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Use Thyme Essential Oils for the Prevention of Salmonellosis

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1. Introduction

Over sixty percent (60%) of poisoning in the world are caused by Salmonella. Salmonellosis is thus become a public health event which justifies the involvement of the World Health Organization (WHO) in the fight against Salmonella (Salm-Surv, 2005). Salmonellosis is a foodborne illness of the most common and widespread. It represents a significant burden to public health and a considerable cost to society in many countries. Each year, millions of cases are reported worldwide, causing thousands of deaths. This disease is caused by the bacterium Salmonella (Salmonella). We know now more than 2500 types, or serotypes of Salmonella. The genus Salmonella, which belongs to the *Enterobacteriaceae* family, is named by Dr. Daniel Elmer Salmon American Veterinary even if the scientist who discovered the type was Theobald Smith, co-worked with Dr. Salmon in the Bureau of Animal Industry (BAI) in 1884 (Brown, 1935).

In 1880 Eberth discovered the causative agent of typhoid fever. The culture of this bacterium was considered in 1884 by Gaffky. The genus Salmonella was used after the bacteriologist Dr. Daniel Salmon had isolated in 1886 a bacterium from the pig (Salmonella choleraesuis), which was considered the cause of swine fever (hog cholera) (Encarta Encyclopedia 2004). In 1896 Widal showed the antigenic diversity of strains of salmonella. Now, more than 2500 Salmonella serotypes were isolated. Since the first observations reported by Eberth until now, the genus Salmonella has continued to have considerable importance in the veterinary and medical domain, both in economic losses due to animal disease, and by the high incidence on humans, typhoid fever and food poisoning salmonella (Bornert, 2000).

2. Taxonomy

Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriale
Family:	*Enterobacteriaceae*
Genre:	*Salmonella*

The nomenclature of salmonella recognizes that the genre has three *Salmonella* species (Le Minor and Popoff, 1987, Reeves et al. Nov.1989):

Salmonella bongori; *Salmonella enterica* or *Salmonella choleraesuis* and *Salmonella subterranea* (Shelob olina et al., 2004).

The second most important species includes six subspecies (Grimont et al., 2000): *Salmonella enterica* subsp. *Arizonae*; *Salmonella enterica* subsp. *Diarizonae*; *Salmonella enterica* subsp. *Enterica*; *Salmonella enterica* subsp. *Houtenae*; *Salmonella enterica* subsp. *Indica* and *Salmonella enterica* subsp. *salamae*.

With this division into species and sub-species actually, 2541 serotypes are recognized officially. These result from multiple combinations of somatic O polysaccharide in nature, flagellar H antigens, protein in nature and, finally, capsular (Vi). Genetic determinants of these factors are stable enough to perform reliable epidemiological surveys. The type of classification based on the O and H antigens is called the Kauffmann-White scheme (Grimont et al., 2000). Names of serotypes should necessarily be written in capitalized block characters (not italics): *Salmonella enterica* subsp. *enterica* serotype Typhimurium. However, the following simplifications are allowed: *Salmonella* Typhimurium or *S*. Typhimurium.

3. Biology of *Salmonella*

Salmonella belongs to the *Enterobacteriaceae* family. On Light microscopy, they appear as Gram-negative, 0,3 to 1µm wide and 1 to 6 µm microns long (Figure 1). They are moving through peritrichous ciliature. Salmonella are mesophilic bacteria, developing at temperatures between 5.2 °C and 47 °C and optimally between 35 ° C and 37 °C, at pH between 4.5 and 9, with water activity (Aw) greater than 0.93.

Salmonella is aero-anaerobic, reduce nitrate to nitrite, can use citrate as single carbon source. She ferments glucose but not lactose or sucrose and she produce gas from glucose (except Salmonella Typhi). Hydrogen sulfide is generally produced from the mid commonly called "triple sugar". The reaction in the oxidase test is negative (Le Minor, 1984 International Commission on Microbiological Specifications for Foods, 1996, Hanes 2003). Like all bacteria stain Gram-negative envelope of Salmonella consists of three elements: the cytoplasmic membrane and outer membrane separated by a periplasmic space consists of peptidoglycan. This structure gives the bacterium its shape and rigidity and allows it to withstand a relatively high osmotic pressure in the environment (Rycroft, 2000)

3.1 Habitats

Salmonella can be isolated from the intestines of many animal species. They are zoonotic agents. The animals are a reservoir and release into the environment is mainly due to fecal contamination (Berends et al. 1996; Murray, 2000; Hanes, 2003). Salmonella can also survive for very long periods in the environment: a few days to 9 months in soil and surface materials Farm Building (wood, concrete, steel, iron and brick), a few months in dry foods not acidified, on stems and leaves of plants and ensiled over a year in the dust (Gray and Fedorka-Cray, 2001). These bacteria can bind to many substrates, such as, boots, brushes, shovels, wheel barrows, clothes... When cleaning and disinfection of livestock housing and feeding it must consider all inanimate material, which can cause a re-infection of the next batch. Can also be contaminated: spider webs, water, byproducts of agro-food, animal feed, the surrounding farms, fishes and birds (Berends et al., 1996). Rodents and insects can also be an important source of *Salmonella* in livestock (Letellier et al., 1999).

3.1.1 Animal reservoir

Serotypes can be classified according to the target animal species. First, some are exclusively adapted to humans, causing serious and very specific diseases. This is essentially *Salmonella* Typhi, Paratyphi, and Sendai, causative agents of typhoid and paratyphoid fevers (Bäumler et al. 1998; Hu and Kopecko, 2003). Second, a number of serotypes can attract animals. Among these are: Choleraesuis, Typhisuis pigs, Abortusequi in horses, sheep Abortusovis, Gallinarum, specific poultry... Finally, most Salmonella serotypes can cross the species barrier. They are present in many animal species, usually in a latent or subclinical disease-causing, and can reach the man, either through food, which is the most common way, either by direct or indirect contact. Any salmonella, with rare exceptions, is potentially dangerous to humans. United States, Salmonella has been associated with collective poisoning from reptiles, which are used as pets (Center for Disease Control, 1999, Mitchell and Shane, 2000). This shows that Salmonella are capable of multiple adjustments and can cause new and various problems to humans, from various sources.

