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Filière : sciences alimentaires

Spécialité : Agro-alimentaire et Contrôle de Qualité

Thème

Contribution to the antimicrobial activity of olive leaves (*Olea europaea L*) Chemlal variety in the region of Chebika(Djelfa)

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Dedication:

To my family, my friends and to everyone who has helped me in life

Ali

Dedication:

To my mother and my brothers

To my beloved father my dear

To my support and my strength after God my friends

To all my teachers from primary to master

To all people consider me a brother

Bouxid

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OLE : Olive leaves extract

FAO : Food and Agriculture Organization

MIC : Minimum inhibitory concentration

MBC : The Minimum Bactericidal Concentration

NB : Nutrient Broth

M-H Agar : Mueller Hinton Agar

ml : milliliter

Y % : The yield

E. coli: Escherichia coli

D1 : first Decimal dilution

D2 :second Decimal dilution

D3 : third Decimal dilution

Summery

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Results and discussion

Abstract

General Introduction

Introduction:

The olive tree (*Olea europaea L.*) has a wide geographical spread in the Mediterranean regions since thousands of years, both cultivated and wild **(Gharabi 2018)**. It is grown globally in tropical and subtropical regions (Fereshteh et al,.2020). The global genetic heritage of olives is characterized by the diversity of varieties **(Muzzalupo et al. 2014)** and includes about 30 genera and 600 species. The genus Olea L. includes more than 30 species distributed throughout the world **(Alireza2020)**. The great variation of climates in Algeria allowed the selection of a large number of Genetic types of olives that adapt well to all climatic conditions **(Abdessemed, 2015)**. Algerian olive cultivation is mainly concentrated in the northern part of the country, where most

orchards (80%) are located in mountainous areas with clay soils **(Abdessemed, 2017).**

In 2020, the area of olive cultivation in Algeria reached more than 430 thousand hectares. Of the 60 million olive trees, more than 40 million are produced with an average productivity of 23 kg of olives per tree. and program has been established to support olive cultivation, among the many measures taken, planting 400,000 hectares of olive trees by 2024 and converting traditional olive groves into high-density and ultra-dense plantations in order to reduce the alternative production cycle. **(Christine Avelin., 2022)**

The olive tree Olea europaea L. has nutritional, economic and environmental importance. **(Gomes et al . ,2012)** There are more than 805 million olive trees around the world, 98% of which are concentrated on the coasts of the Mediterranean Sea.

It is a blessed tree mentioned in several places in the Holy Quran

قَالَ اللَّه تَعَالَى :اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونَةٍ لَّا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُّورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَن يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ (35) سورة النور

Olive leaves are one of the most important byproducts of olive farming, constituting 10% of the entire olive harvest weight and 25 kg/tree during olive tree pruning

olive leaves have rich history of medicinal uses .

The various beneficial properties of olives leaves has drawn the attention of many researchers in various fields , due to their biologically active compounds leaves Recently, antioxidant, hypoglycemic, antihypertensive, antimicrobial, and antiatherosclerotic effects of olive leaves have been reported in various studies**.(Sedef et Sibel.,2009)**.

Currently the impact multidrug-resistant bacteria it has become a global concern to public health .Therefore, a greater attention has been paid to discover new antimicrobial agent the aim of this work is study of olive leaves extract as potentiel antimicrobial agent.

Our work will be divided into two parts. The first part of this work concerns primarily the bibliographic study of the plant. This study begins with a botanical description of the olive tree, followed by. The second chapter explains the main categories of phenolic compounds. The third chapter explains the benefits of olive tree leaves.

The second part of our work will concern the experimental part of this study, with a first chapter presenting the extraction techniques used and the protocols used during the evaluation of the antibacterial activity of our extracts.

The second chapter of the practical part will present the results obtained, followed by a discussion and finally the conclusion.

Chapter 01:

Generalities about the olive tree.

Chapter 1 : Generalities about the olive tree

1- Tree

1.1 History :

Olea europaea L. subsp. europaea var. europaea **(Green, 2002)**, commonly known as olive tree, is one of the main agroecological symbols of the Mediterranean Basin. It has been extensively cultivated for thousands of years in this region, primarily to produce olive oil and/ or table olives **(Breton et al., 2012**). Nuclear and plastid DNA data has shown that the main wild progenitor of the cultivated olive is the wild Mediterranean olive, also known as oleaster (*O. europaea* subsp. *europaea* var. *sylvestris*) **(Besnard et al., 2018)**.

According to archaeological and genetic studies, the domestication of the cultivated olive seems to have occurred after the emergence of major human civilizations in the Middle East ~6,000 years ago, in the Neolithic **(Besnard et al , 2018).**

Olea europaea var. Europea is the main cultivated species belonging to the family, Oleaceae, which includes 30 genera and 600 species

1.2 Taxonomic classification of olive tree

Table 01 : Taxonomic classification of olive tree.

Kingdom	**Plantae**
Division :	Plantae
class :	Magnoliophyta
Order :	Lamiales
Family :	Oleaseae
Genus :	*Olea*
Species :	E.*oleaster* *E.sativa*
Binomial name :	*Oleaeuropaea*

(Selin and Mehmet ,2017)

1.3 Botanical description :

The average height of the olive tree ranges from 20-30 ft (6–9 m) tall. The spread of the tree is 15-25 ft (4.5-7.5 m). Crown has an oval shape.

Chapter 1 : Generalities about the olive tree

a)The trunk:

According to **Beck and Danks (1983)** the trunk is yellowish then changes to very light brown. It is very hard, compact, short, stocky (up to 2m in diameter), and habit of branches

fairly large, tortuous, and smooth.

Figure 01 : Chemlal variety **(original,2023)**

b)The leaves:

Evergreen, opposite, leathery, oblong-ovate, entire and somewhat rolled up,

borne on a short petiole; they are greyish green, dark green below

whitish and single-veined below. Very often they containfats, waxes, chlorophylls, acids (gallic and malic),gums and vegetable fibers.Evergreen, opposite, leathery, oblong-ovate, entire and somewhat

rolled upborne on a short petiole; they are greyish green, dark green belowwhitish and single-veined below. Very often they containfats, waxes, chlorophylls, acids (gallic and malic),gums and vegetable fibers.**(Amouretti, 1985)**

Figure2 : the Chemlal variet **(original,2023)**

c)The flower :

Olive flowers are grouped in an inflorescence comprising a number of flowers, variable from one cultivar to another from 10 to more than 40 per cluster on average. Flowers individual can be hermaphroditic or staminate .**(Loussert and Brousse, 1978)**

Figure 3: flower

Chapter 1 : Generalities about the olive tree

1 .4 .Cultivation of olive :

I .4.1. World olive cultivation area.

According to the FAO, the world area represents 12.8 million hectares in 2020.

Spain is the leading producer with 20% of the cultivation area.

In 2020, Spain holds more than 2.6 million hectares followed by Tunisia with 1.96 million hectares. **(Christine Avelin., 2022)**

1.4.2 Distribution of the world cultivation area in 2020 :

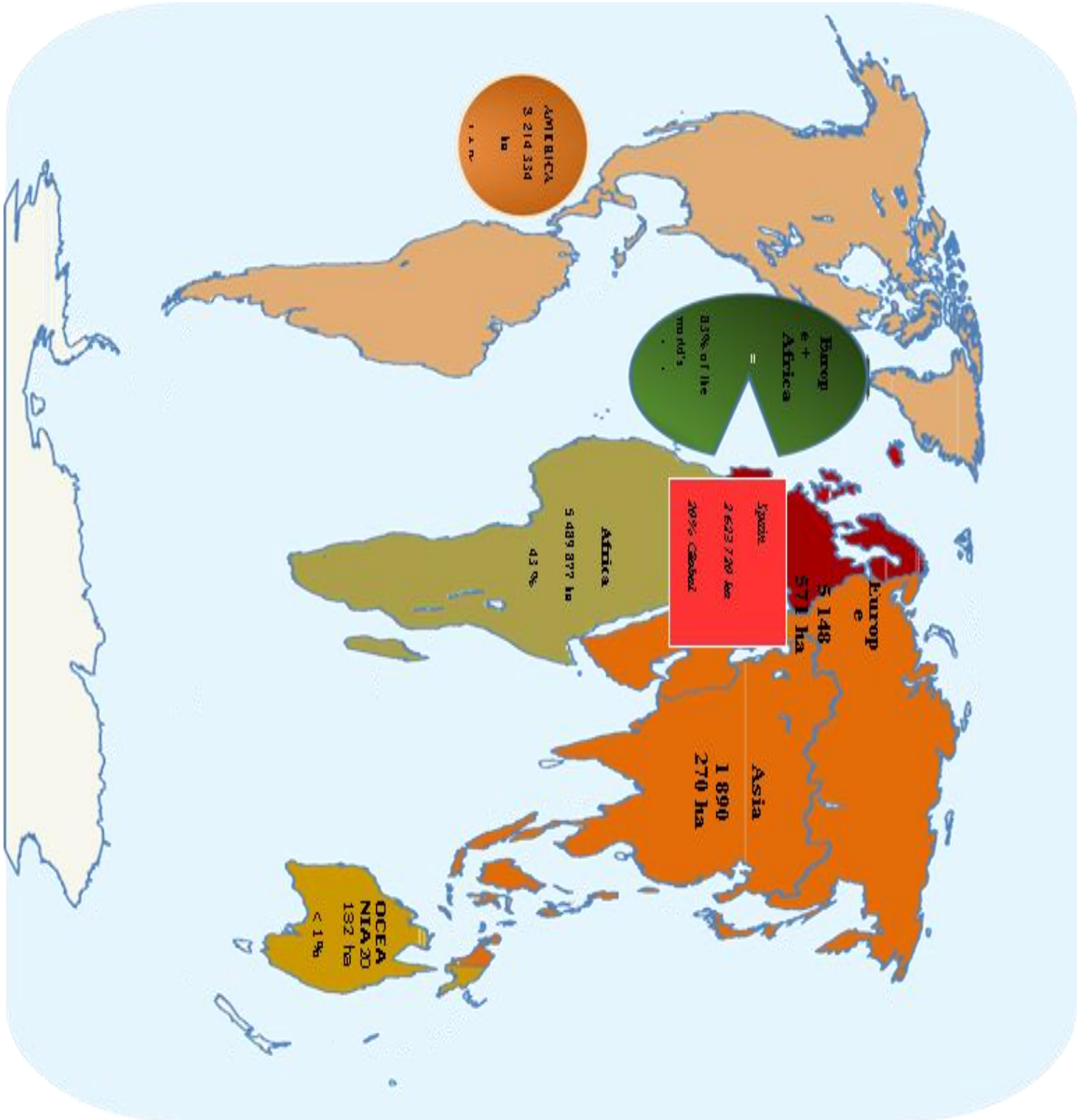

Figure 4 : Distribution of the world orchard in 2020 **(Christine Avelin., 2022)**

Table2: Areas of the main producing countries since 2015to 2020.

