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In vitro, the efficacy of some spice essential oils against foodborne pathogenic bacteria and molds

Dissertation

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Presented by:

Miss. Khemkham Aicha

Committee members*:*

Dr. Azouzi Blel	(Lecturer)	Chairman	University of Djelfa
Dr. Dahia Mostefa	(Lecturer)	Advisor	University of Djelfa
Dr. Laouer Hocine	(Professor)	Examiner	University of Sétif
Dr. Khennouf Seddik	(Professor)	Examiner	University of Sétif

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Dedication

I dedicate this dissertation to:

My beloved mother and father

My sisters and brothers,

My nieces « Aya and Khawla »,

My grandma, and All the family,

To everybody will invest the time to read it.

It was written for you . . .

Acknowledgment

All praises and thanks for the Almighty Allah, the Merciful, the only creator of the universe and source of all knowledge and wisdom, who blessed me with health, thoughts, strength to complete this study.

As the Prophet Mohamed (SAW) said, « *Whoever does not thank people does not thank Allah »,* with this :

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List of Abbreviations

***A.flavus* :** *Aspergillus flavus*

***A.niger* :** *Aspergillus niger*

ASTA: American Spice Trade Assossiation

ATCC : American Type Culture Collection

bc : bactericidal

BC : Before Christ.

***B.cereus* :** *Bacillus cereus*

bs : bacteriostatic

C : Chloramphenicol

°C : Degree Celsius

CFU/ ml : Colony Forming Units /mililiter

cm: centimeter

***E.coli* :** *Escherichia coli*

EO: Essential Oil

EOs: Essential Oils

fc : fungicidal

FOX : Cefoxitin

fs : fungiostatic

g: gram

g/cm^3 : Gram per Cubic Centimeter

GC-MS: Gas chromatography-Mass Spectrometry

GM : Gentamicin

h : hour

HPLC: High Performance Liquid Chromatography

KAN : Kanamycin

***L.monocytogenes* :** *Listeria monocytogenes*

LPS: lipopolysaccharide

MBC: Minimum Bactericidal Concentration

MBCs: Minimum Bactericidal Concentrations

MFC: Minimum Fungicidal Concentration

mg/ml : milligram/milliliter

MHA : Mueller Hinton Agar

MIC: Minimum Inhibitory Concentration

MIQ: Minimum Inhibitory Quantity

ml: milliliter

mm: millimeter

mn : minute

mol/L : mole/liter

NA : Nutrient Agar

NB : Nutrient Broth

nm : nanometer

PDA : Potato Dextrose Agar

PDB : Potato Dextrose Broth

***P.aeruginosa* :** *Pseudomonas aeruginosa*

TE : Tetracyclin

***S.aureus* :** *Staphylococcus aureus*

SD : Standard Deviation

***S.typhi* :** *Salmonella typhi*

v/v : volume/volume

w/v : weight/volume

µg : microgram

µl: microliter

Introduction

Introduction

In the recent years, consumers have become more concerned about the processed food they eat. Synthetic preservatives, which have been used in foods for decades, may lead to negative health consequences (Kommert and Slinter, 2006).

Concerns about the use of antimicrobial agents in food products have been discussed for decades (Gaunt *et al.,* 2005 ; Sondin *et al.*, 2005). The increasing demands for reduced-additive (including antimicrobial agents) and more 'natural' foods, and greater convenience have promoted the search for alternative antimicrobial agents or combinations to be used by the food industry (Lee, 2008 ; Belayar *et al.,* 1999).

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. The gram positive bacterium such as *Staphylococcus aureus* is mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Julseth, 2005). The gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Sondin *et al.*, 2005; Deviando *et al.*, 2009).

Spices and herbs have been added to food since ancient times, not only as flavouring agents, but also as folk medicine and food preservatives (Sipoulou and Ynot, 1995). Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world. They are essentially flavouring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties (Gullocee *et al.*, 2007).

Nowadays, people worldwide are looking towards natural base products since there are no side effects when taken accordingly. Furthermore, there is also an interest in the production of functional, high value, natural products without chemical modification and residues of solvents or additives (Yasir and williams, 2007). This trend in consumer preference increases the demand tremendously with variety products range from essential oils (EOs) (Lee, 2008).

Many studies have been carried out to extract various natural products including the EOs for screening antimicrobial activity (Annissorn, 2005; Dahia *et al.*, 2007). Nowadays, plenty of plants are valued for their antimicrobial activities and medicinal effects in addition to their flavour and fragrance qualities (Hirtoko, 1995).

In this respect, spices, such as *Cinnamomum zeylanicum* (Lauraceae), *Nigella sativa* (Ranunculaceae), *Pimpinella anisum* (Apiaceae), *Syzygium aromaticum* (Myrtaceae), and *Zingiber officinale* (Zingiberaceae) have been reported to possess very good medicinal and antimicrobial properties as those investigated by Sondin *et al.* (2005), Ometta (2006) and Miraglia (2009). The objective of this present study was to assess the potential of EOs from some spices as antimicrobial agents against some food borne pathogenic bacteria and molds.

This dissertation is divided into two parts: The first part, titled "the literature review", and the second part, titled "the experimental part". The first part comprises two chapters, the first chapter gives an overview of selected spices, and the second chapter is devoted to the EOs. The experimental part is dedicated to the study of the antimicrobial activity of the EOs , it comprises three chapters. The first is a presentation of materials and methods, the second gives the obtained results and the last chapter is devoted to the discussion. This dissertation is closed by conclusion and perspectives.

Literature Review

Chapter I :

An overview of Selected Spices

I.1. Definition

Spice is defined as "strongly flavoured or aromatic substance of vegetable origin, obtained from tropical plants, commonly used as a condiment" (Rathore and Shekhawat, 2008).

According to Peter (2001) Spices are defined as products derived from vegetables or mixtures thereof that are free from other material and are used to provide flavorings, seasoning, and aroma to foods.

American Spice Trade Assossiation (ASTA) defines spices « as any dried plant product used primarily for seasoning purposes ». Included are tropical aromatics (pepper, cinnamon, clove, etc.), leafy herbs (basil, oregano, marjoran, etc.), spice seeds (sesame, poppy, mustard, etc.) and dehydrated vegetables (onions, garlic, etc.) (Small, 2006).

I.2. *Cinnamomum zeylanicum* L.

(Cinnamon)

I.2.1 History

In the ancient world cinnamon was more precious than gold. This is not too surprising though, as in Egypt the abundance of gold made it a fairly common ornamental metal. Cinnamon was known in medieval Europe, where it was a staple ingredient, along with ginger, in many recipes. Of this period which still survives. The demand for cinnamon was enough to launch a number of explorers' enterprises. The Sinhalese King paid the Portuguese tributes of 110,000 kilograms of cinnamon annually. From these, come the finest quills (Remis, 1990).

I.2.2. Classification

According to kokillni (1993), the classification is as follows:

Kingdom: Plantae,

Order: Laurales,

Family: Lauraceae,

Genus: *Cinnamomum*,

Species: *Cinnamomum zeylanicum.*

I.2.3. Other Names

Cinnamomum verum

Arabic: qorfa (Baba Aissa, 2000), *English:* cinnamon, *French:* cannelle, *German:* Ceylonzimt, *Italian:* cannella, *Spanish:* canela (Ogura, 2000).

I.2.4. Plant Description

Cinnamon is from a tropical evergreen tree of the laurel family growing up to 7m in its wild state. It has deeply-veined ovate leaves that are dark green on top, lighter green underneath. The bark is smooth and yellowish. Both the bark and leaves are aromatic. It has small yellowish-white flowers with a disagreeable odour that bear dark purple berries (Chugh, 1993).

I.2.5. Spice Description

Cinnamon comes in 'quills', strips of bark rolled one in another (figure 1). The pale brown to tan strips are generally thin. The best varieties are pale and parchment-like in appearance (Ohlan and Blake, 1997).

Figure 1. *Cinnamomum zeylanicum*

I.2.6. Chemical Constituents

Cinnamon bark contains up to 1 to 2 percent volatile oil, tannins, catachins, proanthocyanidins, resins, mucilages, gum, sugar, and calcin-oxide. The major ingredient in the EO is cinnamic aldehyde (cinnamaldehyde) (Iharanate, 2005).

I.2.7. Uses and Benefits

Cinnamon bark is used widely as a spice, it has been used as a flavouring agent since ancient times. The ground spice is used for flavouring baked products such as cakes, biscuits, puddings, chewing gum and deserts. It is used as an ingredient in many medicinal preparations (Vauke, 1995). The bark of Cinnamon is an aphrodisiac, anthelmintic and tonic (Iffert, 1998 ; Kommert and Slinter, 2006). It is useful in the treatment of vata, biliousness, parched mouth, bronchitis, diarrhea, itching, heart disease and urinary disease (Belayar *et al.*, 1999).

I.3. *Nigella sativa* L.

(Black cumin)

I.3.1. History

Nigella sativa was originally discovered in the Egyptian Pharaoh Tutankhamen's tomb (dated about 1325 BC). This is pretty significant because in the ancient Egyptian cultures only the highest of quality items were placed in the tomb to protect the spirit in the after life. Thus, *Nigella sativa* probably had an important role in the ancient Egyptian practices (Remis, 1990).

I.3.2. Classification:

According to kokillni (1993), the classification is as follows:

Kingdom: Plantae

Order: Ranunculales

Family: Ranunculaceae

Genus: *Nigella*

Species: *Nigella sativa*

I.3.3. Other Names

Arabic: Kemoun assoued (Baba Aissa, 2000), *English*: Black cumin, *French:* cheveux de Venus, *German:* Scharzkummel, *Italian:* nigella, *Spanish:* neguilla (Ogura, 2000).

I.3.4. Plant Description

An herbaceous annual of the buttercup family, about 60 cm high. The gray--green leaves are wispy and threadlike. Flowers have five petals bout 2.5 cm wide, white with blue veins and appearing between June and September. They yield a seed capsule with five compartments each topped by a spike. The compartments open when dried to disperse the seeds (Chugh, 1993).

I.3.5. Spice Description

Nigella sativa seeds (figure 2) are small, matte-black grains with a rough surface and an oily white interior. They are roughly triangulate, 1.5 - 3 mm long. They are similar to onion seeds (Ohlan and Blake, 1997).

Figure 2. *Nigella sativa*

I.3.6. Chemical Constituents

According to Luwak (2003) and Gotakh (2006) eight fatty acids (99.5%) and 32 compounds (86.7%) have been identified in *Nigella sativa* fixed and volatile oils, respectively. Other constituents are protein, protein, moisture, ash, crude fibre and carbohydrates. The main compound of the EO is Thymoquinone up to 50%.

I.3.7. Uses and Benefits

Seeds are used as a new source of edible oils and food applications as spice and condiments in cakes, breads, pastries, curries, pickles and in seasoning etc. In the Greco Arab/Unani Tibb system of medicine, black seed has been regarded as a valuable remedy in hepatic and digestive disorders and has been described as a stimulant in a variety of conditions, ascribed to an imbalance of cold humors (Annissorn, 2005). Other researchers have recently also studied its reaction towards cancer, and it is reported to have many anticancer properties (Anridogen,1999). This spice may be more important to Muslims than to Christians and Jews. Prophet Muhammad (SAW) once stated that the black seed can heal every disease except death.

I.4. *Pimpinella anisum* L.

(Aniseed)

I.4.1. History

The early Arabic name was anysum from which was derived the Greek anison and the Latin anisun. It is one of the oldest known spice plants used both for culinary and medicinal purposes since ancient times. Aniseed's carminative properties have been known since antiquity. There is evidence that aniseed was used in Egypt as early as 1500 B.C and it was well known to the Greeks, being mentioned by Dioscorides and Pliny and was cultivated in Tuscany in Roman. In the Middle Ages its cultivation spread to Central Europe (Remis, 1990).

I.4.2. Classification :

According to kokillni (1993), the classification is as follows:

Kingdom: Plantae

Order: Apiales

Family: Apiaceae

Genus: *Pimpinella*

Species: *Pimpinella anisum*

I.4.3. Other Names

Arabic: habet h'laoua (Baba Aissa, 2000), *English*: Aniseed, *French*: anis, *German*: Anis, *Italian*: anice, *Spanish:* anis (Ogura, 2000).

I.4.4. Plant Description

Aniseed is an annual plant, which grows about 0.91m high. It has feathery leaves, the lower leaves are broad, toothed and triangular with upper leaves that are smaller, divided and narrow. It has umbrella-like clusters of dainty, creamy-white flowers and thin roots (Chugh, 1993).

I.4.5. Spice Description

Though the roots and leaves are also edible, it is the seeds that we will concern ourselves here. The seeds are grey-green to brownish, ribbed and ovate, measuring 2 -4 mm long (figure 3). Some seeds retain the fine stalk that passes through the centre of the fruit (Ohlan and Blake, 1997).

Figure 3. *Pimpinella anisum*

I.4.6. Chemical Constituents

According to Mills (2003), *Pimpinella anisum* seeds contain 1.5 - 4% volatile oil and other ingredients, which have a strong seasoning action including coumarins, glycosides, fixed oils. The aroma of the EO is dominated by *trans*-anethole (max. 90%).

I.4.7. Uses and Benefits

Pimpinella anisum has been used as an antispasmodic, antiseptic, aromatic, carminative, digestive, expectorant, stimulant, stomachic and tonic (Mund, 2001). It is also used as an alternative to 'Botox' in reducing wrinkles and fine lines (Julseth, 2005). Aniseed is used to flavour cakes, biscuits and confectionery, as well as breads (Ometta, 2006).

I.5. *Syzygium aromaticum* L.

(Clove)

I.5.1 History

The word ‘clove’ is from the Latin word for ‘nail’ – *clavus*. The clove is native to the North Moluccas, the Spice Islands of Indonesia. It is cultivated in Brazil, the West Indies, Mauritius, Madagascar, India, Sri Lanka, Zanzibar and Pemba. The Chinese wrote of cloves as early as 400 BC. and there is a record from 200 BC of courtiers keeping cloves in their mouths to avoid offending the emperor while addressing him. Arab traders delivered cloves to the Romans 2000 years ago (Remis, 1990).

I.5.2. Classification

According to kokillni (1993), the classification is as follows:

Kingdom: Plantae

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Species: *Syzygium aromaticum.*

I.5.3. Other Names

Eugenia aromaticum or *Eugenia caryophyllata.*

Arabic: qaranfoul (Baba Aissa, 2000), *English:* clove, *French:* clou de girofle, *German:* Gewuzenelke, *Italian:* chiodo di garofano, *Spanish:* clavo de especia (Ogura, 2000).

I.5.4. Plant Description

Clove is an evergreen tree with narrowly elliptic, pinkish (young) to dark green (nature) leaves; up to about 12 m high. Its flowers, when allowed to develop, are red and white, bell-shaped, and grow in terminal clusters (Chugh, 1993).