3.1.2 Salmonella and human

The specific agents of salmonellosis in humans (Salmonella Typhi, Paratyphi, and Sendai) are the agents of typhoid and paratyphoid fevers. Worldwide, the human deaths caused by typhoid fever are estimated at 600,000 per year (Hu and Kopecko, 2003). The cases are mainly listed in the Third World. In developed countries, cases are usually du to imported food. Five percent of patients infected with S. Typhi become chronic carriers, asymptomatic (Mermin et al., 1999). This poses enormous problems if they are employed by food companies.

3.2 Mechanisms of virulence

A considerable number of genes (of the order of hundreds) must be mobilized by Salmonella to counteract the defense mechanisms of the host. All Salmonella serotypes can in theory cause a systemic infection in humans with decreased immune status, although most will generate a febrile diarrhea, vomiting, abdominal pain and in elderly or immuno-défiscients bacteremia, the septicemia and extra intestinal locations, especially vascular (Bäumler et al., 2000). When there is localization of the infection, Salmonella often remain confined to the mesenteric lymph nodes. The first defense mechanisms used by the host are made by the acidity of the stomach and bile salts in the small intestine, which exert a bactericidal effect. Once in the small intestine, Salmonella must as soon as possible adhere to the intestinal mucosa. They will cross at the lymphoid follicles of the ileum (Peyer's patches, located at the bottom of intestinal crypts). At this point in the gut, the epithelium is characterized by the presence among the enterocytes, M cells and the absence of cells secreting mucus. It seems that the fimbriae (adhesins) must be present to allow recognition and binding of Salmonella to Peyer's patches (Dibb-Fuller et al. 1999; Thorns and Woodward, 2000, Vimal et al., 2000).These fimbriae play a critical role in the pathology and the fact that some serotypes are specifically tailored to a particular species. Entry into the Peyer's patches requires the presence of secretion systems of type III. They are encoded by sets of pathogenicity genes ("pathogenicity islands"), known as SPI-1 and SPI-2 (China and Goffaux, 1999; Bäumler et al. 2000; Cornelis, 2000; Jones et al. 2002; Doublet et al., 2005). SPI-1 is normally necessary for passage through M cells of the intestinal mucosa, whereas SPI-2 is involved in the systemic nature of the infection (Hueck, 1998). Subsequent to penetration of salmonella in M cells, the latter will be killed by apoptosis, leading to transmigration across mucosal inflammatory cell type polymorphonuclear (PMN) and acute gastroenteritis.

To survive in the inflammatory process and the development of bactericidal proteins produced by PMN, a set of genes must be activated, especially those in the complex PhoPQ.

4. Biochemical characteristics of salmonella

Salmonella possess the general characteristics of the Enterobacteriaceae and intrinsic differential characters.

4.1 Family characters
Eight main characters determine the Enterobacteriaceae, they are:
1. Bacilli Gram negative;
2. Often through their mobile ciliature peritrichous (rarely stationary), non-spore forming;
3. Bacteria that grow on ordinary media;
4. Aero-anaerobic bacteria optional;
5. Bacilli that ferment glucose with or without gas production;
6. Bacteria that reduce nitrate to nitrite;
7. Bacilli that do not have cytochrome oxidase (Hanes, 2003; ICMSF, 1996);
8. Bacilli that have a catalase.

Some strains do not obey all these characters, in the case of Erwinia, which does not reduce nitrates, Shigella dysenteriae serotype 1 (SD1) that does not have catalase, Salmonella pullorum-galinarum is immobile.

4.2 Differential characters of the genus *Salmonella*
The main biochemical characteristics for identification of Salmonella (Humbert et al., 1998) are:
*   The absence of an active urease, tryptophan or phenylalanine deaminase;
*   Lack of production of indole and acetoin (Voges-Proskauer test negative);
*   The production of hydrogen sulfide from thiosulfate (presence of thiosulfate reductase);
*   The frequent decarboxylation of lysine and ornithine;
*   The growth on Simmons citrate medium.

5. Antigens of Salmonella

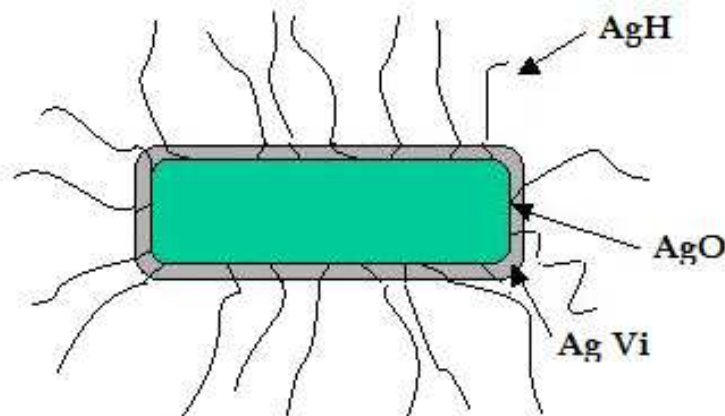Salmonella can have three types of diagnostic antigens of interest (Dumas, 1958).

Fig. 1. Salmonella posses a flagellar antigen (H), somatic (O) and a surface antigen Vi

5.1 Somatic O antigen (Ag O)

The O antigen is an antigen of the wall. O antigens are carried by chains specific lipopolysaccharide (LPS). The O antigen has properties immunizing is a complex containing a protein, a polysaccharide and a phospholipid compound. We distinguish 67 O factors depending on the nature of the sugars used in the construction of oligosaccharide units of the polysaccharide (Humbert et al., 1998). O antigens are composed of a lipid fraction called lipid A is responsible for toxic effects, or basal part of the core and the support of the specific polysaccharide (Gledel and Corbion, 1991). Antigens are classified as major factors O and O factors accessories. The major factors are related to the presence of certain sugars (abequose for O: 4, tyvélose for O: 9) (Humbert et al., 1998). The somatic antigen is stable and it is resistant to alcohol and phenol for two and a half hours at a 100 ° C (Dumas, 1958).

5.2 Flagellar antigen (Ag H)

It is a polymer of flagellin (structural protein of flagella). This antigen is thermolabile, destroyed by heat at 100 ° C by the action of alcohol and by proteolytic enzymes. It is resistant to formalin and loses their agglutinability by antibodies in the presence of alcohol and phenol. Optimum development is achieved on soft liquid media after spending eight hours at 37 ° C (Dumas, 1958). The vast majority of serovars has two genetic systems, and can alternately express two different specificities for their flagellar antigen. It is said that the flagellar antigens of Salmonella are two-phase (Humbert et al., 1998).