Pays	Surfaces en ha					
	2015	2016	2017	2018	2019	2020
Spain	2351370	2521694	2554829	2579000	2601900	2623720
Italy	1147877	1144947	1141893	1142120	1139470	1145520
Tunisia	1624980	1648060	1685301	1534090	1606909	1960000
Maroc	1006491	1008365	1020569	1045186	1073493	1068895
Grèce	821206	797820	792643	963120	903080	906020
Turquie	836935	845542	846062	864428	879177	887077
Syrie	692324	689162	692417	693064	693227	696363
Algria	406571	423683	432959	431009	431506	438828
Portugal	351340	356183	358276	361180	377280	379440
Libye	224513	243179	240386	236026	239864	238759

(Christine Avelin., 2022).

1.4.3 World olive production :

• World production in 2020/2021: 3,010,000 tons

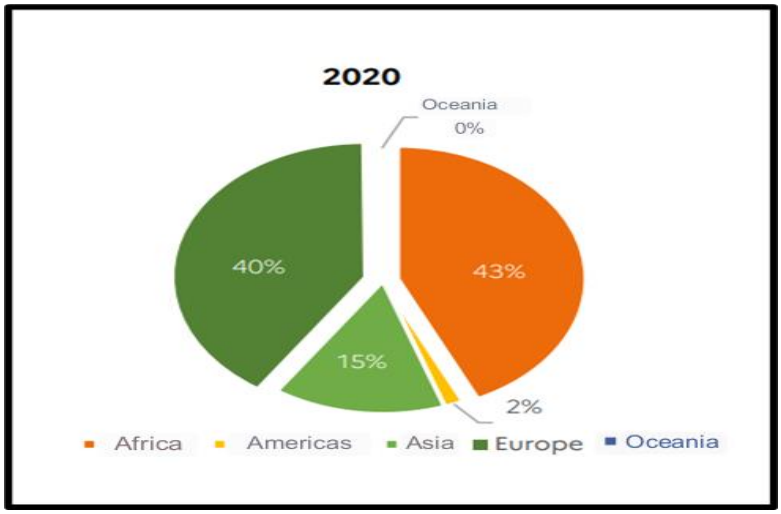

Figure5 : Distribution of surface areas by continent **(Christine Avelin., 2022).**

1.4.4 In algeria :

Chapter 1 : Generalities about the olive tree

In 2020, the Algerian olive-growing area is over 430,000 ha. Out of 60 million olive trees, more than 40 million are in production for an average yield of 23 kg of olives/tree. **(Christine Avelin., 2022).**

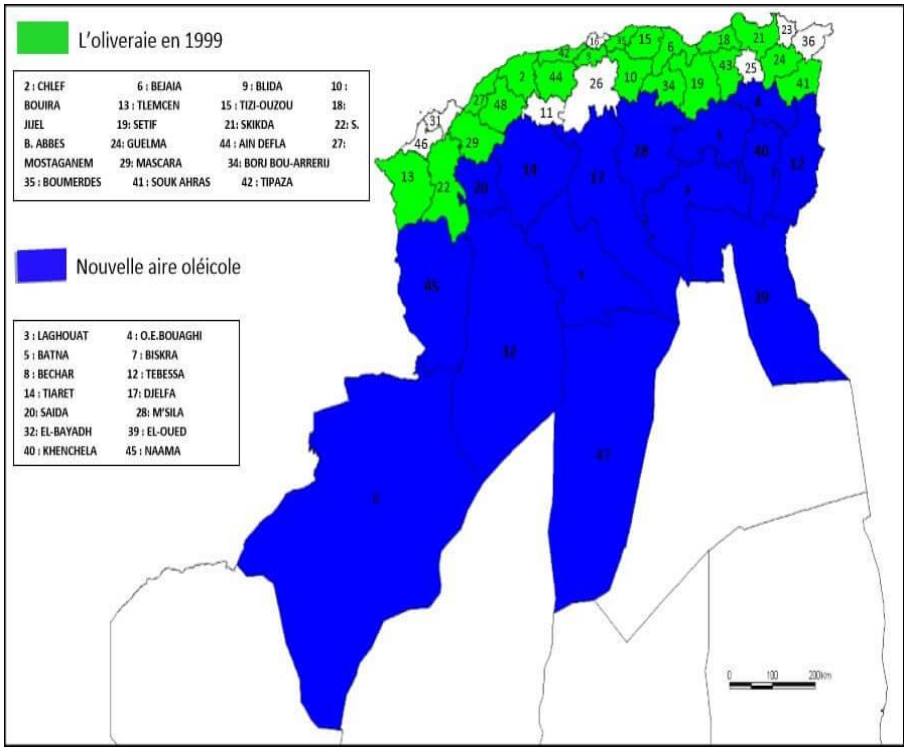

Figure 6: Algeria olive oil map **(ITAFV, 2008).**

Table 3: Main varieties of olive trees grown in Algeria : **(Mendil et Sebai, 2006).**

varieties and synonyms	Origins and diffusion
Azeradj : Aradj, Adjeraz	Kabylie(region ofSedouk-WillayaofBejaïa) occupies 10% of the national olive-growing area, often inassociation with the Chemlal variety, of which it is the pollinator
Chemlal : Achamlal, Achamli, Achemlal	Kabylia: occupies 40% of the Algerian olive orchard
Grosse de Hamma : QelbEthour, cœur de bœuf	Hamma (Constantine), Restricted diffusion

Limli : Imli, limeli	Sidi aiche (Bejaïa) : occupies 8% of the olive groveAlgerian, located on the mountainous slopes of thelower valley of the Soummam to the coast.
Long of Miliana	OriginaryfromMiliana, located in the region Khemis Miliana, Cherchell and the coast of Tenes
Rougette of Mitidja	Plain Mitidja
Sigoise : Tlemcen olive, tell olive	plane of Sig (Mascara) : occupies 25 % of Algerian olive orchard

2. the olive leaves

The olive leaves have a rich history of medicinal uses **(Soni et al., 2006).** There are many references citing the medicinal use of the plant (Olea europaea) in ancient times. The plant is cultivated widely in the Mediterranean region, Arabian Peninsula, the Indian subcontinent and Asia **(Somova et al., 2003).**

Figure 7: Olive (*Olea europaea* L.) leaves

2.1.Olive leaf compounds

2.1.1.Chemical composition

The chemical composition of olive leaves vary depending on origin, proportion of branches on the tree, storage conditions, climatic conditions, moisture content, and degree of contamination with soil and oils. In addition, the structural carbohydrates and nitrogen content in olive leaves depends on factors such as

the variety of the olive tree, climatic conditions, year of harvest, proportion of wood, etc**.(Molina et al,2008)**

2.1.1.1- Primary metabolites :

These are compounds derived from primary metabolism, via the Calvin cycle. Those metabolites such as amino acids, carbohydrates, lipids, proteins and nucleic acids which are necessary for plant survival

2.1.1.2- Secondary metabolites

Phenolic compounds are described as secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These compounds are one of the most widely occurring groups of phytochemicals. They are also of considerable physiological and morphological importance in plants. Structurally, despite their extreme variety, polyphenols possess a common carbon skeleton building block—the C6–C3 phenylpropanoid unit.

Olive leaves contain a large variety of phenolic derivatives and consist of:

- simple phenols (the most common and important low-molecular weight phenolic compounds);
- flavonoids (flavones, flavanones, flavonols, and flavan-3-ols);
- secoiridoids.**(Draganaet al .,2020)**

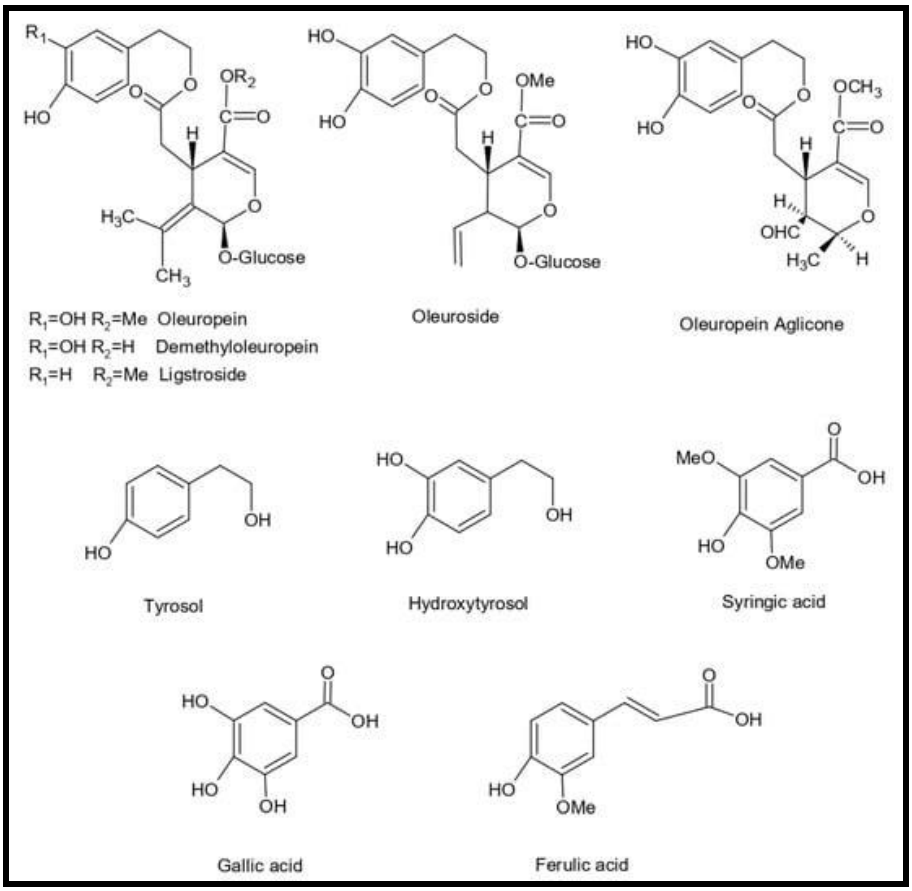

Figure 8: Chemical structures of phenolic compounds already identified in *Olea europaea* L. leaf extract.**(Dragana et al .,2020)**

Chapter 02 :

Polyphenolic Compounds

Chapter 02: Polyphenolic Compounds

1 General information on phenolic compounds :

Polyphenols, a group of heterogenous compounds, refer to natually occurring secondary metabolites largely found in plant species. Their molecular structure is based on a linkage between hydroxyl groups (polar phase) and one aromatic ring . that can be obtained from bacteria, fungi, and marine organisms, but mostly from plants**.(Rahim et al .,2009**). They are a consequence of the reactivity to pathogen attack and the response to insect injuries in the olive tree.According to their chemical structure, phenolic compounds are subdivided into diverse subcategories, including simple phenols, phenolic acids, coumarins, flavonoids, tannins, stilbenes, lignans, quinones, and curcuminoids **(Shavandiet al .,2018)**.