I.5.5. Spice Description

Clove is small (figure 4), tapering, nail-like body, about 12-14 mm long, consisting of four toothed calyx, between which the unopened corolla is seen as a round ball; of a dark reddish brown colour, and hot taste (Ohlan and Blake, 1997).

Figure 4. *Syzygium aromaticum*

I.5.6. Chemical constituents

According to Daniel (2006), Clove contains 14-20% volatile oil, 10-13% tannins, oleanolic acid, vanillin, and a chromene –eugenin. 70-90% of volatile oil is eugenol.

I.5.7. Uses and Benefits

Cloves are used for flavoring foods and as a stomachic and carminative in medicine. It is also found to heal stomach ulcers and inhibit carcinogens by inducing the production of detoxifying agents such as glutathione S-transferase (Belayar *et al.,* 1999). Clove oil is antiseptic and antispasmodic and is often an ingredient of tooth pastes and mouth washes. It is a toothache remedy. It also finds great use in medecines, perfumery, and histological work and for commercial production of vanillin (Kommert and Slinter, 2006).

I.6. *Zingiber officinale* L.

(Ginger)

I.6.1. History

Ginger is native to India and China. It takes its name from the Sanskrit word *stringa-vera,* which means "with a body like a horn", as in antlers. Ginger has been important in Chinese medicine for many centuries, and is mentioned in the writings of Confucius. It is also named in the Koran, indicating it was known in Arab countries as far back as 650 A.D. It was one of the earliest spice known in Western Europe, used since the ninth century. It became so

popular in Europe that it was included in every table setting, like salt and pepper (Remis, 1990).

I.6.2. Classification

According to kokillni (1993), the classification is as follows:

Kingdom: Plantae

Order: Zingiberales

Family: Zingiberaceae

Genus: *Zingiber*

Species: *Zingiber officinale*

I.6.3. Other Names

Arabic: zendjabil (Baba Aissa, 2000), *English:* Ginger, *French:* gingembre, *German:* Ingwer, *Italian:* zenzero, *Spanish:* jengibre, (Ogura, 2000).

I.6.4. Plant Description

A perennial creeping plant, with thick tuberous rhizome, producing an erect stem 30 - 100 cm tall. The lance-shaped leaves are bright green, 15 - 20 cm long, with a prominent longitudinal rib, enclosing conical clusters of small yellow-green flowers marked with purple speckles (Chugh, 1993).

I.6.5. Spice Description

Ginger has a distinctive thickened, branched rhizome (underground stem) which sometimes looks somewhat like a swollen hand (figure 5). The rhizome has a brown corky

outer layer (usually removed before use) and a pale yellow centre with a spicy lemon-like scent (Ohlan and Blake, 1997).

Figure 5. *Zingiber officinale*

I.6.6. Chemical Constituents

The rhizome contains an EO with a high content of mono-, and sesquiterpene derivatives (α- zingiberene). Other constituents are vitamins, carbohydrates, lipids, carboxylic acids, and minerals (Vernin and Parkanyi, 2004).

I.6.7. Uses and Benefits

Ginger is primarily used as a spice, in candied form as confectionery, and for making ginger tea. The rhizome is used in traditional Chinese medicine. For colds, headaches, and vomiting and in European folk medicine as an astringent, carminative, and expectorant (Navajas,1995). In traditional Islamic medicine, the fresh rhizome is used for vomiting, coughs, and flatulence, and the dried rhizome for stomach ache, lumbago, and diarrhea (Iskan *et al.,*2002). Other uses for Ginger Root include the treatment of asthma, bronchitis and other respiratory problems. Ginger Root may be used to help break fevers by warming the body and increasing perspiration and aphrodisiac properties (Bhrak *et al.*, 1990).

Chapter II :

The Essential Oils

II.1. History of Essential Oils

The EOs are considered mankind's first medicine and have been used around the world for centuries. EOs have been used in religious rituals, to treat various illnesses, and for other physical and spiritual needs (Sahin *et al.*, 2003).

Research dates the use of EOs back to 4500 BC. Ancient Egyptians were the first to discover the potential of fragrance, and records demonstrate that oils and aromatics were used for treating illness and performing rituals and religious ceremonies in temples and pyramids (Annissorn, 2005). Oils were used in the embalming process, in medicine and in purification rituals. In 1922, when King Tut's tomb was opened, 50 alabaster jars made to contain nearly 350 liters of oil were discovered. There are also over 200 references to aromatics, incense and ointments in the Old and New Testaments; Frankincense, Myrrh, Cinnamon, Cassia, Rosemary, Hyssop and Spikenard are noted for being used for anointing rituals and healing of the sick (Iskan *et al.*, 2002).

Anridogen (1999), reported that Arabs were the first who developed the techniques for obtaining EO from the naturally occurring organic materials. Arab physician, Avicenna, designed the protocol to extract the EO from the flowers by distillation in the tenth century. He isolated the perfume in the form of oil or attar from the rose flowers and produced rose water. The reintroduction of EOs into modern medicine first began during the late 19th and early 20th centuries. Since that time, EOs have been used traditionally to kill harmful germs, as well as spiritually to balance mood, and dispel negative emotions (Miraglia, 2009).

II.2. Definition of Essential Oils

The EOs also known as ethereal oils, are defined as, the oils obtained by the steam distillation of plants. From the view point of practical applications, these materials may be defined as odiferous bodies of an oily nature, obtained almost exclusively from vegetable organs: flowers, leaves, barks, woods, roots, rhizomes, fruits, and seeds (Annissorn, 2005; Ladegariniae *et al.*, 2006).

The EOs are mixtures of fragrant substances or mixtures of fragrant and odorless substances. A fragrant substance is a chemically pure compound, which is volatile under normal conditions and which owing to its odour (Kommert and Slinter, 2006).

The EOs, or aromatic plant essences, are volatile and fragrant substances with an oily consistency typically produced by plants. They can be liquid at room temperature though a few of them are solid or resinous, and showing different colors ranging from pale yellow to emerald green and from blue to dark brownish red. With a few exceptions, they are lighter than water and have a density between 0.75 and 0.98 g/cm^3. They are different from solid and liquid fatty substances because of their volatility, which increases with rising temperatures (Balz, 1999).

II.3. Sources of Essential Oils

According to Veiruecka (2003) and Handa (2008), the EOs are generally derived from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, lavander), leaves (e.g. mint, lemongrass, jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), bark (e.g. cinnamon, cassia, canella), wood (e.g. cedar, sandal, pine), roots (e.g. angelica, sassafras, vetiver, saussurea, valerian), seeds (e.g fennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), rhizomes (e.g. ginger, calamus, curcuma, orris) and gums or oleoresin exudations (e.g. balsam of Peru, balsam of Tolu, storax, myrrh, benzoin).

II.4. Accumulation of Essential Oils

Depending upon the plant family, EOs may accumulate in specialized secretary structures such as glandular hairs (Labiatae, Verbenaceace, Geraniaceae), modified parenchymal cells (Piperaceae), resin canals (conifers), oil tubes called vittae (Umbelliferae), lysigenous cavities (Rutaceae), schizogenous passages (Myrtaceae, Graminae, Compositae) or gum canals (Cistacae, Burseraceae)(Handa, 2008).

II.5. Biogenesis of Essential Oils

EOs are products of the secondary metabolism of plants during which process the substances of the primary metabolism such as starch, fats and proteins are degenerated. Constituents of EOs are made by two biogenetic processes. During the main process the terpenic substances are made and another process is needed to produce for example coumarins, furocoumarins and phenols (Zieslino, 1999).

Secondary metabolic processes have been studied largely in isolation and relatively little is known about its integration with primary metabolism (Singh *et al.,* 1997). Primary and secondary metabolic processes are intimately interconnected, as the later derives precursors from the primary metabolic reactions (Dubey *et al.*, 2003).

II.6. Essential Oil Extraction Processes

Various extraction methods are used in the manufacture and extraction of EOs, and the method used is normally dependant on what type of botanical material is being used. The most popular extraction methods are water distillation, steam distillation, cold pressing, enfleurage, solvent extraction, supercritical fluid extraction and microwave extraction.

II.6.1. Water Distillation

The water distillation process involves placing the desired plant material in a still and then submerging it in water. The water is then brought to a boil. The heat helps open the pockets containing the plant aromatic molecules so they can be extracted. The vapors cool and condense, the EOs separate from the water and they are collected (Iskan *et al*., 2002).

Grazaliod (1997) reported that the water in this case provides protection for the plant because it acts as a barrier. Less pressure is used as well as a lower temperature than that which is used in the steam distillation method. This extraction method works well with plants that cannot tolerate high heat.

II.6.2. Steam Distillation

Many of the EOs presently used in perfumery are obtained by steam distillation of flowers, leaves, bark, etc. Steam is widely used because of its high latent heat of evaporation, relatively cheaper and widely available (Pyrseger, 1991).

This process involves the use of steam to percolate and vapourise out the EOs from the plant material, with the subsequent condensation of steam and EO prior to their separation. The role of the distiller is to achieve an oil as close as possible to the oil as it exists in the plant. During distillation, only very tiny molecules can evaporate, so they are the only ones which leave the plant (Jofhny, 1995).

II.6.3. Cold Pressing

Armeouf (1995) reported that cold pressed expression, or scarification, is used to obtain EOs for the peels and seeds of citrus, such as bergamot, grapefruit, lemon, lime, mandarin, orange, and tangerine oils. In this process, the outer layer of the fruit peel contains the oil are removed by scrubbing. Then the whole fruit is pressed to squeeze the juice from the pulp and to release the EO from the pouches. The EO rises to the surface of the juice and is separated from the juice by centrifugation.

II.6.4. Enfleurage

The EOs, which are highly volatile and too delicate to withstand the temperature of boiling water, are obtained by the enfleurage method. In this method the EOs are extracted by absorption with fats. The pure and odourless fat in 1:2 proportion in the molten state is spread in thick layer and the flowers petals are spread on the layer and kept in a cool dark room for 24 to 72 hours. The EO gets absorbed in the fat (Peegyre *et al.*, 1992).

II.6.5. Solvent Extraction

Solvent extraction uses very little heat so it is able to produce EOs whose fragrance would otherwise be destroyed or altered during steam distillation. Solvent extraction is used on delicate plants to produce higher amounts of EOs (Chresyre, 2000). In this process, a chemical solvent such as hexane is used to saturate the plant material and pull out the EOs. The plant is removed, the solvent is then boiled off under a vacuum or in a centrifugal force machine to help separate it from the EO. Because the solvent has a lower boiling point than the EO it evaporates and the oil is left. The solvent is cooled back into liquid and reclaimed (Vaeannaz *et al.*, 1997).

II.6.6. Supercritical Fluid Extraction

Carbon dioxide is a new method of extraction using carbon dioxide gas, which is kept under high pressure at a constant temperature. Plants are placed in a stainless steel tank and, as carbon dioxide is injected into the tank, pressure inside the tank builds. Under high pressure, the carbon dioxide turns into a liquid and acts as a solvent to extract the EOs from the plants. When the pressure is decreased, the carbon dioxide returns to a gaseous state, leaving no residues behind (Kaollars, 2002).

II.6.7. Microwave

The Microwave process is a revolutionary method of extraction that reduces the extraction time to as little as a few seconds, with up to a ten-fold decrease in the use of the solvents. The target material is immersed in solvent that is “transparent” to microwaves, so only the target material is heated. Since the microwave tends to heat the inside of the material quickly, the target chemical is expelled in a few seconds. This process allows for direct extraction of fresh material without the need to dry them prior to the extraction (Slloud, 2002).

II.7. Essential Oils Chemistry and Chemical Analysis

The EOs chemistry is very complex in nature as EOs themselves have many chemical ingredients, some play a major part and others a minor part. The most EOs consist of hydrocarbons, esters, terpenes, lactones, phenols, aldehydes, acids, alcohols, ketones, and esters. Among these, the oxygenated compounds (alcohols, esters, aldehydes, ketones, lactones, phenols) are the principal odor source (Sukhdev *et al.*, 2008). The most common method used for the determination and analysis of EO includes Gas Chromatography-Mass Spectrometry (GC-MS) and High Performance Liquid Chromatography (HPLC) (Shahidi and Naczk, 1995).

II.8. Factors Affecting Essential Oil Composition and Yield

Factors that determine the composition and yield of the EO obtained are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence one another. These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage (Ranffouad *et al.,* 2008). Length of exposure to sunlight, availability of water, the presence of fungal diseases and insects (Porsegloke, 2003).

II.9. Antimicrobial Activity

II.9.1. Antimicrobial Agents

Microbiologists differentiate two groups of antimicrobial agents used in the treatment of infectious diseases. 1) Antibiotics, that are natural substances produced by certain groups of microorganisms, and 2) chemotherapeutic agents, which are chemically synthesized (Davidson, 2000). Antibiotics may have a static (inhibitory) effect or cidal (killing) effect on a range of microorganisms (Burt, 2004).

II.9.2. *In Vitro* Tests of Antimicrobial Activity

A number of methods used for evaluation of antimicrobial activity of EOs have been reported in literature. Different assays like disc diffusion assay, well diffusion assay, microdilution assay, measurement of minimum inhibitory concentration (MIC) are often used for measuring the antimicrobial activity of EOs and plants based constituents (Burt, 2004 ; Bakkali *et al*., 2008).

Researchers adapt different experimental protocols to better represent future applications in their particular field. On the other hand, since the outcome of a method can be affected by a number of factors like method used to isolate the plants EOs/extracts, growth phase, the volume of inoculum, culture medium used, pH and temperature of the media and incubation time (Rios *et al*., 1998). Comparison of published results is difficult (Burt, 2004).

Screening of EOs for antimicrobial activity is often done by the disc diffusion assay, in which a paper disc soaked with known concentration of EO is laid on top of an inoculated agar plate. This is generally used as a preliminary check for antimicrobial activity prior to more detailed studies. A number of factors such as the amount of EO placed on the paper discs and the thickness of the agar layer vary considerably between studies (Deviando *et al*., 2009). Generally, the disc diffusion assay is useful for screening between EOs but direct comparison of published data is not feasible (Elgayyar *et al*., 2001). In agar well diffusion assay, well are formed by cutting wells in agar and the EOs are loaded to that wells. This method is mostly sued as a screening method when large numbers of EOs and/or large numbers of bacterial isolates are to be screened (Dorman and Deans, 2000).

Ruberto (2000) and Pintore *et al*., (2002) reported that the most cited and important method in the antimicrobial performance of EOs is the measurement of minimum inhibitory concentration (MIC), which tells us the accurate, exact and reproducible results. In some cases, the minimum bactericidal concentration (MBC) is stated. The strength of the antimicrobial activity can be determined by dilution of EOs in agar or broth.

