoorte R., (1998), the classification of secondary metabolites consists of terpenoids, alkaloids and phenolics Glycosides, tannins and saponins are part of them according to their specific structure

A. Polyphenols and flavonoids

A.1. Polyphenols

Natural polyphenols are plant-derived organic chemicals structurally characterized by the presence of two or more phenol units. The word phenol refers to a molecule formed by attaching an alcohol-type hydroxyl (-OH) group to an aromatic phenyl or benzenoid ring.

According to the White–Bate-Smith–Swain–Haslam definition, the polyphenol term describes the class of generally moderately water-soluble compounds, with molecular weight of 500–4000 Da, >12 phenolic hydroxyl groups, and 5–7 aromatic rings per 1000 Da, where the limits to these ranges are necessarily somewhat flexible. This definition excludes lower molecular weight structures showing potential benefits for human health. In practice, this implies a less rigorous use of the polyphenol term towards the lower molecular weight end of the range. Therefore, there are several thousand compounds in higher plants of potential biological interest with one or more aromatic rings and at least two hydroxyl groups, thus qualifying as polyphenols (Ensa, 2011).

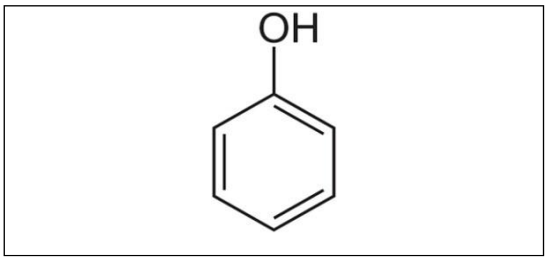


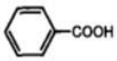
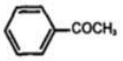
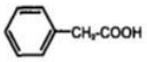
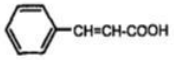
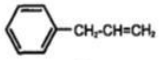
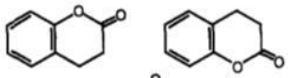
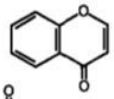
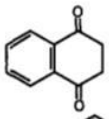
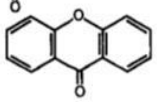
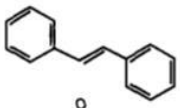
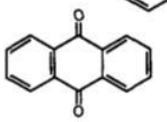
Figure 1 Crude chemical formula of a phenol function

Polyphenols: a single name for a multitude of structures The polyphenols or phenolic compounds designation brings together a vast set of more than 8,000 molecules, divided into ten chemical classes, which have all one thing in common: the presence of at least one 6-carbon aromatic ring in their structure carrying a variable number of hydroxyl (OH) functions. The most numerous representatives and the best known are flavonoids (more than 5,000 molecules isolated) (Hennebelle et al., 2004).

A.1.1. Classification of polyphenols

The structure, the number of aromatic nuclei and the structural elements that link these nuclei are the dominant characters in the classification of polyphenols (Pandey and Rizvi, 2009), the following table shows the main classes of these compounds according to Harborne cited by Bravo (1998).

Table 3 Main classes of polyphenolic compounds (Harborne cited by Bravo, 1998)

Class	Basic Skeleton	Basic Structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naftoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans, neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

A.2. Flavonoids

Lunte (1987) defines flavonoids as follows:

"Flavonids are a large group of plant secondary metabolites based on the structure of 2-phenylbenzopyrone. Thousands of flavonoids are known to exist in nature. They differ from one another in the degree of unsaturation, the pattern of hydroxylation or methylation, and type of sugar attached. The most common flavonoids fall into three general classes: proanthocyanidins, flavonols, and anthocyanidins".

Most recent researches have focused on the health aspects of flavonoids for humans. Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids show potential antiviral activities (Kumar and Pandey, 2013).

It is noteworthy that flavonoids and their chemical derivatives are often less toxic and reveal lower side effects than derivatives produced from other natural compounds. Nevertheless, similar to any chemical, flavonoids can be harmful at high doses (Tarahovsky *et al.*, 2014).

A.2.1. Classification of flavonoids

The basic flavonoid chemical structure is the flavan nucleus, which include anthocyanidins, flavonols, flavones, flavanones, flavan-3-ols and isoflavones differing in the level of oxidation and saturation of the C ring, one of the three rings A,C and B consisting of 15 carbon atoms arranged as (C6-C3-C6) (as illustrated below); two benzene rings (A and B) Combined by an oxygen-containing pyran (C) (Pengelly, 2004; Iriti and Varoni, 2013).

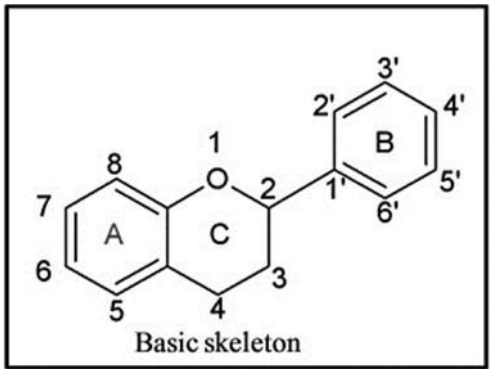
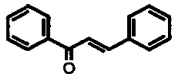
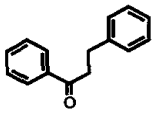
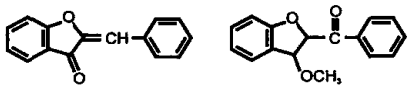
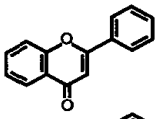
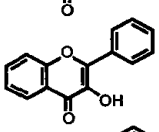
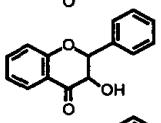
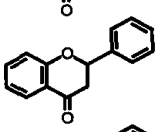
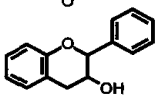
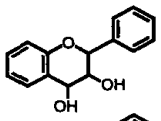
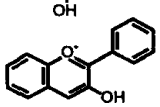
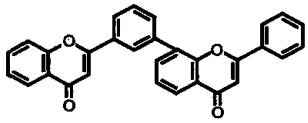
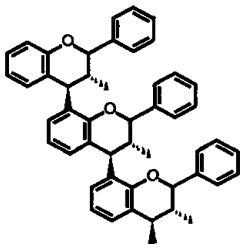


Figure 2 Basic skeleton structure of flavonoids (Panche et al., 2016)

According to Harborne cited by Bravo (1998), flavonoids can be subdivided into 13 classes, as illustrated in the table below:

Table 4 Main classes of polyphenolic compounds (Harborne cited by Bravo, 1998)

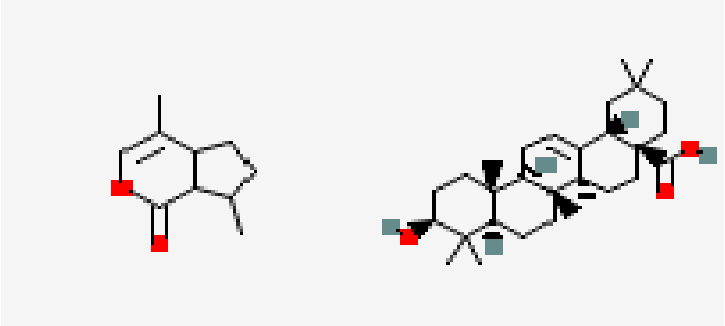
Flavonoid	Basic Structure
Chalcones	
Dihydrochalcones	
Aurones	
Flavones	
Flavonols	
Dihydroflavonol	
Flavanones	
Flavanol	
Flavandiol or leucoanthocyanidin	
Anthocyanidin	
Isoflavonoids	
Biflavonoids	
Proanthocyanidins or condensed tannins	

B. Terpenoids

According to Cheng *et al.*, (2007) and Pengelly, (2004), terpenoids or terpenes comprise one of the most important groups of active compounds in plants and are normally produced in vegetative tissues, flowers, and, occasionally, roots. They play an important role in plant-insect, plant-pathogen, and plant-plant interactions (Baldwin *et al.*, 2006), (Dudareva et al., 2004).

The biosynthesis pathways of monoterpenes, sesquiterpenes, and diterpenes include the synthesis of C5 precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), the synthesis of the immediate diphosphate precursors, and the formation of the diverse terpenoids.

Here are examples of two terpenoids structures; (A): the nepetalactone (a sesquiterpene) and (B) the oleanolic acid (a triterpene):



(A) **(B)**

Figure 3 Chemical structure of nepatalactone (A) and oleanolic acid (B) (PubChem)

B.1. Classification of terpenoids

Since they are built up from isoprene, terpenoids are also known as isoprenoids. They are divided on the basis of their C-skeleton (Graßmann, 2005).

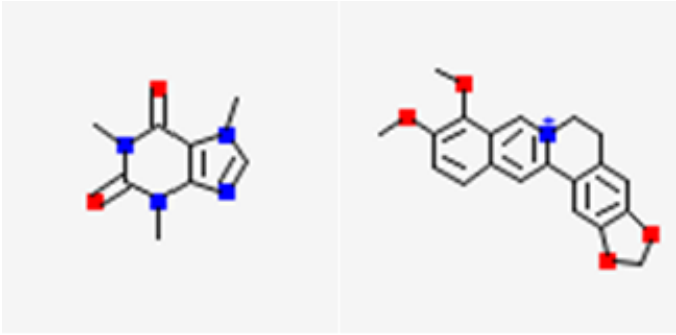
The table below gives a clearer idea about the classification of this class of secondary metabolites:

Table 5 Classes of Terpenoids

Terpenoid classes	Number of C-atoms	Number of isoprene subunits
Monoterpene	10	2
Sesquiterpene	15	3
Diterpene	20	4
Triterpene	30	6
Tetraterpene	40	8
Polyterpene	>40	>8

C. Alkaloids

Typical alkaloids are alkaline organic compounds containing one or more nitrogen atoms, each connected to at least two carbon atoms within a heterocyclic ring system except hormones and vitamins. Amino acids remain direct precursors for most of alkaloids, while few of them are derived from isoprene units. Alkaloids are found in 15 to 30% of all flowering plants while the higher plants are their major source. They are particularly common in certain families such as *Fabaceae*, *Liliaceae*, *Ranunculceae*, *Apocynaceae*, *Solanaceae* and *Papaveraceae*. The most occurring alkaloids are berberine and caffeine (Pengelly, 2004).



(A) **(B)**

Figure 4 Chemical structure of berberine (A) and caffeine (B) (PubChem)

C.1. Classification of alkaloids

Alkaloids are divided into two major divisions depending on their ring structures as follows:

1. Heterocyclic alkaloids: regarded as the most typical.
2. Non-heterocyclic alkaloids: known as protoalkaloids or biological amines, e.g. Colchicine (Pengelly, 2004).

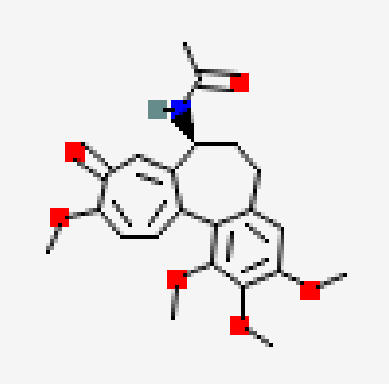
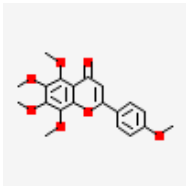
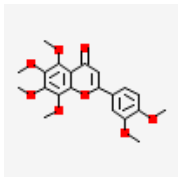
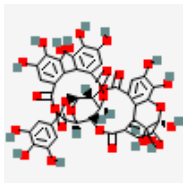
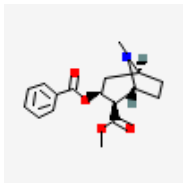


Figure 5 Chemical structure of colchicine (PubChem)

I.4. The contribution of secondary metabolites in medicinal plants therapeutic effect

The therapeutic effect of medicinal plants comes mainly from the bioactive compounds contained therein as shown in the table below:

Table 6 Examples of some bioactive compounds found in medicinal plants and their therapeutic effects.

Metabolite	Famille	Structure (PubChem)	Plants	Therapeutic effect	References
Tangeretin,	Flavone		*Citrus fruits*	Inhibits mutagenesis, thereby prevents carcinogenesis	(Pengelly, 2004)
Nobiletin					
Geranin	Hydrolysable Tannin		*Geranium maculatum, phyllanthusspp*	Cytotoxic, hypolipidaemic	(Pengelly, 2004)
Cocaine	Alcaloid		*Erythroxylon coca*	Blocks nerve conduction upon local application (anesthetic)	(Pengelly, 2004)

Chapter 2:
Biological properties of medicinal plants

Phytochemicals are biologically active chemical compounds that occur naturally in plants and have many disease-preventing functions, these phytochemicals belong to many classes, in particular anticancer, antioxidants, detoxifying agents, immunity-potentiating agents and neuropharmacological agents. It is noteworthy that one phytochemical can belong to many classes at the same time and that one medicinal plant may contain a load of chemicals (Saxena *et al.*, 2013).

Table 7 Bioactive phytochemicals in medicinal plants (M. Saxena, 2013)

Classification	Main groups of compounds	Biological function
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics.	Inhibitors of micro-organisms, reduce the risk of fungal infection.
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid.	Oxygen free radical quenching, inhibition of lipid peroxidation.
Anticancer	Carotenoids, polyphenols, curcumine, flavonoids.	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity.
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols.	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis.
NSA (Non-starch polysaccharides)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins.	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids.
Other classes	Other Alkaloids, terpenoids, volatile flavor compounds, biogenic amines.	Neuropharmacological agents, anti- oxidants, Cancer chemoprevention.

II.1. Antioxidant activity

II.1.1. Reactive oxygen species and oxidative stress

Reactive oxygen species (ROS), such as superoxide radical (O^2-), hydroxyl radical (OH) and peroxyl radical (ROO-), are produced as a part of normal metabolic processes but when the regulation of oxygen metabolism is disturbed, they are produced in an increasing way leading to an unbalance between the production of oxidants (free radicals) and ROS, and the antioxidant defense system. This unbalance can directly or indirectly cause numerous oxidative damages at the molecular level (nucleic acids, proteins, lipids ...), which can significantly affect cellular mechanisms, that may trigger various chronic diseases, such as coronary heart diseases, atherosclerosis, cancer and aging.

