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The antioxidant and antibacterial activity of phenolic extracts and essential oil of *Myrtus communis*

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THE JURY

Chair : Mr. BAHRI Fouad Pr Univ. Mostaganem

Coach : Mr. TERKHI Mohammed Cherif MCA Univ. Mostaganem

Reviewers : Mrs. LAISSOUF MCB Univ. Mostaganem

Co-coach : Melle KEDDAR Fayza PhD candidate Univ. Chlef

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Dedication

In the name of Allah, The Most Gracious, The Most Merciful

All Praise is due to Allah alone, the Sustainer of the entire world

I dedicate this work

To my beloved parents whose enduring love guided me all along

To my brothers and Sisters

To my beloved

To my friends TALEB Ali and MENAD Rahma & FACI Hadja

To all who helped and encouraged me

My deepest gratitude for their support and encouragement

YOUSFI Abdelatif

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Sommary

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Abbreviation

- ANP: acute pyelonephritis.
- ATCC: triphenyle tetrazolium chloride.
- CFU: colony forming unite.
- DMSO: Dimethyl sulfoxide.
- DO: optical dencity
- DPPH°: diphrnyl-1-picryl hydrozyl.
- E.colie: echilechia colie.
- EO: Essontial oils.
- HS: hesadspace.
- HS-SPME: headspace slide-phase microextraction.
- IPP: isopentenyle diphosphate.
- M.communis: myrtus communis.
- MTI: minimum inhibitory concentration.
- PNC: chronic pyelonephritis.
- RNA: acide rubose nucliaire.
- SM: Secondary Metaboiles.
- UCE: urinary cytobacteriological examination.
- UTI: urinary tract infection.
- UV: Ultraviolet.
- VPN: predictive value.

Introduction

INTRODUCTION

Nowadays, urinary tract infections are a real public health problem, as many patients are currently victims of them, especially women, sometimes repeatedly and with a significant risk of complications (H. Leroy, 2012).In some cases, urinary infection can lead to lithiasis of infection. This disease is characterized by the formation of insoluble solid deposits in the excretory system under the influence of bacterial urease.

The eradication of infection is based on adequate and sufficiently prolonged antibiotic therapy to ensure the long-term sterilization of the urinary tract. The introduction of antibiotics into therapeutics has revolutionized the treatment of infectious diseases. Unfortunately, we are now caught up in antibiotic resistance, this problem is a serious threats to global health (M. Chraibi, 2019).

Infections caused by antibiotic-resistant bacteria are difficult and sometimes impossible to treat, making them one of the major public health problems of our time(Tse Sum Bui, 2022). Faced with this problem, the search for new naturally occurring anti-infective substances has become an economic and public health interest. They contain a wide variety of secondary metabolites capable of inhibiting or slowing the growth of bacteria **(**Griffin, *& al;* 1999, Bouyahya, *& al;* 2016). They act in depth by stimulating good reactions without side effects. Their effectiveness depends above all on the choice of these plants and their dosage.

Natural substances and plants in particular represent a huge source of bioactive molecules with often very original structures whose complete and cost-effective synthesis is often difficult to achieve (Djahra, 2014).

Currently, the Algerian population uses a good number of aromatic plants andin the treatment of diseases. For our part, our choice was*Myrtus communis* (Arayhan).

In recent years, this plant has received increasing attention for its propertiesbiological, such as its antioxidant activities (Dellaoui, &al; 2018), anti-inflammatory (Hosseinzadeh & al., 2011), cytotoxic and antimicrobial (Belmimoun *& al*; 2020).

Several studies have shown that myrtle leaf extracts containsignificantly greater amount of total phenolic compounds (Boroujeni, 2018), polyphenols are natural compounds widely distributed in the plant kingdomwhich are of increasing importance in particular because of their beneficial health effects(Koechlin, 2006), the identification and quantification of

polyphenolic compoundsin the leaves of *Myrtus comrnunis* seem interesting from a biological andecophysiological.

This work is part of the research and exploitation of bioactive substancessuch as natural substances with antibacterial activity. The objective of this workis to conduct a study and evaluate in vitro the antimicrobial activity ofextracts from the leaves of *Myrtus communis* against pathogenic strains causing infectionurinary tract.It will also be necessary to characterize the chemical groups to explain the therapeutic effects.We report data on procedures extraction and purification, and identification of compounds polyphenolic in the leaves of *Myrtus communis.*

The objective of this study is to extract phenolic compounds and essential oil of *Myrtus comrnunis* to evaluate their antimicrobial activity in vitro.

This manuscript is divided into two parts:

The first part represented by a bibliographic study divided into three chapters the first one concerning the studied plant *Myrtus comrnunis*, followed by a chapter on the secondary metabolites and a third chapter on urinary tract infection.

The experimental part is the second part of the manuscript composed of two chapters. The first will focus on the equipment and techniques used in this study. The second chapter is devoted to results and discussion. The manuscript ends with a conclusion and perspectives.

Part 1
Bibliography

Chapter I: medicinal plant (Myrtus communis)

I.1. Generality

Myrtle (*Myrtus communis*.) is an aromatic medicinal plant, typical of the coastal areas of the Mediterranean regions, such as North Africa or Southern Europe, but it is also present in South America, Australia, and in some areas of Himalaya (Alipour *& al;* 2014). It belongs to the *Myrtaceae* family, which includes about 3,000 species, and grows spontaneously as an evergreen shrub or a small tree. The plant can reach a height of 2.5 m, with a full head deeply covered by branches and small leaves; flowers are starry, scented, and can be white or pink, whereas berry fruits are edible, small, with a round shape and many seeds inside, generally blue-black, even if some varieties have white-yellow fruits(Figure 01), and ripen in autumn, between October and February. Insects do pollination and birds spread seeds in the environment (Alipour *& al;*2014).

In ancient medical traditions, different parts of myrtle, in particular berries, leaves, flowers, and essential oils, have been extensively used as a remedy for treating cough, gastrointestinal disorders (peptic ulcers, diarrhea, and hemorrhoids), urinary diseases (urethritis), and skin ailments (reddened skin), as well as for inactivating microorganisms and for the wound healing (Aleksic *& al;* 2014).

All these beneficial properties are essentially due the wide range and amount of bioactive compounds, such as polyphenols, flavonoids, anthocyanins, phenolic acids, lignans, tannins, antioxidants, organic acids, fatty acids, and minerals, present in the different parts of the myrtle plants (Aleksic *& al;* 2014). Currently, it is mainly used in culinary practice and in cosmetic, pharmaceutical, and food products: for example, one of the most known derived product is the famous Sardinian sweet liqueur, called "Mirto di Sardegna," that is made from the hydroalcoholic infusion of the berries, contains high amount of anthocyanins and tannins, and is recognized as a geographical indication of the island (EC Reg. no. 110/2008).

However, to the best of our knowledge, only few studies have evaluated the nutritional and phytochemical composition of this plant, as well as its health benefits. The aim of this work is to present and summarize the nutritional and phytochemical composition of the different parts of myrtle plant, as well as their antioxidant capacity. The biological effects of myrtle will be also discussed, paying special attention to the most common human diseases(Aleksic *& al;* 2014).

Figure 01: Details of myrtle (Myrtus communis L.) tree (a), branches (b), leaves, and unripe fruits (c)(Giampieri, F 2020).

I.2.Distribution

Myrtle *M.communis*. is a common part of typical Mediterranean flora. The plant grows abundantly from the northwestern to the eastern Mediterranean, including bordering countries and western Asia, as well as Aegean regions (Baytop, 1997). Myrtle is native to southern Europe, North Africa and west Asia. It is also distributed in Southern America, northwestern Himalaya and Australia. Myrtle is cultivated in gardens, especially in Northwest Indian region, because of its fragrant flowers (Nadkarni, 1989).

Being widespread throughout the Mediterranean region(Figure 2), the species is one of the most important evergreen shrubs in the Mediterranean maquis. In Italy it grows along the coasts and on the internal hills and it is abundant especially on the islands, where it represents one of the most characteristic species (Cannas *& al.* 2013). In Portugal, myrtle grows wild mainly in the central and southern parts of the country. The genus Myrtus, in Tunisia, is represented by only one species, *M. communis*., which grows wild in the coastal areas, the internal hills, and the forest areas of northern Tunisia. Two myrtle varieties are described in old local Tunisian flora: *M. communis* var. italica L. and *M. communis* var. baetica L. (Pottier-Alapetite *& al*. 1979), which possesses the same vegetative characters. The morphological difference between the two varieties regards to size of fruits and leaves. This herb grows spontaneously Iran, Spain, France, Greece, Turkey, Algeria, Morocco, Croatia and Montenegro (Berka-Zougali,*& al*; 2012).