In broth dilution studies a number of different procedures exist for determining the MIC and MBC. The most common methods are that of measurement of optical density and the enumeration of colonies by viable count (Ultee *et al*.,1998 ; Lambert *et al*., 2001). A new microdilution method for determining the MIC of oil based compounds uses the resazurin as a visual indicator of the MIC (Sarker *et al*., 2007).

The diversity of ways of reporting the antimicrobial activity of EOs limits comparison between studies and could lead to duplication of work. One feature of test methods that varies considerably is whether or not a solvent/emulsifier is used to dissolve the EOs/extracts or to stabilise it in waterbased culture media. Several substances have been used for this purpose: methanol/ethanol, dimethyl sulfoxide, n hexane, Tween-20/Tween-80 (Ometta, 2006).

II.9.3. Essential Oils as a Natural Antimicrobial Agents

The EOs and other naturally occurring antimicrobials are attractive to the food industry for the following reasons (Sondin *et al*., 2005): (1) it is highly unlikely that new synthetic compounds will be approved for use as food antimicrobials due to the expense of toxicological testing, (2) there exists a significant need for expanded antimicrobial activity both in terms of spectrum of activity and of broad food applications, (3) food processors are interested in producing “green” labels, i.e., ones without chemical names, and (4) there are potential health benefits that come with the consumption of some naturally occurring antimicrobials.

Recently, EOs of certain plants have been shown to have antimicrobial effects, as well as imparting flavour to foods (Burt, 2004). Some EOs have shown promise as potential food safety interventions when added to processed and raw foods. Some of the most effective natural antimicrobials are extracted from spices and herbs EOs and isolates of the different plant families (Juliano *et al*., 2000; Lambert *et al*., 2001; Burt, 2004). There are many reports in literature regarding the antimicrobial activity of EOs (Pandey *et al*., 2003; Sondin *et al.,* 2005; Kommert and Slinter, 2006).

Among several EOs that may be useful as antimicrobial agents, marjoram oil (*Origanum majorana* L.) may have the greatest potential for use in industrial applications (Deans and Svoboda, 1990; Daferera *et al*., 2000). Sokovic and VanGriensven (2006) evaluated the EOs from *Mentha spicata, Ocimum basilicum, Thymus vulgaris, Origanum vulgare, Salvia officinalis* EOs and their components against three major pathogens of the button mushroom, *Agaricus bisporus*, i.e. the fungi *Verticillium fungicola* and *Trichoderma harzianum* and the bacterium *Pseudomonas tolaasii*. The maximum antimicrobial activity was observed for the *Origanum vulgare* EO.

EOs of *Curcuma longa*, *Satureja sp*., *Rosemaryinus officinalis*, *Thymus vulgaris*, *Salvia officinallis*, *Cinnamomum zeylanicum*, *Origanum vulgare*, *Syzygium aromaticum* and *Camellia sinensis* have been investigated for their inhibitory activity against the foodborne pathogen *Bacillus cereus* (Valero and Salmero'n, 2003 ; Chorianoponlos *et al.*, 2004).

The effects of other EOs such as carrot seed, spearmint, chamomile, orange flower, ginger, against *H. pylori* have also been reported (Kalpoutzakis *et al.*, 2001, Weseler *et al.*, 2005). A variety of EOs from thyme, cinnamon bark, lemongrass, perilla, peppermint, tea tree, coriander, lavender, rosemary, eucalyptus, *Lippia javanica* and *Achillea clavennae* among others on respiratory tract pathogenes has been reported (Skocibusic *et al.*, 2004 ; Viljoen *et al.*, 2005).

II.9.4. The Mode of Antimicrobial Action of Essential Oils

The exact antimicrobial mechanism of EOs is poorly understood. However, it has been suggested that their lipophilic property and chemical structure could play a role (Villoch, 2005). Helander (2003) suggested that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilicity but it has also been proposed that structural properties, such as the presence of the fuctional groups and aromaticity are responsible for the antibacterial activity (Bennett, 2008). It is thought that membrane perforation is the principle mode of action leading to an increase of permeability and leakage of vital intracellular constituents resulting in impairment of bacterial enzyme systems (Sallerio, 2007).

II.10. Toxicity of Essential Oils

As toxicity is dose-dependent, the only risk of toxicity with EOs is concerned with overuse and overdose. dose-dependency also refers to the size of the individual being treated: special care is required when treating a baby or young child as they are much more likely to develop toxicity with a much smaller amount of EO than an adult (Fontackhle, 2005).

Dijoset (2006) reported that EOs have been used in the form of the whole plant for thousands of years for medicinal and cosmetic purposes, but when distilled from the plant they become a hundred times more concentrated. Their physical, physiological and pharmacological effects on the body are therefore increased, and knowledge of safe levels of usage are of paramount importance to a practising aromatherapist.

The majority of EOs when used correctly in aromatherapy treatments represent a negligible risk. However, it should be remembered that EOs are very powerful and concentrated substances, and should therefore be employed with a great deal of care as inappropriate use may cause undesired effects (Busattla, 2008).

II.11. Uses and Benefits of Essential Oils

EOs have become an integral part of everyday life. They are used in a great variety of ways: as food flavorings, as food additives, as food preservatives, as flavoring agents by the cigarette industry, and in the compounding of cosmetics and perfumes (Kivank and Akgul, 1998 ; Sahin *et al*., 2003). Furthermore, they are used in air fresheners and deodorizers as well as in all branches of medicine such as in pharmacy, balneology, massage, and homeopathy. A more specialized area will be in the fields of aromatherapy and aromachology (Yasir and williams, 2007).

In recent years, the importance of EOs as biocides and insect repellents has led to a more detailed study of their antimicrobial potential. EOs are also good natural sources of substances with commercial potential as starting materials for chemical synthesis (Miraglia, 2009)**.**

Experimental Part

Chapter III :

Materials and Methods

Materials and Methods

III.1. Extraction of Essential Oils

III.1.1. Preparation of Spices

The spices samples used in the present study (table 1) were purchased from the local market. In order to obtain the spice EOs, 100 g of each spice were crushed in a mortar and pestle.

Table 1. The selected spices.

Name	Botanical name	Family	Part of plant
Cinnamon	*Cinnamomum zeylanicum*	Lauraceae	Bark
Black cumin	*Nigella sativa*	Ranunculaceae	Seed
Aniseed	*Pimpinella anisum*	Apiaceae	Seed
Clove	*Syzygium aromaticum*	Myrtaceae	Bud
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome

III.1.2. Extraction Procedure

The extraction of EOs was conducted in the laboratory of Biochemsity**,** University of Ziane Achour Djelfa. The ground spices were subjected to waterdistillation (figure 7) for 4 h using a Clevenger-type apparatus (Annissorn, 2005; Miraglia 2009). Briefly, the ground spice was added to round-bottom flask and filled half full with water. Then, the mixture was heated. The heat helps to open the pockets containing the spices aromatic molecules so they can be extracted.

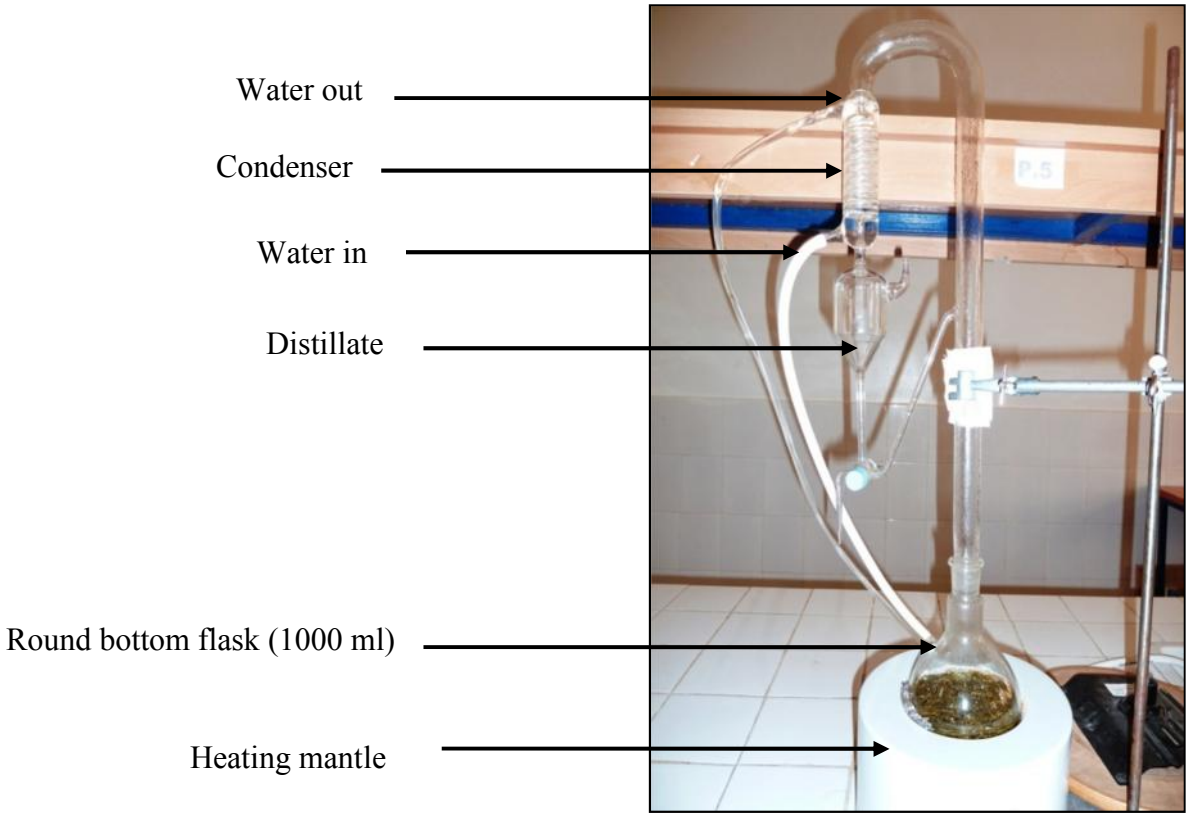


Figure 7. Clevenger apparatus.

The temperature of the process needs to be carefully controlled, just enough to force the ground spice to release the EO, not so hot as it may burn the ground spice. The vapors which contain the EO were passed through a cooling system and condensed into liquid which is the distillate (EO and water).

To separate the EO from water, the distillate was transfered to a separatory funnel and extracted with 20 ml portions of diethyl ether three times, the separatory funnel was stirred and there was an overpressure, the tap was opened for degassing, then, it was placed on the funnel support and the cap was removed to allow to settle the phases.

The organic phase which contains EO and diethyl ether was recovered. Diethyl ether was evaporated overnight. To remove all traces of water in the organic phase, a small amount of an anhydrous magnesium sulfate $MgSO_4$ was added to the organic phase with a simple agitation and filtred through the filter paper.

The EOs were stored in sealed glass bottles, and protected from the light by wrapping in aluminum foil at 4 °C.

III.1.3. Extraction Yield

The extraction yield was achieved by dividing the weight of the collected EO with the weight of the raw material. The calculation for the yield of the EO is as follows (Sondin *et al.,* 2005):

$$\text{Yield (\%)} = \frac{\text{Weight of the collected EO (g)} \times 100\%}{\text{Initial weight of sample (g)}}$$

The process of EOs extraction and recovering are illustrated in the following figure:

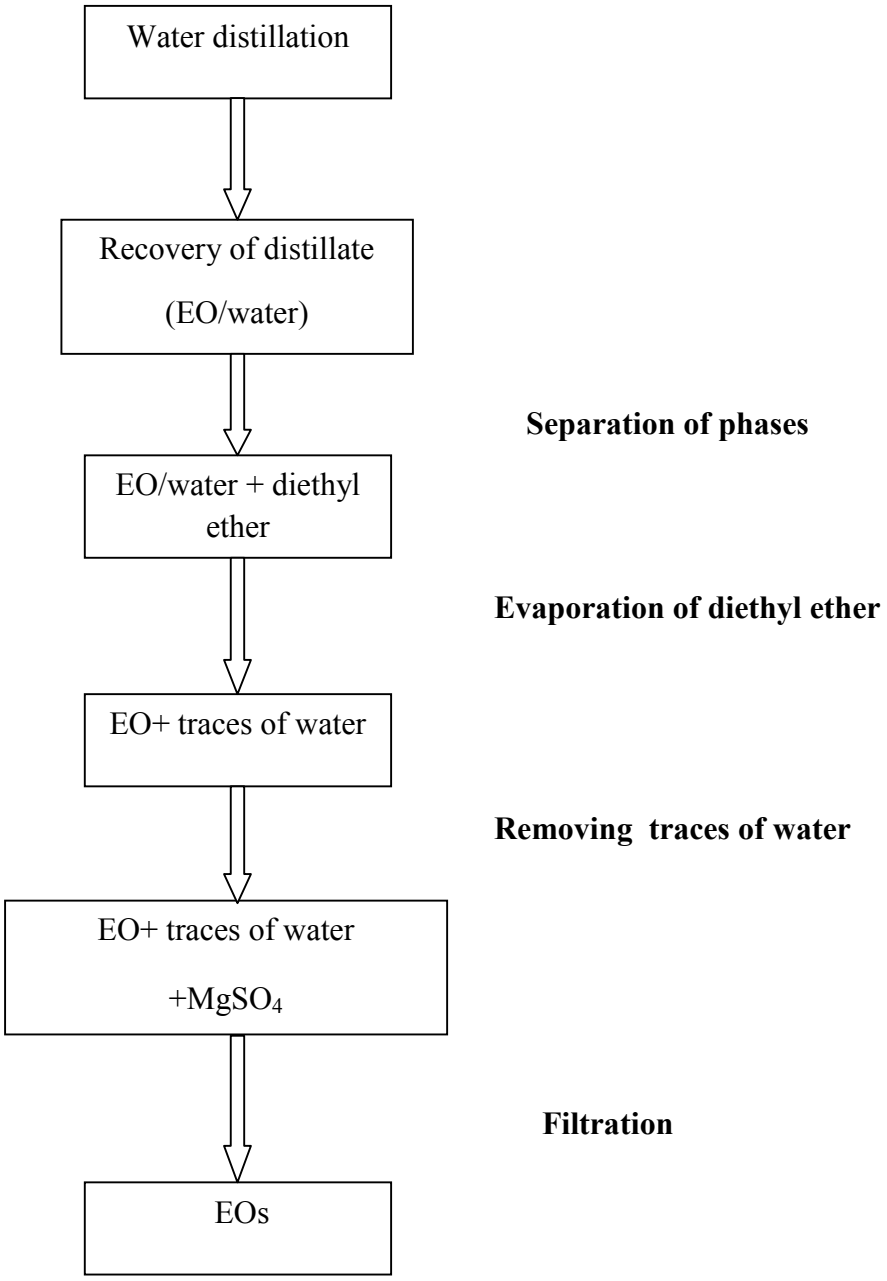


Figure 8. The process of extracting and recovering the EOs

III.2. Assessment of the Antimicrobial Activity

III.2.1. Microbial Strains

The tests for assessing the antimicrobial activity of spice EOs (*Cinnamomum zeylanicum, Nigella sativa, Pimpinella anisum, Syzygium aromaticum* and *Zingiber officinale)* were carried out using six food borne bacterial strains and two molds involved in food poisoning and infectious diseases. The used microbial strains were as follows :

Gram negative bacteria :

Escherichia coli ATCC 25922 (*E.coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P.aeruginosa*), *Salmonella typhi* ATCC 14023 (*S.typhi*).