5.3 The virulence antigen (Vi Ag)

It is an antigen of the envelope; it was identified in three types of serovar: Typhi, Paratyphi C and Dublin, but all strains of these serotypes do not necessarily have this antigen (Humbert et al., 1998). This antigen is considered a surface antigen (Dumas, 1958), it is distinct from the somatic antigen and the flagellar antigen. The Vi antigen makes germs inagglutinable by antibodies O when it is abundant. It does not develop if the cultures are carried out below 25 ° C and above 40 ° C. Heating at 100 ° C destroys the germs and become agglutinating antibodies by O. It is likely glucidolipidopolypeptidique. In addition to these antigens exists in the genus Salmonella, the protein structures from surface pilis pilis which differentiate into common (occurring in mannose-dependent haemagglutination) and sexual pilis (involved in bacterial conjugation) and whose presence is encoded by plasmids (and Gledel Corbion, 1991).

6. Isolation and identification of salmonella

6.1 Isolation of salmonella

Salmonella Typhi, Paratyphi A, B, C are preferably isolated in the blood and feces of typhoid (Dumas, 1958). The *Salmonella* that cause food poisoning or acute gastroenteritis are still being sought in the feces and in food. The detection of *Salmonella* may be direct (bacteriological method) or indirect (serological technique) according to (Humbert et al., 1998). The microbiological analysis of a food is to highlight the microorganisms responsible for the alteration of merchantability and / or health. The analytical methods vary with the type of food, the potential danger it presents, and conservation features, consumption (raw or cooked) and the desired type of germ. Food is supportive environments for the development of a multitude of germs, some of which are pathogenic. Faced with the task of

finding a pathogenic bacteria specie in very small proportion of a product heavily contaminated with the bacteria most various conventional methods of analysis, sampling and isolation have been proposed. Several standards govern the detection of Salmonella in food hygiene (Humbert et al., 1998): the horizontal standards applicable to all types of products (ISO6579 December 1993) at international level and industry standards specific to one type of product (NF V59 - 109 for edible gelatin). The detection of Salmonella in a food according to ISO 6579 has four key steps: The pre-enrichment; Enrichment; Isolation and Biochemical and serological identification.

6.1.1 The pre-enrichment

It's a non-selective phase that uses a rich medium in which the sample is diluted to one tenth (1 / 10) and for which the incubation period is about twenty hours at 35 ° C or 37 ° C (Humbert et al ., 1998). The pre-enrichment allows bacteria to sublethal to recover all of their potential at the end of incubation. The media used are liquids, most often using buffered peptone water or lactose broth (Humbert et al., 1998). For dairy products can be used Ringer's solution or phosphate buffer solution.

6.1.2 Enrichment

Enrichment is designed to minimize the growth of other bacteria associated with the collection and continue the selective breeding of Salmonella. 0.1 ml or 1 ml of the solution pre-enrichment was transferred to one or more enrichment media (10 ml of medium). The enrichment media are classified into three families (Humbert et al., 1998):
– selenite broth;
– broths containing tetrathionate (Muller Kauffmann broth) and
– broth containing malachite green and magnesium chloride.

6.1.3 Isolation

This is a phase that uses selective solid media cast in Petri dishes. The isolation media contains a variety of combination of selective factors (Humbert et al., 1998). *Salmonella* colonies appear as features in their form, color and morphology. Solid media used for isolation are: Rambach medium; Hektoen medium; Salmonella agar - Shigella (SS agar); brilliant green agar and phenol red (VB-RP); xylose lysine Tergitol medium (XLT); Compass mid Salmonella; mannitol lysine, crystal violet, brilliant green; deoxycholate citrate agar lactose sucrose (DCLS); xylose lysine deoxycholate agar (XLD) and Bismuth sulfite agar.

With conventional bacteriological diagnostic methods, other unconventional techniques can be used. These are among others: sensitivity to phage 01, and Felix and Callow and standardized systems (API 20 E, 20 E RAPID, Enterotube Rocks, MIS *Enterobacteriaceae*). The lysis by phage 01 (Felix and Callow) that can be used as a confirmatory test for membership of Salmonella that do not provide positive results with all strains (Gledel and Corbion, 1991).

6.2 Biochemical identification

Biochemical identification of the colonies tried characteristics is done in two steps:
1. The search for characters of the family: often, the Gram stain, the presence of catalase, the absence of cytochrome oxidase, mobility, respiratory type, the mainstream culture

and fermentation of glucose are sufficient to encourage the search for differential characters.

2. The search for differential characters requires pure cultures. Used for this purpose, the reduced rack of le Minor which is a set of five settings: the mid-Hajna Klinger; the Simmons citrate medium; the lysine iron or Taylor medium; the tryptophan-urea medium and mannitol nitrate mobility medium.

The mid-Hajna Kliger (solid medium): The mid-Hajna Kliger has a carmine red color. This medium is only valid for the fermentative bacteria. It is part of the study of carbohydrate metabolism. **The lysine iron medium** or mid-Taylor (purple), enriched with L-lysine and contains low concentrations of glucose. **The Simmons citrate** medium (green), contains citrate as the sole carbon source. Bacteria that are able to use this carbon source, will grow on this agar and cause a pH change at the origin of the middle turn blue. Remains green agar for strains citrate (-). **The urea-indole medium** (liquid medium), the urea -indole or urea -tryptophan medium, is a medium orange, made up of urea and tryptophan. It allows for three enzymatic activities of protein metabolism, including urease, tryptophanase and tryptophan deaminase. **The medium with glycerol** (liquid medium), this medium is green; it is very often added to other settings of Le Minor rack. It helps to distinguish Citrobacter and Salmonella genus. Citrobacter degrades glycerol by causing acidification of the medium (turns to yellow) and Salmonella do not degrade it, the green color is maintained. In summary, the general biochemical characteristics of most serotypes isolated from humans and warm-blooded animals are:

- Lactose (-), ONPG (-)(orthonitrophenyl-β-D-galactopyranoside), H_2S (+), gas (glucose) (+);
- LDC (+)(lysine decarboxylase), ODC (+)(ornithine decarboxylase), ADH (-)(arginine dihydrolase), urease (-), TDA (-)(tryptophane deaminase), indole (-), gelatinase (-) DNase (-);
- No production of acetoin (Voges-Proskauer test (-)), RM (+)(methyl red energy source), Simmons citrate (-), adonitol (-), glycerol (-), galacturonate (-).