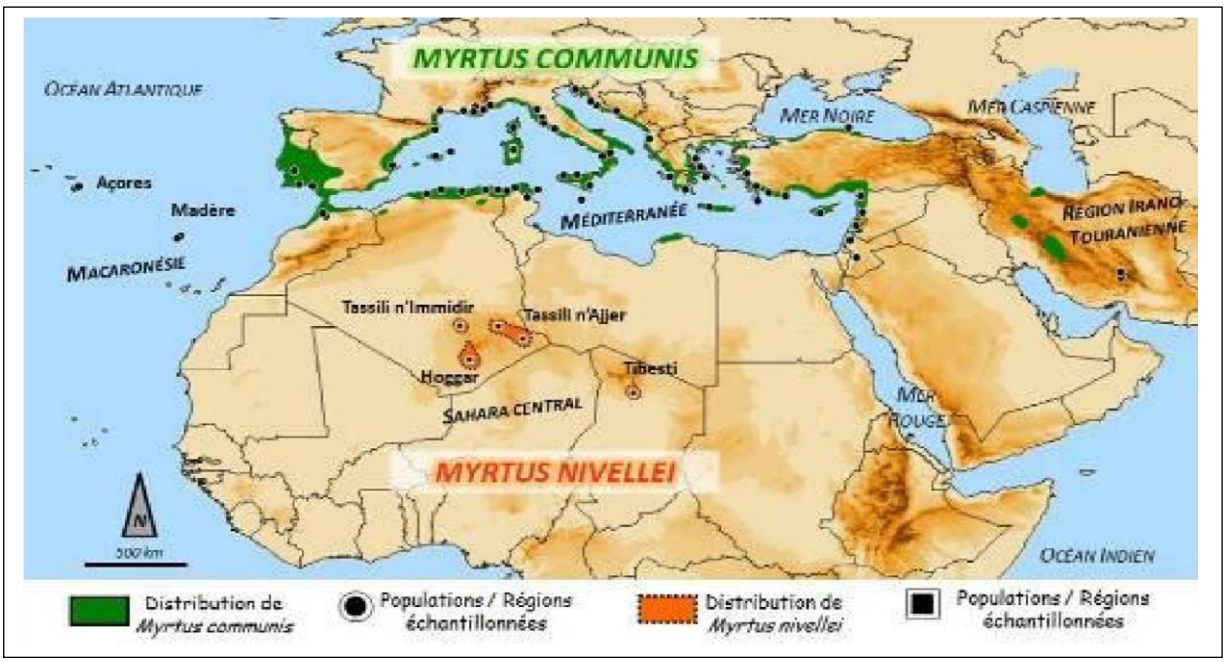

Figure 2 : Distribution du genre *Myrtus* (Migliore, 2011)

I.3. Traditional application

Myrtle has been used since ancient times as a spice, as well as for medicinal and food preparation purposes. Myrtle as a spice finds no wide application because of its bitterness, despite the pleasant odor. The taste is very intense, quite unpleasant and strongly bitter, so its culinary application is limited to the region of origin, such as Italy. In Italy, especially in Sardinia, berries and leaves are used to produce two well-known liquors (Mirto Rosso and Mirto Bianco, respectively) (Messaoud *& al*; 2012). Foods flavored with the smoke of myrtle are common in rural areas of Italy or Sardinia. However, some parts of the plant are used in the food industry, for flavoring meat and sauces, and its berries and leaves are mostly employed for the industrial formulation of sweet liquors with advertised digestive properties.Its leaves are very fragrant and have been extensively used in the perfume and cosmetic industries, particularly in Portugal as well as Turkey (Aleksic, *& al;* 2014).

It is traditionally used as an antiseptic, disinfectant and hypoglycemic agent. In Turkey myrtle leaves as well as fruits have been used as an antiseptic medicine in villages. Similarly, in Italian folk medicine, the fruit of this plant is used in the treatment of many types of infectious disease, including diarrhea and dysentery; the leaves are used as antiseptic and anti-inflammatory agent, as well as a mouthwash, for the treatment of candidiasis. The essential oil obtained from myrtle leaves has been used in the treatment of lung disorders. In traditional medicine, myrtle is frequently consumed as an infusion and decoction (Floch,1983). Generally, in folk medicine, a decoction of leaves and fruits is used orally for the treatment of stomach aches, hypoglycaemia, disbiosis, cough, constipation, poor appetite, as well as also externally for wound healing(Aleksic, & al; 2014).

Different parts of the myrtle plant traditionally have assorted specific applications (Table 1). Infusions made from the leaves and young branches are approved to be stimulant, antiseptic, astringent and hypoglycemic, and they are considered to be a health remedy for asthma, eczema, psoriasis, diarrhea, gastrointestinal disorders and urinary infections (Ziyyat *&al;* 1997). The leaf decoction is used for vaginal washing, enemas and against respiratory diseases, while decoction from the fruits is used as antidiarrheal, antihemorrhoidal agents and in mouth and eyes disease treatment (Ziyyat *& al*; 1997). Flowers are traditionally used against varicose veins, and for preparing capillary lotions **(**Floch, 1983).

Table 01. Application of different *M. communis* .parts.

***M. communis* . part**		**Traditional application**	**References**
Leaves		Food preparation – liquors, flavoring meat and sauces; Perfume and cosmetic preparation – hair tonic and stimulant; Medicine – orally used as antiseptic, anti-inflammatory agent, laxative, analgesic, haemostatic agent and externally for wound healing	Messaoud & *al* ;(2012)
Berries		Food preparation – liquors, flavoring meat and sauces; Medicine – used also orally for infectious disease such as diarrhea and dysentery and externally for skin diseases and wound healing	Messaoud & *a*l ; (2012)
Brunches		Medicine – remedy for asthma, eczema, psoriasis, diarrhea, gastrointestinal disorders and urinary infections, administrated orally; applied by inhalation and externally	Ziyyat & *al* ; (1997)
Flowers		Medicine – against varicose veins and for preparing capillary lotions for external use	Floch,(1983)

I.4. Chemical composition of *(Myrtus communis.)*:

M. communis. main secondary metabolites are polyphenols and essential oils. *Myrtus* species have been reported as very rich in volatile oils phenolic acids, flavonoids tannins, anthocyanin pigments and fatty acids. Previous studies on *M. communis L*. aerial parts have also revealed the presence of several specific chemical compounds. For example, the dried leaves of this herb contain 1,8-cineole (13.5–19.6%), linalool (7.7–15.8%), linalyl acetate (2.5–6%), terpineole, terpinolene, tannins and flavonoid compounds. Leaf and flowers contain essential oils, phenolic acids, flavonoids and tannins. Berries are composed of tannins, anthocyanins (0.2–54%), fatty and organic acids (9–52%), and its content depends on used extraction solvent and/or ripening period. It is evident that the content of these compounds also differs depending on the plant part used (Table 2, Table 3), but generally the most common compounds found in myrtle leaves, steams and flowers are α-pinene (~10–60%) and 1,8-cineole (~12–34%) (Aleksic,*&al*. 2014).

Table 02: Classes of major *Myrtus communis L*. essential oils compounds, their bioactivities and content in different plant parts

Chemical classes	**Chemical subclasses**	**Sublaclasses content in leaf, steam and flower (%)**[a]	**Major myrtle essential oil compounds**	**Major compounds content in myrtle leaf, steam and flower (%)**		**Bioactivity**	**Refernces for bioactivity**
Terpenes	Monoterpene hydrocarbons $C_{10}H_{16}$	L 65–67.5	α-Pinene	57–60	Leaf	Antimicrobial	Kalemba & *al* ;(2003)
		S 30–31	Limonene	9–11	Flower		
		F 40–42.5	Myrcene	0.6–0.8	Flower		
			p-Cymene	2.5–3.5	Steam		
	Sesquiterpene hydrocarbons $C_{15}H_{24}$	L 0.4–0.6	α-Caryophyllene	0.2–0.3	Steam	Antiviral	Djilani & *al* ;(2012)
		S 6.6–7.5	Germacrene-D	2.5	Steam		
		F 2–3.7					
Terpenoids	Oxygenated monoterpenes	L 26	Linalool	4.5–7	Flower-steam	Antmicrobial (mostly antibacterial)	Kalemba & *al* ;(2003)
		S 52–53	Myrtenol	0.1–	Steam		

Chemical classes	Chemical subclasses	Sublaclasses content in leaf, steam and flower (%)[a]	Major myrtle essential oil compounds	Major compounds content in myrtle leaf, steam and flower (%)		Bioactivity	Refernces for bioactivity
				0.6			
		F 33	1,8-Cineole	Dec-33	Flower-steam		
	Oxygenated sesquiterpene		Nerol	0.15	Steam		
			Geraniol	1.5–2	Flower		
		L 0.1–0.8	Caryophyllene oxide	1.5	Steam	Antimicrobial	Kalemba *&al* ;(2003)
		S 0.1–0.2	Spathulenol				
		F 13.5–15		0.6	Flower		
Phenyl-propanoids		L 0.5–0.7	Methyleugenol	4–4.5	Flower	Antioxidant, antimicrobial	Korkina,(2007),
		S 2–2.5					
		F 3.5–4					

Table 03: Classes of *Myrtus communis* L. extracts compounds, their bioactivities and content in different plant parts.