Gram positive bacteria :

Staphylococcus aureus ATCC 25923 (*S.aureus*), *Bacillus cereus* ATCC 14579 (*B.cereus*), *Listeria monocytogenes* ATCC 19115 (*L.monocytogenes*),

Molds :

Aspergillus niger ATCC 6275 (*A.niger*) and *Aspergillus flavus* ATCC 9170 (*A.flavus*).

The strains which are *E.coli*, *P.aeruginosa*, *S.aureus*, *A.flavus* and *L.monocytogenes*, were obtained from Pasteur institute of Algeria.

The strains which are *B.cereus*, *S.typhi* and *A.niger* were obtained from Pasteur institute of Paris.

III.2.2.Culture Media

The culture media used in these tests are:

- Mueller Hinton Agar (MHA)
- Nutrient Agar (NA)
- Nutrient Broth (NB)
- Potato Dextrose Agar (PDA)
- Potato Dextrose Broth (PDB)

Culture media compositions are presented in the annex A.

III.2.3.Turbidity Standard for Inoculum Preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard, was used and prepared as follows: a 0.5 ml of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) was added to 99.5 ml of 0.18 mol / L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension (Chopneeda, 2002).

The correct density of the turbidity standard was verified by using a spectrophotometer to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard. The Barium Sulfate suspension was transferred in 4 to 6 ml aliquots into test tubes with screw-cap of the same size as those used in growing and diluting the bacterial inoculum. These tubes were tightly sealed and stored in the dark at room temperature.

III.2.4. Preparation of Dried Filter Paper Discs

Whatman filter paper n° 1 used to prepare discs of 6 mm and 2.5 cm in diameter, which were placed in a Petri dish and sterilized.

III.2.5. Preparation of Microbial Inoculum

III.2.5.1. Preparation of Bacterial Inoculum

Each culture should be streaked on NA in order to obtain well-isolated discrete colonies. After an incubation of 24 h at 37 ºC, with the Pasteur pipet, colonies were introduced in sterile test tube containing the physiologic water.

The opacity of the bacterial suspension should be equivalent to 0.5 McFarland (10^8 Colony Forming Units CFU/ ml). To make a comparison, the bacterial suspension and $BaSO_4$ turbidity standard were compared against a white background with a contrasting black line (Sipoulou and Ynot, 1995).

III.2.5.2. Preparation of Fungal Inoculum

Suspensions of fungal cells and spores were prepared from pure and young cultures (cultures of 3 days) by scrubbing the Petri dishes. With a volume of 5 ml of sterile physiologic water from these suspensions, which are considered as stock solution, a different dilutions were prepared.

After agitation, the fungal suspensions were standardized by using the spectrophotometer at 630 nm. An optical density of 0.04 is relevant to a concentration of 10^7 spores/ml (Bhrak *et al.*, 1990).

III.2.6. Disc Diffusion Assay

Disc diffusion assay is an old method, but still relevant as it is used in laboratories of bacteriology to measure the antibacterial activity of the antibiotics. For the EOs, this method is called aromatogram equivalent to antibiogram where the antibiotics are substituted by the EOs.

III.2.6.1. Antibiogram Assay

The microorganisms were tested for their sensitivity towards the antibiotics Cefoxitin (FOX: 30 µg), Chloramphenicol (C: 30 µg), Gentamicin (GM: 30 µg), Kanamycin (KAN: 30 µg), and Tetracyclin (TE: 30 µg) by the disc diffusion assay.

After standardization of bacterial inoculum, the cultures were aseptically swabbed on the surface of MHA agar by using sterile cotton swabs.

The antibiotic discs were aseptically placed over the inoculated MHA agar sufficiently separated from each other to avoid overlapping of the inhibition zones. The plates were incubated at 37ºC for 24 h and the diameters of the inhibition zones were measured in mm.

According to Cyfer *et al.* (2008), the sensitivity of microbs is as follows :

For the gram negative bacteria : >16 mm : sensitive, 13-16 mm : intermediate, <13 mm resistant.

For the gram positive bacteria : >18 mm : sensitive, 14-18 mm : intermediate, <14 mm resistant.

III.2.6.2. Aromatogram Assay

The aromatogram is based on using the filter paper discs impregnated with EOs placed on the surface of inoculated agar. After incubation the dimater of inhibition zones are measured in mm. These correspond to zones where the microbs have been inhibited or destroyed by the diffusion of the EO (Borchat and Riensven, 2006).

Within 15 mn after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension, pressed firmly against the inside wall of the tube just above the fluid level and the swab was rotated to remove excess liquid. The swab was streaked over the entire surface of the MHA agar (bacteria) and PDA agar (molds).

This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. The lid was left ajar for 3 to 5 mn to allow for any excess surface moisture to be absorbed before applying the impregnated discs.

In this study, Wattman paper discs of 6 mm were used. These discs were impregnated with 10 µl of EO. These EOs were previously diluted in absolute ethanol : 1/2, 1/5, 1/10 and 1/20 (v/v). Other discs impregnated with 10 µl of ethanol were used as negative control. The discs were then placed on the surface of inoculated agar, after 24 h of incubation at 37 ^{o}C (bacteria) and 5 days at 25 ^{o}C (molds), the diameters of inhibition zones around the discs were measured.

To get reliable results, the experiments cited above were repeated three times, and to ensure comparable experimental conditions, each three discs of similar concentration were placed in the same dish.

III.2.6.3. Determination of the Nature of Essential Oil Inhibition

To determine the nature of EO inhibition as bactericidal or bacteriostatic activity, against the bacteria and fungicidal or fungistatic activity against the molds, a sample from the zone of inhibition was transferred into the test tube containing a NB broth (bacteria) and PDB broth (molds) (Vauke,1995).

After incubation of 24 h at 37°C (bacteria) and 3 days at 25 °C (molds), the test tubes were examined visually. The activity was bacteriostatic if the bacteria re-grow and was considered as bactericidal if the bacteria did not re-grow, fungistatic if the molds re-grow and was considered as fungicidal if the molds did not re-grow.

III.2.7. Agar Dilution Assay

III.2.7.1. Determination of the Minimum Inhibitory Concentration

This method allows to determine the MIC value which is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after incubation (Iskan *et al.*, 2002).

A solution of Tween 80 and physiologic water were sterilized at 120 °C during 15 mn. To this solution, an aliquot of EO was added to obtain a final concentration of 10%. The mixture was agitated during 2 mn in order to disperse the EO in the solution (the stock solution). Two serial of dilution were prepared from stock solution ; each 2 ml of dilution were incorporated to 18 ml of culture media, MHA agar (bacteria) and PDA agar (molds), the mixtures were immediately distributed in two Petri dishes at a rate of 20 ml by Petri dish.

The range of final concentration obtained corresponds to 0.5 - 0.25 - 0.12 - 0.06 and 0.03%. The inoculation of microbial strains was carried out on the surface by streaking from bacterial suspension equivalent to 0.5 Mc Farland and fungal suspension equivalent to 10^7 spores/ml. Each Petri dish of such concentration was relevent to one strain, then the Petri dishes were incubated at 37 °C (bacteria) and 25 °C (molds) for 24 h and 5 days respectively.

III.2.7.2. Determination of the Minimum Bactericidal and Fungicidal Concentration

The MBC and MFC values correspond to the lowest concentration of EO capable of killing 99.9% of intial microbial inoculum. It defines the bactericidal and fungicidal effect of the EO (Kommert and Slinter, 2006).

From the Petri dishes of the agar dilution assay of MICs with zero developement, inoculations were carried out on MHA agar for bacteria and PDA agar for molds. These inoculate Petri dishes were incubated at 37 ^{o}C (bacteria) and 25 ^{o}C (molds) for 24 h and 5 days respectively. The MBC values of EO were deduced from the first dish where no developement was seen.

III.2.8. Microatmosphere Assay

This technique allows to determine the MIQ value which is defined as the smallest quantity of EO which no growth is visible and describes the diffusion of volatile EOs components in closed Petri dishes to monitor the growth of the tested strains (Kilbuck, 2010).

This technique is based on using a paper disc impregnated with EOs and deposited in the center of the Petri dish that contains previously the inoculated strains and then incubated. In this study, the inoculation was carried out by swab as described in the disc diffusion assay on the MHA agar for bacteria and PDA agar for molds, a paper disc of 2.5 cm of diameter, sterilized and deposited on the bottom of lid Petri dish.

For each tested EO (*Cinnamomum zeylanicum*, *Nigella sativa*, *Pimpinella anisum*, *Syzygium aromaticum* and *Zingiber officinale*), An aliquot amount (10, 20, 40 and 80 µl) were applied to the paper discs. The dishes were immediately closed, cover on bottom, and incubated at 37 C^{o} (bacteria) and 25C^{o} (molds) for 24h and 5 days respectively.

Chapter IV :

Results

IV.1. Extraction Yields

Table 2 presents EO yields obtained by water distillation of the five spices. As shown here, each spice contained a little amount of EO. These results demonstrated that *Syzygium aromaticum*, *Pimpinella anisum*, *Cinnamomum zeylanicum*, *Nigella sativa* and *Zingiber officinale* produced 4.0, 1.7, 1.53, 0.9 and 0.5% yields, respectively. Thus, the comparison between the five spices showed that the *Syzygium aromaticum* provided the highest yield with 4% and the *Zingiber officinale* provided the lowest yield with 0.5% of EO.

Table 2. Yields of spice EOs.

N°	Spices	Yield, %
1	*Syzygium aromaticum*	**4%**
2	*Pimpinella anisum*	**1.7%**
3	*Cinnamomum zeylanicum*	**1.53%**
4	*Nigella sativa*	**0.9%**
5	*Zingiber officinale*	**0.5%**

IV.2. Disc Diffusion Assay

IV.2.1. Antibiogram Assay

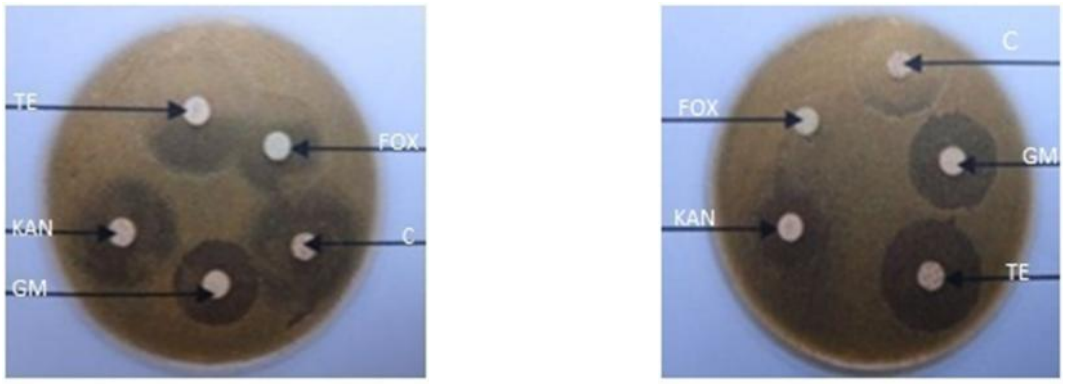
According to the results of antibiogram assay presented in the table 3, *S. aureus* strain (figure 9) was resistant to GM (12 mm) and sensitive to FOX (19 mm), KAN (19 mm), C (24 mm) and TE (25 mm). *B.cereus* was resistant to GM (0 mm), intermediate to FOX (15 mm) and sensitive to KAN (19 mm), C (23 mm) and TE (21 mm). *L.monocytogenes* (figure 9) was resistant to FOX (0 mm) and KAN (10 mm), itermediate to GM (17 mm) and C (17 mm) and sensitive to TE (21 mm). *E.coli* was resistant to GM (10 mm) and TE (0 mm), intermediate to C (15 mm) and sensitive to FOX (17 mm) and KAN (23 mm). *S.typhi* was resistant to all the antibiotics with diameter ranging from 0 to 7 mm and *P.aeruginosa* was also resistant to all the antibiotics with diameter ranging from 3 to 7 mm.

Table 3. The inhibition zones of antibiotics (diameter in mm)

	Disc charge	*S.aureus*	*E.coli*	*S.typhi*	*P.aeruginosa*	*B.cereus*	*L.monocytogenes*
FOX	30 µg	19	17	0	3	15	0
GM	30 µg	12	10	4	3	0	17
KAN	30 µg	19	23	2	5	19	10
C	30 µg	24	15	6	4	23	17
TE	30 µg	25	0	7	7	21	21

Data presented without including the diameter of disc.

S. aureus :Staphyloccocus aureus , E. coli :Esherichia coli, S.typhi : Salmonella typhi, P. aeruginosa : Pseudomonas aeruginosa, B. cereus : Bacillus cereus, L. monocytogenes : Listeria monocytogenes.

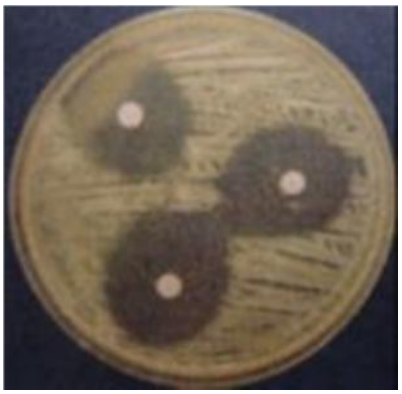


S.aureus *L.monocytogenes*

Figure 9. The antibiogram assay of *S. aureus* and *L. monocytogenes*.

IV.2.2. Aromatogram Assay

The activity of EOs was expressed in the diameter of inhibition zones (figure 10). According to the results of aromatogram assay presented in tables (4, 5, 6, 7 and 8), all the EOs demonstrated an activity against the tested strains. The ethanol as negative control presented no inhibition against the tested strains proving that it is a suitable solvent for testing EOs.



B. cereus (zones of inhibition)
Cinnamomum zeylanicum EO

A. flavus (total inhibition)
Syzygium aromaticum EO

Figure 10. Aromatogram assay.