It should be noted that there are important exceptions. The serotype Typhi does not decarboxylated ornithine, does not grow on a medium composed of Simmons citrate, it is agazogene and produces only trace amounts of H_2S. Serotype Paratyphi A does not decarboxylated lysine and does not grow on Simmons citrate medium. Finally, Salmonella Paratyphi A, Choleraesuis, and Gallinarum do not produce H2S. In this case, the settlements will not have black centers on isolation media consisting of iron citrate and sodium thiosulfate (eg, XLD, Hektoen, SS).

7. Economic importance and societal

The economic importance of these diseases is considerable. In the U.S., economists have estimated the annual costs of salmonellosis between 400 million and U.S. $ 3.5 billion for the entire U.S. economy. They took into account the medical costs and lost productivity (Frenzen et al. 1999; Sarwari et al., 2001). Europe, whereas 95% of salmonellosis is food borne, annual costs range between 560 million and 2.8 billion euros. A single case of salmonellosis is estimated, in turn, to a value between EUR 24 and EUR 3.8 million. This estimate refers to cases where the patient dies of infection (European Parliament and Council of the European Union, 2001). In Africa, there are no data on the annual cost of salmonellosis.

8. Prophylaxis

Currently, even if the food manufacturing is done according to the standards proposed by the WHO, an important part of the fight against zoonoses must be borne by the consumer, who can be considered an integral link in the chain. We must therefore inform about the risks that may result from errors in food handling. Unfortunately, at present, few initiatives have been undertaken in Africa, unlike the situation in Europe and the United States, where politics at this level is a little more proactive. The operation FightBac ® bases its message on a logo simple and easy to understand for educators, children and operators of processing lines and distribution. CSCC logo are constantly reminded that people must wash their food ("Clean"), separate ("Separate"), Cook ("Cook") and cool ("Chill"). Advice is provided for hand washing, cooking food. The FDA in collaboration with the Center for food Safety and Applied Nutrition (CFSAN) has published a brochure about the risk of salmonellosis associated with eggs (FDA 2002). The CDC ("Centers for Disease Control") has published a leaflet on Salmonella Enteritidis, available on the Internet (Center for Disease Control, 2003). Finally, the educated consumers will, no doubt, more likely to seek medical attention, which will encourage feedback and help to reduce the phenomenon of under-reporting of cases.

9. Resistant salmonella

Salmonella is still a topical; it is in any way, a rearguard battle. They are among the first known causes of food borne illness. It is a collective and a real public health problem. Economically, they are crucial, given the casualties they cause. In recent years, problems related to Salmonella have increased significantly, both in terms of the incidence of salmonellosis, that the severity of human cases. While some countries have managed to reverse the upward trend in the incidence of human salmonellosis, new problems were identified. Since the late 90s, Salmonella strains resistant to a range of antimicrobials including major therapeutic agents in human medicine have emerged and are threatening to cause serious public health problem (Mermin et al., 1999). After a very long incubation period, between 7 and 21 days (sometimes up to six weeks), the disease can take many forms (Hu and Kopecko, 2003). The infection may be asymptomatic or cause very mild symptoms in the case of S. Paratyphi or, conversely, cause typhoid fever, severe disease, with fever and sepsis. It mainly affects young children and teenagers (Bäumler et al., 1998). This resistance results from the use of antimicrobials in both human medicine and animal husbandry.

10. Essential oils

10.1 Introduction

The use of essential oils (EO) dates back to the earliest civilizations: first in the East and the Middle East and later in North Africa and Europe (Franchomme et al., 1990). The Hydrosols (aromatic) were used in India over than 7000 years. Between 3000 and 2000 B.C., the Egyptians made used extensively aromatic plants and other plants to treat the sick. the Persians seem to be the first ones who used the hydrodistillation in 1000 B.C. The use of essential oils was a common practice among the Greeks, several books have been published on the subject. Examples of this literature are "Natural History" by Pliny, "The Aphorisms" by Hippocrates, "odor treatment" by Theophrastus and Dioscorides Pedanius wrote a book on herbal medicine (phytotherapy). The Arabs have made a significant improvement in chemistry and in the distillation of oils by inventing the alembic still by Jaber Ibn Hayan. In the late seventeenth and

eighteenth century, more than 10 essential oils were used. In modern history, the therapeutic properties of essential oils have an increasing importance. Aromatherapy has been used to describe the healing properties of essential oils. Actually, we recognize that essential oils have pharmacological, psychological and physiological effects in humans.

Among the plant species estimated by botanists (800 000 to 1 500 00), only 10% are classified as "aromatic". Aromatic plants synthesize and secrete trace amounts of aromatic essence through hair, secretory pockets or channels. Types capable of developing the components of essential oils are distributed in a limited number of families, Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Cupressaceae, Poaceae, Zingiberaceae, such as Piperaceae [Bruneton, 1999]. About 20,000 of plant species in the world are used for food, cosmetics, chemical, pharmaceutical and therapeutic food. Among the 4,000 plant species existing in Morocco, more than 280 plants are currently operating.

The AFNOR NF T 75-006 (AFNOR, 1986) defines the essential oil as "a product made from a vegetable raw material, either by steam or by mechanical means from the exocarp, Citrus, or by dry distillation. Essential oils (EO), also called "essences" are aromatic substances, volatile and oily consistency, contained in plants [Balz, 1986 - Lardry and Haberkorn, 2003]. Most plants contain (Eo), but usually in lower quantities. Only plants known as "aromatic" produce essential oils in sufficient quantity. They are usually concentrated in a particular area of the plant such as leaves, bark or fruit, and generally when they occur in various organs from the same plant, they have different compositions (Conner, 1993). The synthesis and accumulation of essential oils, classified as secondary metabolites, are generally in the specialized histological structures, often located on or near the surface of the plant (Brunechon, 1987): pockets (citrus exocarp) of a storage (eucalyptus), secretory canals or blisters containing resin (conifers), or glands in cuticular (conical epidermal cells on the flowers of Rosaceae), trichomes or secretory glandular trichomes on the leaves of solanaceous or Lamiaceae (Gershenzon, 2000) (Figure 8). It is important to note that several categories of these secretory tissues can coexist simultaneously in the same species, even within the same organ (Fahn, 1979 - Fahn, 1988). For example, to the Lamiaceae family, it is within the secretory hairs, in Myrtaceae in pockets secretory or secretory channels in Asteraceae. Essential oils can be stored in various organs of the plant: flowers (oregano), leaves (lemon grass, eucalyptus), bark (cinnamon), wood (rosewood, sandalwood), roots (vetiver), rhizomes (sweet flag), fruits (star anise) and seeds (caraway). Essential oils are complex mixtures consisting of several compounds, mainly terpenes. Terpenes are formed by one or more isoprene units (Tedder, 1970; Brunechon, 1987), constituting a diverse family both structurally and functionally. Mainly mono-and sesquiterpenes (with 10 and 15 carbon atoms) are the most encountered the diterpenes (20 carbon atoms). Essential oils can also contain aliphatic or aromatic compounds. These terpenoids have an ecological role in plant interactions, such as allelopathic agents. They can be inhibitor of germination, but also during plant-animal interactions, as a protective agent against predators such as insects.