Chemical classes	Chemical classes content in myrtle extracts (%)[a]	Chemical subclasses	Major myrtle extracts compounds	Myrtle organ	Bioactivity	Refernces for bioactivity
Phenolic acids	L 12–15		Gallic acid	Leaf, steam, flower, berry	Antioxidant, antimutagenic, antitumor, antibacterial	Othman & *al* ;(2007)
	S 38–40		Cafeic acid			
	F 38–40		Syringic acid			
	B 8–10		Vanillic acid			
			Ferulic acid			
Tannins	L 79–82 S in traces F 60 B 53–56	Hydrolysable tannins	Gallotannins	Leaf, flower	Antibacterial, anti-cancer, antiviral, inhibition of lipid peroxidation	Funatogawa & *al* ;(2004)

Chemical classes	Chemical classes content in myrtle extracts (%)[a]	Chemical subclasses	Major myrtle extracts compounds	Myrtle organ	Bioactivity	Refernces for bioactivity
		Proanthocyanidins	Delphinidin-3-O-glucoside	Leaf, flower, berry	Antioxidant	Montoro *& al* ; (2006a)
			Petunidin-3-O-glucoside			
			Malvidin-3-O-glucoside			
			Cyanidin-3-O-glucoside			
			Peonidin-3-O-glucoside			
			Delphinidin-3-O-arabinoside			
			Petunidin-3-O-arabinoside			
			Malvidin-3-O-arabinoside			
Flavonoides	L 8–10 S 61–63 F in traces B 35–39	Flavonols	Myricetin	Leaf, steam, berry	Antibacterial, antiviral, antioxidant, anti-inflammatory, anti-allergic, antithrombotic, vasodilatory, anti-mutagenic, neoplastic, anti-cancer	Montoro *& al* ;(2006a)
			Myricetin-3-O-galactoside			
			Myricetin-3-O-ramnoside			
			Quercetin	Steam, Berry		
			Quercetin-3-D-galactoside			
			Quercetin-3-D-rahmnoside			
		Flavanols	Catechin	Leaf, steam, berry		

Chapter II: secondary metabolites

II.1 Secondary metabolites

II.1.1 Definition

Secondary metabolites are compounds that are not required for the growth or reproduction of an organism but are produced to confer a selective advantage to the organism. For example, they may inhibit the growth of organisms with which they compete and, as such, they often inhibit biologically important processes. Fungal secondary metabolites are a major source of medically important compounds, from antibiotics such as penicillin to the anti-cholesterol compound lovastatin. The sequencing of the genomes of *A. nidulans* and other species of Aspergillus revealed that they have many genes predicted, on the basis of sequence, to be involved in secondary metabolism. These genes, moreover, are clustered together, and it has been demonstrated that individual clusters generally encode the genes for a single secondary metabolite biosynthetic pathway. These secondary metabolite biosynthetic clusters are potentially a very important source of medically useful compounds, but most of the clusters are cryptic – not expressed under normal laboratory growth conditions. Molecular genetic methods for activating secondary metabolite genes or entire clusters are being developed, however. This has revealed new A. nidulans secondary metabolites and is establishing A. nidulans as a model system for studying secondary metabolism (Yaegashi & *al*; 2014).

II.1.2 Functions of secondary metabolites

Plant SMs are important compounds that add color, taste, and odor to plants and also mediate plant responses to adverse environmental conditions. A number of factors cause significant perturbations in the production of SM in plants. The endogenous levels of different SMs vary among different plant species and also within the same plant species. A number of cellular and biochemical factors influence the storage and transportation of SM. Developmental factors influence the initiation and subsequent differentiation of particular cellular structures involved in the biosynthesis and storage of SM. Furthermore, the endogenous levels of SM are also influenced by a number of environmental stresses such as nutrient deficiencies, wounding, metal ions, ultraviolet (UV) radiation, light, circadian rhythm, seasonality, salinity, drought, and temperature. Apart from this, the endogenous concentration of SM is associated with the metabolic pathway of the particular SM and growth conditions (Akula,& Ravishankar, 2011). The concentration of SM is also affected by biotic factors (pathogen attack) and thereby mediates plant defense mechanism. For

instance, there exists a significant variation in the levels of phenolics in plants in response to environmental stresses such as light intensity and nutrient availability (Verma *& al;* 2015).

The endogenous levels and storage of SM is also influenced by genetic factors. A number of factors influence the expression of genes related to the biosynthesis of SMs. These factors play an essential role in mediating the biosynthesis, storage, and endogenous concentration of various SMs. In vitro tissue culture may also trigger an increase in the biosynthesis of different SMs via signaling molecules and plant growth regulators. This clearly indicated that the endogenous levels of SM can be changed. Overall, factors that trigger alterations in SMs fall into four categories, viz., environmental factors, morphogenetic factors, ontogenetic factors, and genetic factors. Among these factors, environmental factors are the key determinants for the fluctuations in plant SMs (Verma *& al*; 2015).

II.1.3 Secondary metabolites of plants

Plant secondary metabolites represent highly economically valuable products. These are used as high value chemicals such as drugs, flavors, fragrances, insecticides, dyes, etc. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have in vitro antimicrobial properties. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. About 25,000 terpenoids are known as secondary compounds and are derived from the five-carbon precursor isopentenyl diphosphate (IPP). In total, around 12,000 known alkaloids are identified, and they possess one or more nitrogen atoms which are biosynthesized from amino acids. The 8000 known phenolic compounds are synthesized either through the shikimic acid pathway or through the malonate/acetate pathway (Rodney &*al.*2000).

Many alkaloids are used in medicine, usually in the form of salts. Some examples include vinblastine which has antitumor properties; quinine which has antipyretics and antimalarial properties (Reyburn *& al*; 2009); and reserpine which can be used to treat high blood pressure. Alkaloids are regarded as reserve materials for protein synthesis, as protective substances discouraging animal or insect attacks, and as plant stimulants or regulators or simply as detoxification products. Alkaloids currently in clinical use include the analgesics morphine and codeine, the anticancer agent vinblastine, the gout suppressant colchicine, the muscle relaxant tubocurarine, the antiarrhythmic ajmalicine, the antibiotic sanguinarine, and the sedative scopolamine.

In vitro studies have shown that natural phenols have antimicrobial (antiviral, anti-inflammatory, and vasodilatory actions .It protects the plant against adverse factors which threaten its survival in an unfavorable environment, such as drought, physical damage or infections. Resistance of plants to UV radiations is due to the phenolic compounds especially the phenylpropanoids present in them. Phenolic compounds act as antioxidants protecting cells from oxidative stress scavenging of free radicals by hydrogen atom donation. The action of phenolic as neuroprotective, fungicidal, bactericidal compounds and their anti-atherosclerosis effects, and anticancer (Olsson *& al*;2004**)** activity is well documented.