For the effect of *Cinnamomum zeylanicum* EO (table 4), the results showed that *S. aureus* was the most inhibited bacterium with a big zones of inhibition (44.17±1.83, 26.5±1.59 and 19.83±0.75) at the dilutions (1/2, 1/5 and 1/10), followed by *B. cereus* and *L. monocytogenes* which were inhibited with an approximate values (38.00±1.55) and (36.08±1.78) respectively. However, *P. aeruginosa* was the less inhibited bacterium with small zones of inhibition (9.48±1.66, 07.00±0.99 and 04.08±0.33) at the dilutions (1/2, 1/5 and 1/10), followed by *E. coli and S. typhi* which were inhibited with an approximate values (14.67±0.52) and (15.77±0.52), respectively. Besides, *A. flavus* and *A. niger* were completely inhibited at the dilutions (1/2 and 1/5).

Table 4. The effect of the EO of *Cinnamomum zeylanicum*

dilution / strain	1/2	1/5	1/10	1/16	1/20	ethanol
S. aureus	44.17±1.83	26.5±1.59	19.83±0.75	06.55±0.50	03.23±0.20	0
E. coli	14.67±0.52	11.83±0.89	10.00±0.89	08.00±0.9	06.15±0.33	0
P. aeruginosa	9.48±1.66	07.00±0.99	04.08±0.33	01.99±0.15	0	0
B. cereus	38.00±1.55	21.14±0.71	15.00±0.60	06.08±0.45	02.78±0.29	0
S. typhi	15.77±0.52	12.83±0.75	09.33±0.41	07.15±0.36	05.00±0.22	0
L. monocytogenes	36.08±1.78	20.02±1.05	12.88±0.76	05.49±0.45	0	0
A. niger	90	90	55.06±2.16	35.02±1.77	0	0
A. flavus	90	90	65.88±3.21	41.12±1.89	0	0

Data presented as mean ± SD, excluding the diameter of disc

For the effect of *Nigella sativa* EO (table 5), the results showed that *S. aureus* was the most inhibited bacterium with a big zones of inhibition (49.05±1.89, 37.22±1.56 and 19.78±1.03) at the dilutions (1/2, 1/5 and 1/10), followed by *L. monocytogenes* and *B. cereus* which were inhibited with an approximate values (37.77±1.53) and (35.26±1.45) respectively. However, *P. aeruginosa* was the less inhibited bacterium with small zones of inhibition (16.03±0.99), (10.06±0.46) and (07.39±0.32) at the dilutions (1/2, 1/5 and 1/10) followed by *E. coli* and *S. typhi* which were inhibited with an approximate values (20.02±0.82) and (21.08±1.44) respectively. Besides, *A. flavus* was more inhibited than *A. niger* with zones of inhibition (67.86±1.98) and (50.38±1.66) respectively at the dilution (1/2). The same observation had been noticed for the other dilutions.

Table 5. The effect of the EO of *Nigella sativa*

dilution / strain	1/2	1/5	1/10	1/16	1/20	ethanol
S. aureus	49.05±1.89	37.22±1.56	19.78±1.03	08.55±0.79	03.77±0.33	0
E. coli	20.02±0.82	11.99±0.52	08.65±0.32	03.08±0.19	0	0
P. aeruginosa	16.03±0.99	10.06±0.46	07.39±0.32	05.12±0.21	0	0
B. cereus	35.26±1.45	26.08±1.08	13.22±0.86	06.36±0.56	0	0
S. typhi	21.08±1.44	14.06±0.88	09.22±0.48	03.59±0.29	0	0
L. monocytogenes	37.77±1.53	29.59±1.09	15.98±0.80	6.35±0.25	0	0
A. niger	50.38±1.66	37.02±1.06	21.44±0.82	09.08±0.45	03.02±0.12	0
A. flavus	67.86±1.98	49.66±1.46	35.26±0.98	28.13±0.53	09.06±0.42	0

Data presented as mean ± SD, excluding the diameter of disc

For the effect of *Pimpinella anisum* EO (table 6), the results showed that *S. aureus* was the most inhibited bacterium with a big zones of inhibition (35.26±0.98, 21.04±0.73 and 14.77±0.51) at the dilutions (1/2, 1/5 and 1/10), followed by *L. monocytogenes* and *B. cereus* which were inhibited with an approximate values (30.55±0.97) and (29.06±0.67) respectively. However, *P. aeruginosa*, *E. coli* and *S. typhi* were inhibited with an approximate values (15.02±0.93), (16.05±0.99) and (17.49±0.79), respectively at the dilution (1/2). Besides, *A. flavus* was more inhibited than *A. niger* with zones of inhibition (52.04±1.76) and (45.68±1.65) respectively at the dilution (1/2). The same observation had been noticed for the other dilutions.

Table 6. The effect of the EO of *Pimpinella anisum*

dilution / strain	1/2	1/5	1/10	1/16	1/20	ethanol
S. aureus	35.26±0.98	21.04±0.73	14.77±0.51	07.06±0.43	0	0
E. coli	16.05±0.99	11.45±0.69	06.98±0.43	0	0	0
P. aeruginosa	15.02±0.93	09.33±0.53	05.89±0.32	0	0	0
B. cereus	29.06±0.67	17.04±0.43	08.35±0.31	0	0	0
S. typhi	17.49±0.79	11.57±0.65	07.39±0.43	0	0	0
L. monocytogenes	30.55±0.97	19.95±0.75	11.83±0.45	04.66±0.31	0	0
A. niger	45.68±1.65	28.68±0.89	09.99±0.37	0	0	0
A. flavus	52.04±1.76	39.88±1.55	18.99±0.89	05.79±0.29	0	0

Data presented as mean ± SD, excluding the diameter of disc

For the effect of *Syzygium aromaticum* EO (table 7), the results showed that *S. aureus* was the most inhibited bacterium with a big zones of inhibition (55.03±2.61, 44.33±2.08 and 30.67±1.50) at the dilutions (1/2, 1/5 and 1/10), followed by *B. cereus* and *L. monocytogenes* which were inhibited with an approximate values (46.55±1.69) and (45.89±1.78) respectively. However, *P. aeruginosa* was the less inhibited bacterium with small zones of inhibition (17.50±0.54), (13.83±1.17) and (09.50±1.22) at the dilutions (1/2, 1/5 and 1/10), followed by *E. coli* and *S. typhi* which were inhibited with an approximate values (28.09±1.65) and (29.01±1.76) respectively. Besides, *A. flavus* and *A. niger* were completely inhibited at the dilutions (1/2 and 1/5).

Table 7. The effect of the EO of *Syzygium aromaticum*

dilution / strain	1/2	1/5	1/10	1/16	1/20	ethanol
S. aureus	55.03±2.61	44.33±2.08	30.67±1.50	09.00±0.98	02.35±0.09	0
E. coli	28.09±1.65	16.55±0.99	10.55±0.56	03.93±0.21	0	0
P. aeruginosa	17.50±0.54	13.83±1.17	09.50±1.22	0	0	0
B. cereus	46.55±1.69	39.08±1.03	27.66±0.59	15.75±0.36	07.54±0.19	0
S. typhi	29.01±1.76	25.83±1.83	18.33±1.63	0	0	0
L. monocytogenes	45.89±1.78	37.89±1.45	25.76±1.29	15.43±0.82	04.88±0.42	0
A. niger	90	90	34.67±2.66	13.50±2.88	0	0
A. flavus	90	90	46.00±3.74	27.33±1.86	0	0

Data presented as mean ± SD, excluding the diameter of disc

For the effect of *Zingiber officinale* EO (table 8), the results showed that *S. aureus* was the most inhibited bacterium with a big zones of inhibition (38.07±2.33, 30.88±1.99 and 22.69±0.88) at the dilutions (1/2, 1/5 and 1/10), followed by *B. cereus* and *L. monocytogenes* which were inhibited with an approximate values (30.92±1.70) and (29.00±1.55) respectively. However, *P. aeruginosa* was the less inhibited bacterium with small zones of inhibition (14.00±0.90), (12.03±0.59) and (05.65±0.39) at the dilutions (1/2, 1/5 and 1/10) followed by *S. typhi* and *E. coli* which were inhibited with an approximate values (18.01±1.57) and (18.90±1.86), respectively at the dilution (1/2). Besides, *A. flavus* was more inhibited than *A. niger* with zones of inhibition (60.79±1.99) and (55.19±1.85) respectively at the dilution (1/2). The same observation had been noticed for the other dilutions.

Table 8. The effect of the EO of *Zingiber officinale*

dilution / strain	1/2	1/5	1/10	1/16	1/20	ethanol
S. aureus	38.07±2.33	30.88±1.99	22.69±0.88	17.64±0.23	11.09±0.15	0
E. coli	18.90±1.86	14.09±1.01	08.04±0.79	04.68±0.20	0	0
P. aeruginosa	14.00±0.90	12.03±0.59	05.65±0.39	05.09±0.19	0	0
B. cereus	30.92±1.70	21.89±0.99	13.88±0.60	06.98±0.25	0	0
S. typhi	18.01±1.57	14.03±0.95	09.00±0.50	0	0	0
L. monocytogenes	29.00±1.55	18.00±0.80	12.36±0.46	0	0	0
A. niger	55.19±1.85	30.22±0.98	10.55±0.45	0	0	0
A. flavus	60.79±1.99	35.08±1.02	16.99±0.53	05.33±0.31	0	0

Data presented as mean ± SD, excluding the diameter of disc.

To get more data, the efficacy of EO dilutions against the tested strains was so remakable (figures 11, 12, 13, 14 and 15), in which, the EO was more active when it was more concentrated, and this activity decreased with the higher dilution, whatever the tested strain. So this activity is inversely proportional to the dilution.

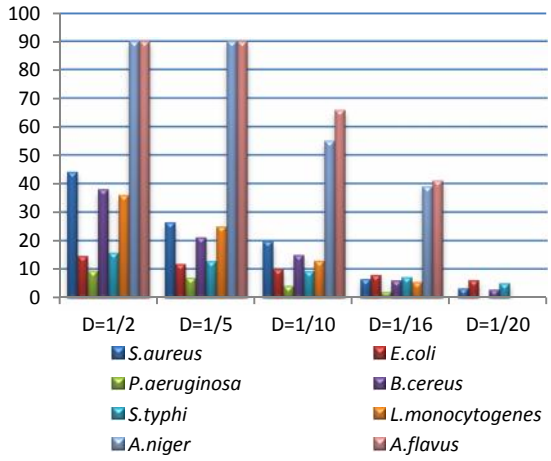


Figure 11. *Cinnamomum zeylanicum* EO dilution.

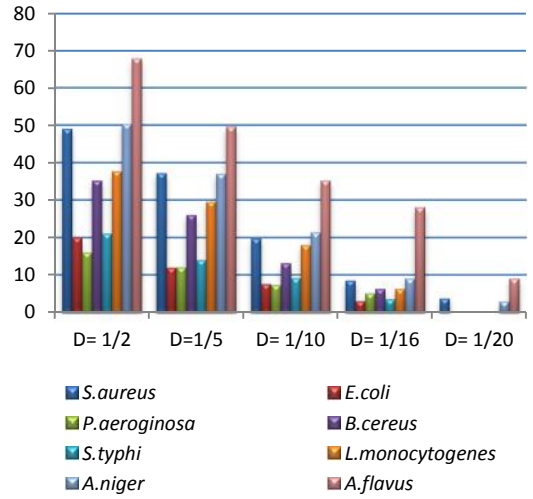


Figure 12. *Nigella sativa* EO dilution.

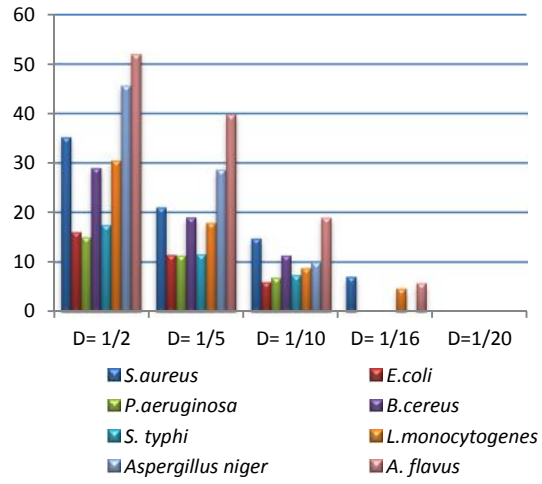


Figure 13. *Pimpinella anisum* EO dilution.

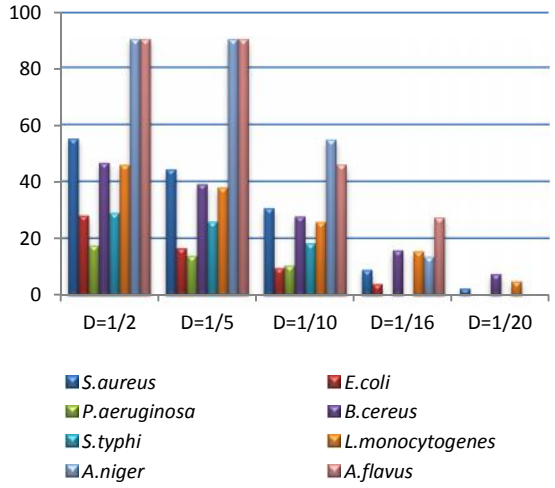


Figure 14. *Syzygium aromaticum* EO dilution.

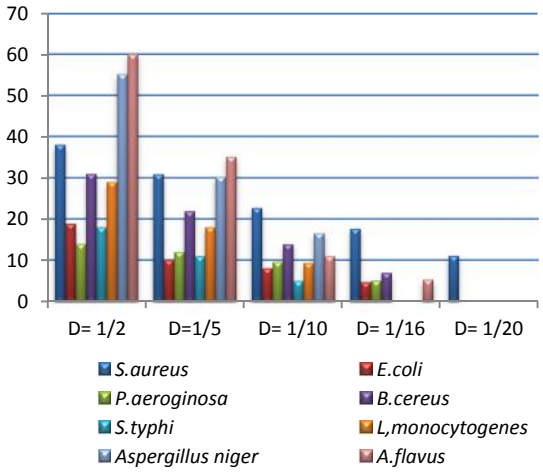


Figure 15. *Zingiber officinale* EO dilution.

The efficacy of EOs against each tested strain at the lowest dilution (1/2) was in the following order :

For *S. aureus* (figure 16)

Syzygium aromaticum > Nigella sativa> Cinnamomum zeylanicum> Zingiber officinale> Pimpinella anisum

For *P. aeruginosa* (figure 17)

Syzygium aromaticum> Nigella sativa > Pimpinella anisum> Zingiber officinale> Cinnamomum zeylanicum.

For *B. cereus* (figure 18)

Syzygium aromaticum> Cinnamomum zeylanicum> Nigella sativa> Zingiber officinale> Pimpinella anisum.