They are also involved, through their characteristic odors in the attraction of pollinators (Langenheim, 1969). Some plants may have an odor similar due to a common molecule present in significant amounts in the essential oil. According to the economic environment, it would be profitable to produce plant species may provide an essential oil with high molecular compound, and therefore generally better (Tedder, 1970).

Herbs that produce essential oils have been the subject of various researches particularly in the field of perfumery. A range of products to smell more or less pronounced depending on the concentration of volatile compounds collected from the essential oils produced by steam distillation or expression of the peel fruit (Tedder, 1970;Brunechon, 1987).
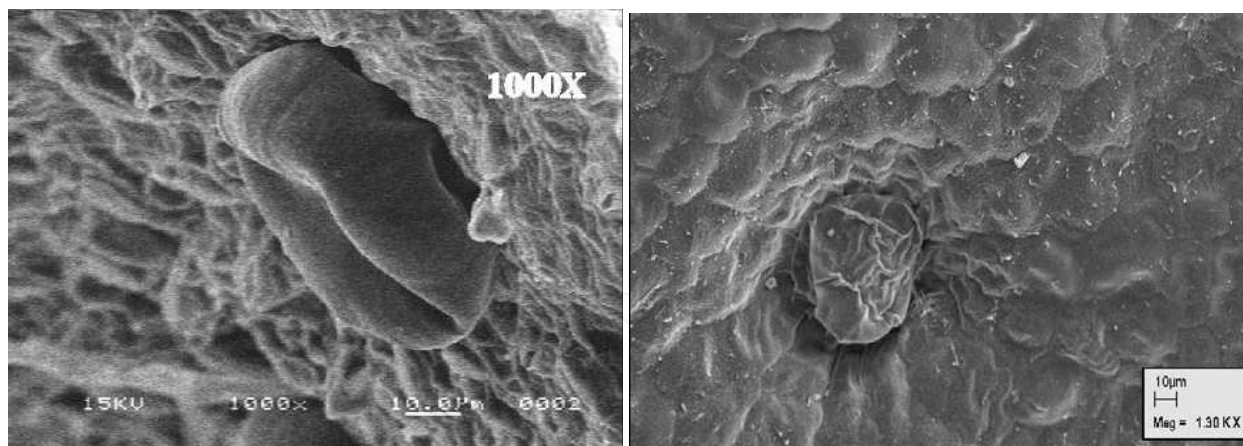
Fig. 2. Glandular trichomes of mint gardens observed in electron microscope scan (1000 ×) before (a) and after (b) extraction by steam distillation [Lucchesi, 2005].

10.2 Chemical composition of essential oil

Essential oils are composed of a complex mixture. These compounds mainly belong to two families of chemicals: terpene compounds and aromatic compounds.

The terpene compounds are hydrocarbons of general formula $(C_5H_8)_n$ formed from isoprene units (Figure 3) and are represented in essential oils, mainly monoterpenes, sesquiterpenes and rarely few diterpenes. These compounds may be acyclic, monocyclic, bicyclic or tricyclic [Paris, 1981].
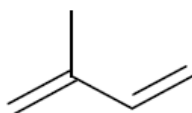

Fig. 3. Isoprene.

The aromatic compounds are derived from phenylpropane, which are characteristic of certain species, such as cinnamaldehyde in cinnamon essential oil, eugenol in the cloves, anethol and aldehyde anasique, in the essential oils of anise and fennel (Fig. 10) (Paris, 1981).

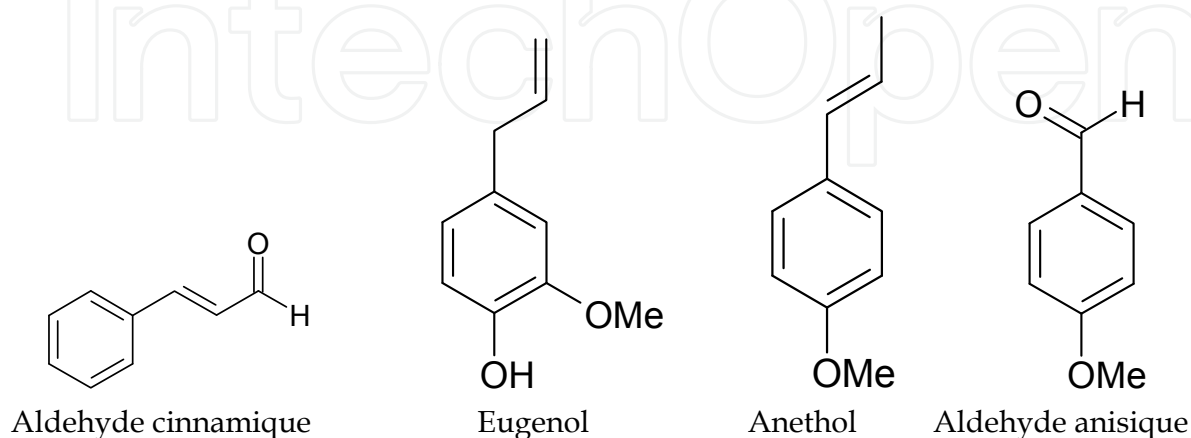Aldehyde cinnamique   Eugenol   Anethol   Aldehyde anisique

Fig. 4. Examples of aromatic compounds.