Terpenoids are commercially important fragrance and flavoring agents. Prenol and α-bisabolol are used in fragrance due to fruity odor and sweet floral aroma, respectively. Mono and sesqui terpenes are basis of natural perfumes and also of spices and flavorings in the food industry. The roles of terpenoids as pharmaceutical agents with activities such as antibacterial and antineoplastic are still under investigation. There are examples of diterpenes that exhibited in vitro cytotoxic, antitumor, and antimicrobial activities. Terpenes are vital for life in most organisms exerting metabolic control and mediating inter and intra species interactions, for example, manufacture compounds in response to herbivory or stress factors, and it has also been shown that flowers can emit terpenoids to attract pollinating insects and even attract beneficial mites, which feed on herbivorous insects. Cheng *& al; (*2007) have reported that terpenes may act as chemical messengers influencing the expression of genes involved in plant defensive functions or influence gene expression of neighboring plants

II.1.4 Classification of secondary metabolites

Over 2,140,000 secondary metabolites are known and are commonly classified according to their vast diversity in structure, function, and biosynthesis. There are five main classes of secondary metabolites such as terpenoids and steroids, fatty acid-derived substances and polyketides, alkaloids, nonribosomal polypeptides, and enzyme cofactors (McMurry, 2015).

II.1.4.1. Terpenoids and steroids

They are major group of substances derived biosynthetically from isopentenyl diphosphate. Currently, over 35,000 known terpenoid and steroid compounds are identified. Terpenoids have different variety of unrelated structures, while steroids have a common tetracyclic carbon skeleton and are modified terpenoids that are biosynthesized from the triterpene lanosterol.

II.1.4.2. Alkaloids

There are over 12,000 known compounds of alkaloids, and their basic structures consist of basic amine group and are derived biosynthetically from amino acids.

II.1.4.3. Fatty acid-derived substances and polyketides

Around 10,000 compounds are identified and are biosynthesized from simple acyl precursors such as propionyl CoA, acetyl CoA, and methylmalonyl CoA.

II.1.4.4. Nonribosomal polypeptides

These amino acids derived compounds are biologically synthesized by a multifunctional enzyme complex without direct RNA transcription.

II.1.4.3. Enzyme cofactors

Enzyme cofactors are nonprotein, low-molecular enzyme component (McMurry, 2015).

II.1.5 Sources of secondary metabolites

The major sources of secondary metabolites are plants (80% of secondary metabolite), bacte-ria, fungi, and many marine organisms (sponges, tunicates, corals, and snails) (Table 04) (Bérdy, 2005).

Table 04: Approximate number of known natural metabolites(Thirumurugan, *& al*;2018).

Source	All known compounds	Bioactives	Antibiotics
Natural products	Over one million	200,000–250,000	25,000–30,000
Plant kingdom	600,000–700,000	150,000–200,000	~25,000
Microbes	Over 50,000	22,000–23,000	~17,000
Algae, lichens	3000–5000	1500–2000	~1000
Higher plants	500,000–600,000	~100,000	10,000–12,000
Animal kingdom	300,000–400,000	50,000–100,000	~5000
Protozoa	Several hundreds	100–200	~50
Invertebrates	~100,000	NA	~500
Marine animals	20,000–25,000	7000–8000	3000–4000
Insects/ worms/ *etc.*	8000–10,000	800–1000	150–200
Vertebrates (mammals, fishes, amphibians, *etc.*)	200,000–250,000	50,000–70,000	~1000

II.1.6. Production of secondary metabolites from plants

II.1.6.1. Conventional

The conventional method of secondary metabolite production relies on extraction of metabolite, not production, from the tissues of plant by different phytochemical procedures like solvent, steam, and supercritical extraction. The recent developments in biotechnological methods like plant tissue culture, enzyme and fermentation technology have facilitated in vitro synthesis and production of plant secondary metabolites. The major processes include.(Thirumurugan, *& al*; 2018)

II.1.6.2. Immobilization

Cell or biocatalysts are confined within a matrix by entrapment, adsorption or covalent linkage. On addition of suitable substrate and provision on optimum physico chemical parameters, the desired secondary metabolites are synthesized. Immobilization with suitable bioreactor system provides several advantages, such as continuous process operation, but for the development of an immobilized plant cell culture process, natural or artificially induced secretion of the accumulated product into the surrounding medium is necessary.(Thirumurugan *& al*; 2018).

II.1.6.3. *In vitro* tissue, organ, and cell culture

Plant cell and tissue cultures can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots, meristems, etc., both for multiplication and extraction of secondary metabolites. Shoot, root, callus, cell suspension, and hairy root culture are used to synthesize metabolite of interest. Metabolites which are localized in multiple tissues can be synthesized through unorganized callus or suspension cultures. But when the metabolite of interest is restricted to specialized part or glands in host plant, differentiated microplant or organ culture is the method of choice. Saponins from ginseng are produced in its roots, and hence in vitro root culture is preferred for saponin synthesis. Similarly, antidepressant hypericin and hyperforin are localized in foliar glands of Hypericum perforatum, which have not been synthesized from undifferentiated cells (Smith MAL *& al*; 2002).

The quantum of secondary metabolite production in cell cultures can be enhanced by treating plant cellset al with biotic and/or abiotic elicitors. Methyl jasmonate, fungal carbohydrates, and yeast extract are the commonly used elicitors. Methyl jasmonate is an

established and effective elicitor used in the production of taxol from Taxus chinensis andginsenoside from Panax ginseng (Kim, *& al*; 2004). The most recently evolved and designed metabolic engineering can be employed to improve the productivity.

The production of metabolites through hairy root system based on inoculation with Agrobacterium rhizogenes has garnered much attention of late. The quality and quantity of secondary metabolite by hairy root systems is same or even better than the synthesis by intact host plant root. In addition, stable genetic make up, instant growth in plant tissue culture media san phytohormones provides additional scope for biochemical studies. Root tips infected with A. rhizogenes are grown on tissue culture media [Murashige and Skoog's (MS) Gamborg's B5 or SH media] lacking phytohormones. Srivastava and Srivastava (Shrivasava,*& al;* 2006) have recently summarized the attempts to adapt bioreactor design to hairy root cultures; stirred tank, airlift, bubble columns, connective flow, turbine blade, rotating drum, as well as different gas phase reactors have all been used successfully. Genetic manipulation in hairy root culture for secondary metabolite production is being tried out. The established roots are screened for higher growth and production of metabolites. Transgenic hairy roots generated though Agrobacterium rhizogenes have not only paved way for plantlet generation but also for synthesis of desired product through transgenic hairy root cultures.

II.2. Essential oils

II.2.1 Definition

Essential oils are concerted, hydrophobe liquids which is unstable fragrance mixes from plant. Essential oils are otherwise called unstable, etherneal oil or just as the "oils of" the plants which they were separated, for example, oil of cloves. Oil is "essentials" as in it conveys a particular fragrance, or pith, plants (Williamms,*& al*; 1993).

Essential oil is often alluded to as the "life forces" of plant. These "vital" is extricated from blossoms, leave, stem, root, seed, barks, and natural product skins. The measure of key oil can be collected from anywhere in the range of 0.001 percentage to 9 percentage of the aggregate. Oil has powerful antimicrobial components, containg extensive variety of helpful

contents. This type of oil is frequently utilized for their flavour and restorative property, in a large determination of items, for example, nourishments, medication, and beatufiers. Just unadulterated oils contain a full range of intensifies that shabby impersonations basically can't copied (Williamms,*& al*; 1993).

II.2.2Organoleptic and Physical Characteristics of Essential Oils

Essential Oils are usually lucid and mobile liquids, but a few are solid, such as orris, or semisolid, such as guaiac wood, at room temperature. The majority of EOs are colorless or pale yellow, although a few are deeply colored, such as blue chamomile, and European valerian, which is green (Tisserand & Young, 2013). The typical odor of EOs depends on the organs, species, and origins of plants. They are volatile oils with a high refractive index and optimal rotation, as the result of many asymmetrical compounds. The relative density of EOs is commonly lower than that of water, but several exceptions exist. EOs are usually recognized as hydrophobic, but they are largely soluble in fats, alcohols, and most organic solvents. Moreover, they have sensitivity to being oxidized to form resinous products through polymerization (Li *& al*., 2014).

II.2.3 Taxonomy of Essential Oil–ProducingPlants

EO-bearing plants belong to various genera distributed in around 60 families. The major plant families are well known for their ability to produce EOs of medicinal and industrial value, and include Alliaceae, Apiaceae, Asteraceae (Compositae), Lamiaceae (Labiatae), Myrtaceae, Poaceae, Cupressaceae, Lauraceae, Pinaceae, Zingiberaceae, and Rutaceae. All of the EO-producing plant families are rich in terpenoids. At the same time, plant families, such as Apiaceae (Umbelliferae), Lamiaceae, Myrtaceae, Piperaceae, and Rutaceae, more frequently contain phenylpropanoids. EOs can be obtained from many different parts of plants, including flowers (rose), leaves (peppermint), fruits (lemon), seeds (fennel), grasses (lemongrass), roots (vetiver), rhizomes (ginger), wood (cedar), bark (cinnamon), gum (frankincense), tree blossoms (ylang–ylang), bulbs (garlic), and dried flower buds (clove) (Tisserand,*& al*; 2013).