For *S. typhi* (figure 19)

Syzygium aromaticum> Nigella sativa> Zingiber officinale> Pimpinella anisum > Cinnamomum zeylanicum.

For *L. monocytogenes* (figure 20)

Syzygium aromaticum> Nigella sativa> Cinnamomum zeylanicum> Pimpinella anisum >Zingiber officinale.

For *E. coli* (figure21)

Syzygium aromaticum> Nigella sativa> Zingiber officinale> Pimpinella anisum > Cinnamomum zeylanicum.

For *A. niger* (figure 22)

Syzygium aromaticum / Cinnamomum zeylanicum> Zingiber officinale> Nigella sativa> Pimpinella anisum.

For *A. flavus* (figure 23)

Syzygium aromaticum / Cinnamomum zeylanicum> Nigella sativa> Zingiber officinale> Pimpinella anisum.

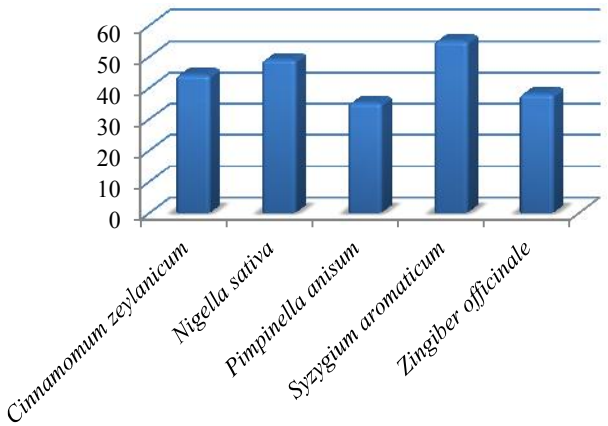


Figure 16 : The effect of EOs against *S. aureus*

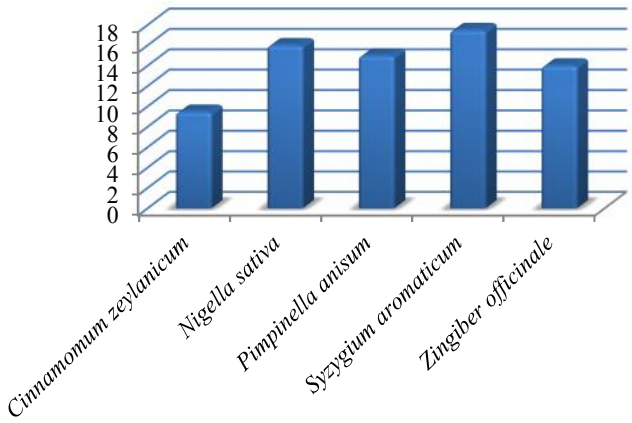


Figure 17 : The effect of EOs against *P. aeruginosa*

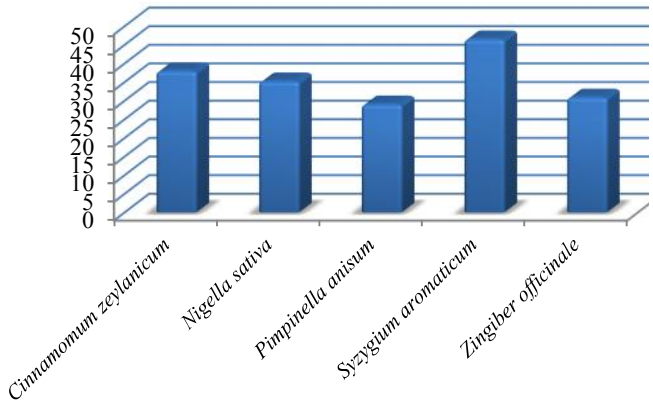


Figure 18 : The effect of EOs against *B. cereus.*

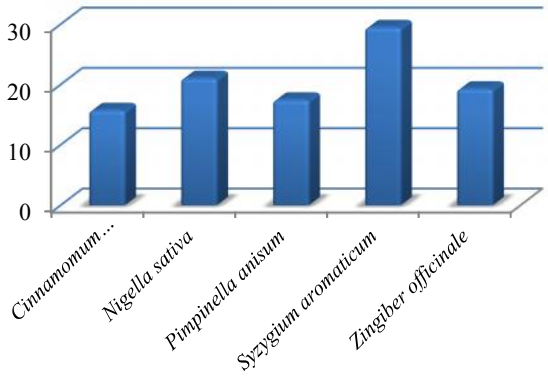


Figure 19 : The effect of EOs against *S. typhi.*

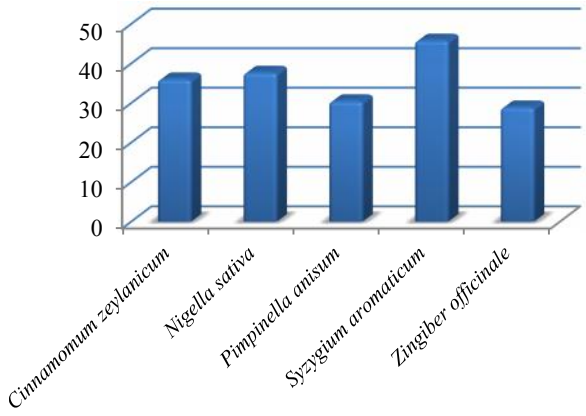


Figure 20 : The effect of EOs against *L. monocytogenes.*

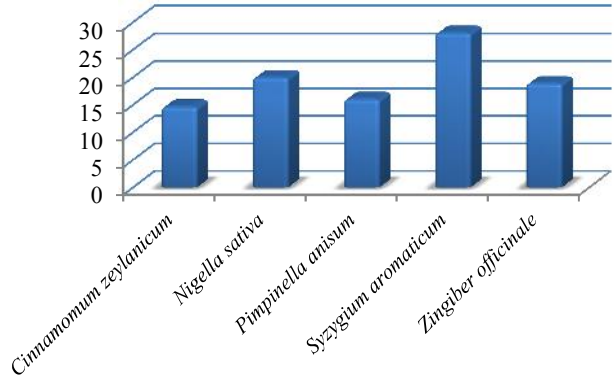


Figure 21 : The effect of EOs against *E. coli.*

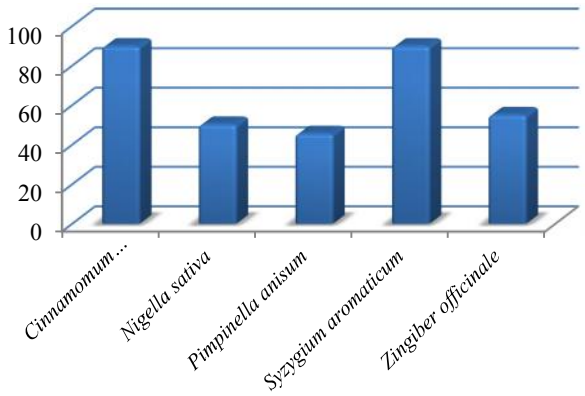


Figure 22 : The effect of EOs against *A. niger.*

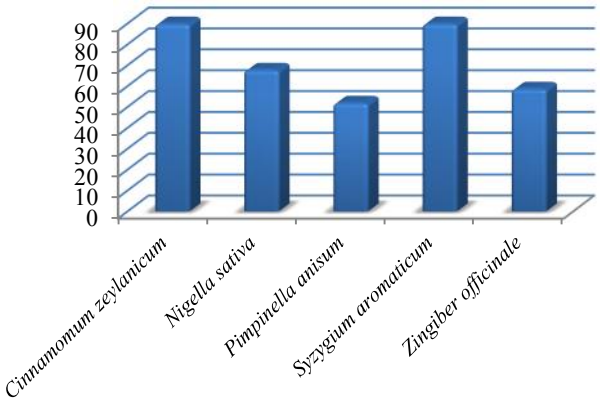


Figure 23 : The effect of EOs against *A. flavus.*

IV.2.3. Determination of the Nature of Essential Oil Inhibition

According to the results presented in the table 9, both *Syzygium aromaticum* EO and *Cinnamoum zeylanicum* EO demonstrated the same results with bactericidal activity against the bacteria and a fungicidal activity against *A. niger* and *A. flavus.* The *Nigella sativa* EO and *Pimpinella anisum* EO showed the same effect against the tested bacteria, in which, a bactericidal activity against the bacteria and fungiostatic activity against the *A. niger* and *A. flavus.*The *Zingiber officinale* EO showed a bactericidal activity against the tested bacteria, except for *L. monocytogenes* where the activity was bacteriostatic and fungiostatic activity against the *A. niger* and *A. flavus.*

Table 9. Determination of nature of EO inhibition

	Dilution	***Cinnamomum zeylanicum***	***Nigella sativa***	***Pimpinella anisum***	***Syzygium aromaticum***	***Zingiber officinale***
S. aureus	1/2 1/5 1/10 1/20	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc
E. coli	1/2 1/5 1/10 1/20	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc
P. aeruginosa	1/2 1/5 1/10 1/20	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc
B. cereus	1/2 1/5 1/10 1/20	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc
S. typhi	1/2 1/5 1/10 1/20	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc
L. monocytogenes	1/2 1/5 1/10 1/20	bc bc bc bc	bc bc bc bc	bc bc bc bc	bc bc bc bc	bs bs bs bs
A. niger	1/2 1/5 1/10 1/20	fc fc fc fc	fs fs fs fs	fs fs fs fs	fc fc fc fc	fs fs fs fs
A. flavus	1/2 1/5 1/10 1/20	fc fc fc fc	fs fs fs fs	fs fs fs fs	fc fc fc fc	fs fs fs fs

bs : bacteriostatic effect, bc :bactericidal effect, fc : fungicidal effect, fs : fungiostatic effect.

IV.3. Agar Dilution Assay

IV.3.1. Determination of the Minimum Inhibitory Concentration

According to the results of the MIC values presented in the table 10, the tested EOs demonstrated an inhibitory effect against all the strains. The gram positive bacteria which are *S. aureus, B. cereus and L. monocytogenes* showed high sensitivity towards the EOs of *Cinnamomum zeylanicum*, *Nigella sativa, Pimpinella anisum, Syzygium aromaticum*, and *Zingiber officinale* with values of MIC ranging from 0.03% to 0.25%. However, the gram negative bacteria showed a sensitivity towards the EOs with values of MIC ranging from 0.12% to 0.5%. The molds *A. niger* and *A. flavus* exhibited also highest sensitivity towards the tested EOs at MIC values ranging from 0.03 to 0.12 %. *P. aeruginosa* showed a higher and similar values of MIC (0.5%) for all the tested EOs, except for the *Syzygium aromaticum* EO (0.25%).

According to these results, the efficacy of EOs against the tested strains was in the following order:

- For the gram positive bacteria :

S. aureus : *Syzygium aromaticum*/*Cinnamomum zeylanicum*> *Zingiber officinale* > *Nigella sativa* > *Pimpinella anisum*.
B. cereus : *Syzygium aromaticum*> *Cinnamomum zeylanicum* / *Nigella sativa* > *Zingiber officinale* / *Pimpinella anisum.*
***L. monocytogenes* :** *Syzygium aromaticum*/*Cinnamomum zeylanicum*> *Zingiber officinale*/ *Nigella sativa* > *Pimpinella anisum.*

- For the gram negative bacteria :

***E. coli* :** *Syzygium aromaticum*/*Cinnamomum zeylanicum* > *Zingiber officinale* / *Nigella sativa* > *Pimpinella anisum.*
***P. aeruginosa* :** *Syzygium aromaticum* > *Cinnamomum zeylanicum /Zingiber officinale/ Nigella sativa* / *Pimpinella anisum.*
***S. typhi* :** *Syzygium aromaticum* / *Nigella sativa*> *Cinnamomum zeylanicum/ Zingiber officinale* >*Pimpinella anisum.*

- For the molds :

***A. niger* :** *Syzygium aromaticum*/*Cinnamomum zeylanicum* > *Zingiber officinale* >*Nigella sativa* / *Pimpinella anisum*

***A. flavus* :** *Syzygium aromaticum*/*Cinnamomum zeylanicum* > *Zingiber officinale* / *Nigella sativa* > *Pimpinella anisum*

Table 10. MIC values of EOs

	***Cinnamomum zeylanicum*%**	***Nigella sativa*%**	***Pimpinella anisum*%**	***Syzygium aromaticum*%**	***Zingiber officinale* %**
S. aureus	0.03	0.12	0.25	0.03	0.06
E. coli	0.12	0.25	0.5	0.12	0.25
P. aeruginosa	0.5	0.5	0.5	0.25	0.5
B. cereus	0.06	0.06	0.12	0.03	0.12
S. typhi	0.25	0.12	0.5	0.12	0.25
L. monocytogenes	0.06	0.12	0.25	0.06	0.12
A. niger	0.03	0.12	0.12	0.03	0.06
A. flavus	0.03	0.06	0.12	0.03	0.06

IV.3.2.The Minimum Bactericidal and Fungicidal Concentration

From the results of the tested EOs presented in the table 11, the gram positive bacteria were affected by values of MBC ranging from 0.06% to 0.5 %.However, the gram negative bacteria were affected by values of MBC ranging from 0.12 % to 0.5%. Besides, *P. aeruginosa* was affected by high and similar values of MBC (0.5%). *A. niger* and *A. flavus* were affected by the lowest values of MFC (0.03%) for *Syzygium aromaticum* EO and *Cinnamomum zeylanicum* EO.

Table 11. MBC and MFC values of the EOs

	***Cinnamomum zeylanicum*%**	***Nigella sativa*%**	***Pimpinella anisum*%**	***Syzygium aromaticum*%**	***Zingiber officinale*%**
S. aureus	0.06	0.12	0.25	0.06	0.06
E. coli	0.12	0.5	0.5	0.25	0.5
P. aeruginosa	0.5	0.5	0.5	0.5	0.5
B. cereus	0.06	0.12	0.25	0.06	0.12
S. typhi	0.5	0.25	0.5	0.25	0.5
L. monocytogenes	0.06	0.25	0.5	0.06	0.5
A. niger	0.03	0.5	0.5	0.03	0.5
A. flavus	0.03	0.25	0.5	0.03	0.25

IV.4. Microatmosphere Assay

The results of the microatmosphere assay presented in the tables (12, 13, 14, 15 and 16) demonstrated the activity of the vapor of EOs against the tested strains.

For *Cinnamomum zeylanicum* EO (table 12) :

- *S. aureus*, *B. cereus* and *L. monocytogenes* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

- *E. coli*, *P. aeruginosa* and *S. typhi* were completely inhibited at 20 µl, 40 µl and 80 µl, except for the aliquot of 10µl where a development was seen (figure 24), the MIQ values were 20 µl.