1.1Simple phenols :

Simple phenols are compounds that presenting an aromatic ring with one or more hydroxyl groups linked.(**Kougan et al., 2013) 25** Representative examples of simple phenols include catechol,.hydroquinone, resorcinol, and phloroglucinol, as illustrated in Figure 1

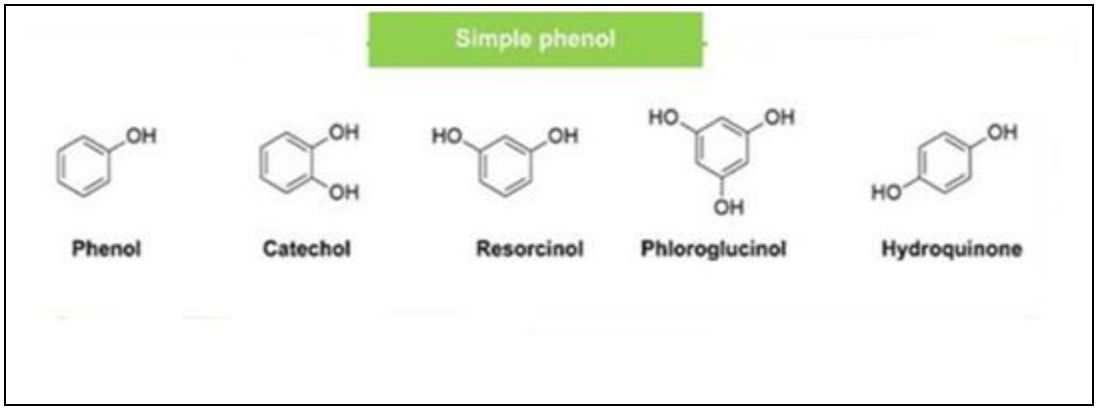

Figure 9 : examples of simple phenols**.(Kougan et al., 2013)**

1.2. phenolic acids :

are coumond that Consists of a carboxylic acid linked to an aromatic ring.Based on their structure, phenolic acids comprise two key classes, including benzoic acid and cinnamic acid derivatives. **(Mulleret al .,2019)**

a) Hydroxybenzoicacids

Hydroxybenzoicacids are benzoicacidssubstitutedwith a hydroxyl group. Alternatively, they can beviewed as phenolsthat are substitutedwith a carboxylicacidfunctional group thatisdirectlybonded to the phenolring .

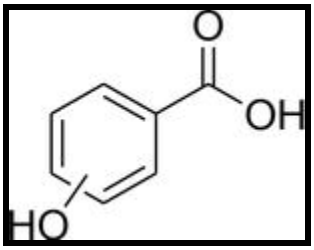

Figure 10 : General structures of hydroxyl-substitutedbenzoicacids. **(Hamad ,2021)**

b) Hydroxycinnamicacids

When the carboxylicacidfunctional group isseparatedfrom the phenol ring by a C=C bond, phenolicacids are described as hydroxycinnamicacids

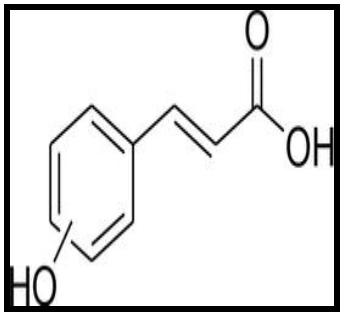

Figure 11 : General structures of hydroxyl-substitutedcinnamicacids. **(Hamad ,2021)**

1.3 Coumarin

Coumarin (1-benzopyran-2-one) derivatives are chemical compounds of the benzopyrone class, comprised of fused benzene and α-pyrone rings, that can be discovered in bacteria, fungi, and plants**.** **(Hoult et Payá ,1996)**. To date, over 1300 coumarin types have been described and obtained through

plant extraction or microbial synthesis. **(Costa et al,2016)** Natural coumarins can be categorized into four primary classes, including simple coumarins, pyrano coumarins, furanocoumarins, and bicoumarins (dicoumarin), as illustrated in. **(Wu et al ,2020)** These compounds are mostly isolated from plants, nonetheless, some of them can also be produced by microorganisms **(Lacy ,2004)**

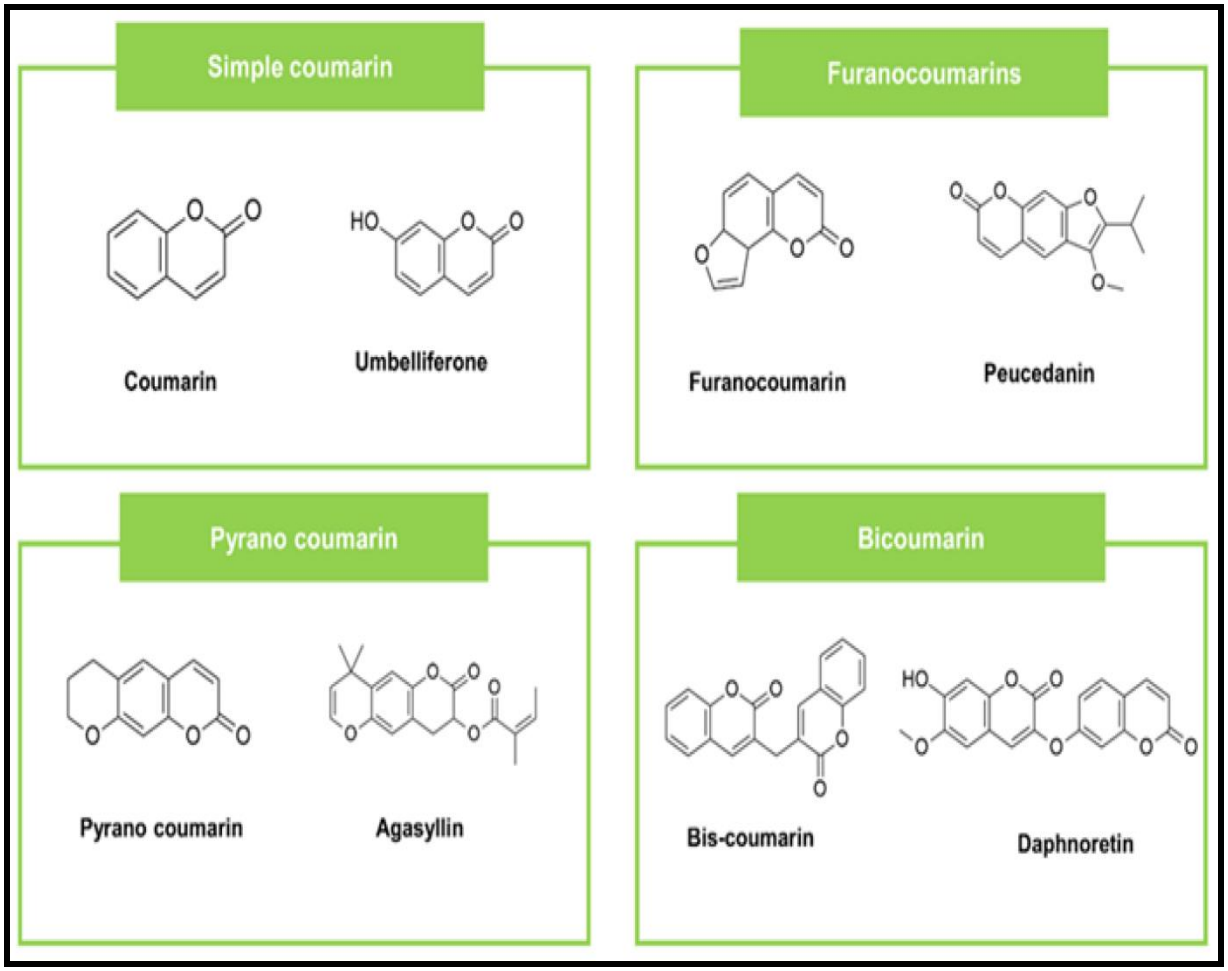

Figure 12: Representative chemical structures of different classes of coumarin and nature-derived coumarin.**(Matoset al .,2020)**

1.4 Flavonoids :

Flavonoids are a wide class of polyphenolic compounds based on a basic structure of 2-phenyl chroman.**(Nabaviet al .,2020)**On the other side, isoflavonoids own a basic structure of 3-phenyl-chroman which is biogenetically derived from the 2-phenyl chroman skeleton of flavonoids.

(Miadoková, 2009). Until now, more than 8000 flavonoid derivatives have been recognized in nature, as both free state and conjugated state, as ester or glycosidic derivatives. **(Mukherjee, 2019)** .Flavonoids are generally discovered in plant sources, but they may innately occur in certain microalgae and fungi.**(Orsat et al .,2017)**As described by Jin et al., based on the oxidation degree of the main heterocycle, flavonoids are categorized into seven subclasses: flavonols, flavones, isoflavones, anthocyanidins, flavanones, flavanols, and chalcones .as illustrated in figure 13