II.2.4 Essential Oil Extraction Methods

EOs are complex mixtures of low–molecular weight (usually less than 500 Da) compounds. Analytical procedures for EOs from medicinal plants include two steps: distillation or extraction, which takes at least several hours, and analysis, which is completed after 15 min. There are several extraction methods for EO extraction, comprising steam distillation, hydrodistillation, organic solvent extraction, expression, enfleurage, microwave-assisted distillation, microwave hydrodiffusion and gravity, high-pressure solvent extraction,

supercritical carbon dioxide extraction, ultrasonic extraction, solvent-free microwave extraction, and the phytonic process (Farhat,*& al*; 2010).

On a commercial scale, steam distillation is a preferred method for the extraction of EO. Distillation is often performed by prolonged heating and stirring in water or a solvent using the Clevenger, Dean–Stark, or Likens–Nikerson apparatus. Distillation consumes more than 70% of the total energy and time for the process, with a high consumption of solvent. Steam distillation has several disadvantages. High temperatures and water can cause chemical modifications of EOs. Highly volatile components and some water-soluble components are lost through steam distillation. On the other hand, when using solvent extraction it is almost impossible to obtain a solvent-free product. Under steam distillation conditions and even conventional solvent extraction, monoterpenes are famously susceptible to chemical changes and losses of more volatile constituents during the removal of the solvent (Presti,*& al*; 2005).

Therefore, other techniques should be developed, with the aim of reducing the sample preparation step and the disadvantages of steam distillation. Moreover, the properties of the EOs extracted through different methods have been found to vary depending on the method used.

Due to the residues existing in the extracts obtained by conventional solvents, which can pollute foods and fragrances, using a combination technology of an organic solvent with a low boiling point and steam distillation could replace this method. Organic solvents with low boiling ranges could overcome many of the problems that conventional solvents cause in the process of oleoresin extraction, including the difficult separation of the product from the extraction solvent and the high remnants of solvent. The application of organic solvents with low boiling points in the extraction of oil and EOs has been reported on previously (Carrín *& al*, 2008).

Headspace solid-phase microextraction (HS-SPME) is a simpler and more rapid procedure for extraction of the volatile fraction of aromatic plants in comparison with hydrodistillation, which is time consuming and needs a large amount of sample. HSSPME analysis allows for a qualitative estimate of volatile compounds using a small quantity of material (Paolini, *& al*;2008). HS-SPME has also been used for the characterization of the chemical variability of aromatic plants and for the study of volatile fractions emitted by species without EOs. During hydrodistillation, the most volatile compounds and watersoluble compounds are lost in the gaseous phase and in the hydrolate phase, respectively; whereas,

with HS extraction, it is the fiber affinity of each compound that monitors the sampling of the volatiles (Benyelles,*& al*; 2014).

II.2.5 Chemistry of Essential Oils

There are two main groups of metabolites that can be found in nature: primary and secondary metabolites. Primary metabolites are universal compounds, present in all living organisms, and include proteins, carbohydrates, lipids, and nucleic acids. Secondary metabolites are found only in some species and are classified as terpenoids, shikimates, polyketides, and alkaloids. EOs are composed of different chemical compounds. The constituents of plant EOs fall mainly into two distinct chemical classes: terpenes and phenylpropanoids. Although terpenes and their oxygenated derivatives (terpenoids) are more frequent and abundant in EOs, certain species contain high quantities of shikimates; namely, phenylpropanoids, and when these compounds are present, they provide a specific odor and flavor to the plant (Baser;*& al*; 2015).

II.2.6. Classification of Essential Oil Composition

The constituents of plant EOs fall mainly into two distinct chemical classes: terpenes and phenylpropanoids. Terpene compounds can be divided into two main categories: terpenes with a hydrocarbon structure, mainly the mono-, sesqui-, and diterpenes and their oxygenated derivatives, for instance, alcohols, oxides, aldehydes, ketones, phenols, acids, esters, and lactones (Fernandez,*& al*; 2013).

II.2.7. Essential Oil Properties and Pharmacologic Effects

Natural products and their derivatives are important sources of novel therapeutic molecules. A large numbers of EOs have potential to be used in the medicinal industry. In particular, EOs obtained from the Apiaceae, Lamiaceae, Myrtaceae, Poaceae, and Rutaceae families are important from the point of view of medicinal applications. Anise seed oil, caraway, cumin, oregano, clove, tea tree, coriander, sage, summer savory, sweet basil, fennel, thyme, lemon balm, peppermint, and German chamomile are some examplesof important EOs (Raut *& al,* 2014). Apart from these, a few more families, such as Cupressaceae, Hypericaceae (Clusiaceae), Fabaceae (also known as Leguminosae), Liliaceae, Pinaceae, Piperaceae, Rosaceae, Santalaceae, and Zygophyllaceae, are of considerable potential (Raut,*& al*; 2014).

EOs have been prescribed since ancient times for a variety of health problems by practitioners of traditional systems of medicine all over the world. They are used in the pharmaceutical industry as alternative medicines and natural therapies. The composition of EOs in these plants and their biological activities, which have been used in traditional medicine, should be investigated scientifically to improve the quality of healthcare (Rehman *& al*., 2016).

There have been numerous studies that claim that the biological properties of EOs and their aromatic components have pharmacologic effects, including antimicrobial, antiviral, antibacterial, antifungal, insecticidal, antioxidant, anticancer, antimutagenic, antidiabetic, antiinflammatory, antihypertensive, vagolytic, immunomodulatory, and antiprotozoal effects (Boukhatem,*& al*; 2013).

There is a need to explore EOs from the members of these families for various purposes, particularly for their medicinal properties. The EOs from members of the Apiaceae family are well known for their antibacterial, antifungal, anticancer, and antiviral activities. Furthermore, many genera that are well known for their chemotherapeutic, antiviral, antimicrobial, antimutagenic, antioxidant, and antiinflammatory properties belong to the Lamiaceae family. These are also useful against intestinal disorders and bronchitis (Hamid, *& al*; 2011).

A wide variety of EOs are known to possess antimicrobial properties, and in many cases this activity is due to the presence of active constituents, mainly to isoprenes, such as monoterpenes, sesquiterpenes, and related alcohols, along with other hydrocarbons and phenols. In particular, terpene hydrocarbons and oxygenated terpenes exhibit pronounced antimicrobial activity. Medicinally active components of EOs, such as citral, geraniol, and geranyl acetate, have shown antimicrobial and anticancer properties (Bedi,*& al*; 2008).

On the other hand, the lipophilic character of the hydrocarbon skeleton of EOs and the hydrophilic character of their functional groups are important in the antimicrobial action of the components of EOs. Therefore, a ranking of activity has been proposed, which is as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons**)**. For example, some EOs containing phenolic structures are highly active against a broad spectrum of microorganisms (Kalemba,*& al*; 2003).

Chapter III: Urinary Tract Infection

III.1. Definition

Normal urine is sterile, the first cubic centimeters of urine emitted are sometimescontaminated by the saprophytic flora of the urethra and possibly the vagina. Urinary tract infection occursdefines the presence of an abnormal germ in the urine at a concentration greater than105 germs/ml (Carlet & Guibert 1989), generating an inflammatory response and signs andsymptoms of varying nature and intensity depending on the terrain. It associates at least one of the signs fever (> 38°C), urinary urgency, pollakiuria, urinary burns orsuprapubic pain, in the absence of other infectious or non-infectious cause, to positive uroculture.The relevance of clinical and biological data is to be assessed according to the differentsituations (Vildé, 2002).

III.2. Anatomy of the urinary system

The purpose of the urinary system is to ensure the purification of the blood, it extracts the waste from the blood circulating that result from the metabolism and ensures their discharge to the outside in the form of urine (Figure 03) (Hachimi 2007).