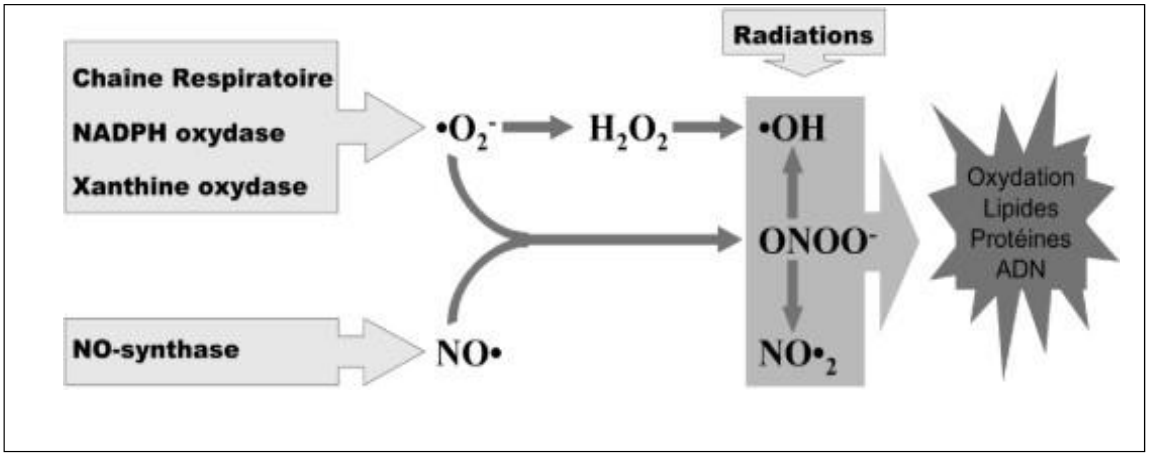


Figure 6 Origins and targets of ROS (Cano et al., 2007)

While the superoxide radicals seem not very reactive, the hydroxyl radical is the most aggressive species and therefore the most damaging of oxidative stress. However, these species remain potentially toxic via their reactions with hydrogen peroxide or nitric oxide, respectively generating hydroxyl radicals and peroxynitrite anions, both are very harmful to biological materials (Madhavi et al., 1996) (Wong et al 2006).

Most of the toxicity of hydrogen peroxide comes from its ability to generate the hydroxyl radical ·OH in the presence of metal cations such as Fe^{2+} known as the Fenton reaction (Gardès-Albert *et al.*, 2003):

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + OH$$

II.1.2. Defense mechanisms against oxidative stress

A biological antioxidant has been defined as any substance, when occurring at low concentrations in regard to those of an oxidizable substrate, it significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1995). However, a redox reaction still generally happens, even in the presence of an antioxidant., unless an antioxidant prevents the generation of an oxidizing species. The difference is that the oxidizing species reacts with the antioxidant instead of the "substrate," i.e., the oxidant is reduced by the antioxidant, thus; electron-donating antioxidants can be described as reductants, and inactivation of oxidants by reductants can be described as redox reactions. In this regard, therefore, "total antioxidant power" may called total reducing power (Benzie and Strain, 1999).

Antioxidant activity is a complex procedure usually occurring through several mechanisms and is influenced by many factors, making them hard to be fully described with only one method. Therefore, it is crucial to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (Schlesier *et al.*, 2002; Aruoma, 2003; El Jemli *et al.*, 2016).

II.1.3. Polyphenolic compounds and flavonoids as antioxidants

Polyphenolic compounds (including the ubiquitous group of flavonoids) are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Early researches have focused on the role of polyphenols in attenuating oxidative damage, being the most abundant form of exogen antioxidant in the human diet (Mubarak *et al.*, 2012).

The natural antioxidants have recently attracted a considerable attention of researchers. In this respect, as they contain a mixture of different chemical compounds, medicinal plants are being regarded as an easily available and potent source of antioxidants (Saiah et al., 2016).

The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of indirect and direct

antioxidant activity through radical scavenging and metal ion chelation ability respectively (Fig.7) (Kasprzak et al., 2015).

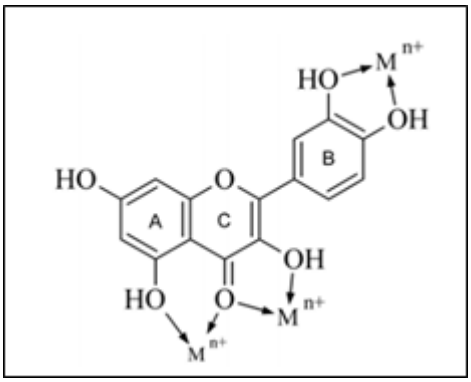


Figure 7 Possible chelating sites of quercetin

The B ring hydroxyl configuration is the most significant determinant of scavenging of ROS (Fig.8) because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical (Kumar and Egbuna, 2019).

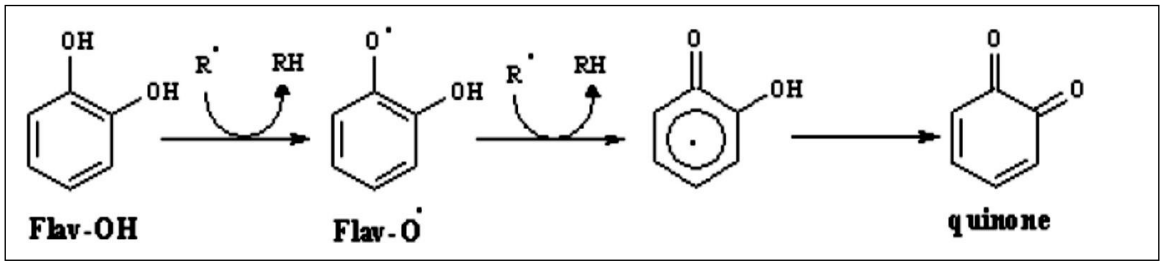


Figure 8 ROS scavenging by the flavonoids

II.1.3.1. The correlation between the phenolic content and the antioxidant activity

Crude antioxidant activity correlated positively to total phenolic concentration *in vitro* for a wide range of foods, but further researches should be carried out *in vivo*, since the antioxidant activity may be affected by metabolism and bioavailability. Several studies: El Jemli *et al.*, (2016), Saiah et al., (2016) and many others have worked on the correlation between plants phenolic content and antioxidant activity that gave positive results.

II.1.3.2. Structure–activity relationship of flavonoids

Many studies have shown the existence of structure-activity relationships (SAR) in the case of flavonoids. Thus, it has been shown that the activities of flavonoids and

their metabolites depend essentially on the number and position of their functional groups. The structural elements necessary to obtain an optimal antioxidant activity was established by several authors (Wolfe et Liu., 2008; Mercader et al., 2008; Khlebnikov, 2007 ; Sroka et al., 2005 ; Afanas et al., 2001 ; Van Acker et al., 1996) cited by Hadj Salem, (2018). These structural elements are as follows:

- The presence of a catechol function on cycle B
- The presence of a hydroxyl group in position 3
- The presence of an enone pattern at the C-cycle level.
- O-methylation.

The figure below illustrates what has been explained above:

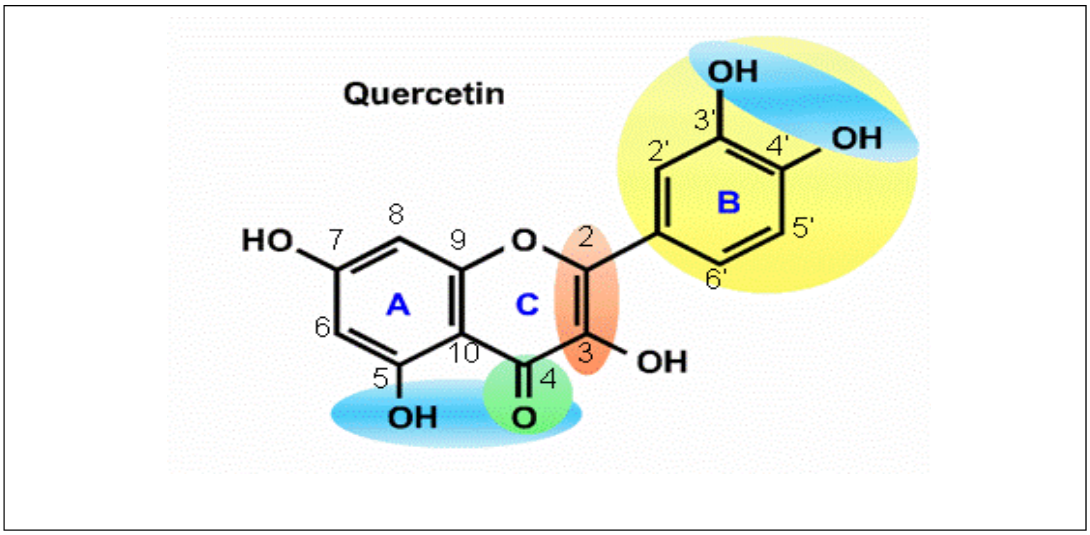


Figure 9 Quercetin active sites for an antioxidant activity

II.2. The antimicrobial activity

Antibiotic resistance has become an extremely critical issue in public health. It caused a crisis in many hospitals around the world in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients (Ahmad and Beg, 2001; Capita and Alonso-Calleja, 2013) In order to prevent this phenomenon, research for new antimicrobial substances from other sources such as plants has been deemed necessary.

Traditionally used medicinal plants produce a variety of compounds with known therapeutic properties. Substances that can either inhibit the growth of pathogens or kill

them and have little or no toxicity to host cells are considered candidates for the development of new antimicrobial drugs. In recent years, the antimicrobial properties of medicinal plants have been increasingly reported in different parts of the world, hence the importance of directing research towards the discovery of new pathways that are a source of inspiration for new plant-based drugs, in the form of secondary metabolites including phenolic compounds used as antimicrobial agents in popular medicine (Ahmad and Beg, 2001).

II.2.1. The correlation between the phenolic content and the antimicrobial activity

According to Mori et al., (1987), Nishino et al., (1987), Barnabas and Nagarajan, (1988) and Tsuchiya et al., (1996), cited by Rauha *et al.*, (2000), polyphenolic compounds are widely known as antioxidants and the attention has also been paid to their antimicrobial activity, but no dramatic evidence of their effectiveness has been reported.

II.2.2. The structure-activity relationship of phenolic content and antimicrobial activity

It should be noted that in many studies like reported in Rauha *et al.*, (2000) and da Silva *et al.*, (2006), the antimicrobial potential of the plants may be due to the secondary metabolites present in their extracts that have antibacterial properties.

The data obtained in the study done by Friedman et al., (2003) on the antibacterial activities of phenolic benzaldhehydes and benzoic acids against Escherichia coli and two others pathogens species indicate that the benzaldehydes are more active than the benzoic acids and that bactericidal activities are influenced by both the number and the position of the OH and OCH_3 groups on the benzene rings.

Polyphenols have been reported to exhibit antibacterial activities with distinguished characteristics in their reactivity with proteins related polyamides polymers. The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes. Phenolic compounds notably proanthocyanidins (often called condensed tannins) is vulnerable to polymerization in air through oxidization reactions. Therefore, an important factor governing their toxicity is their polymerization size. Oxidized

condensation of phenols may result in the toxification of microorganisms. On the other hand, polymerization can result in the detoxification of phenols. This supports the fact that polyphenols may be responsible for the antimicrobial activities of extracts of the screened plants (Saiahetal.,2016).

Chapter 3:
Biochemical analysis principles

III.1. Quantification of the total phenolic content

Principle

The total phenolic contents in the above four extracts were measured using the Folin–Ciocalteu method (Singleton and Rossi, 1965). The Folin–Ciocalteu reagent is a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) that reacts with phenols and non-phenolic reducing substances to form chromogens. The latter can be detected spectrophotometrically, since in alkaline conditions the oxoyungstate and oxomolybdate formed in this redox reaction display a blue coloration proportional to the concentration of polyphenols (Apak et al., 2018).

III.2. Quantification of the flavonoid content

Principle

According to (Djeridane *et al.*, 2006); the flavonoids content in extracts was determined spectrophotometrically by the $AlCl_3$ method.

The principle for determining the flavonoid level in the aluminum chloride method is the formation of a complex between aluminum chloride with keto groups on C-4 atoms and hydroxy groups on C-3 or C-5 atoms adjacent to the flavon and flavonol groups. The compound used as the standard for determining the level of flavonoids is quercetin, as quercetin is a flavonoid group of flavonols that has keto groups on C-4 atoms and also hydroxyl groups on neighboring C-3 and C-5 atoms. The maximum wavelength absorption is measured in the range of approximately 400 to 800 nm. To determine the level of flavonoids, the addition of potassium acetate is to detect the presence of 7-hydroxyl groups while the incubation treatment for 30 minutes is carried out before the measurement is planned so that the reaction takes place perfectly, thus providing maximum color intensity (Azizah et al., 2014).

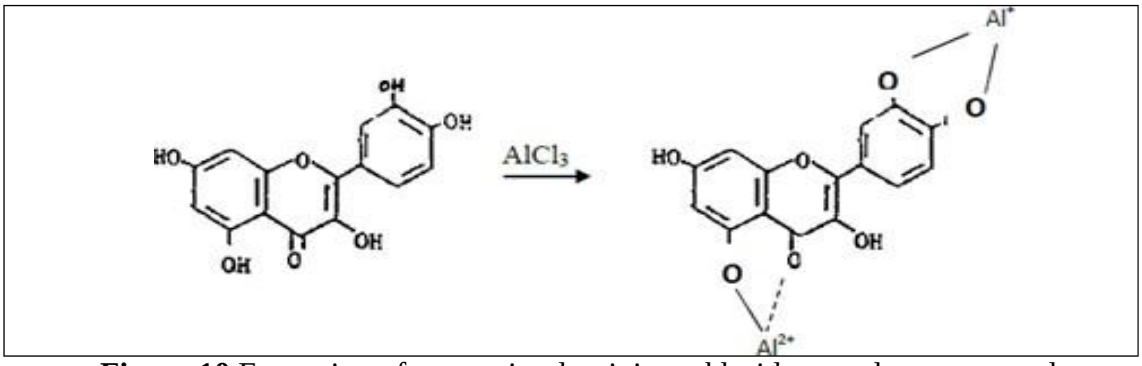


Figure 10 Formation of quercetin-aluminium chloride complex compounds

III.3. The Ferric Reducing Antioxidant Power assay (FRAP)

Principle

The FRAP assay was originally developed by Benzie and Strain, (1999) to measure reducing power in plasma. But the assay subsequently has also been adapted and used for the assay of antioxidants in botanicals. Reducing power of the tested extracts were measured by the method of Oyaizu, (1986) with a slight modification. According to this method, the extract which has reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous "the Perl's Prussian blue complex" that has an absorption maximum at 700 nm. Increased absorbance of the reaction mixture indicates greater reduction capability (Singhal et al., 2014).

Antioxydant

Potassium ferricyanide + Ferric chloride ⟶ Potassium ferrocyanide + ferrous chloride

$K_4[Fe(CN)_6] + FeCl_3 \longrightarrow K_3[Fe(CN)_6] + FeCl_2$

III.4. The hydroxyl radical scavenging assay activity

Principle

In plants, the hydroxyl radical is generated in illuminated chloroplasts, one electron reduction of H_2O_2 yields H_2O and hydroxyl radical (OH), the strongest oxidant produced in biological systems. Generation of OH from H_2O_2 is catalyzed by transition metals, particularly iron and copper. In normal circumstances, concentrations of these

oxygen radicals are likely to remain low (Smirnoff and Cumbes, 1989; Castro and Freeman, 2001).