- *A. niger* (figure 25) and *A. flavus* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

Table 12. The MIQ values of the EO of *Cinnamomum zeylanicum*

	0 µl	**10 µl**	**20 µl**	**40 µl**	**80 µl**
S. aureus	+	-	-	-	-
E. coli	+	+	-	-	-
P. aeruginosa	+	+	-	-	-
B. cereus	+	-	-	-	-
S. typhi	+	+	-	-	-
L. monocytogenes	+	-	-	-	-
A. niger	+	-	-	-	-
A. flavus	+	-	-	-	-

+ : presence, - : absence.

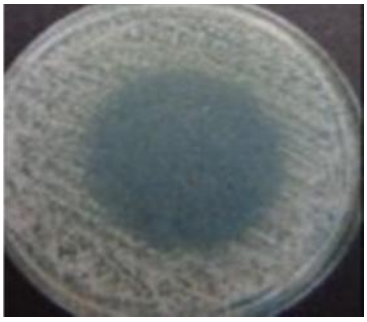


Figure 24. Presence of *E.coli*

(10µl of *Cinnamomum zeylanicum* EO).

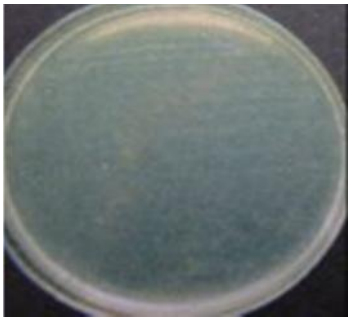


Figure 25. Absence of *A.niger*

(10µl of *Cinnamomum zeylanicum* EO).

For *Nigella sativa* EO (table 13) :

- *S. aureus*, *B. cereus* and *L. monocytogenes* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

- *E. coli* was completely inhibited at 20 µl, 40 µl and 80 µl, except for the aliquot of 10µl where a development was seen (the MIQ value was 20 µl), *P. aeruginosa* and *S. typhi* were completely inhibited at 40 µl and 80 µl, except for the aliquots of 10µl and 20 µl where a development was seen (the MIQ values were 40 µl).

- *A. niger* and *A. flavus* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

Table 13. The MIQ values of the EO of *Nigella sativa*

	0µl	**10µl**	**20 µl**	**40µl**	**80µl**
S. aureus	+	-	-	-	-
E. coli	+	+	-	-	-
P. aeruginosa	+	+	+	-	-
B. cereus	+	-	-	-	-
S. typhi	+	+	+	-	-
L. monocytogenes	+	-	-	-	-
A. niger	+	-	-	-	-
A. flavus	+	-	-	-	-

+ : presence, - : absence.

For *Pimpinella anisum* EO (table 14) :

- *S. aureus*, *B. cereus* and *L. monocytogenes* were completely inhibited at all the used aliquots where the MIQ values were 10 µl

- *E. coli*, *P. aeruginosa* and *S. typhi* were completely inhibited at 20 µl, 40 µl and 80 µl, except for the aliquot of 10µl where a development was seen (the MIQ values were 20 µl).

- *A. niger* and *A. flavus* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

Table 14. The MIQ values of the EO of *Pimpinella anisum*

	0 µl	**10 µl**	**20 µl**	**40 µl**	**80 µl**
S. aureus	+	-	-	-	-
E. coli	+	+	-	-	-
P. aeroginosa	+	+	-	-	-
B. cereus	+	-	-	-	-
S. typhi	+	+	-	-	-
L. monocytogenes	+	-	-	-	-
A. niger	+	-	-	-	-
A. flavus	+	-	-	-	-

+ : presence, - : absence.

For *Syzygium aromaticum* EO (table 15) :

- *S. aureus*, *B. cereus* and *L. monocytogenes* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

- *E. coli*, *P. aeruginosa* and *S. typhi* were completely inhibited at 20 µl, 40 µl and 80 µl, except for the aliquot of 10µl where a development was seen (the MIQ values were 20 µl).

- *A. niger* and *A. flavus* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

Table 15. The MIQ values of the EO of *Syzygium aromaticum*

	0 µl	**10 µl**	**20 µl**	**40 µl**	**80 µl**
S. aureus	+	-	-	-	-
E. coli	+	+	-	-	-
P. aeruginosa	+	+	-	-	-
B. cereus	+	-	-	-	-
S. typhi	+	+	-	-	-
L. monocytogenes	+	-	-	-	-
A. niger	+	-	-	-	-
A. flavus	+	-	-	-	-

+ : presence, - : absence.

For *Zingiber officinale* EO (table 16) :

- *S. aureus*, *B. cereus* and *L. monocytogenes* were completely inhibited at all the used aliquots where the MIQ values were 10 µl.

- *E. coli*, *P. aeruginosa* and *S. typhi* were completely inhibited at 40 µl and 80 µl, except for the aliquot of 10 µl and 20 µl where a development was seen (the MIQ values were 40 µl).

- *A. niger* and *A. flavus* were completely inhibited at all the used aliquotes where the MIQ values were 10 µl.

Table 16. The MIQ values of the EO of *Zingiber officinale*

	0µl	**10µl**	**20 µl**	**40µl**	**80µl**
S. aureus	+	-	-	-	-
E. coli	+	+	+	-	-
P. aeruginosa	+	+	+	-	-
B. cereus	+	-	-	-	-
S. typhi	+	+	+	-	-
L. monocytogenes	+	-	-	-	-
A. niger	+	-	-	-	-
A. flavus	+	-	-	-	-

+ : presence, - : absence.

Chapter V :

Discussion

V. 1. Extraction Yields

There are many variables which dictate percent yield, including: weather conditions, soil, elevation, harvest time, distillation time, pressure and temperature of distillation (Vauke *et al.,* 2003). According to (Gaunt *et al.*, 2005), The differences shown in the yield extraction of EOs might be caused by different factors including nutritional status of the plants as well as other environmental factors, different extraction techniques and genetic factors.

Literature revealed that EO contents depend not only on temperature, relative humidity, but also duration of sunshine, air movement and rainfall (Deviando *et al.*, 2009; Ometta, 2006). Julseth (2005) reported that the yield of *Pimpinella anisum* EO and *Cinnamomum zeylanicum* EO were 0.6% and 0.7% respectively which are lower than the present results. Kacem and Meraihi (2006) reported that the yield of *Nigella sativa* EO was 0.4 % which is lower than the obtained result.

This result is in agreement with those of Sanyder (1998) who found the yield of EO from *Syzygium aromaticum* to be ranging from 3.1 - 7.8 %. Vauke (1995) and Ometta (2006) examined the EO contents of *Cinnamomum zeylanicum* and *Cinnamomum cassia* to be 0.79% and 0.56%, respectively. Bourkett and Readie (1998) reported the yield of *Nigella sativa* EO to be 1.3%, which is considerably higher than the value determined in this present study.

Iskan *et al.* (2002) reported the yield EO of *Zingiber officinale* to be 0.37%. While in other research, *Zingiber officinale* and *Zingiber zerumbet*, the yields were found to be 0.7% and 0.93%, respectively (Bhrak *et al.*, 1990). Vauke *et al.* (2003) and Gaunt *et al.* (2005) reported the yield of the EOs obtained by water distillation of *Cinnamomum zeylanicum* and *Syzygium aromaticum* to be 1.7% and 6.3%, respectively. Iffert (1998) reported 1.05% yield of *Cinnamomum zeylanicum* EO extracted by hydrodistilation method, which is considerably lower than the result presented here.

V. 2. Disc Diffusion Assay

V.2.1. Antibiogram Assay

The inhibition zones of antibiotics for *S. aureus* are close to those reported by Cyfer *et al.* (2008) and Nuranta (2001). However, these results are in disagreement with those reported by Bell (2005) who used antibiotics superior than 30 µg/ disc.

For *E. coli*, the results obtained in this study are in agreement with those obtained by Losupthawee (1997) in which he studied the sensitivity of gram negative bacteria towards 21 antibiotics. *S. typhi* was resistant to the antibiotics with diameter of inhibition ranging from 0 to 7 mm, these results are in disagreement with those reported by Choechuen (1993).

Chopneeda (2002) reported that the inhibition zones of C, GM were 17 mm and 25 mm for *P.aeruginosa* which are higher than the present results. For *L. monocytogenes*, the inhibition zones of KAN, TE and GM found in this study were close to those reported by Cyfer *et al.* (2008).

V.2.2. Aromatogram Assay

The spice EOs of this study exhibited inhibitory activity against foodborne bacteria and molds. This is in agreement with many previous studies (Gupta *et al*. 2005; Ometta, 2006) but, there are many methods used for determining antimicrobial activity. This situation led to several difficulties such as comparing results from different laboratories, determining antimicrobial effectiveness, and evaluating inhibition zones (Deviando *et al.*, 2009).

The only strain that demonstrated the smallest inhibition zones towards the tested EOs was *P. aeruginosa.* Many research works confirm that *P. aeruginosa* is most resistant towards EOs because of the cell wall structure (Hirtoko, 1995 ; Vauke *et al*. 2003 ; Julseth, 2005). The inhibition zones of *Nigella sativa* EO for *P. aeruginosa* and *E.coli* are smaller than those found by Bourkett and Readie (1998) which are 29 mm and 35 mm respectively. The inhibition zones of *Pimpinella anisum* EO for *A.niger* and *B.cereus* are higher than those reported by Mund (2001). Sondin *et al*. (2005) reported that the inhibition zones of *Zingiber officinale* EO against *S. aureus* and *E. coli* were 39 mm and 15 mm respectively which are approximate to the values of the present study.

The differences in the efficacy can be explained first of all, by natural differences from the EOs, because they come from different plant species and families (Apiaceae, Lauraceae, Myrtaceae, Ranunculaceae and Zingiberaceae). The differences in the chemical composition of the EOs and the different experimental conditions could also constitute an explanation (Martinez and Jordal, 2003).

Literature reported that Gram positive bacteria are more sensitive to the EOs than Gram negative bacteria (Sipoulou and Ynot, 1995 ; Sahin *et al*., 2003; Gullocee *et al*., 2007). The reason would be that lipopolysaccharide (LPS) layer of Gram negative bacteria in outer membrane having high hydrophobicity and acts as a strong barrier against hydrophobic molecules (Mund, 2001). It can pass through cell wall of Gram positive bacteria easier than the Gram negative bacteria because cell wall of the Gram positive contained peptidoglycan and lack of outer membrane (Gupta *et al*. 2005).

Iffert (1998) reported that *Cinnamomum zeylanicum* EO reduced significantly *E. coli* strains. Similar research on other bacteria carried out by Bhrak *et al*. (1990) showed that *Cinnamomum zeylanicum* EO and *Syzygium aromaticum* EO had strong inhibitory actions, while *Brassica juncea* and *Allium sativum* EOs had only slight antimicrobial activity.

Our results were in accord with the results of Annissorn (2005) who reported that *Nigella sativa* EO exhibited good antimicrobial activity against the positive gram bacteria. However, present results are in contrast to those reported by Anridogen (1999), who reported no antimicrobial activity of *Nigella sativa* EO against *S. aureus*. Such difference may be due to the used culture media and the solvent of dilution.

In other reports (Iskan *et al*., 2002; Ladegariniae *et al.,* 2006) the results revealed that the selected EOs showed an antimicrobial activity in direct contact assay. Among these EOs, *Origanum vulgare*, *Syzygium aromaticum,* and *Thymus vulgaris* , which they exhibited an effective antibacterial activity, in particular against *L. monocytogenes* and *B. cereus*, with inhibition zones of 42 mm, 39.2 mm and 36 mm for *L. monocytogenes*, 30 mm, 39 mm and 35.5 for *B. cereus*. Vauke (1995) reported that *Cinnamomum zeylanicum* EO and *Cinnamomum cassia* EO against the same gram negative bacteria and with the same amount (10 µl/ paper disc), had inhibition zones smaller than 20 mm as presented in this study.

Fabiano (2004) tested the EOs of *Zingiber officinale* and *Nigella sativa* against *A. flavus* and *A. niger*. He found these EOs to be a slight inhibitors which are in disagreement with the present results. According to Sanyder (1998), similar observations were made where *Syzygium aromaticum*, *Cinnamomum zeylanicum* and *Brassica juncea* EOs were recognised as strong antifungal agents, while *Mentha longifolia* as weak ones.

The potent antimicrobial activity of *Syzygium aromaticum* EO and *Cinnamomum zeylanicum* EO can be predominantly attributed to eugenol and cinnamaldehyde. These are the phenolic components of *Syzygium aromaticum* and *Cinnamomum zeylanicum* EOs, which render them effective against the tested strains. This was confirmed by Belayar *et al.* (1999), where eugenol and cinnamaldehyde limitd the growth of *B. cereus* by inhibiting the production of certain enzymes needed for its growth.

Kivank and Akgul (1998) reported that the most active components of the EOs were phenols, followed by aldehydes and Ketones. However, the correlation between the composition and activities of EOs have not been brought to satisfying conclusion yet, it still under study (Miraglia, 2009).

Thymoquinone, major compound in *Nigella sativa* EO was reported to be the responsible of the antimicriobial activity of this EO (Gotakh, 2006). Burt (2004) reported that Antimicrobial activity of an EO is attributed mainly to its major compounds, although the synergetic or antagonistic effect of one compound in minor percentage of mixture has to be considered . It is clear that the antimicrobial activity of *Pimpinella anisum* EO is mainly due to anethole (Lamarta, 2009). According to Navajas (1995) , zingiberene was found to be the most active compound against bacteria and molds.

V.2.3. Determination of the Nature of Essential Oil Inhibition

In the determination of the nature of inhibition, both *Syzygium aromaticum* EO and *Cinnamomum zeylanicum* EO had a bactericidal activity against the tested bacteria and these results are in agreement with those reported by Belayar *et al*. (1999). However, Vauke (1995) reported a bacteriostatic activity of *Cinnamomum zeylanicum* EO against *Bacillus cereus* and *Bacillus subtillus* which is in disagreement with the present study. Besides, these two EOs had a fungicidal activity against the molds and these results support other works (Iffert, 1998 and Sanyder,1998).

On the other hand, The *Nigella sativa* EO had a bactericidal activity against the bacteria and fungiostatic activity against *A. niger* and *A. flavus* which is in agreement with those reported by Anridogen (1999). *Pimpinella anisum* EO had a bactericidal activity against the bacteria wich is in disagreement with those observed by Mund (2001) and fungiostatic activity against the molds which are in agreement with those found by Gupta *et al.* (2005). The *Zingiber officinale* EO had a bactericidal activity against the tested bacteria, except for *L. monocytogenes* where the activity was bacteriostatic and fungiostatic activity against the *A. niger* and *A. flavus* which are in disagreement with those found by Iskan *et al*. (2002) for *L. monocytogenes* and in agreement with those reported by Fabiano (2004) for the molds.