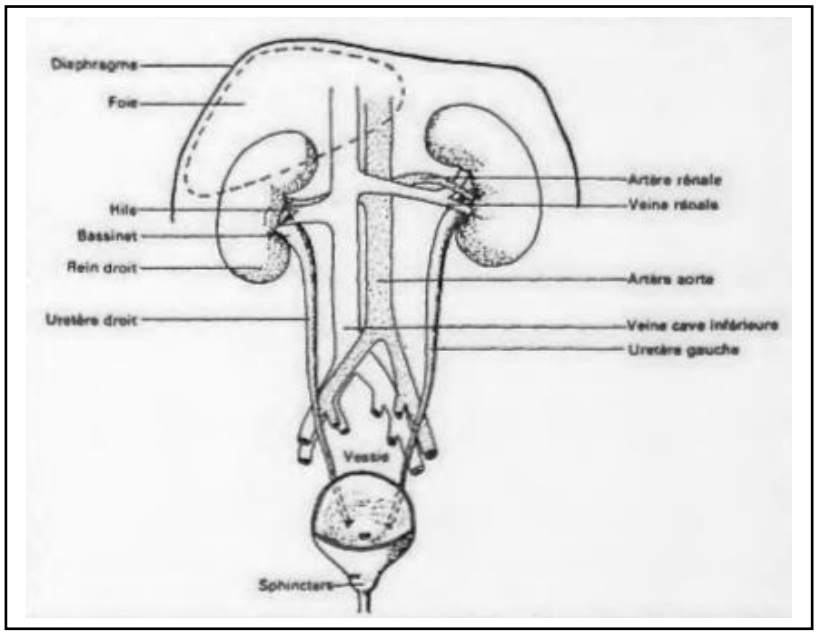

Figure 03: Anatomy of the urinary tract (Wallart, 2011).

III.3. Kidneys

There are two kidneys, one right and one left. Their shape is that of a bean (Figure 04). They weigh about 140 grams, 12 cm long, 6 cm wide and 3 cm thickness. Their color is red,

their consistency firm with a smooth and regular surface. They are located symmetrically on either side of the spine (first lumbar spine), in the abdominal cavity, behind the peritoneum (extra-peritoneal organs). The kidneys occupy each a box called the renal box. The kidney seen in cut consists of two parts a central part "medullary substance" and a peripheral part "the substance The inner edge of each kidney is hollowed out from a deep cavity the sinus with a orifice called the renal hilum. The bottom of the sinus is crossed by projections that are the papillae. The microscope kidney consists of small juxtaposed elements which are the nephrons or tubes uriniferous. The nephron is the fundamental unit of the kidney, each nephron understands different parties:

- The Malpighian corpuscle: the initial segment of the nephron.

- The Malpighi Glomerulus: network of arterial capillaries.

- The Contoured Tube or Proximal Tube.

- The loop of Henle.

- The collecting tube of Bellini, which opens at the top of the papilla.

Renal vascularization is provided by the renal vessels. Each kidney receives an arteryrenal coming from the abdominal aorta which branches and leaves through the renal vein to the vena cavalower (Alain and Sylvie, 2007).

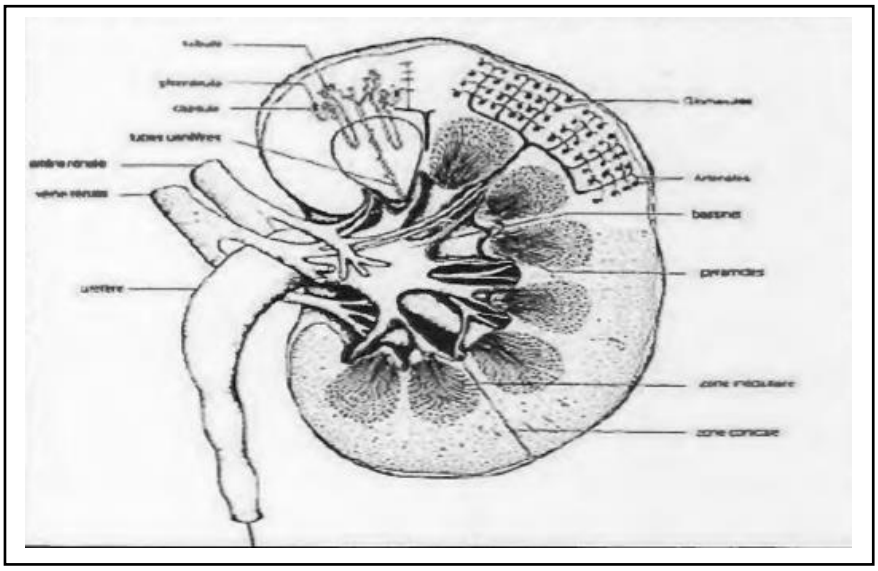

Figure 04: A cross-section of a kidney (Wallart, 2011)

III.4.The urinary tract

The urinary tract consists of all the ducts that the urine passes through from thekidneys to the outer middle, they include: calyxes, bassinet, ureter, bladder and the urethra (Abdllah &*al*., 2009).

III.4.The chalices

These are hollow tubes of two types: the small calyxes are inserted around the pyramids ofMalpighi and cap their tops, they collect the urine emitted by these pyramids. The youngcalyxes unite and form wider tubes, the large calyxes that arenumber of three: upper, middle and lower (Abdllah,*& al;* 2009).

III.4.2.The bassinet

As a result of the union of the large calyxes, it constitutes at the level of each kidney areservoir that collects the urine secreted and discharges it into the ureter. The basin is an organcontractile which aids in the progression of urine in the urinary tract (Abdllah,*& al*;2009).

III.4.3.The ureter

It is a very long duct which goes from the basin to the bladder, it is 25cm long and 3 to5 mm diameter, it is contractile, animated by peristaltic ripples allowing theflow of urine to the bladder. The ureter first descends into the lumbar region, where itis applied directly to the posterior wall of the abdomen, then it penetrates the smallof which it skirts the wall outside, in this region it comes in contact with the rectum inback and genital tract forward, finally it penetrates into the bladder from which it crosses the wall according toan oblique path of lem about, it abuts on the posterior-inferior side of the bladder, theorifices of both ureters are approximately 2cm apart from each other (Abdllah,*& a*l; 2009).

III.4.4. Bladder

It is a reservoir in which urine accumulates in the interval of urination .When it is empty, the bladder is flattened from top to bottom and when it is full, it becomesovoid with large posterior and inferior extremity. Bladder capacity is variable, needurine is felt for an average capacity of about 300 ml, this is the capacityphysiological, but the bladder is very extensible and its maximum capacity may be greater.The bladder is located in the small pelvis, of which it is the anterior organ, just behind thepubis and pubic symphysis, back it

responds to the rectum in humans, uterus andvagina in the woman, at the top she is covered by the peritoneum and comes into contact, by herintermediate with the intestinal loops. The vesical wall has three orifices: the orifices of thetwo ureters and a median orifice, that of the urethra, these three orifices draw a triangle to whichis called a trigon. The bladder wall has a layer of muscle fibresCalled mucosa, it is the contraction of the detrusor that ensures the evacuation of the bladder (Abdllah,&*al*; 2009).

III.4.5.The urethra

It is the excretory canal of the bladder, its appearance is different in both sexes:

- In humans: the urethra is 16 cm long on average and has different pathsportions, from its origin at the level of the bladder, it's enforce in the prostate crosses thisgland, it is the prostatic urethra coming out of the prostate, it is surrounded by a muscle, theribbed sphincter of the urethra, the contraction of which resists the need to urinate. The urethrathen crosses the perineum, it is the perineal urethra, finally it enters an erectile organ, theSpongy body, it is the spongy urethra, from which it follows the entire length of the penis.The urethra ends at the anterior end of the shaft, at the level of the gland by an orifice, theurethral meatus.

-In women: the urethra is very short, its length does not exceed 3 cm, it descends forward of thevagina and opens to the front of the vulva. It also has a striated sphincter thatprovides voluntary continence (Abdllah,&*al*; 2009).

III.5. Types of urinary tract infections

III.5.1. Acute cystitis

Acute cystitis is usually the witness of a low location, to the bladder while knowing that itmay be the only sign of renal parenchymal infection. This is the most common manifestationof the urinary tract infection (UTI). It combines urinary burning, apain like permictional urethral burns, dysentery that shows an obstacleto the flow of urine and the emission of turbid foamy urine with sometimes depositswhitish.

Moreover, the episode of cystitis is preceded by a few days of digestive disorders withconstipation or alternating diarrhea/constipation (Fries, 1992).

III.5.2. Acute prostatitis

Prostatitis is an acute bacterial inflammation of the prostate gland. Itassociates flu-like syndrome (fever > 39°C, chills, myalgia) with disordersirritative (pollakiuria, dysuria) or obstructive (acute urinary retention). Touchrectal is painful and shows a enlarged prostate, regular, with sometimes aurethral discharge. Digestive signs such as constipation or alternating diarrhea/constipation and anorexia are often associated and may be in the foreground. It acts as asevere infection that may lead, in the absence of treatment, to severe sepsis, septic shockor a prostate abscess. Lumbar pain, usually unilateral interstitial tissue(Vorkaufer, 2011).