Several *in vitro* methodologies for determination of HO• scavenging capacity are available, mostly based on Fe^{3+}+EDTA+H_2O_2+ ascorbic acid system to generate a constant flux of HO- radicals (Magalhães *et al.*, 2008).

III.5. The hydrogen peroxide assay activity

Principle

Hydrogen peroxide (H_2O_2) is generated *in vivo*. It is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate (Priyanka *et al.*, 2013)

One of the most common methods for assessing the scavenging capacity against this molecule is based on the intrinsic absorption of H_2O_2 in the UV region at 230 nm (Magalhães *et al.*, 2008).

III.6. The antibacterial and the antifungal activity

Principle

The principle of antimicrobial activity of plant extracts is to carry out a microbial culture on Muhler-Hinton (MH) for bacterial strains and Sabouraud for Fungal strains, in the presence of filled discs with a quantity of plant extracts. If they have antimicrobial activity, an inhibition zone will be observed around the disk due to the diffusion of samples.

The bacterial and the fungal strains were used to assess the antibacterial and antifungal properties of the test samples, against 5 microorganisms of significant importance.

Polyphenols, especially flavonoids and tannins, are known for their toxicity to microorganisms. The mechanism of toxicity may be related to the inhibition of

hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins (Cowan, 1999).

Part two

Experimental part

Chapter 1: Material and methods

The present study was devoted to the extraction of different parts of a medicinal plant and the quantification of its total polyphenols and flavonoids content on the one hand, and on the other hand, the investigation of the *in vitro* biological screening of its antioxidant, antimicrobial and antifungal activities in order to establish a correlation between both of them and a possible structure activity relationship.

I.1. Materials

I.1.1. Plant materials

The plant used in the present study is a wild medicinal plant, that was harvested during the summer of 2019 in Ali Mendjeli, Constantine. Different parts of the plant were investigated to obtain four extracts namely:

- E1: Roots.
- E2: Total aerial part.
- E3: Flowers.
- E4: Leaves and branches.

I.1.2. Laboratory equipment

All used chemicals are of analytical quality and are listed in the appendix.

Other laboratory equipment used is:

- UV spectrophotometer.
- Precision balance.
- Microbiology incubator.
- Water bath.
- Magnetic stirrer.
- Rotary evaporator.
- Beakers.
- Flasks.
- Funnel.
- Test tubes.
- Test tube rack.
- Filter paper.
- Pipettes.

- Petri dish.
- Tweezers.
- Spatula.
- Round-bottom flasks.

I.1.3. Microbial material

References of the bacterial strains tested	Reference of the fungal strain
Escherichia coli **ATCC 25922**	*Trichodermaharzianum Rifai*
Klebsiella pneumonie **ATCC 700603**	
Staphylococcus aureus **ATCC 25923**	
Pseudomonas aeruginosa **ATCC 27853**	

I.2. Methods of study

As mentioned above, four different parts of the wild medicinal plant are the subject of our practical part. Experimental is divided into three stages as follows: Preparation of the plant samples

I.2.1. Preparation of the plant samples

After the plant is harvested and perfectly cleaned, it is put to dry in a place away from light and humidity. Once it is completely dried, the plant is ground into a powder using a mortar and a pestle.

I.2.2. Maceration of the plant samples

Maceration is an extraction process that consists of immersing a plant in a liquid (e.g. alcohol) and maintaining this contact for a period of time at room temperature. It is highly required to extract thermosensitive compounds.

The choice of the solvent of extraction depends highly on the polarity of the compounds contained in the plant.

Table 8 Maceration of the plant samples

Sample simples	Solvent	Manipulation steps
E1	Ethanol	• A grind of each of the 4 parts of the previously dried plant, is put in a sterile jar of shaded glass and filled with ethanol with a ratio of 1/3 (plant powder/solvent). • The whole content is then stirred, tightly closed and left to macerate at room temperature in the shade for a month. • Each macerate is then filtered using a Whatman paper N°4, and evaporated to give the crud ethanol extract.
E2	Ethanol	
E3	Ethanol	
E4	Ethanol	

I.2.3. Evaporation

Evaporation of maceration solvent is done using a rotating evaporator set at 40°C under reduced pressure, maintained with a vacuum pump. This resulted in four dry raw extracts, in the form of a dark brown reddish gum for E1 and a green brownish one for E2, E3 and E4 accumulated on the edges of the round-bottom flask.

The whole gum is recovered by scratching the edges of the flask using a spatula. This is the crude extract to be conserved aseptically in the freezer for future uses in the quantitative analysis.

To calculate the mass of this crude extract we need to weigh the round-bottom flask empty than containing the gum, the difference between both is the mass used to calculate the yield according to the following formula:

Yield %= (Crude extract mass/powder mass) *100

I.2.4. Dilution samples

A range of seven dilutions (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128) was established for the four extracts, starting from the mother solution (1mg/1ml ethanol), to obtain the concentrations [1.9, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, 1000] µg/ml, as shown in the following figure:

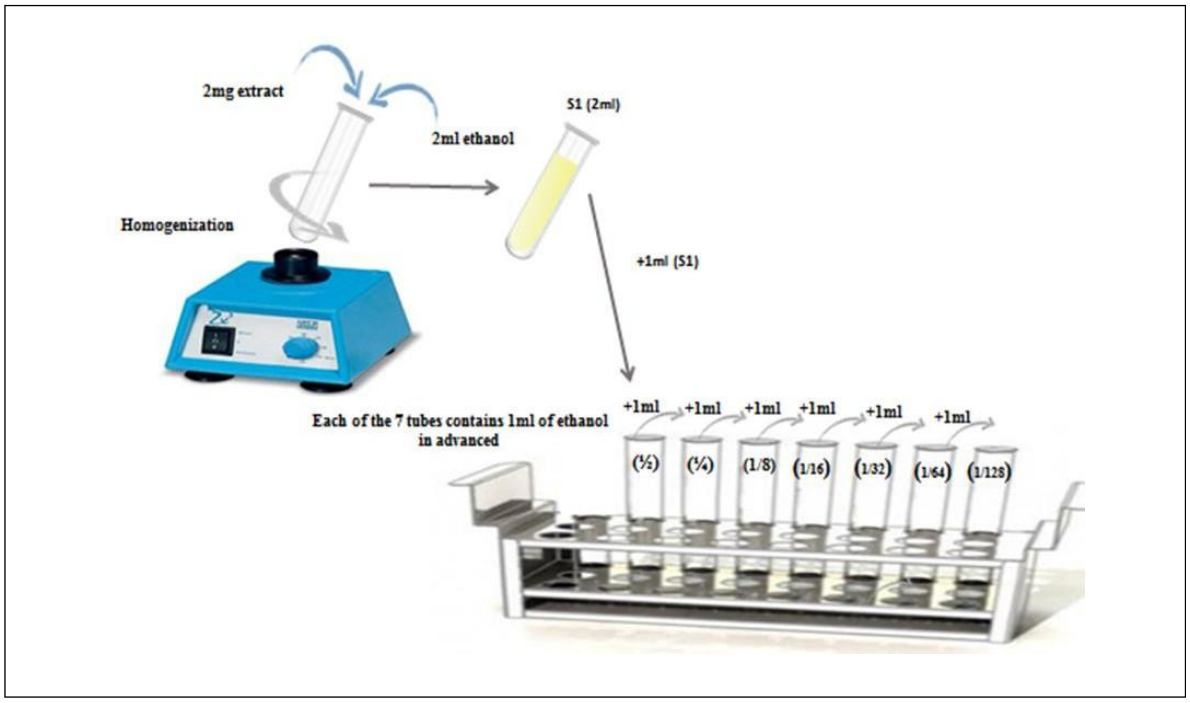


Figure 11 Preparation of the mother solution & the dilution range

I.3. Quantitative analysis

I.3.1. Quantification of the total polyphenols content

Protocol

0.2 ml of each sample was firstly mixed with 1ml of diluted Folin–Ciocalteu reagent (1/10 H_2O) by vortexing. After that, 0,75ml of Sodium carbonate (Na_2CO_3) (7,5%) are added. Then, the reaction mixtures are further incubated for 2 hours at room temperature in the dark, and finally, the absorbed optical density is recorded at the wavelength of 765 nm.

I.3.2. Quantification of the total flavonoid content

Protocol

0,4 ml of diluted sample with 1ml ethanol is separately mixed with 1 ml of 2% aluminum chloride methanolic solution ($AlCl_3$). After incubation at room temperature for 15 min, the absorbance of the reaction mixture is measured at 430 nm with spectrophotometer.

I.4. Biological activities analysis

I.4.1. The antioxidant activity analysis

In this study, three complementary tests were used to assess the antioxidant activity: the ferric reducing antioxidant power (FRAP), the hydrogen peroxide activity and the hydroxyl radical scavenging assays, as the two last activities are linked and produced in chains, so to cut this chain at one's level or another is beneficial.

I.4.1.1. The FRAP assay

Protocol

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method. Briefly, 0.1 ml of each of the extracts at different concentrations, 0.4 ml of phosphate buffer (pH 6.6), and 0.5ml of potassium ferricyanide $K_3Fe(CN)_6$ (1%) are mixed and incubated at 50°C for 20 min, to reduce ferricyanide into ferrocyanide. The reaction is stopped by adding 0.5 ml of 10% trichloroacetic acid (TCA). Finally, it is mixed with 0.4 ml of distilled water and 0.1 ml of Ferric chloride ($FeCl_3$) (0.1%) and the absorbance is measured at 700 nm. Ascorbic acid is used as positive control.

I.4.1.2. Hydroxyl radical scavenging assay

Protocol

Hydroxyl radical scavenging activity of the extracts is determined according to the salicylic acid method reported by Smirnoff and Cumbes, (1989).

The reaction mixture contained 0.4 ml of each extract and concentration, 0,24 ml iron sulfate $FESO_4$ (22.24 mg/10 H_2O) , 0,2 ml H_2O_2 (0,3µl H_2O_2/199,7µl H_2O) , 0,8 ml acid salicylic (4mg/10 ml H_2O), then it was incubated at 37°C for 30 minutes in a water bath, after that 0,36 ml H_2O was added. The absorbances of the mixtures are measured at 510 nm using a (UV/Vis) spectrophotometer. The ascorbic acid is used as the positive control compound.

I.4.1.3. Hydrogen peroxide scavenging assay

Protocol

The ability of the plant extracts to scavenge hydrogen peroxide is estimated according to the method reported by (Ruch, Cheng and Klaunig, 1989) with minor modifications. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (1 M pH 7.4) (0.63 ml H_2O_2 + 199.4 ml phosphate buffer pH 7.4). 1ml of each sample of the concentration range is added to 0,1ml of hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm is determined after 10 minutes of incubation. Ascorbic acid is used as a standard.

I.4.2. The antibacterial and antifungal activity analysis

The antimicrobial sensitivity and resistance tests of our extracts were carried out according to the Agar disk-diffusion testing developed in 1940 (Heatley, 1944).

Protocol

A range of dillution is prepared for each extract starting from a mother solution with a concentration of 1mg/ml.

The culture media are prepared by pouring the agar in petri dishes; Muhler Hinton for bacterial strains and Sabouraud for fungal ones.

Near the Bunsen burner, the filter paper discs (Whatman No. 1, 6 mm diameter) are impregnated with each extract and then applied to the surface of the agar plates which have been seeded by spreading the microbial suspension. The seeding is carried out in such a way to ensure a homogeneous distribution of the bacteria/fungi. The petri dishes are put to solidify at 4°C for 4 hours, then are incubated during 24 hours at the appropriate temperature 37C° in the incubator.

Antimicrobial activity is determined in terms of the diameter of the inhibition zone produced around the discs. The resulting inhibition zone diameter was measured in millimeter using a ruler.

Chapter 2:
Results and discussion

II.1 Yield results and discussion

The results of yields for the four extracts Roots (E1), aerial part (E2), Flowers (E3) and leaves and branches (E4) are reported in the table and illustrated in the histogram below:

Table 9 Yields of the four parts of the plant

Extracts	Powder mass	Extract mass	Yield
E1	174,4 g	3 g	1,76 %
E2	110,2 g	2,3 g	2,09 %
E3	32,8 g	1 g	3,05 %
E4	130 g	2 g	1,54 %

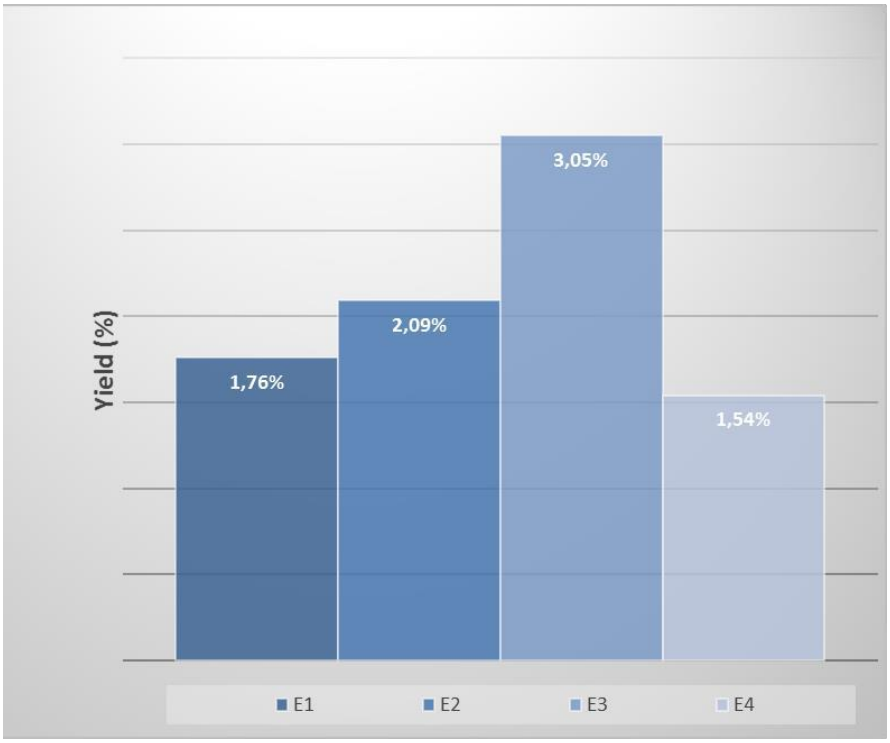


Figure 12 Yields of the four extracts in percentage (%)

These results show that the E3 (flowers) have the best yield (3.05%) among the other extracts making it a target for future studies, followed by E2 (aerial part) then E1 (roots) and finally E4 (leaves and branches) (2.09, 1.76, 1.59 % respectively).

II.2. Quantitative analysis

Quantification of the total phenolic compounds content (TPC) and the total flavonoid content (TFC):

The values of the total polyphenolic compound content and the flavonoid content of the four extracts of the wild medicinal plant E1, E2, E3 and E4 respectively, are expressed in µg equivalent:

- Of gallic acid per mg of dry extract (µg GAE/mg DE), using the linear regression equation of its calibration curve (Fig.9) for the determination of polyphenols.
- Of quercetin per mg of dry extract (µg QE/mg DE), using the linear regression equation of its calibration curve (Fig.10) for the determination of flavonoids.