This difference in nature of inhibition between the studied species could be explained by the fact that, the tested EOs pertained to different families, containing different chemical compositions (Gaunt *et al.,* 2005).

V.3. Agar Dilution Assay

V.3.1. Determination of the Minimum Inhibitory Concentration

The results showed that the EO had a substantial inhibitory effect against all the tested strains, noted by growth inhibition. *B. cereus* showed a MIC value 0.06% of *Cinnamomum zeylanicum* EO similar to the result reported by Iffert (1998). The highest inhibitory activity was observed with *Cinnamomum zeylanicum* EO and *Syzygium aromaticum* EO (the lowest MIC of 0.03%) which are in agreement with those reported by Borchat and Riensven (2006). On the other hand, *P. aeruginosa* was the most resistant bacteria with a high values of MICs for all the EOs which support the results found by other researchers (Smith, 1999 ; Branen, 2005).

The MIC value of *Pimpinella anisum* EO for *S. aureus* is lower than that found by Mund (2001) and Iskan *et al.* (2002). Sipoulou and Ynot (1995) reported that the MIC values of *Nigella sativa* EO and *Zingiber officinale* EO against the Gram positive bacteria are ranging from 0.2 to 0.35 % which are higher than those observed in the present study. However, the MIC value of *Zingiber officinale* EO was 0.6 % for *A. flavus* and *A. niger* which supports the results found by Fabiano (2004).

In their detailed investigations, Jacyuet *et al.* (1999) demonstrated the results of the antimicrobial effect of different EOs on Gram positive and Gram negative bacteria, as well as on the mold *A.flavus*. Among other EOs studied, they determined the MIC values of *Pimpinella anisum* EO, which were between 0.15 and 2.5% for the bacteria: *Acinetobacter baumanii, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Salmonella thyphimurium, Serratia marcescens* and *Staphylococcus aureus,* while for the mold *A.flavus* the MIC value was 0.5%.

The differences in MIC values can be explained by different concentrations of antibacterial and antifungal components in the EOs, but also in the application of methods for determining MIC values (Yasir and williams, 2007 ; Gullocee *et al.,* 2007) . This is why data obtained by Jacyuet and Coworkers (1999) cannot be compared to our data, because they used a dilution of the EO in broth and we used a dilution in agar. According to Mund (2001), MIC values are also influenced by a number of variables, such as the composition of the culture medium, inoculum density.

V.3.2. Determination of The Minimum Bactericidal and Fungicidal Concentration

The MBCs of *Syzygium aromaticum* EO and *Nigella sativa* EO in this study were close to those reported by Sondin *et al.* (2005) and Vauke *et al.* (2003). Ometta (2006) reported that the MBCs of *Cinnamomum zeylanicum* EO and *Pimpinella anisum* EO were 0.15% and 0.7% respectively which are higher than the present results for *L. monocytogenes*. On the other hand, *E. coli* and *B. cereus* showed a values of MBC lower than the present results. These differences, due to several reasons such as different growing environment , different extracting methods of EOs and the determination method of these values (Yasir and williams, 2007). MFCs of *Syzygium aromaticum* EO and *Cinnamomum zeylanicum* EO are similar to those reported by Kommert and Slinter (2006) and Borchat and Riensven (2006).

Navajas (1995) reported that the MBCs of *Zingiber officinale* EO were 0.45%, 0.25% for *S.aureus* and *B.cereus* respectively, and the MFC was 0.35 % for *A.flavus* which are higher than the present results. The MFC of *Nigella sativa* EO for *A. niger* of this study is higher than that reported by Bourkett and Readie (1998). However, the MBCs of *Nigella sativa* EO for *E. coli* and *P. aeruginosa* are similar to those reported by Anridogen (1999).

V.4. Microatmosphere Assay

This technique had been used to determine the antimicrobial activity of the EO vapours in order to use them as atmospheric disinfection agents. Previous studies carried out on the inhibitory effect of EO vapours are limited which makes it difficult to compare and confirm results. Most researchs that had been performed are based on the agar diffusion method and have not considered the possible antimicrobial effect by the vapour from the EOs (Kilbuck, 2010).

The results of this study demonstrated that the volatile vapours of all the EOs had a stronger inhibitory activity (10 µl of MIQ) against the Gram positive bacteria than the Gram negative bacteria, this is in agreement with the results reported by (Suzuki and Dainis, 2005) who tested the vapours of EOs of *Cuminum cyminum, Carum carvi* and *Cinnamomum zeylanicum* against both Gram positive and negative bacteria.

The five EOs had a similar effect against both gram positive bacteria and molds, but in the direct contact (aromatogram), the effect of EOs was more strong against the molds than the bacteria which are in agreement with those reported by Udagawa and Chandani (2004).

In general, the volatile phase contains a high quantity of light compounds, more volatile and a low quantity of compounds relatively low volatils (Kilbuck, 2010). According to Suzuki and Dainis (2005), the volatility of compounds of EO depends on their polarity, vapour pression and the boiling point.

It has been noted that the inhibitory effect of EO can differ between the volatiles and the direct contact with microorganisms (Eumkeb and Waff, 2005). The inhibitory effect of some EOs on fungi have been reported to be greater when the oil vapours are used (Lebai, 2003).

Lee (2008) tested the EO vapours of *Cinnamomum zeylanicum*, *Nigella sativa* and *Pimpinella anisum* against *B. cereus* and *P. aeruginosa* but in comparison to the present study his results were negative. This may be because Lee (2008) used only 1 µL of EO for each treatment. The antifungal activity of the volatile phase of EOs had been reviewed by Cavanagh (2009) and confirmed that many EOs possess strong activity against a wide range of fungi.

The exact mechanism of action of EO is unclear, but some reports agree that fungi grow mainly on the surface of the agar medium and might be more susceptible to direct vapour contact than the disc diffusion assay (Sondin *et al*. 2003). An other explanation could be the fact that the antimicrobial activity of volatile compounds results from the combined effect of direct vapour absorption on microorganism and indirect effect through the medium that absorbed the vapour (Kilbuck, 2010). The effect of EOs was seen similar with value of 10 µL for the molds which is in agreement with those reported by Jakkar (2003), but lower than those observed by Kilbuck, (2010), especially for *Nigella sativa* EO and *Pimpinella anisum* EO. However, the MIQ values of *Zingiber officinale* EO reported by Eumkeb and Waff (2005) for the Gram negative bacteria are 25 µL which are higher than those observed in the present study.

Liseton *et al.* (1998) reported in similar study that the MIQ values of *Syzygium aromaticum* EO for *S. aureus*, *B. cereus* and *L. monocytogenes* are in agreement with those observed in the present study.

Conclusion and Perspectives

Conclusion and perspectives

The objective of this work was to investigate *in vitro* the antimicrobial activity of EOs of *Cinnamomum zeylanicum*, *Nigella sativa*, *Pimpinella anisum*, *Syzygium aromaticum* and *Zingiber officinale*. A number of conclusions can be drawn from the work reported here.

The results of the present study indicated that spice EOs possess antibacterial activity towards gram negative and gram positive bacteria where the gram negative bacteria were more resistant than the gram positive bacteria. *Syzygium aromaticum* EO was the most effective EO against all the tested bacteria.

For all the EOs, the molds were found to be more sensitive than bacteria and *A.flavus* was more sensitive than *A.niger*, except for *Syzygium aromaticum* and *Cinnamomum zeylanicum* EOs that were both completely inhibited . *S.aureus* was the most sensitive bacterium expressed by the largest zone of inhibition. While, *P.aeruginosa* was the most resistant bacterium expressed by the smallest zone of inhibition.

These spice EOs act through their natural inhibitory mechanisms by either inhibiting or killing the strains completely. The gram negative bacteria were inhibited by MIC values higher than the gram positive bacteria and molds. The values of MBC for the gram negative bacteria were also higher than those for gram positive bacteria with a little exception for *L.monocytogenes*. However, the lowest values of MFC were observed for *Syzygium aromaticum* and *Cinnamomum zeylanicum* EOs. The EOs were so active in vapour phase , all the EOs had a similar MIQ values for the gram positive bacteria and molds, which were also lower than those observed for the gram negative bacteria.

The present investigation provides support to the effectiveness of antimicrobial activity of the EOs tested especially in the light of the current trend in finding alternative remedies that are effective against increasing numbers of food pathogenic bacteria and molds. With the increasing awareness of people towards natural food and natural therapies, spice EOs may act as the most obvious alternative.

Results obtained in this study are only first step in seeking substances from natural source and biologically active. Based on these results the following perspectives are suggested :

1. Further studies are necessary in order to determine whether these spice EOs could be applied in a manner that will be bactericidal and fungicidal but not phytotoxic.
2. Chemical compounds in EOs having significant positive or negative effects should be studied in detail for their specific effects.
3. Evaluate these compounds alone and in combination to identify their activities.
4. A large variety of microbial strains should be tested with spice EOs to validate the activities, especially for the antibiotic-resistant bacteria.
5. *In vivo* studies and clinical trials would be needed to justify and further evaluate the potential of EOs as reliable antimicrobial agents.
6. More detailed studies of the mechanism of actions of these EOs will be of great help in utilizing their full potential in pharmaceutical, cosmetics and aromatherapy industries.
7. Standardisation of methods used in assessment of antimicrobial activity of EOs.

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Annex A

Mueller Hinton Agar (MHA)

- 30.0% beef infusion
- 1.75% casein hydrolysate
- 0.15% starch
- 1.7% agar
- pH adjusted to neutral at 25 °C.

Nutrient Broth (NB)

- Peptone 10.0 g
- Meat extract 10.0 g
- Sodium chloride 05.0 g
- Distilled water 1000 mL
- pH 7.2±0.2

Nutrient Agar (NA)

- The nutrient agar was prepared by adding 1.5% agar in nutrient broth and was autoclaved.

Potato Dextrose Broth (PDB)

- Potatoes peeled and diced into small pieces 200g
- Glucose 20g
- Distilled water 1000 mL

Potato Dextrose Agar (PDA)

- The potato dextrose agar (PDA) was prepared by adding 1.5% agar to potato dextrose broth and then sterilized by autoclaving.

Abstract :

The present work aims to evaluate the antimicrobial activity of five spice EOs, namely *Cinnamomum zeylanicum*, *Nigella sativa, Pimpinella anisum, Syzygium aromaticum and Zingiber officinale.* All these EOs have been extracted by water distillation. The antimicrobial activity of these EOs was tested against six bacteria and two molds, which are responsible for many health-related problems, by using disc diffusion assay, agar dilution assay and microatmosphere assay. The results showed that the Gram positive bacteria were more sensitive than gram negative bacteria where *P.aeruginosa* was the most resistant bacterium. However, *S.aureus* was the most sensitive bacterium. *Syzygium aromaticum* EO was the most effective against all the strains. The MIC values of EOs for gram positive bacteria were (0.03% to 0.25%), for the gram negative bacteria were (0.12% to 0.5%) and for the molds were (0.03% to 0.12%). The MBC values of EOs for gram positive bacteria were (0.06% to 0.5%), for the gram negative bacteria were (0.12 % to 0.5%). The lowest values of MFC were observed for *Syzygium aromaticum* EO and *Cinnamomum zeylanicum* EO (0.03%).The MIQ values of EOs for gram positive bacteria and molds were 10µl, for the gram negative bacteria were 20µl and 40µl.

Key words : essential oils, spices, antimicrobial activity.

Résumé :

Ce présent travail vise à évaluer l'activité antimicrobienne des HEs de cinq épices. à savoir: *Cinnamomum zeylanicum*, *Nigella sativa, Pimpinella anisum, Syzygium aromaticum* et *Zingiber officinale*. Ces HEs ont été extraites par hydrodistillation. L'activité antimicrobienne de ces HEs a été testée contre six bactéries et deux moisissures, qui sont responsables de plusieurs problèmes liés à la santé, en utilisant la méthode de diffusion en disc, de dilution en gélose et la méthode de microatmosphère. Les résultats ont montré que les bactéries à gram positif étaient plus sensibles que les bactéries à gram négatif où *P.aeruginosa* était la plus résistante. Cependant, *S.aureus* était la plus sensible. HE de *Syzygium aromaticum* était le plus efficace contre toutes les souches. Les valeurs CMI des HEs pour les bactéries à gram positif (0.03% à 0.25%), pour les bactéries à gram négatif (0.12% à0.5%) et pour les moisissures (0.03% à 0.12%). Les valeurs CMB des HE pour les bactéries à gram positif (0.06% à 0.5%), pour les bactéries à gram négatif (0.12% à 0.5%). La valeur la plus petite de CMF (0.03%) a été observée pour HE de *Syzygium aromaticum* et HE de *Cinnamomum zeylanicum*. Les valeurs de QMI pour les bactéries à gram positif et moisissures étaient 10µl, pour les bactéries à gram négatif étaient 20µl et 40µl.

Mots clés : huiles essentielles, épices, activité antimicrobienne.

ملخص

هذا العمل يهدف إلى تقييم النشاط المضاد للميكروبات للزيوت الاساسية لخمس توابل : *Cinnamomum zeylanicum, Nigella sativa, Pimpinella anisum, Syzygium aromaticum ,Zingiber officinale*. استخلصت هذه الزيوت الاساسية بواسطة التقطير المائي. اختبر النشاط المضاد للميكروبات لهذه الزيوت الاساسية ضد ست سلالات بكتيريا و فصيلتين من العفن متسببة في عدة مشاكل مرتبطة بالصحة. وذلك باستعمال طريقة 'disc diffusion agar dilution و microatmosphere. اظهرت النتائج ان البكتيريا غرام موجب اكثر حساسية من البكتيريا غرام سالب. *P.aeruginosa* هي البكتيريا اكثر مقاومة . في حين *S.aureus* هي الاكثر حساسية . الزيت الاساسي ل *Syzygium aromaticum* هو الاكثر فاعلية ضد الميكروبات. قيم MICs للزيوت الاساسية من اجل البكتيريا غرام موجب كانت (0.03 % الى 0.25 %), من اجل البكتيريا غرام سالب كانت (0.12% الى 0.5 %) ومن اجل العفن كانت (0.03% الى 0.12%). قيم MBCs للزيوت الاساسية من اجل البكتيريا غرام موجب كانت (0.06% الى 0.5%). من اجل البكتيريا غرام سالب كانت (0.12% الى 0.5%). القيمة الصغرى ل MFC لوحظت في الزيت الاساسي ل *Syzygium aromaticum* و *Cinnamomum zeylanicum* (0.03%) . قيم :MIQ من اجل البكتيريا غرام موجب و العفن كانت 10µl و من اجل البكتيريا غرام سالب كانت 20µl و 40µl .

الكلمات المفتاحية زيوت اساسية, توابل, النشاط المضاد للبكتيريا.