III.5.3. Acute Pyelonephritis (ANP)

It is an acute inflammation of the bassinet with involvement of renal parenchyma by streaksgeneral suppurative interstitial nephritis. It most often manifests as awell-typed semiology: fever of sudden onset at 39°C or more often with a chillunique said solemn sometimes the beginning is progressive with an isolated 38° C hyperthermia, acontinuous lumbar or bilateral pain with renal colic-type radiation.Urinary signs may be important with dysuria and pollakiuria, but these signs are onlystill not present. The associated signs are variable: headache, anorexia and constipation(Kouakou, 1984).

III.5.4. Chronic Pyelonephritis (PNC)

These are chronic renal parenchymal alterations secondary to episodes of ANC or repeated episodes of chronic renal parenchymal infection. The infection enters the interstitial tissue, and PNC combines inflammatory infiltration lesions and tissue sclerosis interstitial (Ogundaini, 1999).

III.6. Microbial agents responsible for urinary tract infections

The microorganisms most frequently found in patients with infectionare described as uropathogens.

III.6.1. Gram-negative bacilli

Most urinary tract infections are due to the upward spread ofbacteria of intestinal origin hence the predominance of enterobacteria in which:

- Escherichia coli: The majority of studies show that E.coli is the germ most involved ininfectious disease. According to studies, it is responsible for 40% to 70% of infectionsin hospitals and about 70% in cities. E. coli also accounts for 25% to 40% ofGram-negative bacilli isolated from bacteremia (Bourjilat,&*al*; 2009).

-Proteus mirabilis: is also among the enterobacteria often isolated in the urine. It is5-10% of uncomplicated urinary tract infections (Lamnaouer, 2002).

-Klebsiella: The genus Klebsiella causes complicated urinary infections or nosocomialat a very high percentage.

-Pseudomonas aeruginosa: This species J is responsible for unnary infectionscomplicated, resulting from contamination by endourinary instrumental maneuvers (Dhote and Paugam, 1999).

III.6.2. Gram Positive Cocci

Urinary Cocci Gram-positive infections are rare. Staphylococcus saprophyticus is found(coagulase-negative or white staphylococcus) which is responsible for 10-15% ofacute urinary tract infections and Streptococci (Enterococci and Group B Streptococci) thatare responsible for uncomplicated urinary tract infections (Dhote and Paugam, 1999; Lamnaouer, 2002; Ekoumou, 2003).

III.6.3. Other sprouts

Parasites such as Trichomonas vaginalis and Schistosoma haematobium may be associatedcystitis, as well as fungi such as Candida albicans. Othersexually transmitted microorganisms such as Chlamydia trachomatis, Ne isseriagonorrhea and Herpes simplex virus (Fakae,&*al*;2000).

III.7 Pathophysiology

III.7.1. Colonization and Infection

Many factors promote the colonization of bladder urine, the absence of mechanism ofdefense of urine and bacterial adhesion to urothelium. Colonization is of two types:

-non-specific adhesion due to hydrophobic and/or electrostatic interactions

-specific bacterial adhesion due to elements represented by Pili orsome adhesives and Fimbrae that attach to surface receivers located on theurothelial cells. Fimbrae are rigid filaments of 3 to 7 mm diameter and 2 to 3 mmlong. These are protein structures arranged concentrically on certain bacteria.These fimbrae bind to specific receptors of the urothelial cell membrane. Thisability of adhesion ensures bacteria an increased resistance to their elimination by the flowurinary tract and by this mechanism promotes their extension from the peri-urethral area to the bladder andbassinet (Kaunan,1988; Mottet,1990).

III.7.2. Constitution of the infection

III.7.2.1. The Ascending Path

Bacteria can enter the bladder in three ways: bacteria present inthe urethra are introduced into the bladder during the survey, i.e., bacteria colonizing the urethrareach the bladder thanks to the mucous film that envelops the probe, or the bacteriacan reach the bladder through the light of the probe itself. This is the mode of access tothe most common bladder. These bacteria are introduced into the probe light on occasiona disconnection from the drainage system.

The probe by draining urine exerts pressure on the bladder mucosa and canalseriurethral which results in the occurrence of infection of the prostate and epididymis.The balloon of the probe irritates the bladder mucosa to the point of encroaching on it causing a foreign-body reaction that will promote bacterial multiplication (Kouadio, 1992).

III.7.2.2. Descending Track

It is the primary seeding of the kidney by blood and the secondary migration of germsin the urine. Septicemia Staphylococcus aureus, Salmonella, E. coli, Candida andRenal localization with urinary tract infection is possible (Konan, 1995).

III.7.3. Special case in women

The frequency of UTI in women is explained by the urethra which is short (3cm), broad, straight,near the peri-anal area, the frequency of sexual intercourse that promotes the opening of the meatusurethral, transit disorders (constipation, diarrhea) and by certain habits such as takingoestroprogestative (florevaginal modification), prolonged maceration (clothingfit) or a perineal toilet performed from back to front (Yabi, 2006).

III.8. Diagnosis

III.8.2. Urinary Cytobacteriological Examination (UCE)

The suprapubic puncture provides the most representative samples of intravesical urine. Other methods of sampling (on-the-fly samplingdirect puncture of the specific operculum of the urinary tube, collected by urinary sounding inincontinent women, in men by penile cases), less invasive and adapted todifferent clinical situations, are usable with an acceptable level of reliability. For thesesampling conditions may affect the level of contamination of theremoval (need for proper grooming of the external genitals in the absence ofprobe, disinfection of the probe lid). Adequate conditions of transport andstorage are even more important to respect (speed less than 2 hours at temperatureenvironmental) in order to avoid contamination, which is a hindrance to the interpretation of theurine cytobacteriology (UCE). Retention of urine at 4°C for 24 hours isan alternative with no influence on bacteriuria.

The limit of quantification of bacteria and urinary yeast by the usual method is equalto 103 CFU/ml. Consequently, bacteriuria or candiduria is to be consideredif it is 103 CFU/ml under strict conditions of sampling, transport and analysisurine. The qualitative term pyuria due to its vagueness must be abandoned in favour ofa quantitative measurement of leukocytes (leukocyte). Leukocyte has no interest in thepatient tested (D- II). In a symptomatic patient without a probe, the association of a bacteriuria103 CFU/ml to leukocytturia 104/ml is strongly suggestive of infection (A - II) (Bonacorsi, 2016).

CONCLUSION

In this work, we are interested in the study of the antimicrobial effect of methanol extracts from leaves and stems and the essential oil of *Myrtus communis*, a plant widely used in traditional medicine throughout the world, has been studied.

We proceeded our work by calculating the yield of the extract of the leaves and stems as well as the essential oil. The yield obtained from the phenolic extracts of the dry leaves of *M communis* is 38.7%, while for the stems, the extraction yield was 18.2% and 0.47% for EO.

We evaluated in vitro the antibacterial power of these extracts on six (6) bacterial strains: *Escherichia* ATCC 25922 */coli Escherichia coli* (clinical)/ *Staphylococcus aureus* ATCC25923/*Staphylococcus aureus* (clinical)/ *Pseudomonas aeruginosa*ATCC27853*Pseudomonas aeruginosa* (clinic)/ *Proteus mirabilis*ATCC23659/*klebsiella pneumoniae* ATCC70603and yeast; *Candida albicans*ATCC 10230/*Candidaalbicans* (clinical*)*, microbial germs, commonly found inurinary tract infection and urinary lithiasis.

The method used and that of blank discs impregnated with extracts of this plant.For all the microorganisms tested, the effect of the phenolic extracts of the leaves is more important than that of the stems. The extract of the leaves exerts a remarkable inhibitory effect with all clinical germs. This results in inhibition zones with a diameter of 22mm with *Candida albicans*, 16mm with *E.coli* and 13mm with *Pseudomonas aeurigenosa*. For the extracts of the stems, we have recorded zones of inhibition rather important namely 12mm with *Staphylococcus aureus* and Candida albicans The effect of the essential oil of *Myrtus communis* is not as important as the phenolic extracts of the leaves and stems. One of the

inhibition zones was recorded only with *Staphylococcus aureus* (13 mm) and *E coli* (11 mm). This is probably due to the methose of the extraction or to the harvest season of the plant.