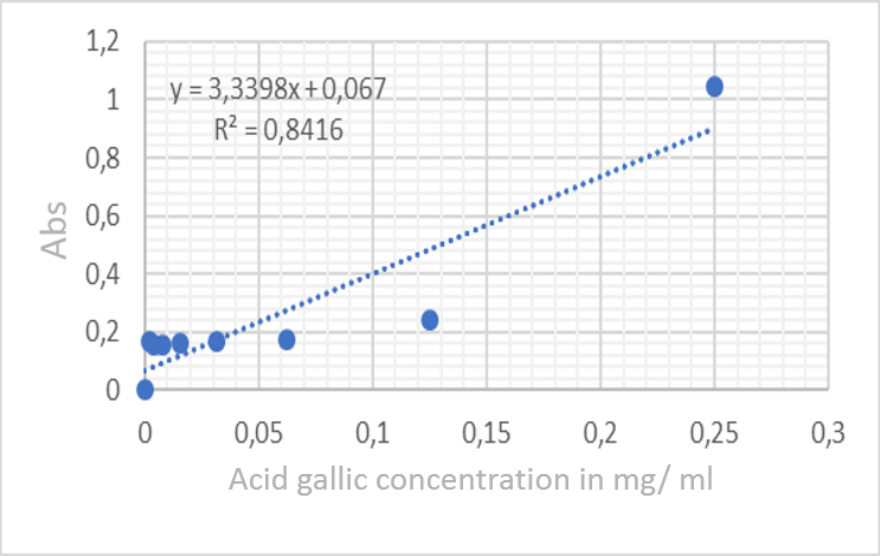


Figure 13 Calibration curve of gallic acid (µg GAE/mg DE)

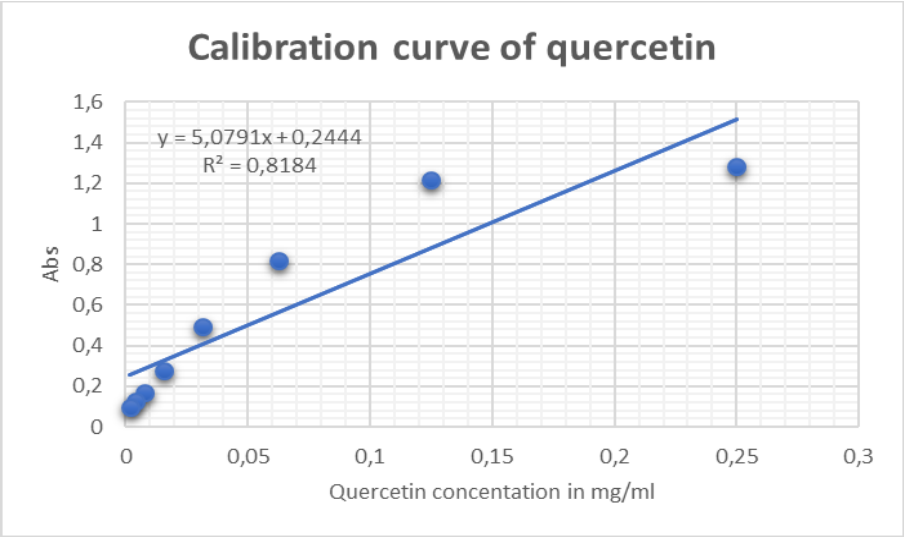


Figure 14 Calibration curve of the quercetin (µg QE/mg DE)

II.2.1. The total phenolic compound content and discussion

The results of the total phenolic compounds content are reported in the table and the curve below:

Table 10 Total phenolic content (µg GAE/mg DE) in the four extracts with the different dilutions

TPC (µg AGE/ mg DE)	7,8 µg/ml	15,6 µg/ml	31,25 µg/ml	62,5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
E1	23,15 ±0.00	24,91 ±0.00	26,86 ±0.00	31,80 ±0.01	37,37 ±0.00	53,33 ±0.01	55,84 ±0.00	76,23 ±0.00
E2	31,80 ±0.00	28,89 ±0.01	38,54 ±0.00	29,34 ±0.00	42,10 ±0.00	56,53 ±0.00	89,05 ±0.02	138,30 ±0.00
E3	26,11 ±0.00	161,93 ±0.01	39,49 ±0.00	47,91 ±0.00	69,98 ±0.00	106,56 ±0.00	158,3 0±0.01	278,40 ±0.01
E4	26,41 ±0.00	26,11 ±0.00	32,34 ±0.00	38,24 ±0.00	50,93 ±0.01	61,50 ±0.01	90,87 ±0.01	144,29 ±0.00

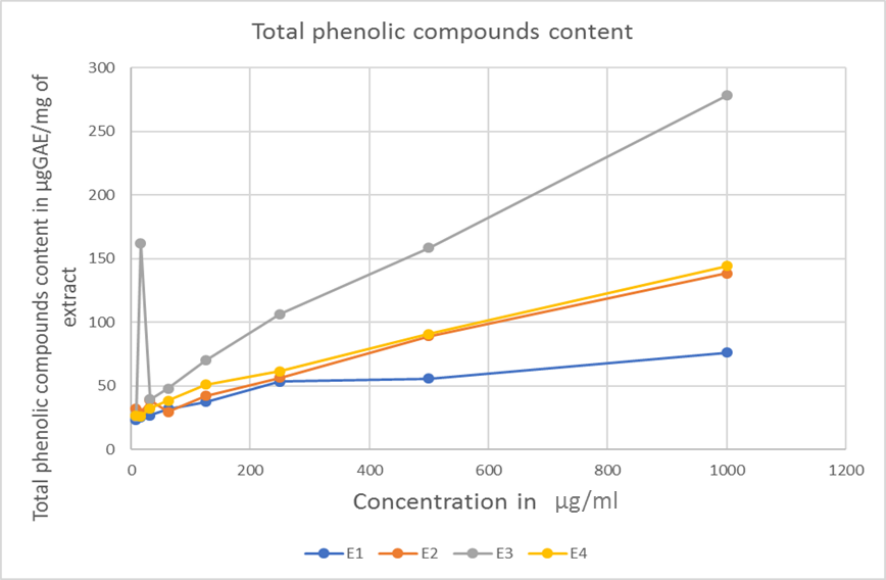


Figure 15 Total phenolic compounds content

II.2.2. The total flavonoid content and discussion

The results of the total flavonoid content are reported in the table and the curve below:

Table 11 Total flavonoids content (µg QE/mg DE) in the four extracts with the different dilutions

TFC (µg QE/ mg DE)	7,8 µg/ml	15,6 µg/ml	31,25 µg/ml	62,5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
E1	24,1 ±0.00	26,46 ±0.00	21,74 ±0.00	25,28 ±0.01	27,19 ±0.00	27,53 ±0.01	26,95 ±0.00	26,05 ±0.00
E2	14,96 ±0.06	34,57 ±0.04	34,38 ±0.03	33,14 ±0.05	21,66 ±0.00	12,58 ±0.00	15,32 ±0.00	16,40 ±0.00
E3	25,18± 0.00	22,86± 0.00	22,07 ±0.00	21,32 ±0.00	8,01 ±0.00	5,65 ±0.00	36,05 ±0.00	67,57 ±0.00
E4	23,21 ±0.00	18,68 ±0.00	21,05 ±0.00	20,44 ±0.00	ND	17,58 ±0.00	3,98 ±0.00	9,06 ±0.01

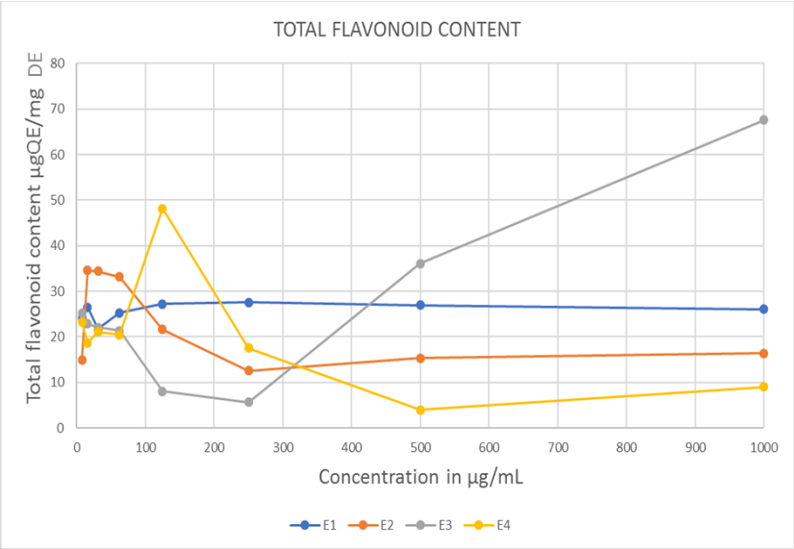


Figure 16 Total flavonoids content

Table 12 Total phenolic and flavonoid contents of the four extracts (1000 µg/ml**)**

Extracts	Total phenolic compounds content (µgGAE/mg of extract)	Flavonoids content (µgQE/mg of extract)
E1	76,23±0.00	27,53±0.01
E2	138,30±0.00	34,57±0.04
E3	278,40±0.01	67,57±0.00
E4	144,29±0.00	23,21±0.00

Histograms allow us to compare the values of the total phenolic compounds and flavonoid content of the four extracts:

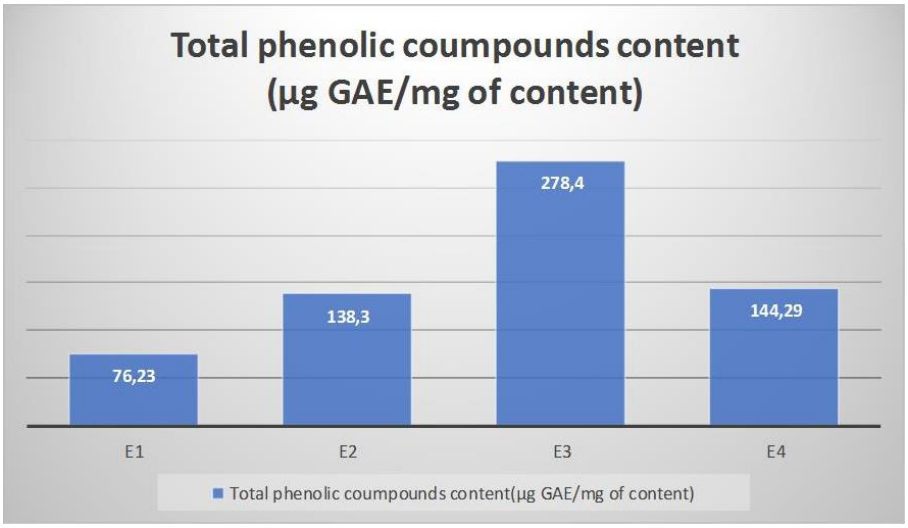


Figure 17 Total phenolic compounds content expressed in µg GAE/mg of content (DE)

The total phenolic content in Fig.17 shows the highest concentration in polyphenols in extract E3 (flowers) with a value of (278,40±0.01) DE; followed by a relatively low value of (144,29±0.00), (138,30±0.00) µg EGA/mg DE for E4 (leaves and branches) and E2 (aerial part). As we can see in the figure; the extract that contains the lowest concentration equal to (76,23±0.00) µg GAE/mg DE is E1 (roots).

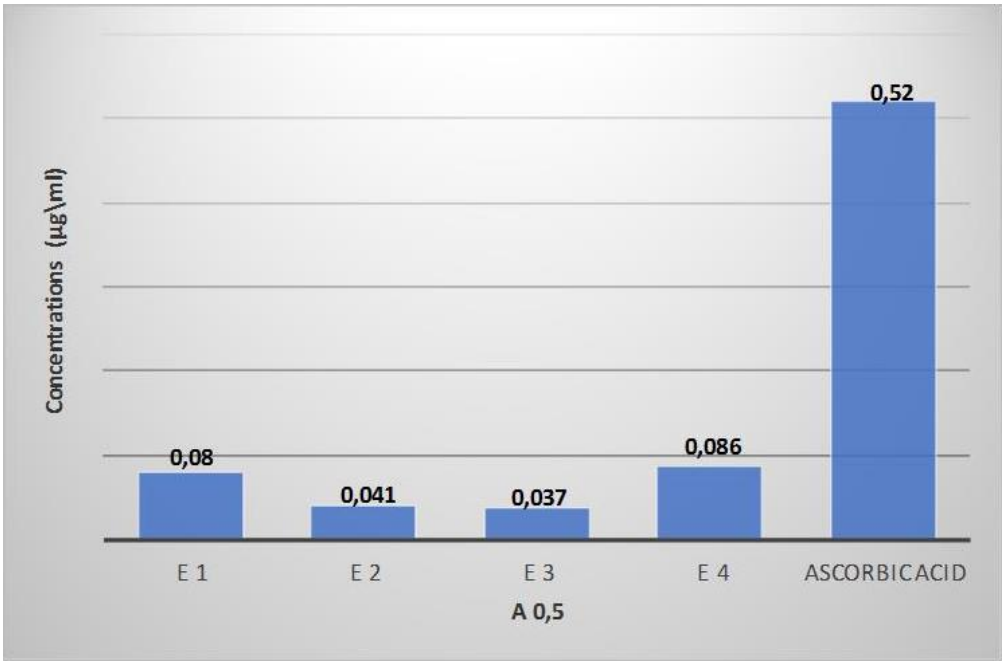


Figure 18 Total flavonoids content expressed in µg QE/mg content (DE)

Similarly to the phenolic content, the extract E3 (flowers) contains the highest concentration of flavonoids with a value of (6,57±0.00) µg QE/mg DE followed by E2 (aerial part) (34,57±0.04) µg QE/mg DE, E1(roots) (27,53±0.01)) µg QE/mg DE and finally E4 with (23,21±0.00) µg QE/mg DE as the lowest concentration.

II.3. Evaluation of biological activities

II.3.1. Study of the antioxidant activity

The antioxidant activity of different parts of the plant is evaluated by the following methods: Reducing power, hydroxyl radical, and the hydrogen peroxide activity.

II.3.1.1. The Ferric Reducing Antioxidant Power test results and discussion

The reducing capacity of the samples is studied from the transformation of potassium ferricyanide (Fe^{3+}) into potassium ferrocyanide (Fe^{2+}). This method is often used as an indicator of electron donor activity, which is an important mechanism of phenolic antioxidant action and can be strongly correlated with other antioxidant properties (Dorman *et al.*, 2003).