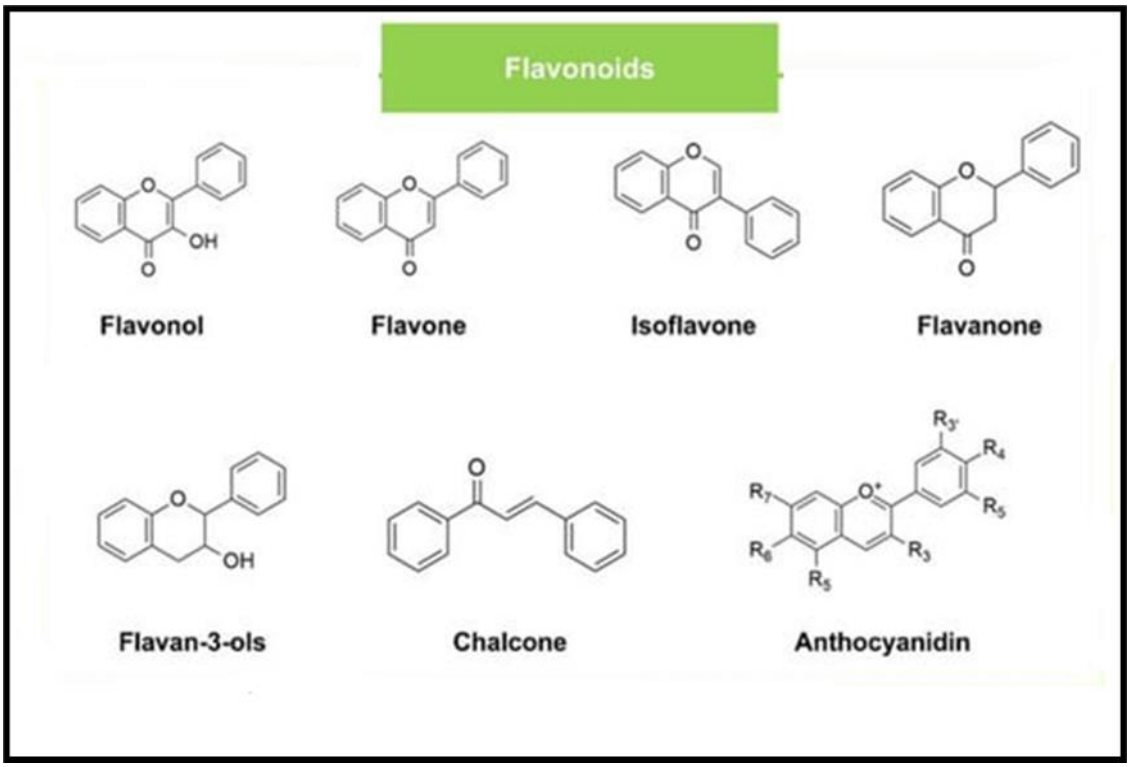

Figure 13: Representative chemical structures of different classes of flavonoids.

1.5 Stilbenes :

Stilbenes are phenolic compounds in which two phenol units are linked by two-doubly bonded carbons. Examples of stilbenes include resveratrol, pterostilbene and piceatannol as shown in figure 14

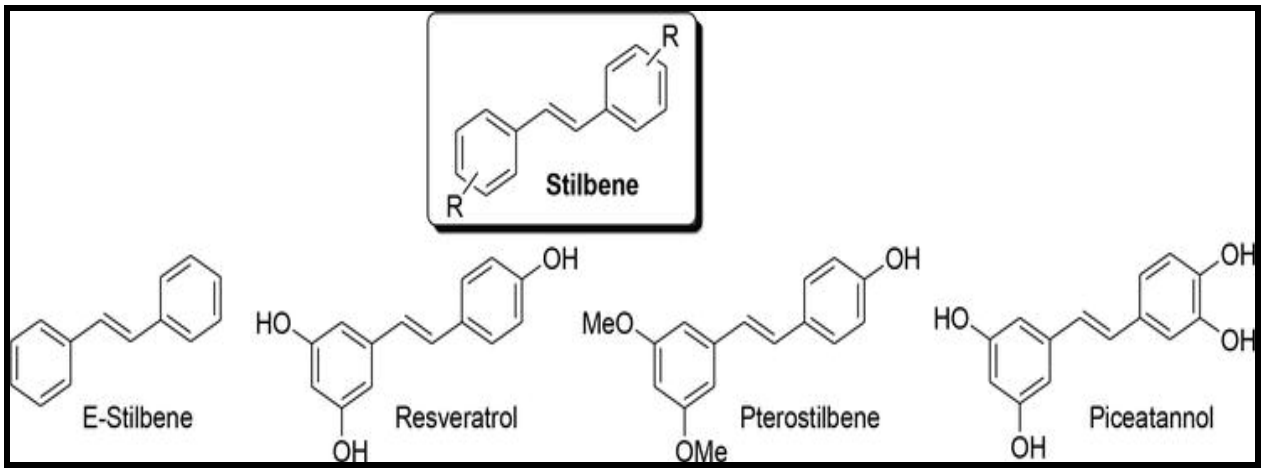

Figure 14 : Stilbenes **(Hamad ,2021)**

Hamad H. Al Mamari

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1.6 Lignans :

Lignans consist of two phenolunits linked by four carbons. Example sincludematairesinol, secoisolariciresinol and pinoresinol.

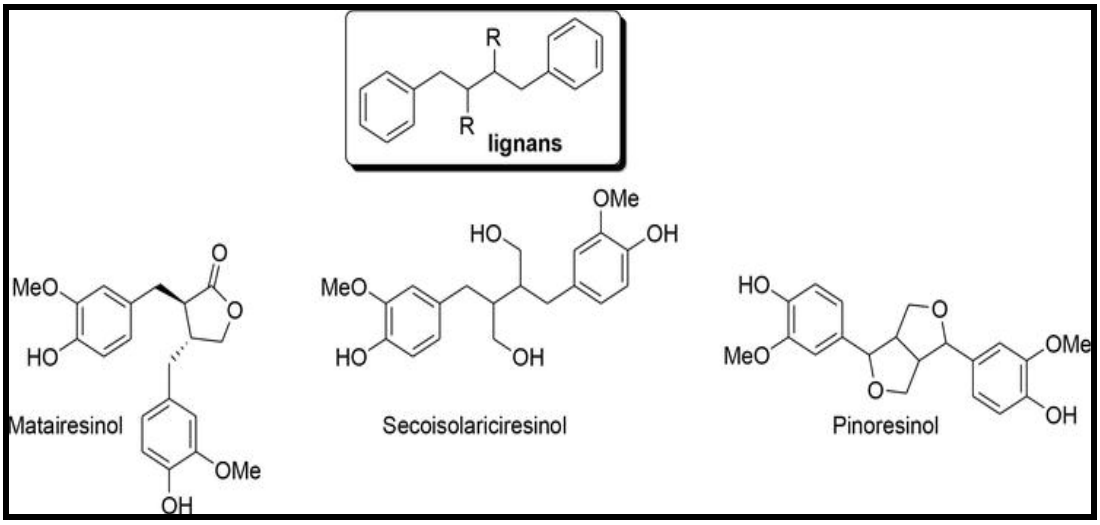

Figure15 : General structure of lignans and examples.

Chapter 02: Polyphenolic Compounds

1.7 Lignins :

Ligninsconsist of phenolunits or phenolic compounds that are linkedwitheachother by carbonchains. Lignins are high molecularweightpolymers.

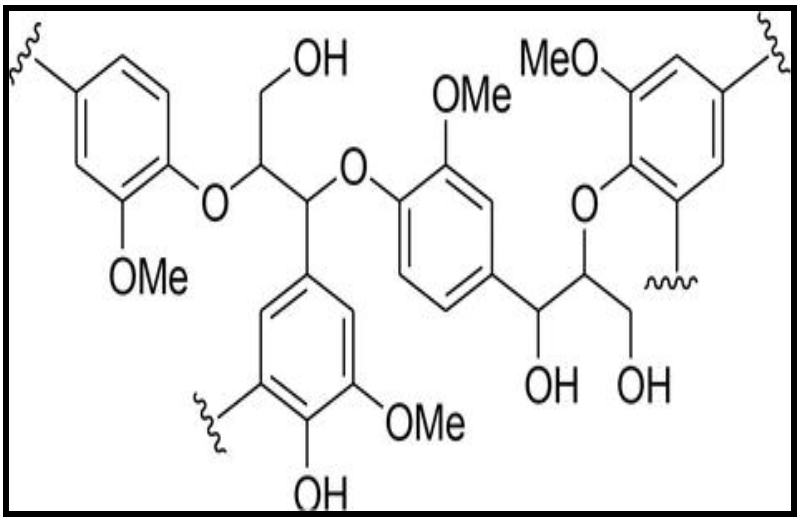

Figure 16: A segment of lignins.**(Hamad ,2021)**

Chapter 03 :

Olive leaves extract benefits

Chapter 03: Olive leaves extract benefits

1. Olive leaves:

Olive leaves contain a large variety of phenolic derivatives and consist of :

- simple phenols (the most common and important low-molecular weight phenolic compounds).
- flavonoids (flavones, flavanones, flavonols, and flavan-3-ols).
- secoiridoids.

There are five groups of phenolic compounds principally present in olive leaves: oleuropeosides (oleuropein and verbascoside); flavones (luteolin-7-glucoside, apigenin-7-glucoside, diosmetin-7-glucoside, luteolin, and diosmetin); flavonols (rutin); flavan-3-ols (catechin), and substituted phenols (tyrosol, hydroxytyrosol, vanillin, vanillic acid, and caffeic acid). The most abundant compound in olive leaves is oleuropein, followed by hydroxytyrosol, the flavone-7-glucosides of luteolin and apigenin, and verbascoside. Hydroxytyrosol is a precursor of oleuropein, and verbascoside is a conjugated glucoside of hydroxytyrosol and caffeic acid. **(Sedef et sible,2009)**

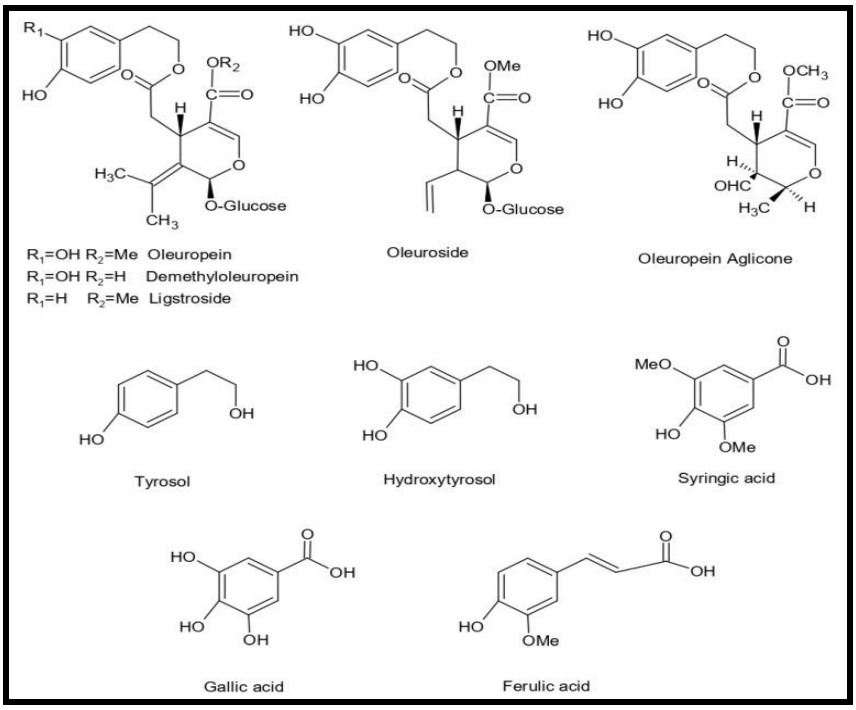

Figure17 : Chemical structures of phenolic compounds already identified in *Olea europaea L*. leaf extract. **(Dragana et al 2020)**

Chapter 03: Olive leaves extract benefits

Oleuropein:

(3,4-dihydroxyphenyl) ethanol (hydroxytyrosol) ester with α-glucosylatedelenolic acid, is one of the iridoid monoterpenes. Oleuropein belongs to a specific group of coumarin-like compounds, named secoiridoids. The main demonstrated biological activities of oleuropein are antioxidant and anti-inflammatory effects as well as the ability to treat oxidant and inflammatory-related diseases such as cardiovascular disease, hepatic disorder, obesity, and diabetes. Oleuropein shows anti-clastogenic activities, free-radical scavenging properties, and inhibits the development of different tumors cell types.