The same happened with reference strains (Figure 11). Phenolic extracts from M. communis leaves shows more antimicrobial activitythat of the stems of the same plant vis-à-vis the germs tested. These extracts are proven to be active against Gram bacteria – known for their strong resistance tobiocides including *Proteus mirabilis* (18mm), *Klebsiella pneumonia* (17mm) and *Pseudomonas aeruginosa* (15mm).

All of these bacterial strains tested were found to be sensitive to the extracts studied. Recorded Minimum Inhibitory Concentrations (MIC) range from 0.009 to 0.7mg/ml while the Minimum Bactericidal Concentrations (MBC) range from 0.01 to 0.3 mg/ml. For the majority of microorganisms tested, this activity is essentially bactericidal in nature.

These activities are related to the quality of the phenolic compounds detected in this plant. Qualitative and quantitative research of these compounds in *Myrtus communis* showed that the methanol extract was the richest in phenolic substances.

The effect of the essential oil of *Myrtus communis* is not as important as the phenolic extracts of the leaves and stems. Inhibition zones were recorded only with *Staphylococcus aureus* (13 mm) and *E coli* (11 mm). This is probably due to the extraction method,or to the harvest season of the plant or to the low concentration of essential oil. So we have to think about increasing the doses of the different extracts. Plant composition alsovery important according to the vegetative cycle or geographical origin. This would establish the best harvest period for the most important parts of the plant.

We also determined the antioxidant activity of the extracts phenolic leaves and stems .The results of the anti-free radical test with DPPH was significant and very high (87. 30% leaves, 84.51% stems).

All these results obtained in vitro are only a first step in the search for biologically active naturally occurring substances, an in vivo study isdesirable, to obtain a more in-depth view of the antimicrobial activities of extracts of this plant.

This work has provided a scientific basis for the use of Myrtus Communis in the treatment of microbial pathologies.

III.8.1. The Urine Test Strip

The urinary test strip is the first easy and quick in-office examination. Itallows to guide the diagnosis. It is to be carried out in front of all urinary functional signs, orfever without a point of appeal, especially in children, and in the follow-up of a pregnancy, adiabetes,or high blood pressure. It is important to check before using astrip its expiry date. When there is evidence of urinary tract infection, it shouldtake urine from a clean, dry specimen and then soak the urine strip inurine (1 second). It is advisable to keep it horizontal to eliminate excess andwait at least one minute to read the results. Then, you have to understand thereactive with the colorimetric range present on the bottle at the indicated times.

In the urinary tract infection, two tests are stained: the leukocyte-esterase test whose thresholddetermination is 104 leukocytes/ml and the nitrite test which has as threshold 105 germs/ml, the positivity of this test depends on the urine taken (pH too acidic, or urine stay < 4hin the bladder) and the relevant germ (nitrite-free).

Urine Test Strip is said to be negative when leucocyturia and nitrites are negative and said to be positive if a leucocyturia or nitrites are detected.

The urinary strip has a good predictive value (VPN) 99.4%, that is to say iteliminates the diagnosis of urinary tract infection. The positive predictive value is that 33.5% isto say that a positive urine test strip does not confirm a urinary tract infection.

To confirm the diagnosis, a cytobacteriological examination must be carried out in search of agerm, especially for complicated cystitis, pyelonephritis, prostatitis,Pregnancy-related urinary tract infections (Vorkaufer, 2011).

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Appendices

Appendix 01: Growing Media

Mueller Hinton Agar

Ingredients	Gram/litre
Meat infusion	300 ml
Casein peptone	17 ,5g
Starch	1,5g
Agar	17g

Nutrient broth

Ingredients	Gram/litre
Peptones	10g
Beef extract	1g
Yeast extract	2g
Sodium chloride	5g

pH final 6.8 ± 0.2 at 25°C

Gélose nutritive

Ingredients	Gram/litre
Tryptone	5,0g
Meat extract	1,0g
Yeast extract	2,0g
Sodium chloride	5,0g
Bacteriological agar agar	12,0g

Appendix 02: Sterile T.T.C.Solution

Formula 2-3-5-triphenyl-2H-tetrazolium chloride + distilled water

TTC concentration varies by formula

Storage: The tubes will keep between 2 and 8°C in the dark until the expiry date indicated on the package. The TTC is photolabile and becomes yellow under the effect of light.

Appendix 03: BUCHI R-210 Rotary Evaporator Data Sheet.

Reference	BUC-23011A000
Display	Temperature, water/oil
Type of elevator	Motorisé
Rotation speed	20-280 Tour/minute
Power consumption	1360 W
Ball size	50-4000 ml
Maximum weight of the ball	3 kg
Dimensions (L H P)	550×575×415 mm
Weight	19 – 21 kg with bath
Bath volume	4 liters
Bath temperature range	20 – 180 °C
Pressure	+/- 2°C
Dimension of the heating bath (L H P)	285 ×240×300
Weight of the heating flask	4kg
IP protection	IP 21
Conformité	THIS
Feeding	100 -240 V / 50 – 60 Hz

ABSTRACT

In this work, we have studied the antibacterial effect of the phenolic compounds and essential oils of *M.communis* , this plant widely used in traditional medicine to all over the world.The antimicrobial activity of *Myrtus communis* extracts was evaluated in vitro using Aromatogram method (by discs). The bacteria tested are commonly encountered in urinary tract infections and lithiasis of infection. All these strains were found to be sensitive to the extracts studied. The Recorded minimum inhibitory concentrations (MIC) range from 0.7 to 0.1mg/ml while minimum bactericidal concentrations (MBC) range from 10.5 to 30 mg/ml. This activity is essentially bactericidal in nature. The results of the anti-free radical test with DPPH of raw extract of the leaves of Myrth revealed a percentage of inhibition of the radical DPPH, significant and very high 87. 30% and for stems extract to 84.51%.

Keywords:*Myrtus communis*., phenolic extract, organic extracts, polyphenols, antimicrobial activity.

Resume

Dans ce travail, nous avons étudié l'effet antibactérien des composés phénoliques et des huiles essentielles de *M.communis* , cette plante largement utilisée en médecine traditionnelle dans le monde entier.L'activité antimicrobienne des extraits de *Myrtus communis* a été évaluée in vitro à l'aide de la méthode Aromatogram (par disques). Les bactéries testées sont couramment rencontrées dans les infections des voies urinaires et les lithiases d'infection. Toutes ces souches se sont révélées sensibles aux extraits étudiés.Les concentrations minimales inhibitrices (CMI) enregistrées varient de 0,7 à 0,1 mg/ml, tandis que les concentrations minimales bactéricides (MBC) varient de 10,5 à 30 mg/ml. Cette activité est essentiellement bactéricide dans la nature.Les résultats du test anti-radicaux libres avec DPPH d'extrait brut des feuilles de Myrth ont révélé un pourcentage d'inhibition du DPPH radical, significatif et très élevé de 87. 30% et pour les tiges de 84,51%.

Mots-clés : Myrtus communis. , extrait phénolique, extraits organiques, polyphénols, activité antimicrobienne.

خلاصة

هذا النبات M.communis في هذا العمل، درسنا التأثير المضاد للبكتيريا للمركبات الفينولية والزيوت الأساسية لـ المستخدم على نطاق واسع في الطب التقليدي في جميع أنحاء العالم. تم تقييم النشاط المضاد للميكروبات لمستخلصات (عن طريق الأقراص). عادة ما تواجه البكتيريا Aromatogram في المختبر باستخدام طريقة Myrtus communis التي تم اختبارها في التهابات المسالك البولية وداء الحصى. تم العثور على كل هذه السلالات حساسة للمستخلصات التي من 0.07 إلى 0.01 مجم/مل بينما تتراوح التركيزات (MIC) تمت دراستها. تتراوح التركيزات المثبطة الدنيا المسجلة من 7.5 إلى 30 مجم/مل. هذا النشاط هو في الأساس بكتيريا في الطبيعة. كشفت نتائج اختبار (MBC) البكتيرية الدنيا الجذري، DPPH عن نسبة مئوية من تثبيط Myrth للمستخلص الخام لأوراق DPPH الجذور المضادة للحرية باستخدام كبيرة وعالية جدًا 87. 30٪ ومستخلص السيقان إلى 84.51٪.

الكلمات الرئيسية: المستخلص الفينولي، المستخلصات العضوية، البوليفينول، النشاط المضاد للميكروبات Myrtus communis