Table 13 Antioxidant activity (Absorbances) by the reducing power assay

Extracts	0,0019 µg/ml	0,0039 µg/ml	0,0078 µg/ml	156 µg/ml	31,25 µg/ml	625 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	A0,5µg/ml
E1			1,25 ±0.05	1,4 ±0.08	1,34 ±0.28	1,21 ±0.07	1,14 ±0.02	1,15 ±0.02	1,42 ±0.05	1,23 ±0.09	0,08
E2			1,38 ±0.03	1,3 ±0.05	1,22 ±0.03	1,30 ±0.02	1,36 ±0.03	1,32 ±0.02	1,46 ±0.1	1,36 ±0.01	0,041
E3			1,3 ±0.01	1,15 ±0.00	1,16 ±0.01	1,38 ±0.00	1,32 ±0.03	1,17 ±0.04	1,17 ±0.01	1,18 ±0.05	0,037
E4			1,34 ±0.00	1,28 ±0.00	1,4 ±0.00	1,16 ±0.00	1,43 ±0.04	1,29 ±0.02	1,27 ±0.04	1,45 ±0.14	0,086
Ascorbic acid	1,88 ±0.00	1,00 ±0.02	0,99 ±0.00	0,79 ±0.00	0,97 ±0.01	0,94 ±0.01	0,98 ±0.01	1,04 ±0.01			10.2

*A0.50: The concentration at absorbance 0.5.

The results of the previous table are represented using comparative curves as shown below:

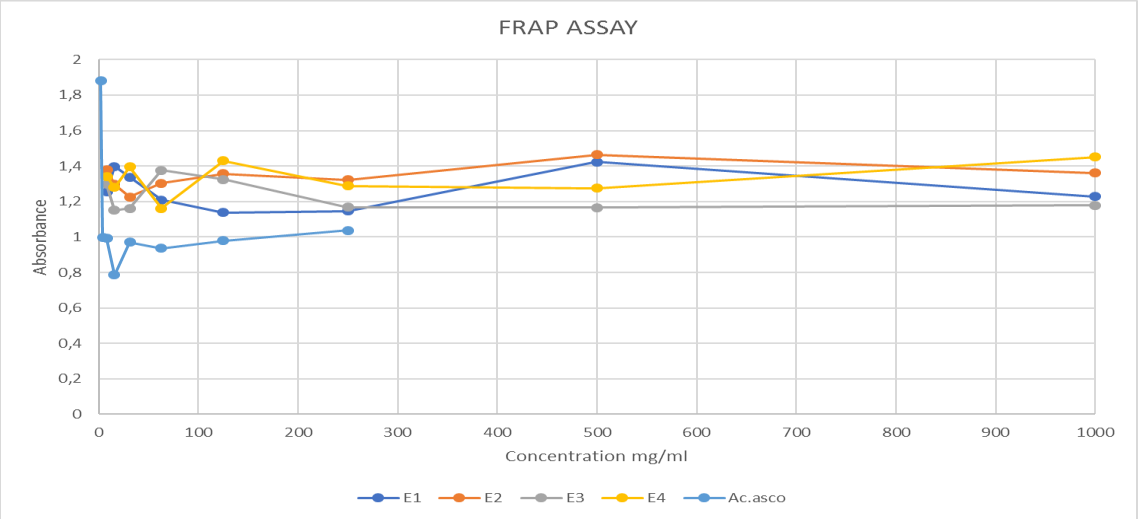


Figure 19 Reducing activity of the four extracts and the ascorbic acid

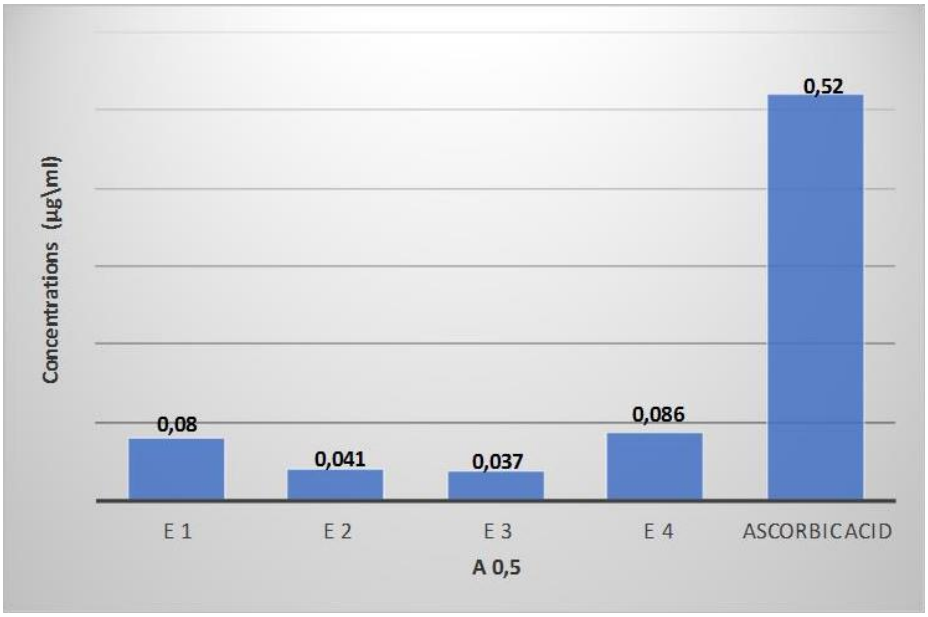
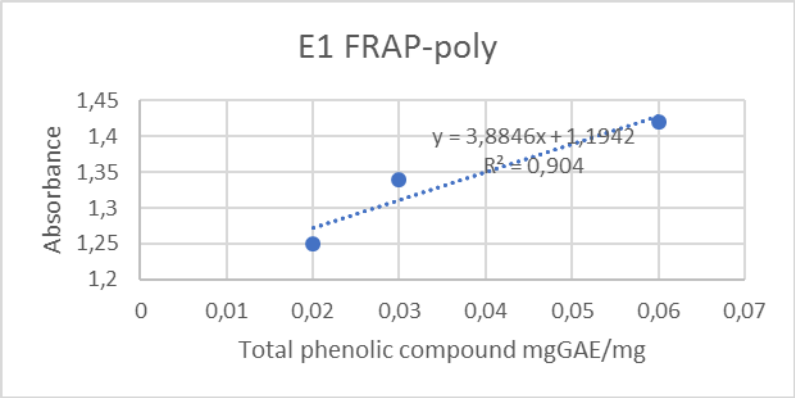


Figure 20 A 0,5 values of the different extract and the standards for the FRAP assay

The flower extract (E3) has the most interesting potential among the other extracts with the lowest A0.5 of the order of 0.037µg/ml. For the aerial part (E2), the roots (E1) and the leaves and branches (E4) extracts, they respectively present less potential with values of 0.041, 0.08 and 0.086 µg/ml. All of them presenting less reducing activity than the standard which has an A0.5 equal to 0.52µg/ml.

II.3.1.1.1. The correlation FRAP/TPC



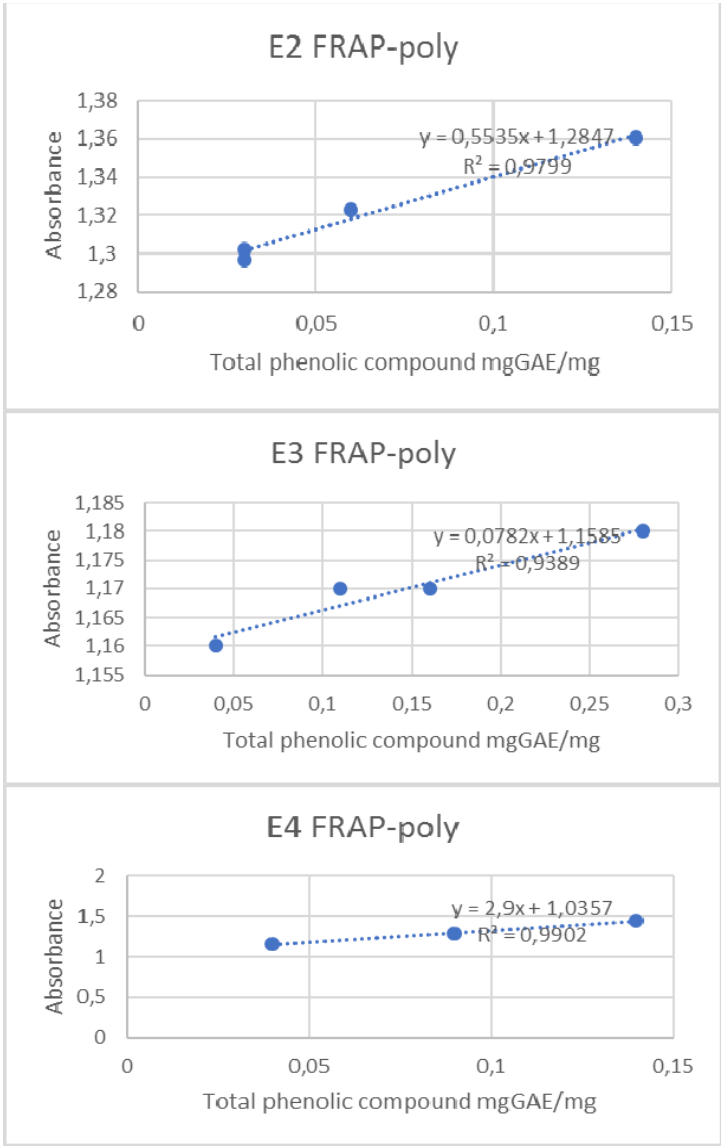


Figure 21 Correlation between TPC of the four extracts and the FRAP assay results

According to the 4 curves, the ferric reducing power of the four tested extracts exhibit a high correlation with the phenolic content with correlation factors that varies form (0.904-0.9902). This explains the fact that the ferric reducing power increases with the increasing of the phenolic content and gives an idea about the contribution of the phenolic compounds in the antioxidant activity of the different parts of our medicinal plant.

II.3.1.2. The hydroxyl radical scavenging assay results and discussion

The results of the hydroxyl radical scavenging are reported in the table below as absorbances:

Table 14 Antioxidant activity by the hydroxyl radical scavenging assay

Extracts	1,9 µg/ml	3,9 µg/ml	7,8 µg/ml	15,6 µg/ml	31,25 µg/ml	62,5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	A0,5µg/ml
E1			0,33 ±0.00	0,31 ±0.00	0,32 ±0.00	0,33 ±0.00	0,35 ±0.01	0,35 ±0.00	0,34 ±0.00	0,44 ±0.00	>1000
E2			0,29 ±0.00	0,31 ±0.00	0,4 ±0.00	0,41 ±0.00	0,43 ±0.01	0,56 ±0.00	0,7 ±0.00	0,96 ±0.00	184,28
E3			0,09 ±0.00	0,09 ±0.00	0,11 ±0.00	0,12 ±0.00	0,17 ±0.00	0,24 ±0.00	0,32 ±0.00	0,57 ±0.00	829,22
E4			0,33 ±0.00	0,33 ±0.00	0,34 ±0.00	0,31 ±0.00	0,36 ±0.00	0.34 ±0.00	0,38 ±0.00	0,78 ±0.01	649,46
Ascorbic acid	0,56	0,57	0,55	0,54	0,51	0,45	0,16	0,08			1,69

The results of the previous table are represented using comparative curves:

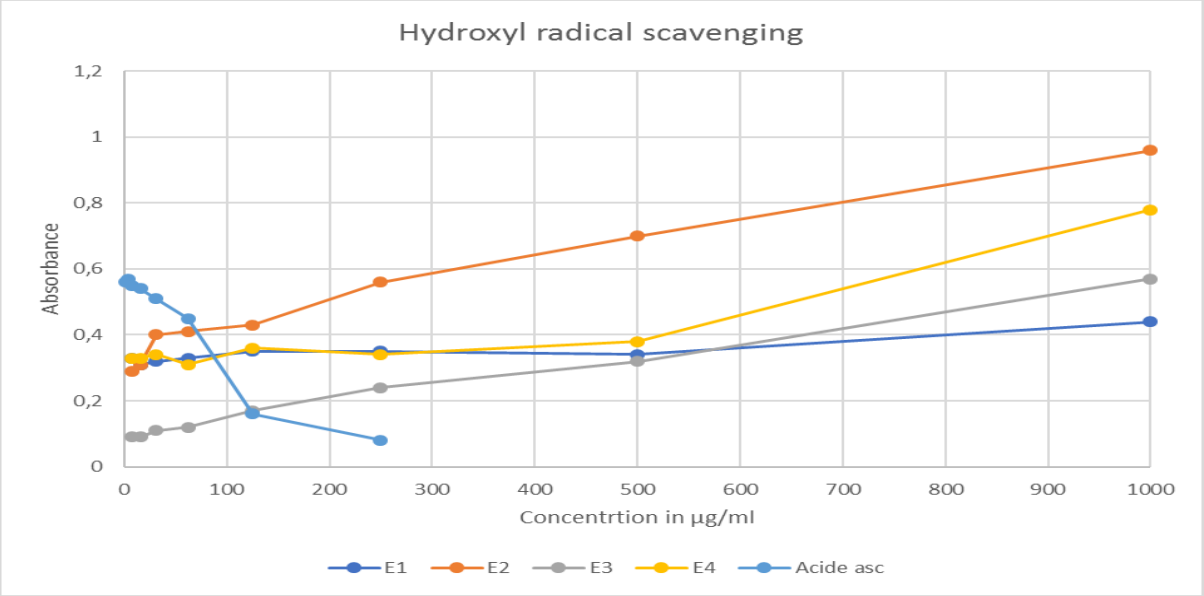


Figure 22 Absorbances of the hydroxyl radical scavenging activity for the four extracts and the ascorbic acid

The values are illustrated in the histogram below in order to facilitate the comparison of the A0.5 values of the four extracts with the standard (the ascorbic acid) followed by their discussion:

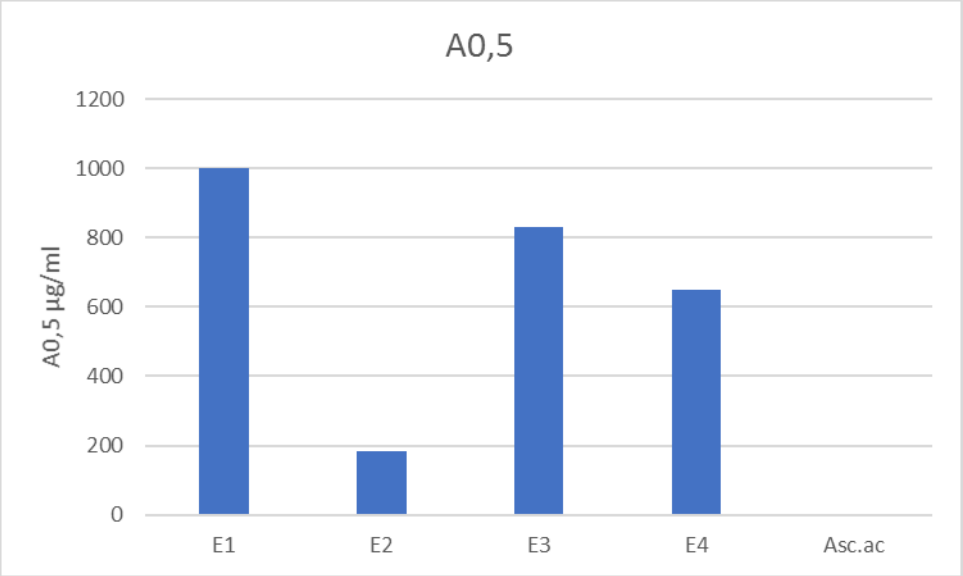
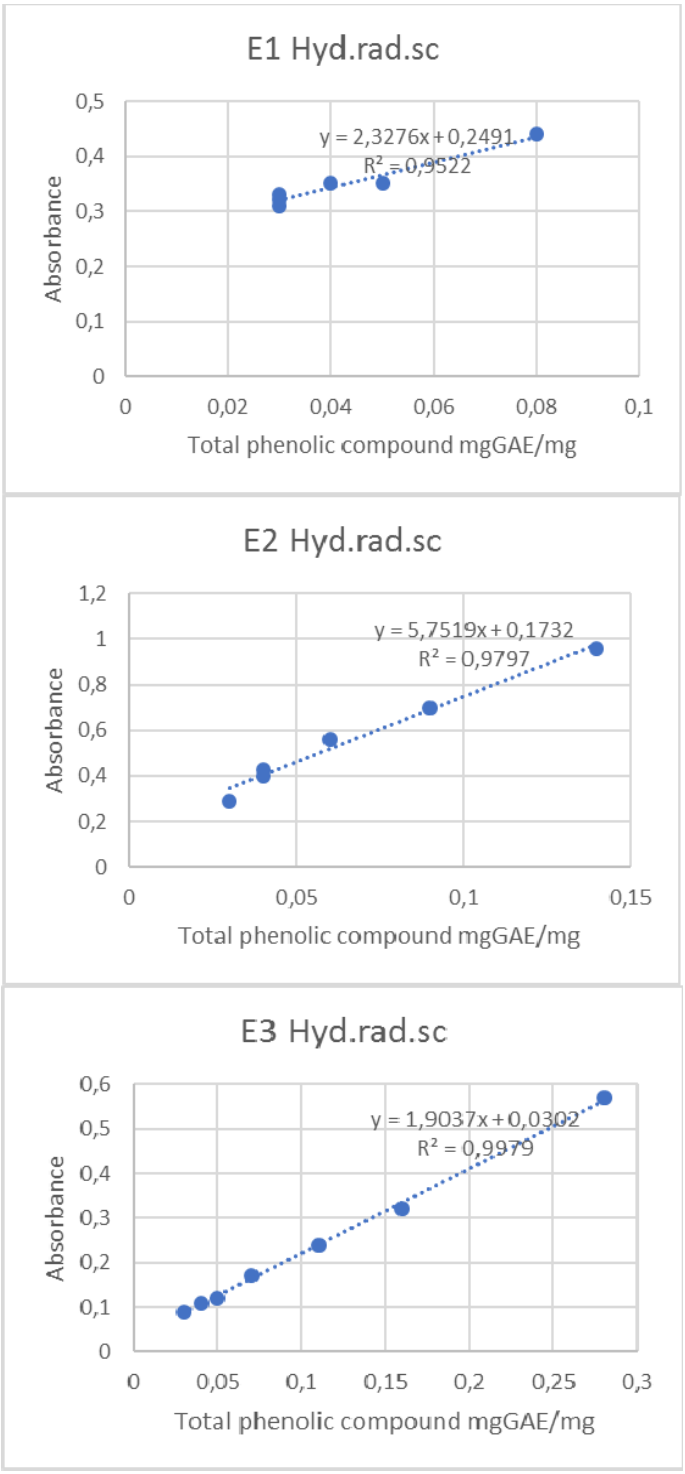


Figure 23 A 0,5 values of the four extracts and the ascorbic acid for the hydroxyl radical scavenging assay

For the Hydroxyl radical scavenging assay; the extract of the aerial part E2 showed the lowest A0.5 (184,28 µg/ml) and the closest to the one of ascorbic acid (1,69 µg/ml). This gives it the highest antioxidant potential among the other extracts that express higher values of A0.5, and thus have a lower antioxidant potential as followed: The leaves and branches extracts E4 with (649,46 µg/ml) followed by the flowers ones E3 (829,22 µg/ml) and then the roots' E1 with a very high A0.5 of more than 1000 µg/ml. This makes it the extract with the lowest antioxidant potential.

II.3.1.2.1. The correlation hydroxyl radical scavenging/TPC



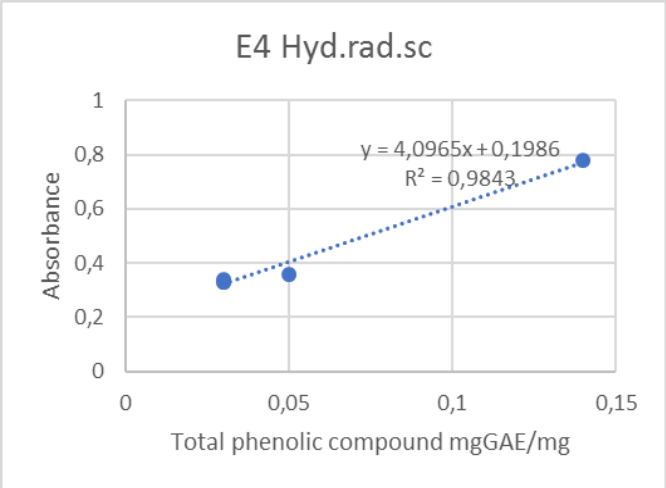


Figure 24 Correlation between TPC of the four extracts and the hydroxyl radical assay results

The hydroxyl radical scavenging results showed a very high correlation with the total phenolic compounds content in the four extracts, with a correlation coefficient that varies form (0.9522-0.9979). This indicates the correlation between the two parameters and shows the obvious contribution of the phenolic compounds in the antioxidant potential observed in the different extracts from the medicinal plant studied.

II.3.1.3. The hydrogen peroxide activity assay results and discussion

Contrary to the FRAP assay and the hydroxyl radical scavenging assay results, the hydrogen peroxide results are presented as percentage of inhibition and not as absorbances:

Table 15 Antioxidant activity by the hydrogen peroxide assay

Extracts	1,9 µg/ml	3,9 µg/ml	7,8 µg/ml	15,6 µg/ml	31,25 µg/ml	625 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	IC 50 µg/ml
E1			76,08 ±0.01	56,25 ±0.00	82,4 ±0.00	95,65 ±0.01	100 ±0.03	100 ±0.02	100 ±0.02	100 ±0.01	5,13
E2			69,77 ±0.01	55,29 ±0.00	80,862 ±0.01	92,94 ±0.01	94,83 ±0.02	100 ±0.04	100 ±0.02	100 ±0.01	5,59
E3			69,81 ±0.01	77,08 ±0.01	71,69 ±0.01	87,731 ±0.01	68,38 ±0.54	100 ±0.01	100 ±0.02	100 ±0.04	5,59
E4			82,49 ±0.02	58,67 ±0.01	82,83 ±0.01	82,63 ±0.00	100 ±0.03	40,08 ±1.16	100 ±0.01	100 ±0.01	4,73
Ascorbic acid	45,38 ±0.00	64,25 ±0.02	77,46 ±0.00	72,53 ±0.00	91,07 ±0.00	89,58 ±0.00	100±0.00	100 ±0.06			2,56

*IC50: The half maximal inhibitory concentration.

The results of the previous table are represented using comparative curves:

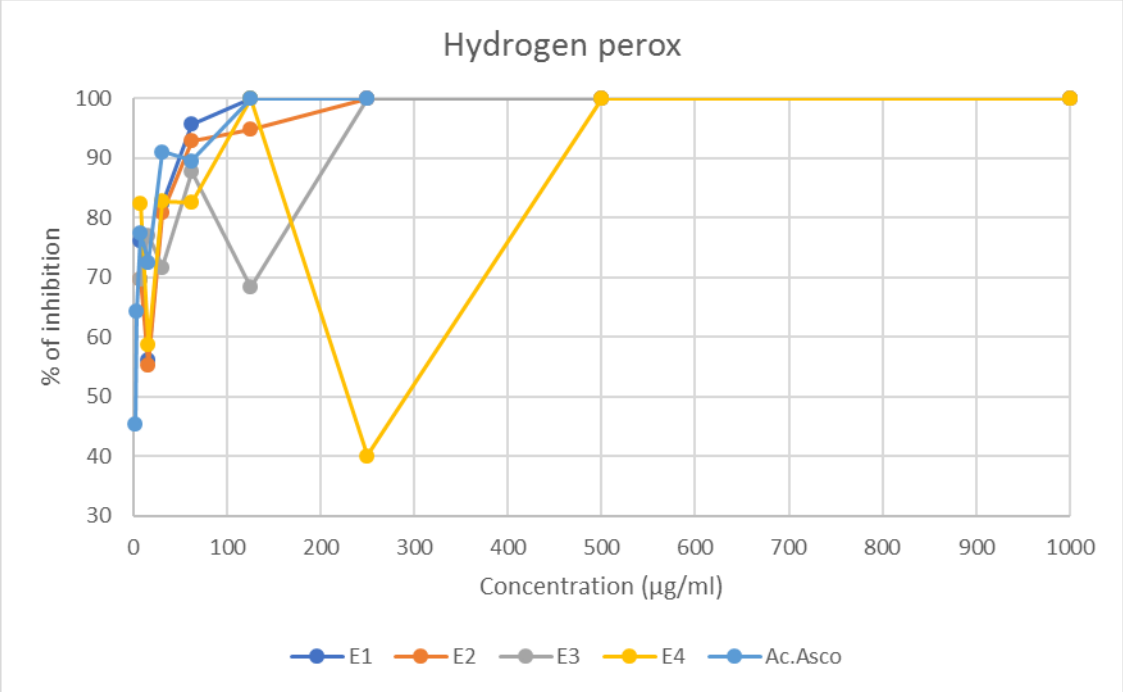


Figure 25 Absorbances of the hydrogen peroxide activity for the four extracts and the ascorbic acid

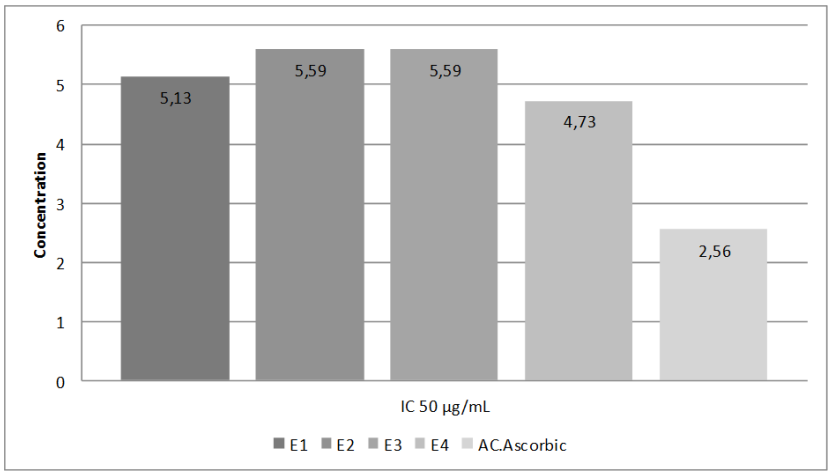
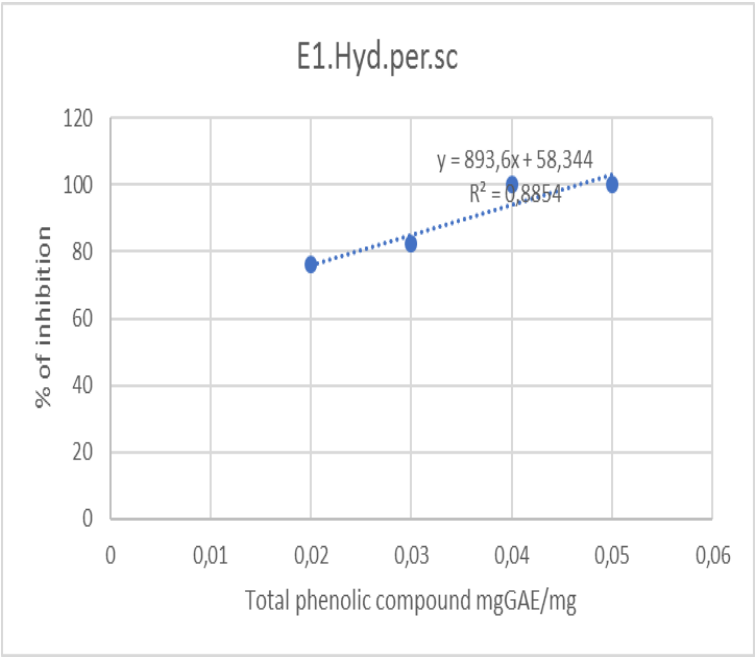


Figure 26 IC 50 values of the different extracts and the ascorbic acid for the hydrogen peroxide assay

All plant's parts exhibit approximatively similar inhibition ability of hydrogen peroxide species with IC50= 5.13, 5.59, 5.59, 4.73 µg/ml respectively, which are nearly close to that observed with the ascorbic acid (IC50=2.56 µg/ml).

II.3.1.3.1. The correlation hydrogen peroxide activity/TPC



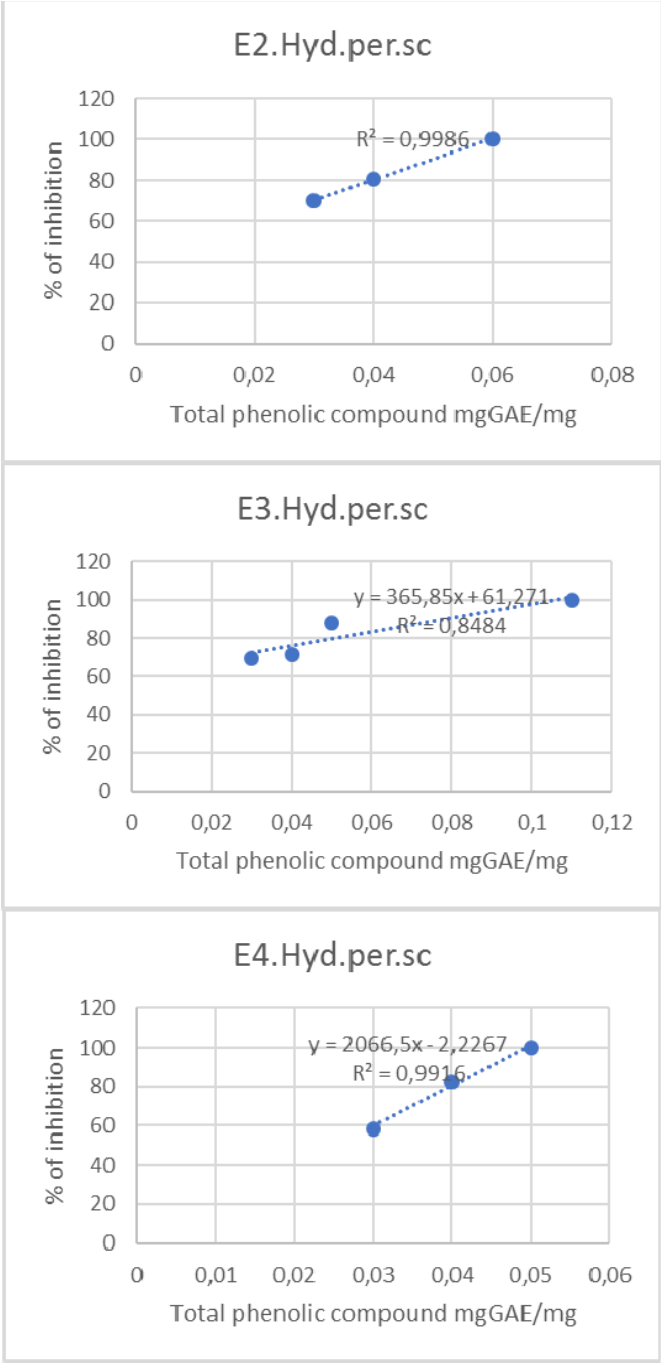


Figure 27 Correlation between TPC of the four extracts and the hydrogen peroxide assay results

According to the 4 curves, the hydrogen peroxide activity of the four extracts of our medicinal plant showed a high correlation with the phenolic content with a coefficient of correlation that varies form (0.8484-0.9986).