Additionally, olive leaves contain a significant number of flavonoids, which represent the most common and widely distributed group of olive leaves polyphenols. **(Jemai et al, 2008)** reported that they can be present in the aglycone form (apigenin, diosmetin, luteolin, quercetin,) or in the glycosylated form (luteolin-7-*O*-rutinoside, luteolin-7-*O*-glucoside, luteolin-5-*O*-glucoside, quercetin-7-*O*-rutinoside)

Luteolin (3′,4′,5,7-tetrahydroxyflavone) : member of the flavonoid family. inhibits chromosome alterations and presents antioxidant effects, anti-tumorigenic properties, and anti-inflammatory activities. Additionally, luteolin potentially controls colon cancer in multiple aspects

2.Olive leaf extract :

Olive leaf extract is a liquid with dark brown color and bitter in taste. Olive leaf extract is signified as a part of natural medicine with a wide range of health benefits. It has been used traditionally as an herbal supplement because it contains polyphenolic compounds with beneficial properties such as increasing energy levels, lowering blood pressure, and supporting the cardiovascular and immune systems. (**Dragana et al ,2020)**

Olive leaf extract contains many different compounds collectively termed olive biophenols, which are thought to give the extract its different therapeutic properties. The main component of all the constituents of olive leaf extract, oleuropein, has antimicrobial, antioxidative, antiviral, antiatherogenic, cardioprotective, and antihypertensive properties. Oleuropein belongs to a specific group of coumarin-like compounds, named secoiridoids.

Chapter 03: Olive leaves extract benefits

2.1 Olive leaves extract active components and biological activities :

Table 4 : Olive leaves extract active components and biological activities.

Phenolic compound	Biologicalactivities	References
-Secoiridoids (oleuropein), -Simple phenols (Hydroxytyrosol, tyrosol), -Phenolicacids (Cinnamicacid, Gallicacid, Syringicacid, p-coumaricacid, Ferulicacid, Caffeicacid, Vanillicacid), -Flavonols (Rutin, Quercetin), -Flavanol (Catechin), and -Flavone (Luteolin)	*Antioxidantactivity*	**Khadem , et al 2019**
-Flavones (Luteolin, Luteolin-4′-O-glucoside, Apigenin, Apigenin-7-Oglucoside), and -Flavonols (Rutin, Quercetin)	*Antioxidant and anticancer impacts*	**Wang , et al 2018**
-Secoiridoids (Oleuropein), Phenolicacids (Verbascoside), -Flavones (Luteolin, glucoside)	*Neuroprotectiveeffects*	**Giacometti,et al 2018**
-Secoiridoids (Oleuropein), and -Simple Phenols (Hydroxytyrosol)	*Antioxidanteffect*	**da Rosa et 2020**
-Secoiridoids (Oleuropein, ligstroside, secologanoside) -Simple Phenols (hydroxytyrosol glucoside), -Flavones (luteolin glycosides), and -Phenolicacids (Verbascoside)	Antioxidant effects and cytoprotection in kidney cells	**Ranieri et al 2019**
-Secoiridoids (Oleuropein), -Phenolicacids (Verbascoside),	Antifungaleffect	**Muzzalupo et**

and -Flavones (Luteolin-4-O-glucoside, Luteolin-7-glucoside)		**al 2020**
-Secoiridoids (Oleoside, Oleuropein, Oleuropeindiglucoside, Oleuropein, aglycone), -Flavones (Apigenin, Luteolin 7-O-glucoside, Chrysoeriol-7-O-glucoside), -Flavonols (Rutin), -Phenolicacids (Verbascoside), -Simple phenols (Hydroxytyrosol), and other compounds (Elenolicacid glucoside)	Antioxidant and anti-asthmatic impacts	**Rouibah et al 2022**
-Secoiridoids (Oleuropein)	Anticancer impact	**Ruzzolini et al 2020**
-Secoiridoids (Oleuropein), -Simple Phenols (Tyrosol), -Phenolic acids (Vanillic acid, gallic acid), and -Flavanols (Catechin)	Antibacterial and anti-inflammatory impacts	**Qabaha et al 2018**

3.Antibiotics :

Antibiotics are chemicalsubstances , The termantibiotic has itsorigin in the wordantibiosis (i.e. against life). obtainedfromvariousspecies of microorganisms (bacteria, fungi, actinomycetes) thatsuppress the growth of othermicroorganisms and eventuallymay destroy them. The probable points of differenceamongst the antibioticsmaybephysical, chemical, pharmacologicalproperties, antibacterialspectra, and mechanism of action. They have made it possible to cure diseases caused by bacteria, such as pneumonia, tuberculosis, and meningitis, and theysave the lives of millions of people around the world.However, overuse of antibiotics has led to the loss of theire fficacybecause of the increase in microbialresistance **(Mayers.,et al 2009 .**

Chapter 03: Olive leaves extract benefits

Currently,its impact is considerable with treatment failure sassociated with multidrug-resistant bacteria and it has become a global concern to public health .Therefore, a greater attention has been paid to discover new antibiotics.

4.Olive leaves extract antimicrobial activities :

OLE has been shown to have antimicrobial activities against foodborne pathogens such as *Staphylococcus aureus, E. coli, Salmonella* spp., and *L. monocytogenes* **(Techathuvanan et al., 2014)**

According to **(Liu et al.,(2017)** oleuropein and verbascoside have great impact against *Salmonella enteritidis* .

antimicrobial activity against *Escherichia coli* of polyphenols, such as oleuropein, rutin, hydroxytyrosol, and caffeic acid

Klebsiella Pneumoniae is a gram-negative bacterium. Even though it is present in the normal flora of the mouth, skin, and intestine, it can be the cause of damaging changes to human and animal lung if aspirated, especially to the alveoli resulting in bloody sputum. Many researchers determined that oleuropein, rutin, and hydroxytyrosol have great impact against *Klebsiella Pneumoniae* **(Sudjana et al,2009).**

Escherichia coli is a group of bacteria that usually lives in the intestine of humans and animals and helps to keep our guts healthy. Howevern ,Pathogenic strains of Escherichia coli can be ingested with contaminated food, such ascontaminated beverages, including water, unpasteurized milk, and fruit juices. Escherichia coli can infect anyone specified groups of people including children, older adults and people with weakened immune systems are more at risk of developing symptoms than others. It has been published a wide range of studies regarding the great antimicrobial activity against Escherichia coli of polyphenols, such as oleuropein, rutin, hydroxytyrosol, and caffeic acid. **(Sudjana et al,2009).**

Chapter 03: Olive leaves extract benefits

4.1Mode of antibacterial action of oleuropien :

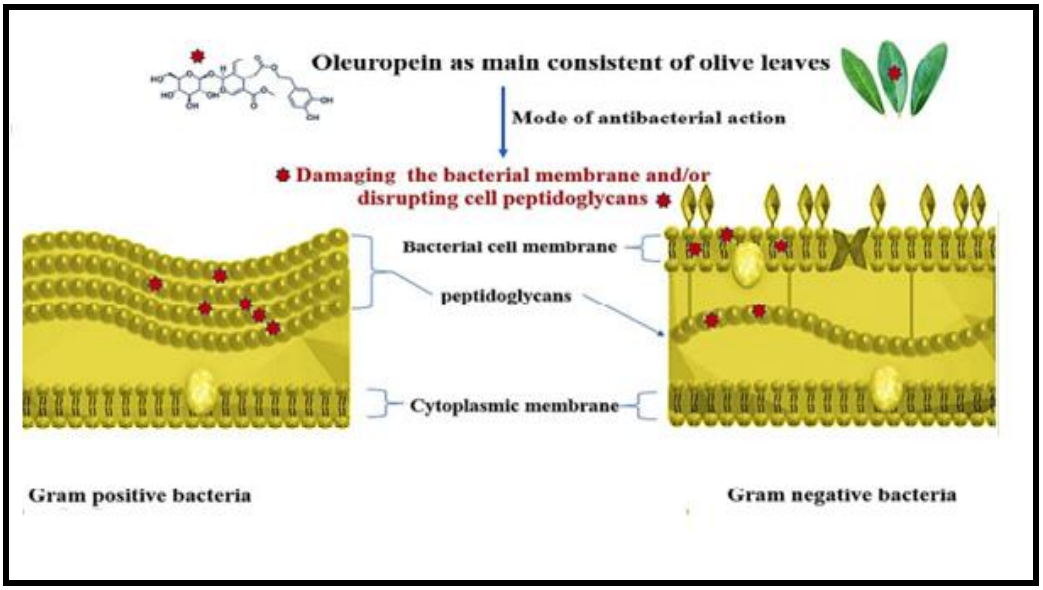

Figure 18 : Mode of antibacterial action of oleuropein as main component of olive leaves; oleuropein inhibit both Gram negative and Gram positive bacteria propagation via damaging the bacterial membrane and/or disrupting cell peptidoglycans.**(Sammy et al,2022)**

Part two :

Experimental part

Chapter 01:
Material and methods

experimental work :

This experimental work consists of evaluating the antimicrobial effect of polyphenolic extracts of Arceuthobium oxycedri on available pathogenic germs, with a view to understanding the type of inhibitory action that the main bioactive compounds of Arceuthobium oxycedri can exert.
The work is composed of four main parts:

- Extraction by maceration.
- Liquid-liquid extraction
- Evaluation of the effect of polyphenolic extracts on human pathogenic microorganisms
- Determination of the minimum inhibitory concentration (MIC)

Material and Methods

1.Material and methods :

1.1Plant material:

The plant material consists of olive leaves (*Olea europea L*) variety Chemlal which were collected during the month of march 2023 from the region of of chebiaka in Djelfa city After drying for several days, at room temperature and away from sunlight in a well-ventilated place, to preserve the integrity of the molecules as much as possible, the dried leaves were finely crushed in a blender.