The majority of the components of EO are monoterpenes; they represent 90% of most essential oils. They are volatile usually easily driven by steam, have often pleasant odor (Lamart, 1994). By the diversity of their structure, they can be classified into several groups (Bakkali, 2008) (Table 3). Several factors may be responsible of the chemical polymorphism of essential oils. The most important are climate, soil, time harvest and method of storage and retrieval. Genetic factors (Echeverrigaray, 2001) and the growth cycle (Hance, 2003) may also influence this variability…(Bakkali, 2008). The use of essential oils is of great interest in many areas. Thus, the species most studied for their antibacterial and antifungal properties (biological activities) belong to the Lamiaceae family: Thyme, oregano, savory, lavender, mint, rosemary, sage and hyssop.

10.3 Biosynthesis of essential oil

Essential oils are very complex natural mixtures that can hold about 20-60 components with very different concentrations. The main group composed by terpenoids or isoprenoids is a family of secondary metabolites widely distributed in the plant kingdom. More than 22000 compounds have been identified (Connolly 1992). Their classification is based on the number of repetition of the basic unit of isoprene: hemiterpene (C5), monoterpene (C10), sesquiterpene (C15), diterpene (C20), sesterpene (C25), triterpene (C30), tetraterpene (C40) and polyterpene. The major terpene components of essential oils are monoterpene and sesquiterpene. Isoprenoid biosynthesis of essential oils can be simplified into three phases: Isopentenyl diphosphate (IPP) Biosynthesis, condensation of IPP units and formation of prenyl diphosphates and Conversion of prenyl-diphosphates. The condensation of isopentenyl diphosphate (nucleophilic entity) to dimethylallyl diphosphate (electrophilicentity) leads to geranyl diphosphate (GPP, C10), precursor of monoterpenes. A further condensation type head-to-tail of IPP on the GPP leads to farnesyl diphosphate (FPP, C15), the precursor of sesquiterpenes. The prenyl transferases allow prenyl chain elongation by addition of one molecule of IPP.
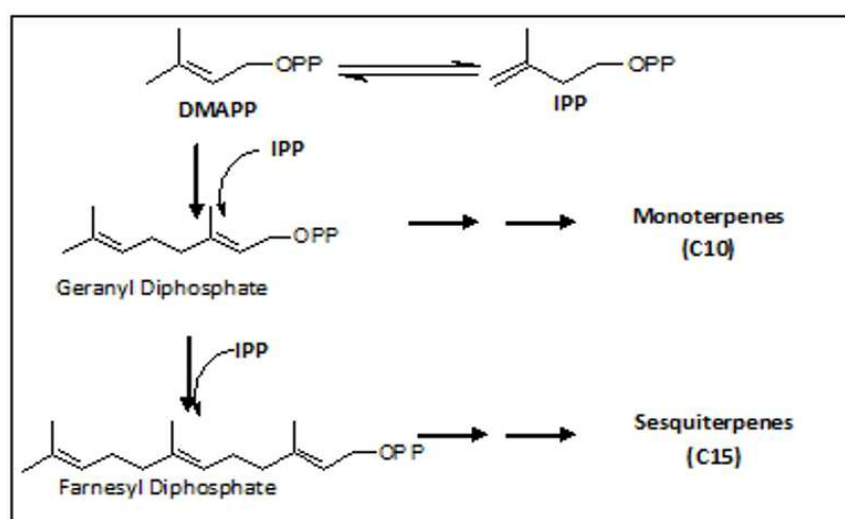

Fig. 5. Biosynthetic precursors of the main metabolic constituents of essential oils

Conversion of prenyl- diphosphates: Under the action of terpene synthases, the acyclic isoprene precursors thus obtained may undergo transformations for the formation of other compounds by hydroxylation, oxidation, inter-conversion alcohol /aldehyde, methylation of the hydroxyl and carboxyl, acylation...
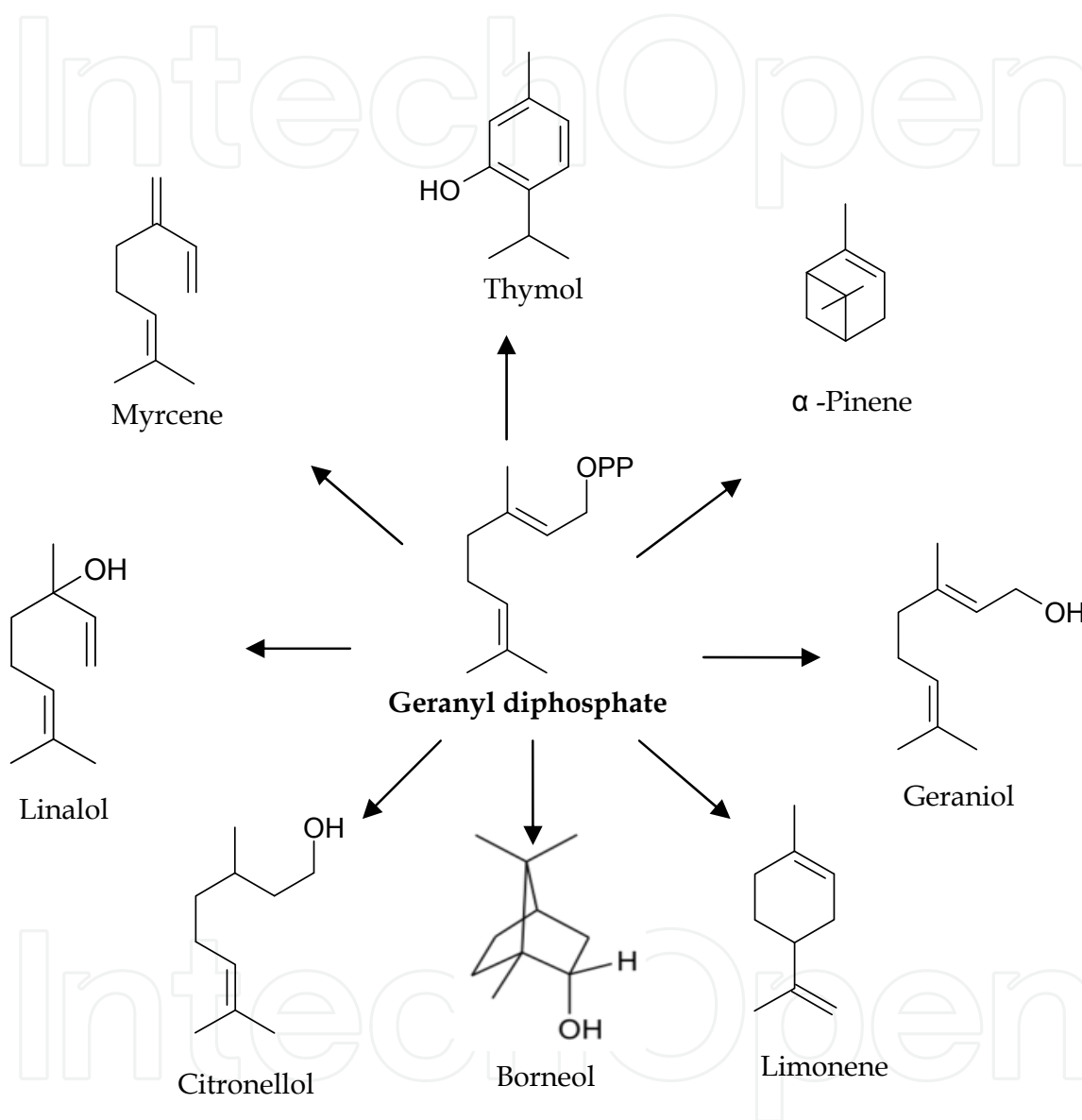
Fig. 6. Structures of monoterpenes derived from geranyl diphosphate.