II.3.2. Study of the antibacterial and antifungal results and discussion

II.3.2.1. Antibacterial test

Table 16 Zones of growth inhibition (mm) showing antibacterial activity of the four extracts and the antibiogram test

Compounds	*E.coli*	*P.aeruginosa*	*K. pneumonia*	*S. aureus*
E1	**15.00 ±0.50**	**12.00 ±0.00**	**N.D**	**N.D**
E2	**20.00 ±0.50**	**N.D**	**N.D**	**N.D**
E3	**12.00± 0.00**	**N.D**	**N.D**	**N.D**
E4	**N.D**	**N.D**	**N.D**	**N.D**
NA	**42.00**	**0.00**	**28.00**	**22.00**
GN	**38.00**	**24.00**	**18.00**	**-**
DMSO	**0.00**	**0.00**	**0.00**	**0.00**

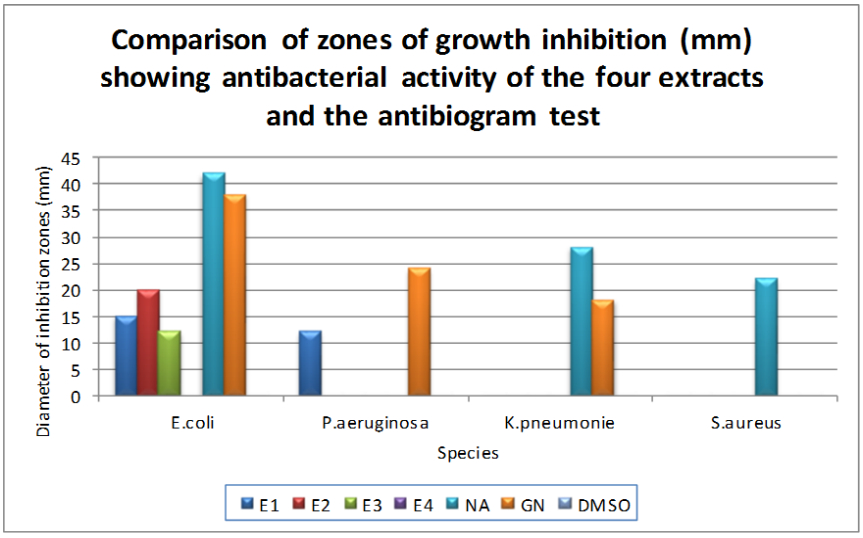


Figure 28 Comparison of zones of growth inhibition (mm) showing antibacterial activity of the four extracts and the antibiogram test

The diameters' results of the growth inhibition zones expressed in mm have shown a variation in the antibacterial properties of the different parts of the plant and revealed that the inhibition zones for:

- Extract E1 (roots) are present only for the following strains: *E.coli* with a highest diameter equal to 15.00 ± 0.50, followed by 12.00 ± 0.00 for *P.aeruginosa*. Meanwhile, the *K. pneumonia and S. aureus* did not show any zone of inhibition, which expressed their resistance.
- Extract E2 (the aerial part) showed the inhibition zone just for *E.coli* with a diameter equal to 20.00±0.50 while the inhibition for the three other stains was null.
- Extract E3 (flowers), a zone of inhibition was observed only for *E.coli* with a diameter of 12.00±0.00. The other strains showed a resistance toward this extract.
- In contrast, concerning the extract E4 (Leaves and branches) no antibacterial activity was observed against the four strains.

Gentamicin (10 µg/ disc) and Nalidixic (30 µg/ disc) were used as positive control.

II.3.2.2. Antifungal activity test

Table 17 Zones of growth inhibition (mm) showing antifungal activity of the four extracts

Antifungal Species	Zones of growth inhibition (mm) of the antifungal activity of the four extracts			
	E1	E2	E3	E4
Trichoderma harzianum Rifai	20±0.60	20±0.40	10±0.00	10±0.00

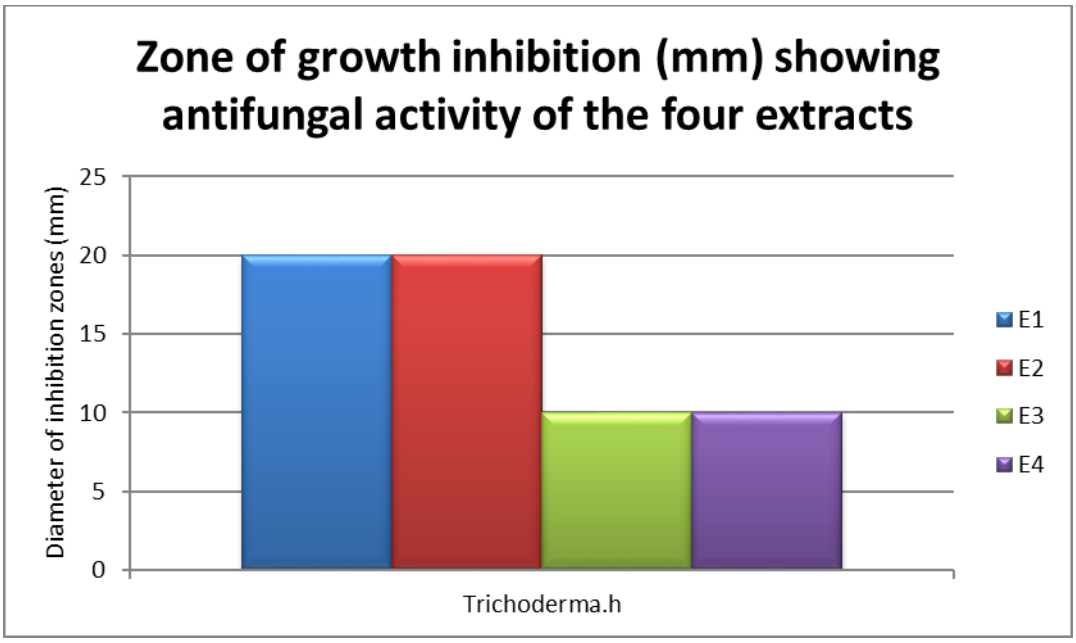


Figure 29 Zones of growth inhibition (mm) showing antifungal activity of the four extracts

The diameters results of the growth inhibition zones showing antifungal activity against *Trichoderma harzianum Rifai* reveal that the zones of inhibition for the extract E1 (the roots) and the extract E2 (the aerial part) have the highest and the same diameter of 20.00 which is a very good activity, however the extract E3 (the flower) and the extract E4 (the leaves and branches) showed a modest activity with a diameter equal to 10.00.

Conclusion and perspectives

This study first aimed at valuing and promoting Algeria's medicinal plants in order to facilitate people's access to improved traditional medicines with less side effects and toxicity risks. In order to validate the traditional use of the wild plant species used in the present study, and look for alternatives to synthetic chemicals, this research has been conducted based on the quantitative determination of total polyphenols, total flavonoids and the assessment of the antioxidant, antibacterial and antifungal properties of this plant.

This work allowed us at first place to master universal analytical techniques, from the extraction to quantitative analysis of secondary metabolites from different parts of the wild medicinal plant used (the roots, the aerial part, the flowers and the leaves and branches) and its valorization by the evaluation of its antioxidant, antibacterial and antifungal potential.

First, the quantitative dosage of the total phenolic compounds, by the Folin-Ciocalteu method revealed the flower extract as being the richest extract in polyphenols, followed by the leaves and branches extract, then the aerial part extract and finally the roots extract, with respective values of : (278.40±0.01), (144.29±0.00), (138.30±0.00) and (76.23±0.00) µg GAE/mg.

Similarly, the results of the quantitative determining of total flavonoids by the aluminum chloride method have also shown the flower extract as the richest extract in flavonoids. Followed by the roots extract, then the aerial part extract and finally the leaves and branches extract, with respective values of: (67.57±0.00), (26.05±0.00), (16.40±0.00) and (9.06±0.01) µg QE/mg DE.

Subsequently, a series of biological tests were conducted *in vitro* to assess the antioxidant potential of the four extracts studied by three methods (The FRAP, the hydroxyl radical scavenging and the hydrogen peroxide assays), exhibiting the following results:

The FRAP assay gave positive results, therefore the two following tests were assessed showing better results for hydroxyl radical scavenging assay than for the hydrogen peroxide assay.

For the antibacterial activity, three out of four extracts (the aerial part, the flowers and the roots extracts) revealed an activity against ***E.coli*** while this latter remained resistant to the leaves and branches extract. As for the second species ***P.aeruginosa,*** there is only one extract (that of the roots) among the four extracts that demonstrated antibacterial activity. While, the other two species ***K.pneumonia*** and ***S.aureus*** were resistant to all plant extracts.

Regarding the antifungal activity the plant has demonstrated a great antifungal potential towards the species ***Trichoderma. H,*** especially for the root and the aerial part extracts.

Through this study, a correlation between the total phenolic content, the flavonoid content and the antioxidant potential was established and turned out to be probably due to the plant richness in phenolic compounds, flavonoids and other secondary metabolites, making it a promising source for treatment of many diseases by using it to synthetize new phytomedicines.

As perspectives, we judge necessary the following points:

- To conduct preclinical studies *in vivo* to prove results obtained *in vitro*.

- Additional biological tests will be necessary to ascertain new and beneficial activities of this plant.

- Consequently, phytochemical investigations should be planned to identify and characterize active principles, and assess toxicity by laboratory assays.

- Undertake theoretical computations and *in silico* studies of molecular interactions between bioactive compounds structures and observed therapeutically effect SAR.

Bibliography

Ahmad, I. and Beg, A. Z. (2001) 'Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens', Journal of Ethnopharmacology. Elsevier, 74(2), pp. 113–123. doi: 10.1016/S0378-8741(00)00335-4.

Ali-Shtayeh, M. S. et al. (1998) 'Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area', Journal of Ethnopharmacology, 60(3), pp. 265–271. doi: 10.1016/S0378-8741(97)00153-0.

Ali, B. H. et al. (2008) 'Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research', Food and Chemical Toxicology. Elsevier Ltd, pp. 409–420. doi: 10.1016/j.fct.2007.09.085.

Ansari, S. and Siddiqui, A. (2012) PISTACIA LENTISCUS: A REVIEW ON PHYTOCHEMISTRY AND PHARMACOLOGICAL PROPERTIES.

Apak, R., Capanoglu, E. and Shahidi, F. (eds) (2018) Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications. United kingdom: John Wiley and Sons Ltd.

Aruoma, O. I. (2003) 'Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods', in Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis. Elsevier, pp. 9–20. doi: 10.1016/S0027-5107(02)00317-2.

Azizah, D. N., Kumolowati, E. and Faramayuda, F. (2014) 'PENETAPAN KADAR FLAVONOID METODE AlCl3 PADA EKSTRAK METANOL KULIT BUAH KAKAO (Theobroma cacao L.) Dyah Nur Azizah, Endang Kumolowati, Fahrauk Faramayuda', Des, pp. 45–49.

Azwanida, N. (2015) 'A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation', Medicinal & Aromatic Plants. OMICS Publishing Group, 04(03). doi: 10.4172/2167-0412.1000196.

Baba AIssa, F. (1990) 'Baba Aïssa Farid - Les plantes médicinales en Algérie.pdf', p. 181.

Baldwin, I. T. et al. (2006) 'Volatile signaling in plant-plant interactions: "Talking

trees" in the genomics era', Science, 311(5762), pp. 812–815. doi: 10.1126/science.1118446.

Benzie, I. F. F. and Strain, J. J. (1999) 'Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration', Methods in Enzymology. Academic Press Inc., 299, pp. 15–27. doi: 10.1016/S0076-6879(99)99005-5.

Capita, R. and Alonso-Calleja, C. (2013) 'Antibiotic-Resistant Bacteria: A Challenge for the Food Industry', Critical Reviews in Food Science and Nutrition. Taylor & Francis Group , 53(1), pp. 11–48. doi: 10.1080/10408398.2010.519837.

Castro, L. and Freeman, B. A. (2001) 'Reactive oxygen species in human health and disease', Nutrition, pp. 161–165. doi: 10.1016/S0899-9007(00)00570-0.

Cheng, A. X. et al. (2007) 'Plant terpenoids: Biosynthesis and ecological functions', Journal of Integrative Plant Biology, 49(2), pp. 179–186. doi: 10.1111/j.1744-7909.2007.00395.x.

Cowan, M. M. (1999) Plant Products as Antimicrobial Agents, CLINICAL MICROBIOLOGY REVIEWS.

Cronquist, A. (1988) The evolution and classification of flowering plants. 2nd edn. New York Botanical Garden.

Djeridane, A. et al. (2006) 'Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds', Food Chemistry, 97(4), pp. 654–660. doi: 10.1016/j.foodchem.2005.04.028.

Dorman, H. J. D. et al. (2003) 'Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs', Food Chemistry, 83(2), pp. 255–262. doi: 10.1016/S0308-8146(03)00088-8.

Dudareva, N., Pichersky, E. and Gershenzon, J. (2004) 'Biochemistry of plant volatiles', Plant Physiology, pp. 1893–1902. doi: 10.1104/pp.104.049981.

El Jemli, M. et al. (2016) 'Radical-Scavenging activity and ferric reducing ability of

juniperus thurifera (L.), J. oxycedrus (L.), J. phoenicea (L.) and Tetraclinis articulata (L.)', Advances in Pharmacological Sciences, 2016. doi: 10.1155/2016/6392656.

Ensa, R. L. (2011) 'AMERICAN JOURNAL OF FOOD AND NUTRITION Using medicinal plants in Algeria'. doi: 10.5251/ajfn.2011.1.3.126.127.

Francesco Capasso, Timothy S. Gaginella, Giuliano Grandolini, A. A. I. (2003)

Phytotherapy: A Quick Reference to Herbal Medicine - Francesco Capasso, Timothy S. Gaginella, Giuliano Grandolini, Angelo A. Izzo - Google Livres. Edited by H. Fank Krabbes.