1.2-Microorganisms used :

1.3Microbial biological materials:

The antibacterial activity of the extracts studied was tested on strains recovered after isolation

7 bacterial strains that has been tested

Table 5 : bacterial strains

Strain	**Type of bacteria**	**Type of sample**
- *Escherichia coli*	- Bacillus Gram –	- Pus
- *Pseudomonas aeruginosa*	- Bacillus Gram –	- ECBU (urine)
- *Proteus mirabilis*	- Bacillus Gram–	- CSF
- *Serratia Marcescens*	- Bacillus ,Gram –	Intraperitonitis collection
- *Klebsiella pneumoniae*	- Bacillus, Gram –	- Urine
- *Streptococcus spp*	- Cocci .Gram +	- Blood culture
- *Citrobacter Freundii*	- Bacillus , Gram –	- Pleural fluid

1.4Culture media:

Depending on the methods used and the strains; the culture media mainly used are:

- Müeller Hinton Agar;
- Nutrient Agar;
- Brain Heart Infusion Broth (BHIB)

4- chemical solvents:

Table 6 : materials and products

Appliances	Chemical products	Other
Heating mantle Autoclave Magnetic stirrer Balance Incubator Oven Balance Spectrophotometer Rotary evaporator	Methanol Ethanol Ether Chloroform Ethyl acetate 1-butanol Anhydrous magnesium sulfate Distilled water	Support Spatula filter paper Petrie dishes Paper discs Cuvettes Swabs Rack forceps Bunsen burner Micropipette Tip

2 Methods for preparing olive leaves extracts :

2.1.Aqueous extraction (Maceration):

Maceration (solid-liquid extraction) is an operation which consists of diluting plant matter (crushed material) in aqueous methanol to extract the active ingredients (phenolic compounds and flavonoids). The extraction method is based on the protocol described by **(Vuorela, 2005).**

To extract the polyphenols from the plant studied, we macerated 125 g of plant powder in a hydro-alcoholic mixture (methanol/water 35/15; V/V) with stirring from time to time using a magnetic stirrer at room temperature, and protected from light. The operation is repeated three times after each maceration, with the solvent being renewed every 24 hours, the whole is filtered on filter paper. The

hydroalcoholic extracts are combined and evaporated under reduced pressure at a temperature below 45°C until a viscous solution is obtained.

2.2.Liquid-liquid extraction:

Liquid-liquid extraction is the simplest separation method. It passes in one second through the metabolites (solutes) dissolved in the liquid phase immiscible with the first liquid phase. In practice, the solute is generally in the aqueous phase. Organic solvents are used to extract them. This step allows separation of polyphenols according to structure and degree of polymerization; confront them with several solvents from low polarity to high polarity **(Lide, 1996).**

diethyl ether,Chloroform ,ethyl acetate,butan-1-ol

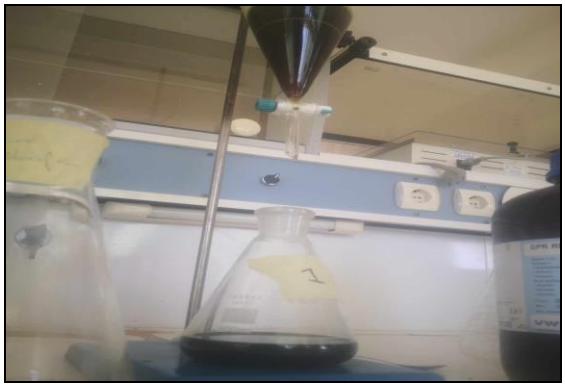

Figure 19 : decontation **(original,2023)**

3.Yield calculation

The yield of an extraction is calculated by the ratio between the mass of the extract obtained and the mass of the processed plant raw material. This yield is expressed as a percentage and calculated by the following formula:

Extraction Yield Formula

EY % =EW/OW 100

- EY : the Extraction Yield (%)
- EW : the extracted weight (g)
- OW : the original weight (g)

4. Antimicrobial activity of plant extracts :

4.1.Preparation of culture media:

a) Nutrient agar (NA):

Nutrient Agar is an ordinary non-selective medium generally used for the growth of bacterial strains**.**according to the technical sheet of the nutrient agar box We prepared a quantity of 1L mixed on a magnetic stirrer with 38g of dehydrated nutrient agar powder (according to the technical sheet of the Nutrient agar box) in an Erlenmeyer the mixture obtained is sterilized in the autoclave at 121°C for 15 minutes.

Figure 20: Preparation of nutrient agar medium **(original,2023)**

b) Mueller Hinton agar (MHA) :

Mueller Hinton agar is a standardized solid medium recommended for studying the sensitivity of bacteria to antimicrobial agents by the diffusion method or agar dilution. We prepared the quantity of 1L mixed with 38g of dehydrated Muller Hinton agar powder (according to the technical sheet of the Muller Hinton agar box), before carrying out sterilization in the autoclave at 121°C for 15 minutes.

Figure21 : Preparation of Mueller Hinton medium **(original,2023)**

4.2.Preparing the discs:

The discs are prepared from Wattman No. 1 paper, with a diameter of 6 mm. Then they are put in a test tube, and sterilized in an autoclave and stored until use.

4.3.Transplanting bacterial strains

The different bacterial strains were subcultured using the streak method, then incubated in an oven at 37°C for 18 to 24 hours in order to obtain a young culture and isolated colonies (bacterial cells in their exponential growth phase).

4.4.Preparation of the bacterial suspension:

In order to obtain a bacterial suspension, sterile screw cap tubes containing sterile physiological water were inoculated from the pure and young bacterial cultures (on GN) subsequently prepared; these tubes were vortexed to properly disperse the clumps of bacteria, a few drops of this culture was diluted 5ml in sterile physiological water to obtain a suspension

4.5.Disk diffusion method on agar (antibiogram):

The method is recommended by Clinical and Laboratory Standards Institute (CLSI)

a) in solid medium :

The agar diffusion method is the equivalent of the antibiogram where the antibiotics are replaced by our studied extracts.

Material and Methods

This method is carried out by depositing sterile discs (6mm in diameter, Wattman No. 1 paper) impregnated with the extracts studied on a Mueller Hinton (M.H) agar medium previously poured into petri dishes and seeded with 1ml of tested microorganisms (107 cfu/ml).

After incubation, the results are read by measuring the diameter (in mm) of the inhibition zone (clear zone free of colonies around the disc) using a caliper.

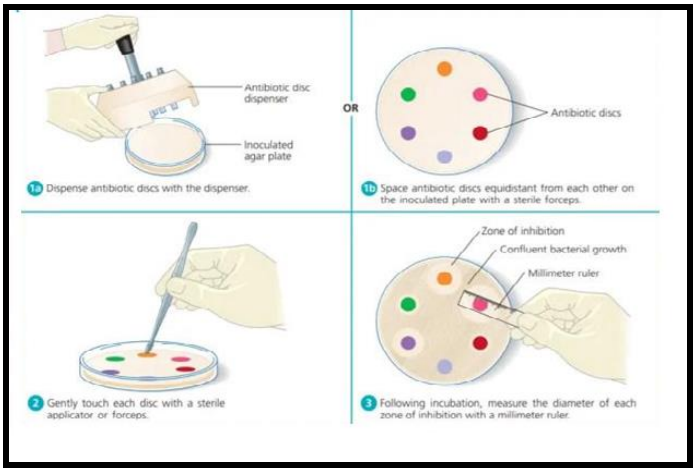

Figure 22 : Kirby Bauer Disc Diffusion Method

The sensitivity of the different strains to the extracts is classified, according to the diameter of inhibition, according to the following criteria:

• Not sensitive (-): Ø < 8 mm;

• Sensitive (+): Ø between 9-14 mm;

• Very sensitive (++): Ø 15-19 mm;

Extremely sensitive (+++): Ø > 20 mm . **(Ponce et al., 2003).**

Figure23 : Size determination **(original,2023)**

5. Determine the nature of antibacterial activity (bactericidal or bactericidal):

5.1. Determination of the Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration (MIC) in general is the lowest concentration of antimicrobial capable of inhibiting any visible growth after an incubation time of 18 to 24 hours. Here its determination was made from the measurement of the turbidity induced by the growth of the germs studied. The MIC will therefore correspond to the lowest concentration for which there is absence of turbidity. Therefore it is the tube where its final and initial optical densities are equal.

We use the dilution method in a liquid medium, this is carried out only for the most active concentration in sensitivity.

Figure 24:serial dilution **(original ,2023)**

5.2.Determination of the Minimum Bactericidal Concentration (MBC):

The minimum bactericidal concentration (MBC) is defined as the lowest concentration at which bacteria are destroyed

For its determination, make a subculture from the experimental tubes determined having presented total inhibition on the nutrient agar in streaks then incubated at 37°C for 24 hours. Observe with the naked eye the presence or absence of bacterial growth.

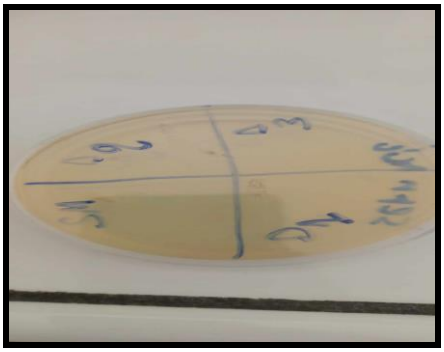

Figure 25: example of bactericidal activity **. (original,2023)**

Chapter 02:
Results and discussion

Results and discussion

Results and discussion :

In this part we will first present the principles of the experiments we carried out, and then we will attempt to discuss these results in comparison with similar studies

1.Extraction yield:

Successive extractions using solvents of increasing polarity make it possible to separate the compounds from the plant material according to their degree of solubility in the extraction solvent. The yield expressed as a percentage is calculated As below:

$$\mathbf{EY}\ \% = EW/OW * 100$$

- EY : the Extraction Yield (%)
- EW : the extracted weight (g)
- OW : the original weight (g)

the yield (%) of the crude extracts of olive leaves (Olea europaeaL) Chemlal variety

Table 7 : the Extraction Yield

Matériel végétal	Extrait	Rendement
125g	ethyl ether extract	2.15%
	Chloroformic extract	0.88%
	Ethyl acetate extract	4.26%
	Butanolic extract	1.58%
	Aqueous extract	13.70%

In general, dry extract yields vary depending on extraction parameters: temperature, extraction solvent, particle size and solvent diffusion coefficient (Majhenic L., 2007). In our study, the extract had Aqueous extract the best yield (13.7%), followed by ethyl acetate (4.26 %) , ethyl ether extract

(2.15%), n-butanolic (1.58 %) and Chloroformic extract (0.88 %).