10.4 Quality control

According to French and European Pharmacopoeia, the control of essential oils can be made by the miscibility of ethanol, the index of refraction, the optical rotation and gravity. The color and smell are also important parameters. The quantitative and qualitative chromatographic profile of an essential oil helps to know exactly the chemical

composition and search for any traces of other undesirable products such as pesticides or chemicals added.

A pure and natural essential oil is characterized by its composition strictly 'vegetable', unlike gasoline, synthetic or "natural identical" fully reconstituted from synthetic chemicals. In aromatherapy, the use of such profiles is essential to differentiate within a species changes induced by chemical factors influencing the plant biosynthesis, such as sunshine, altitude, nature and composition of the soil. Indeed, the observation of a clump of thyme and knowledge of its origin is not sufficient to characterize its essential oil. For example, an essential oil of Thymus vulgaris can be thymol chemotype, linalool, geraniol or thuyanol. Botanically, it is the same plant family Lamiaceae (or Labiatae).

10.5 Economic characteristics of EO

The plant is a concentrate of active ingredients (enzymes, polysaccharides, alkaloids, terpenes, tannins, resins etc.) and often requires large quantities of plants for a few drops of essential oil (100 kilos per 200 grams of EO thyme). If scarcity is one of their attractions, it also makes the price, hence the importance of good conservation: the dark (dark bottles), air and heat. In Morocco, the development of natural resources is the integration of non-food plants in the Moroccan economy, especially their conservation and development in food, cosmetics, industrial and therapeutic. Moroccan products in the areas of essential oils and aromatic extracts are well known on the world market. For some products, Morocco has a privileged position; he is the sole or primary producer, in the case of mint, chamomile wild, thyme, Atlas cedar, etc. For other products, production Moroccan faces other competitors; this was the case for example of rosemary undergoing tough Tunisian and Spanish competition. In other cases, the position of Morocco on the world market is rather high, in the case of myrtle, mint and so on. (Benjilali, 1986).

10.6 Methods of extraction of EO

There are several techniques of extraction of essential oils (Sallé, 2004). They can be extracted simply by cold pressing, distillation, by volatile solvent or supercritical fluid such as carbon dioxide. Distillation by steam distillation of water (still) is the method most practiced in the industry. The essential oils obtained are generally low polarity products, volatile, fragrant and usually less dense than water (Valnet, 2005). They are soluble in most organic solvents and poorly soluble in water. (Sallé, 2004). Steam-hydrodistillation is the most common method of recovery of essential oils. Under the action of water vapor, gas is released by the plant tissue and effect of temperature, volatiles products are driven by the steam. The essential oils are recovered by condensing (Bruneton, 1993). Hydrodistillation (steam water 'in situ') method involves by immersing the plant material in water which is then boiled (Bruneton, 1999). The enfleurage, distillation assisted by microwaves, extraction with solvents, extraction with a supercritical fluid are among the methods used to recover the EO of aromatic and medicinal plants (Sallé,2004, Brian, 1995).

11. Biological activity of thymus EO

11.1 Source of *Salmonella sp*

Salmonella sp. was isolated from soil argan Marrakech (Morocco). The identification was performed according to the methods described above. Inocula were prepared from liquid

cultures by 18 h, diluted with saline so as to contain about 108 cells / ml, at a density of between 0.08 and 0.1 at 625 nm (Careaga and al. 2003; Joffin and Leyral,2001). We evaluated the sensitivity of this strain against the essential oils of *thymus broussonetii* and *thymus maroccanus* from the region of Marrakech (Morocco). We used the Mueller Hinton medium for our study. The antimicrobial activity of essential oils, we used the method of diffusion from antibiotic susceptibility discs of pure essential oil (Jacob et al., 1979). The media poured in Petri dishes are inoculated with 1 ml of bacterial suspension of 108 cells / ml and excess inoculum was removed by aspiration (Shunying et al., 2005). The essential oil is deposited in the volume of 10 ml on Whatman sterile paper discs of 6 mm in diameter. In parallel, we use cookies to check the growth of the strain tested. The petri dishes were left 30 min at room temperature to allow complete diffusion of the product (CA-SFM, 1993). The antibacterial activity was determined in terms of diameter of inhibition zone around the discs recorded after 24 h of incubation at 37 ° C. The test is performed in three repetitions in the same experimental conditions.

11.2 Essential oils of T. broussonetii and T. maroccanus

Thymus (thyme) is an aromatic and medicinal plant belonging to the *Lamiaceae* family. This plant is native to the Mediterranean region. (Duke, 1989; Zargari, 1990; Newall et al., 1996). *Thymus broussonetii* Boiss and *Thymus maroccanus*. are an endemic plants of Morocco. Its species are used for traditional medicine for the treatment of various illnesses (Bellakhdar, 1997; Sijelmassi, 1993). The studies of essential oil of *T. broussonetii* have been published indicating the antimicrobial, anti-inflammatory, the immunological, behavioural effects and antitumor properties (Lattaoui et al., 1994; Lattaoui and Tantaoui-Elaraki, 1994, Ismaili et al. 2001; 2002; 2004, Elhabazi et al., 2006, Jaafari et al, 2007).