Friedman, M., Henika, P. R. and Mandrell, R. E. (2003) 'Antibacterial Activities of Phenolic Benzaldehydes and Benzoic Acids against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica', Journal of Food Protection, 66(10), pp. 1811–1821. doi: 10.4315/0362-028X-66.10.1811.

Gardès-Albert, M. et al. (2003) Mécanismes biochimiques Espèces réactives de

l'oxygène Comment l'oxygène peut-il devenir toxique ?

Gioxari, A. et al. (2011) 'Pistacia lentiscus resin regulates intestinal damage and inflammation in trinitrobenzene sulfonic acid-induced colitis', Journal of Medicinal Food, 14(11), pp. 1403–1411. doi: 10.1089/jmf.2010.0240.

Graßmann, J. (2005) 'Terpenoids as Plant Antioxidants', Vitamins and Hormones, 72, pp. 505–535. doi: 10.1016/S0083-6729(05)72015-X.

Hadj Salem, J. (2018) Extraction , identification , caractérisation des activités

biologiques de flavonoïdes de Nitraria retusa et synthèse de dérivés acylés de ces molécules par voie enzymatique

Halliwell, B. and Gutteridge, J. M. C. (1995) 'The definition and measurement of antioxidants in biological systems', Free Radical Biology and Medicine, 18(1), pp. 125–126. doi: 10.1016/0891-5849(95)91457-3.

Hartmann, T. (2007) 'From waste products to ecochemicals: Fifty years research of plant secondary metabolism', Phytochemistry. Pergamon, pp. 2831–2846. doi: 10.1016/j.phytochem.2007.09.017.

Heatley, N. G. (1944) 'A method for the assay of penicillin', Biochemical Journal. Portland Press Ltd., 38(1), pp. 61–65. doi: 10.1042/bj0380061.

Hennebelle, T., Sahpaz, S. and Bailleul, F. (2004) 'Polyphénols végétaux, sources, utilisations et potentiel dans la lutte contre le stress oxydatif', Phytothérapie. Lavoisier, 2(1), pp. 3–6. doi: 10.1007/s10298-004-0003-8.

Iriti, M. and Varoni, E. (2013) 'Chemopreventive Potential of Flavonoids in Oral Squamous Cell Carcinoma in Human Studies', Nutrients. MDPI AG, 5(7), pp. 2564–2576. doi: 10.3390/nu5072564.

Kasprzak, M. M., Erxleben, A. and Ochocki, J. (2015) 'Properties and applications of flavonoid metal complexes', RSC Advances. Royal Society of Chemistry, 5(57), pp. 45853–45877. doi: 10.1039/c5ra05069c.

Khan, M. Y. et al. (2007) Olea europaea: A Phyto-Pharmacological Review, Pharmacognosy Reviews. Available at: http://www.phcogrev.com (Accessed: 17 May 2020).

Kumar, G., Rao, B. and Bhaskara Rao, K. V (2011) 'A Review on Pharmacological and Phytochemical Properties of Zingiber officinale Roscoe (Zingiberaceae)', Article in Journal of Pharmacy Research, 4(9), pp. 2963–2966.

Kumar, S. and Egbuna, C. (eds) (2019) Phytochemistry: An in-silico and in-vitro Update, Phytochemistry: An in-silico and in-vitro Update. Singapore: Springer Singapore. doi: 10.1007/978-981-13-6920-9.

Kumar, S. and Pandey, A. K. (2013) 'フラボノイドレビュー', TheScientificWorldJournal, 2013, p. 162750. doi: 10.1155/2013/162750.

Lunte, S. M. (1987) 'Structural classification of flavonoids in beverages by liquid chromatography with ultraviolet-visible and electrochemical detection', Journal of Chromatography A, 384(C), pp. 371–382. doi: 10.1016/S0021-9673(01)94684-9.

Magalhães, L. M. et al. (2008) 'Methodological aspects about in vitro evaluation of

antioxidant properties', Analytica Chimica Acta, 613(1), pp. 1–19. doi: 10.1016/j.aca.2008.02.047.

Miraj, S., Azizi, N. and Kiani, S. (2016) 'A review of chemical components and pharmacological effects of Melissa officinalis L'.

Mubarak, A. et al. (2012) 'Polyphenol composition of plum selections in relation to total antioxidant capacity', Journal of Agricultural and Food Chemistry, 60(41), pp. 10256–10262. doi: 10.1021/jf302903k.

Ouelbani, R. et al. (2016) 'Ethnobotanical investigations on plants used in folk medicine in the regions of Constantine and Mila (North-East of Algeria)', Journal of Ethnopharmacology. Elsevier Ireland Ltd, 194, pp. 196–218. doi: 10.1016/j.jep.2016.08.016.

Oyaizu, M. (1986) 'Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine.', The Japanese Journal of Nutrition and Dietetics. The Japanese Society of Nutrition and Dietetics, 44(6), pp. 307–315. doi: 10.5264/eiyogakuzashi.44.307.

Pandey, K. B. and Rizvi, S. I. (2009) 'Plant polyphenols as dietary antioxidants in human health and disease', Oxidative Medicine and Cellular Longevity. Hindawi Limited, pp. 270–278. doi: 10.4161/oxim.2.5.9498.

Pengelly, A. (2004) The Constituents of Medicinal Plants. 2nd edn. United kingdom: CABI publishing.

Phillipson, J. D. (2001) 'Phytochemistry and medicinal plants', Phytochemistry, 56(3), pp. 237–243. doi: 10.1016/S0031-9422(00)00456-8.

Priyanka, B. et al. (2013) 'International Research Journal of Pharmaceutical and Applied Sciences (IRJPAS) EVALUATION OF ANTI OXIDANT ACTIVITY OF ETHANOLIC ROOT EXTRACT OF ALBIZIA LEBBECK (L .) BENTH', 3(2), pp. 93–101.

Rauha, J. P. et al. (2000) 'Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds', International Journal of Food Microbiology,

56(1), pp. 3–12. doi: 10.1016/S0168-1605(00)00218-X.

Ruch, R. J., Cheng, S. jun and Klaunig, J. E. (1989) 'Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea', Carcinogenesis, 10(6), pp. 1003–1008. doi: 10.1093/carcin/10.6.1003.

Saiah, H., Allem, R. and El Kebir, F. Z. (2016) 'Antioxidant and antibacterial activities of six Algerian medicinal plants', International Journal of Pharmacy and Pharmaceutical Sciences, 8(1), pp. 367–374.

Saxena, M. et al. (2013) 'Phytochemistry of Medicinal Plants', Journal of Pharmacognosy and Phytochemistry, 1(6), pp. 168–182.

Schlesier, K. et al. (2002) 'Assessment of antioxidant activity by using different in vitro methods', Free Radical Research. Free Radic Res, 36(2), pp. 177–187. doi: 10.1080/10715760290006411.

da Silva, J. F. M. et al. (2006) 'Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities', Food Chemistry, 99(3), pp. 431–435. doi: 10.1016/j.foodchem.2005.07.055.

Singhal, M., Paul, A. and Singh, H. P. (2014) 'Synthesis and reducing power assay of methyl semicarbazone derivatives', Journal of Saudi Chemical Society, 18(2), pp. 121–127. doi: 10.1016/j.jscs.2011.06.004.

Singleton, V. L. and Rossi, J. A. (1965) 'Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents', American Journal of Enology and Viticulture, 16(3).

Smirnoff, N. and Cumbes, Q. J. (1989) 'Hydroxyl radical scavenging activity of compatible solutes', Phytochemistry, 28(4), pp. 1057–1060. doi: 10.1016/0031-9422(89)80182-7.

Tarahovsky, Y. S. et al. (2014) 'Flavonoid-membrane interactions: Involvement of flavonoid-metal complexes in raft signaling', Biochimica et Biophysica Acta - Biomembranes. Elsevier B.V., 1838(5), pp. 1235–1246. doi: 10.1016/j.bbamem.2014.01.021.

Thirumurugan, D. et al. (2018) 'An Introductory Chapter: Secondary Metabolites', in

Secondary Metabolites - Sources and Applications. InTech. doi: 10.5772/intechopen.79766.

Verpoorte, R. (1998) 'Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development | Robert Verpoorte - Academia.edu', Division of Pharmacognosy, 3, pp. 232–238.

Volkers Schulz, Varro Eugene Tyler, R. H. (2012) Rational Phytotherapy: A Physician's Guide to Herbal Medicine. Springer S.

Waterman, Emily; Lockwood, B. (2007) Active Components and Clinical Applications of Olive Oil.

Yang, L. et al. (2018) 'Response of plant secondary metabolites to environmental factors', Molecules, 23(4), pp. 1–26. doi: 10.3390/molecules23040762.

Appendices

Appendix 1

Laboratory equipment

UV spectrophotometer SHIMADZU UV-1280 : is an instrument that determine the amount of analyte in a sample by the sample's ability or inability to absorb light at a ceratin wavelength. It uses light to measure chemical concentrations. The basic principle behind this method is that : "Each compounds absorbs or transmit light over a certain range of wavelength".

Absorption spectroscopy is directly proportional to the concentration of the analyte in the sample.

Rotary evaporator BUCHI B-490 : is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation.

Microbiology incubator Memmert: An incubator provides conditions for optimal growth of microbiology cultures. Natural or forced air convection maintains a constant, even temperature in a range from a few degrees above room temperature to as high as 100C.

Other devices were used such as :

Water bath <u>Memmert</u>

Precision balance <u>Sartorius</u>

Appendix 2

Reagents and chemicals

Sodium carbonate (Na_2CO_3, 7,5 %)

Na_2CO_3	7,5 g.
H_2O	100 ml.

Folin Ciocalteu (FCR)

FCR	1ml.
H_2O	9ml.

Aluminum chloride ($AlCl_3$, 2 %)

$AlCl_3$	2g.
H_2O	100 ml.

Potassium ferricyanide ($K_3Fe(CN)_6$, 1 %)

$K_3Fe(CN)_6$	1g.
H_2O	100ml.

Ferric chloride ($FeCl_3$,0.1 %)

$FeCl_3$	0,1g.
H_2O	100ml.

Trichloroacetic acid (TCA, 10 %)

TCA	1g.
H_2O	10ml.

Iron sulfate ($FeSO_4$)

$FeSO_4$	22,24 mg.
H_2O	10 ml.

Hydrogen peroxide H_2O_2 (20 mM)

H_2O_2	30 µl.
H_2O	19,94 ml.

Salicylic acid (3mM)

$C_7H_6O_3$	4mg.
H_2O	10 ml.

Abstracts

Abstract

In the framework of enhancing medicinal plants of the Algerian and the Mediterranean flora, this study investigates phytochemical screening of different parts' extracts of an Algerian medicinal plant: roots, stems, branches, flowers and aerial parts. It also highlights the quantification of the main secondary metabolites; total polyphenols and flavonoids and their correlation with antioxidant activity *in vitro*. Biological tests have shown encouraging results for the antioxidant activities namely: reducing power, hydrogen peroxide and hydroxyl radical scavenging, as well as for the antibacterial and antifungal activities which was carried out by the diffusion method by discs on agar medium, where the effectiveness of these extracts has been demonstrated against three five and bacterial referential strains.

Keywords: Algerian medicinal plants, Polyphenols, Flavonoides, Antioxidant activity, antibacterial activity, antifungal activity.

Résumé

Dans le cadre de la valorisation des plantes médicinales de la flore algérienne et méditerranéenne, la présente étude investie un criblage phytochimique des extraits des différentes parties: racines, tiges et branches, fleurs et partie aériennes d'une plante à usage médicinale et met en évidence la quantification des principaux métabolites secondaires ; les polyphénols et flavonoïdes totaux et leur corrélation avec l'activité antioxydante *in vitro*. Les tests biologiques initiés ont montré des résultats encourageants pour les activités antioxydantes suivantes : pouvoir réducteur, hydrogène peroxyde et hydroxyl radical scavenging. Ainsi que pour l'activité antimicrobienne qui a été réalisée par la méthode de diffusion par disques sur milieu gélosé où l'efficacité de ces extraits a été prouvée contre cinq souches, fongique et bactériennes référenciées.

Mots clés : Plantes médicinales algériennes, Poly phénols, Flavonoïdes, Activité antioxidante, activité antimicrobienne.

الملخص

تسلط الضوء هذه الدراسة على النباتات الجزائرية ذات الاستعمال الطبي من خلال إجراء فحص كيميائي نباتي لمستخلصات أجزاء مختلفة لنبات طبي: الجذور والسيقان والزهور والأجزاء الهوائية. كما تركز على تكميم الأيضات الثانوية الرئيسية؛ البوليفنول الكلي والفلافونويد وعلاقتهما بالنشاط المضاد للأكسدة في المختبر. أظهرت النتائج البيولوجية نتائج مشجعة للأنشطة المضادة للأكسدة على غرار: قدرة الإرجاع، بيروكسيد الهيدروجين وكسح جدر الهيدروكسيل وكذلك للنشاط المضاد للبكتيريا والفطريات الذي تم باستخدام طريقة الانتشار بواسطة أقراص في وسط جيلوزي حيث تم إثبات فعالية هذه المستخلصات ضد خمس سلالات فطرية وبكتيرية.

كلمات مفتاحية: النباتات الجزائرية ذات الاستعمال الطبية، البوليفنولات، الفلافونويدات، النشاط المضاد للأكسدة، النشاط المضاد للبكتيريا، النشاط المضاد للفطريات.

Presented by : *Khadidja Yasmine ARIANE*
Zeyneb AISSANI

Academic year : 2019/2020

Polyphenolic Potential and in vitro Biological Screening of Antioxidant, Antibacterial and Antifungal Activities of an Algerian Wild Medicinal Plant

End of cycle dissertation for obtaining a master degree in Applied Biochemistry.

Abstract

In the framework of valuating medicinal plants of the Algerian and Mediterranean flora, this study investigates phytochemical screening of different parts' extracts of an Algerian medicinal plant: roots, stems, branches, flowers and aerial parts. It also highlights the quantification of the main secondary metabolites; total polyphenols and flavonoids and their correlation with antioxidant activity *in vitro*. Biological tests have shown encouraging results for the antioxidant activities namely: reducing power, hydrogen peroxide and hydroxyl radical scavenging, as well as for the antibacterial and antifungal activities which were carried out by the diffusion method by discs on agar medium where the effectiveness of these extracts has been demonstrated against five fungal and bacterial referential strains.

Keywords : Algerian medicinal plant, Polyphenols, flavonoids, antioxidant activity, antibacterial activity, antifungal activity.

Research laboratory: Laboratoire d'Obtention de Substances Thérapeutiques (LOST).

Evaluation jury :

Jury president : Amel Daffri (MCB – UFM Constantine 1)
Supervisor : *Toma Nardjes* Mouas (MCA – UFM Constantine1)
Examiner : Zeyneb Boucherit (MAA– UFM Constantine 1)

Dissertation defense date : 28th, June 2020