Results and discussion

2.Evaluation of antibacterial activity

The antibacterial activity of the extracts obtained is determined by the diffusion method in agar medium. It allowed us to highlight the antibacterial power against our seven gram-positive and gram-negative bacteria.

The results obtained are collected in chart below :

a) Aqueous extract

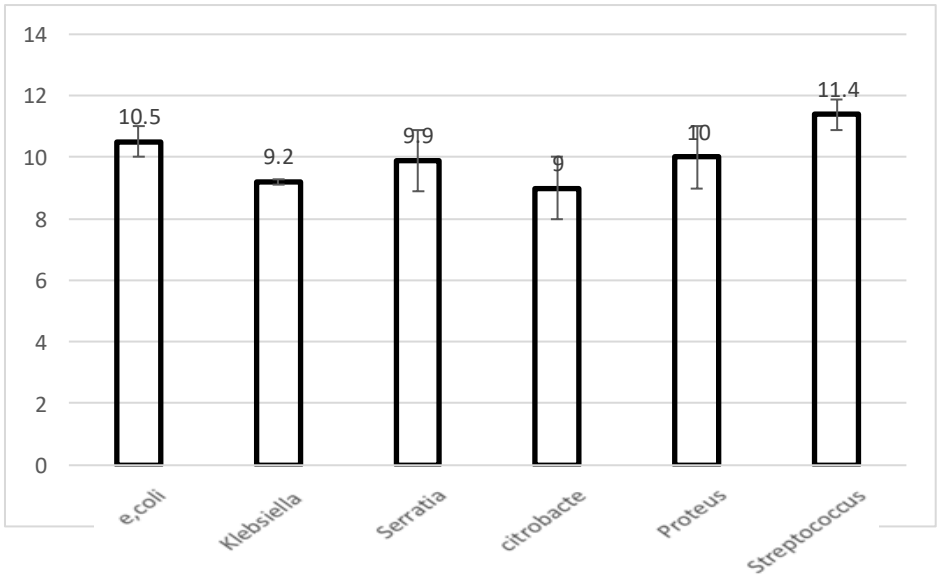

Figure 26: Inhibition diameter of Aqueous extract against tested strains

Results and discussion

b) Ethyl acetate extract :

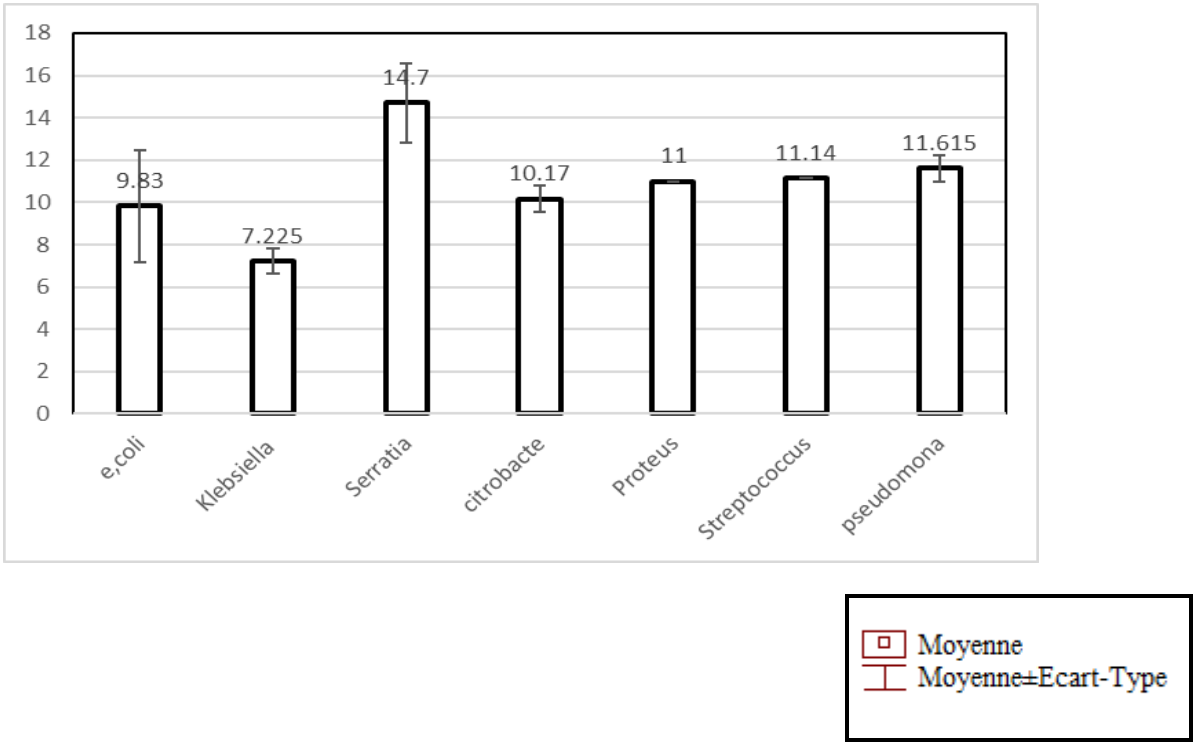

Figure 27 : Inhibition diameter of Ethyl acetate exrtract against tested strains
(The vertical bars represent standard deviations)

c)Ehyl ether extract :

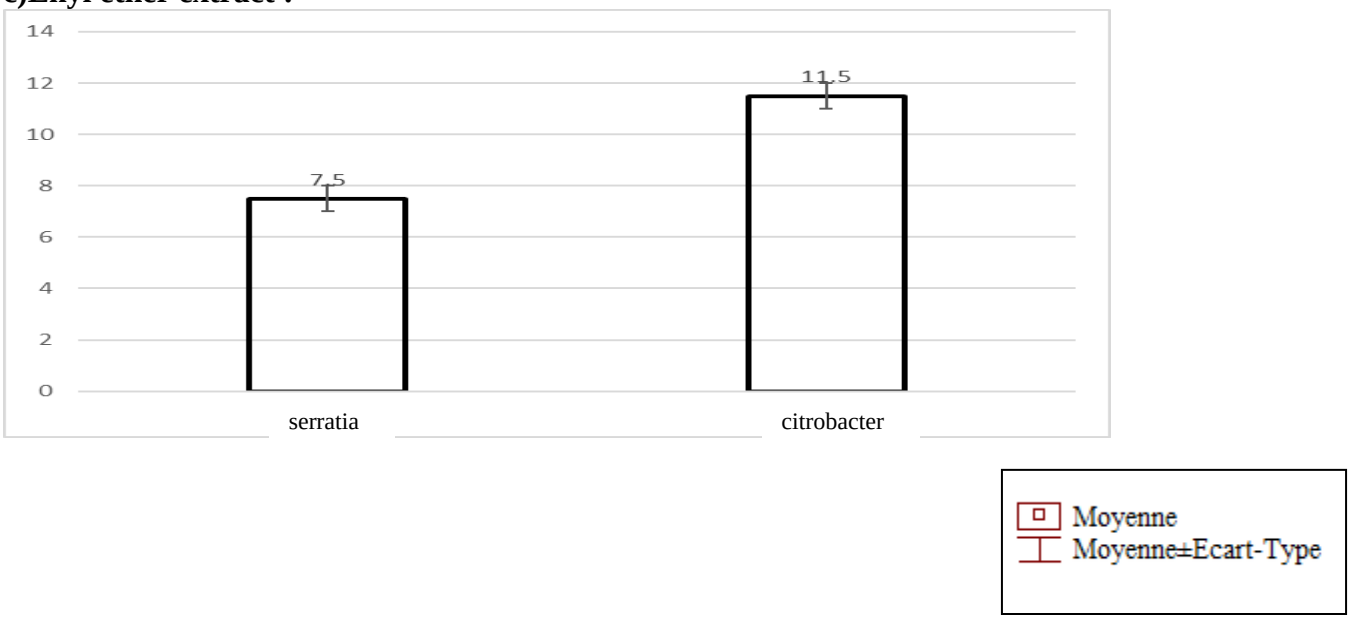

Figure 28: Inhibition diameter of Ethyl acetate exrtract against tested strains

d) Chloroform extract :

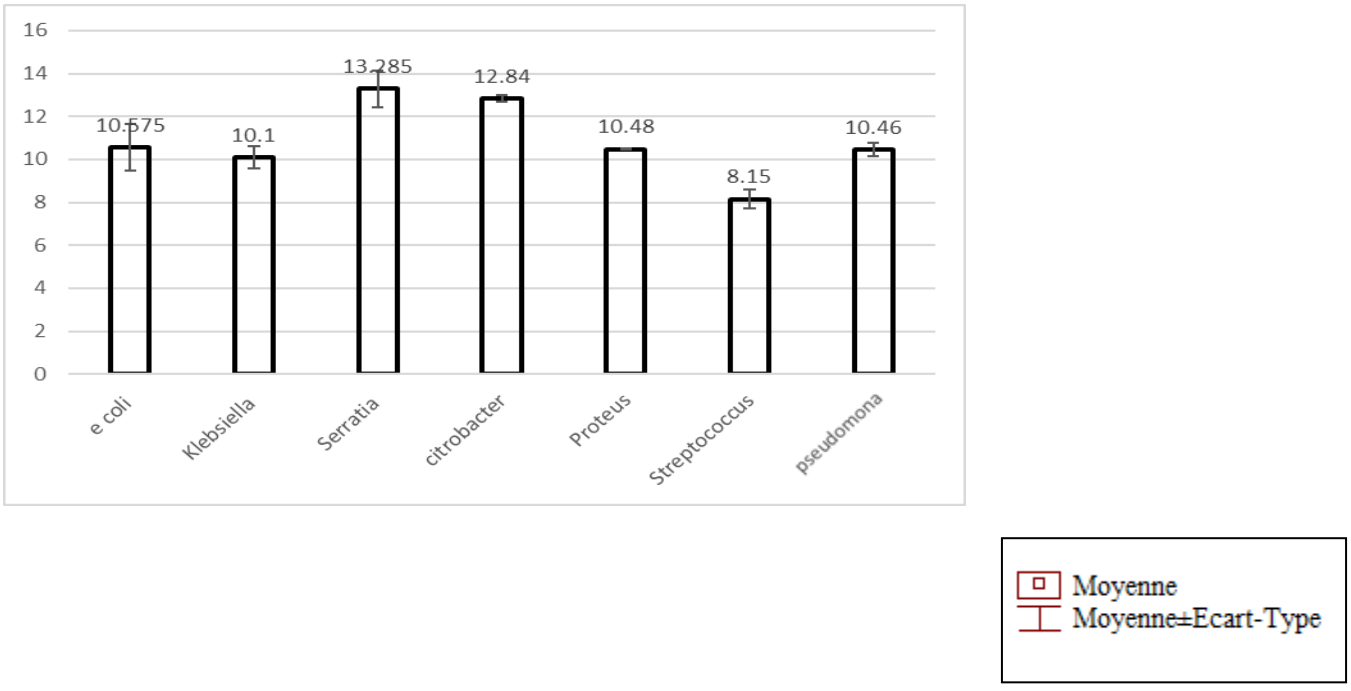

Figure 29 : Inhibition diameter of Ethyl acetate exrtract against tested strains

Butan-1-Ol Extract :

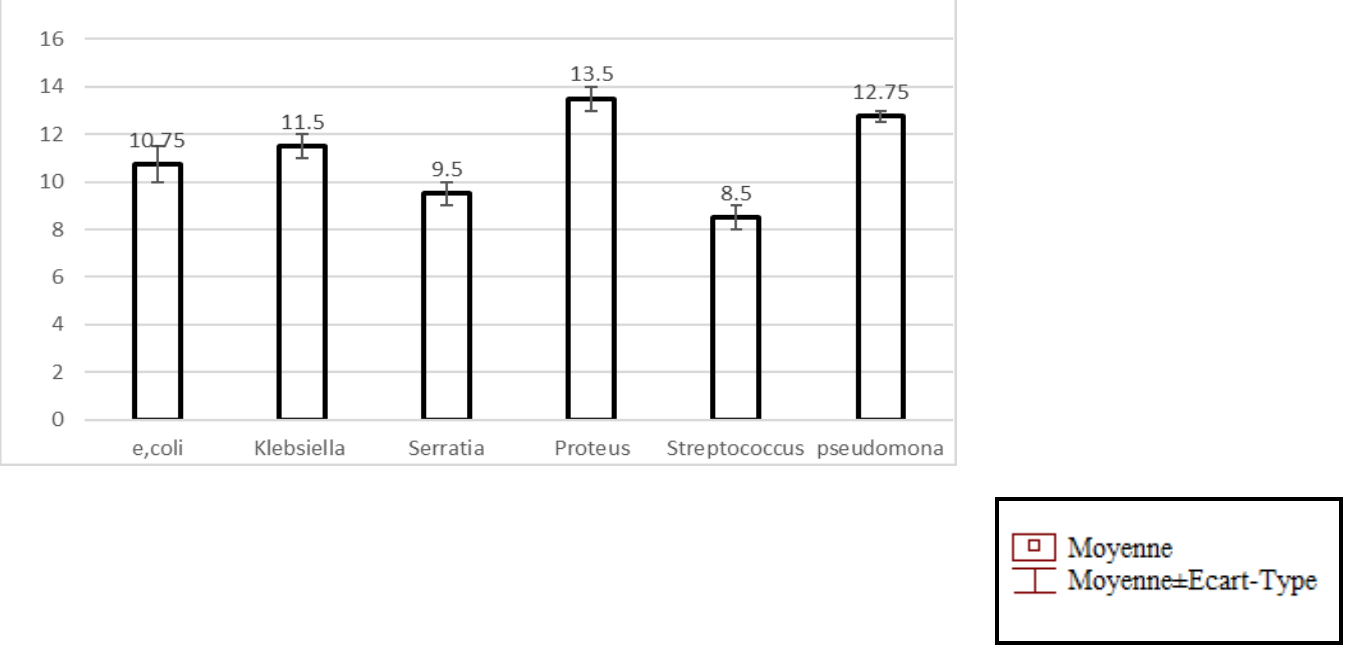

Figure 30 : Inhibition diameter of Ethyl acetate exrtract against tested strains

3.Evaluation of antibacterial activity:

3.1. Results of the CMI and the CMB:

The determination of MIC and CMB were carried out only for the strains which showed average sensitivity to concentrations

table 8: Aqueous Extract

Extract Mg/ml	*Escherichia coli*	*Klebsiella pneumoniae*	*Serratia marcescens*	*Citrobacter freundii*	*Streptococcus pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
SM	+	+	+	+	+	+	-
D1	+	+	+	+	+	+	-
D2	+	+	+	+	+	+	-
D3	+	+	+	+	+	+	-

Results and discussion

Table 9 : Ethyl Ether Extract

Extract Mg/ml	*Serratia marcescens*	*Citrobacter freundii*
SM	+	+
D1	+	+
D2	+	+
D3	+	+

Table 10: Butan-1-Ol Extract

Extract Mg/ml	*Escherichia coli*	*Klebsiella pneumoniae*	*Serratia marcescens*	*Streptococcus pneumoniae*	*Proteus mirabilis*
SM	-	+	+	+	+
D1	-	+	+	+	+
D2	-	+	+	+	+
D3	-	+	+	+	+

Table 11: Chloroform Extract

.EXTRact Mg/ml	*Escherichia coli*	*Klebsiella pneumoniae*	*Serratia marcescens*	*Citrobacter freundii*	*Streptococcus pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
SM	+	+	+	+	+	-	+
D1	+	+	+	+	+	-	+
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+

Table 12 : Ethyl acetate extract

Extract Mg/ml	*Escherichia coli*	*Klebsiella pneumoniae*	*Serratia marcescens*	*Citrobacter freundii*	*Streptococcus pneumoniae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
SM	+	+	-	+	-	-	+

D1	+	+	-	+	+	+	+
D2	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	+

Table 13 : summary of MIC vlues of the five OLE mg/

Strrainss/OLE	Aquos	Butanolic	Acetat	Chloroforrm	Dethyl either
Proteus mirabilis.	/	/	1	0.5	/
Serratia Marcescens	/	/	1	/	/
Pseudomonas aeruginosa	0.125	/	/	/	/
Escherichia coli	/	1.25	/	/	/
Streptococcus spp	1	/	1	/	/
Serratia Marcescens	/	/	/	/	/
Citrobacter Freundii	/	/	/	/	/

Table 14 : summary of MBC values of the two OLE on the four strain tasted.

Extract strain	Aquos	Acetat
Escherichia coli	/	+
Serratia Marcescens	/	-
Pseudomonas aeruginosa.	+	-
Streptococcus spp	+	+

Results and discussion

Test carried out :

Positive: bacteriostatic effect (presence of bacterial growth);

Negative: bactericidal effect (absence of bacterial growth)

The acetate extract has a Streptococcus pneumoniae *Serratia marcescens* The Minimum Bactericidal Concentration is1 Mg/ml,

Proteus mirabilis The Minimum Bactericidal Concentration is 1 Mg/ml

Streptococcus spp. The Minimum Bactericidal Concentration is 1 Mg/ml

The Aqueous extract has a bacteriostatic activity against Pseudomonas aeruginosa The Minimum Bactericidal Concentration is 0.125Mg/ml.

Antibacterial activity depends on the physicochemical characteristics of the phytobiotic compounds and the strains used. The antibacterial power of crude plant extracts is dependent on their chemical compositions **(Ben Sassi Aet al., 2007).**

In addition to their chemical composition, antibacterial activity is linked to the polarity of bioactive substances. The less polar extracts, such as ether, are always less sensitive compared to polar extracts because the OH hydroxyl groups are more active against microbial agents **(Chabot Set al., 1992).**

The choice of pathogenic strains also influences the antibacterial power of the extracts. In general, Gram(-) bacteria are more resistant than Gram(+) bacteria, thanks to the structure of their outer membrane **(Pool EK., 2001).**

In our case, all the extracts showed a more or less significant antibacterial power against Gram negative bacteria. This power depends on the nature of the extract, its concentration and the type of strains used. The effect of an extract is probably due to the synergy between many components which, when separated, become individually inactive.

Conclusion

conclusion

Conclusion :

Recently, the use of medicinal plants in herbal medicine has received great attention in biomedical research

The objective of this study is to study the antibacterial activity of the five extracts of olive leaves Olea europaea L. chemlal variety with increasing polarity: diethyl ether, chloroform ethyl acetate and n-butanol against seven strains. Wich they are :

- ***Citrobacter Freundii***
- ***Streptococcus spp***
- ***Klebsiella pneumoniae***
- ***Proteus mirabilis***
- ***Serratia Marcescens***
- ***Escherichia coli***
- ***pseudomonas aeruginosa***

The acetate extract has a bactericidal activity in concentration of 0.1 Mg/ml against pseudomonas aeruginosa and, Serratia Marcescens and bacteriostatic effect against Escherichia coli and Streptococcus spp.

Aqueous extract :has a bacteriostatic activity against proteus.in all of the four Different

concentration According to these results, Aqueous extract can be used as an antimicrobial agent for *proteus,Mirabilis.*

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ملخص

تشكل النباتات الطبية مصدرًا غنيًا ومتنوعًا المكونات الحيوية والتي لها العديد من الاستخدامات في عدة مجالات وخاصة الصيدلانية منها .وفي ظل طاهرةاكتساب البكتيريا للمقاومة ضد بعض المضادات الحيوية. لذلك نحن مهتمون بدراسة فعالية خمس مستخلصات من اوراق الزيتون من صنف شملال ضد سبعة بكتيريا ممرضة .كا عامل ضد للبكتيريا .وقد ظهرت نتائج ايجابية حيث مستخلص acetate له فعل قتل لبكتتيريا ***Pseudomonas aeruginosa*** و ***Serratia Marcescens***

Abstract

Medicinal plants constitute a rich and diverse source of biological components that have many uses in several fields, especially pharmaceutical ones. Under pure conditions, bacteria acquire resistance to some antibiotics. Therefore, we are interested in studying the effectiveness of five extracts from olive leaves from the Chemlal variety against seven pathogenic bacteria. As an antibacterial agent. Positive results have emerged, as acetate extract has a killing effect on *Pseudomonas aeruginosa* and *Serratia Marcescens* bacteria.

Résume

Les plantes médicinales constituent une source riche et diversifiée de composants biologiques qui ont de nombreuses utilisations dans plusieurs domaines, notamment pharmaceutiques. Dans des conditions pures, les bactéries acquièrent une résistance à certains antibiotiques. Nous souhaitons donc étudier l'efficacité de cinq extraits de feuilles d'olivier de la variété Chemlal contre sept bactéries pathogènes. En tant qu'agent antibactérien. Des résultats positifs ont été obtenus, car l'extrait d'acétate a un effet mortel sur les bactéries ***Pseudomonas aeruginosa*** **et** ***Serratia Marcescens.***