T. broussonetii and *T. maroccanus* were collected at flowering stage in July 2006 in a High Atlas mountain respectively, in "Ait Ourir" and in "Essaouirra" from the region, Centre and Southwest of Morocco. The inflorescences, leaves and stem were separated by hand. Samples were dried at the shade.

11.3 Chemical composition of essential oils of T. broussonetii and T. maroccanus

The identification of 93.1% (36 compounds) of the chemical composition of the essential oil of some leaf of *T. broussonetii* (figure 7) is shown in Table 2. This part produces a yield of 1.6% essential oils. The chemical identity of this oil shows that it consists mainly of monoterpene hydrocarbons which represent more than half of this oil (53.3%). In this class of monoterpenes, p-cymene (21.0%), the α-pinene (11.8%) and camphene (8.5%) are the most important compounds. The class of oxygenated monoterpenes is second with 33%, represented mainly by borneol (16.5%) and thymol (11.3%). The sesquiterpene hydrocarbon represent 6.6% and consist mainly leden (3.2%). The spathulénol is the only oxygenated sesquiterpene identified in this part of the plant with a percentage of 0.2%.

The identification of 96.7% of the chemical composition of the essential oil of some leaf of T. maroccanus (figure 8) is shown in Table 3. The yield of essential oils of this part is 1%. The monoterpene hydrocarbon species represent 49.5% of the leaves of this species. The α-pinene (11.6%) and p-cymene (25.3%) are the most important compounds in this class. The oxygenated monoterpenes represent 37.9%, they consist mainly of carvacrol (33%). The sesquiterpene hydrocarbon represents 6.6% and consists of 2% β-caryophyllene. The oxygenated sesquiterpenes represented only 0.4% of this oil.

Fig. 7. *T. broussonetii*

Fig. 8. *T. maroccanus*

	IRa	IRb	%
Monoterpenes hydrocarbon			**53.3**
Tricyclene	921	1001	0.3
α-Thujene	924	-	0.3
α-Pinene	934	1012	11.8
Thuja-2,4(10)-diene	937	1112	0.1
Camphene	944	1051	8.5
Sabinene	966	1105	0.2
β-Pinene	971	1093	1.9
Myrcene	984	1141	2.1
α-phellandrene	998	1185	0.2
3-δ-carene	1006	1128	0.1
α-terpinene	1010	1158	1.0
p-cymene	1017	1244	21.0
Limonene	1024	1176	2.2
γ-Terpinene	1052	1220	2.5
Terpinolene	1079	1254	0.2
Oxygenated Monoterpenes			**33.0**
(E)-Sabinene hydrate	1054	1423	0.3
Linalol	1085	1510	0.2
Camphre	1122	1466	0.1
Isoborneol	1140	1651	tr
Borneol	1148	1651	16.5
Terpinen-4-ol	1161	1554	0.4
Dihydrocarvone 1	1171	1557	0.4
Dihydrocarvone 2	1179	1579	0.1
Carvenone	1233	1676	tr
Thymol	1268	2124	11.3
Carvacrol	1280	2151	3.7
Sesquiterpenes hydrocarbon			**6.5**
α-Cubebene	1348	1431	tr
β-Bourbonene	1382	1488	0.1
α-Gurjunene	1408	1497	0.1
β-caryophyllene	1416	1558	0.3
Aromadendrene	1437	1570	2.1
allo-Aromadendrene	1456	1605	0.4
γ-Muurolene	1469	1683	0.2
Ledene	1491	1655	3.2
Calamenene	1508	1784	tr
Oxygenated Sesquiterpènes			**0.2**
Spathulenol	1561	2059	0.2

IRa: Index of retention in non-polar chromatographic column HP-1
IRb: Index of retention in the polar chromatographic column HP-20
tr: trace

Table 2. Percentage and chemical composition of the EO part of the leaf of *T. broussonetii*

	IRa	IRb	%
Monoterpenes hydrocarbon			**49.5**
Tricyclene	921	1001	tr
α-Thujene	924	-	0.8
α-Pinene	934	1012	11.6
Thuja-2,4(10)-diene	937	1112	0.1
Camphene	944	1051	0.8
β-Pinene	971	1093	0.4
Myrcene	984	1141	1.4
α-phellandrene	998	1185	0.3
α-terpinene	1010	1158	1.1
p-cymene	1017	1244	25.3
Limonene	1024	1176	2.7
γ-Terpinene	1052	1220	4.6
Terpinolene	1079	1254	0.1
Oxygenated Monoterpenes			**37.9**
(E)-Sabinene hydrate	1054	1423	0.2
Linalol	1085	1510	2.3
Camphre	1122	1466	0.2
Borneol	1148	1651	0.8
Terpinen-4-ol	1161	1554	0.5
Dihydrocarvone 1	1171	1557	0.3
Dihydrocarvone 2	1179	1579	0.2
Carvone	1233	1676	0.2
Thymol	1268	2124	0.4
Carvacrol	1280	2151	33.0
Eugenol	1328	-	tr
Sesquiterpenes hydrocarbon			**6.6**
α-Cubebene	1348	1431	tr
α-ylangene	1370	1461	tr
Copaene	1375	1463	0.1
β-Bourbonene	1382	1488	0.1
β-Patchoulene	1386	-	tr
β-caryophyllene	1416	1558	2.0
β-Cubebene	1425	1667	tr
Aromadendrene	1437	1570	1.4
α-Humulene	1449	-	0.1
allo-Aromadendrene	1456	1605	0.2
D-Germacrene	1474	1667	tr
β-Gurjunene	1484	1681	0.2
Ledene	1491	1655	1.0
γ-Cadinene	1504	-	1.3
Calamenène	1508	1784	0.1
(Z)-α-Bisabolène	1532	-	tr
Guaiazulène	1652	-	tr

	IRa	IRb	%
Sesquiterpènes oxygénés			**0.2**
Spathulénol	1561	2059	0.2
Caryophyllène oxyde	1567	1920	0.1
Globulol	1571	-	tr
Autres			**2.4**
Octen-3-ol	964	1357	1.3
Octen-3-one	980	1225	0.2
Carvacryl acetate	1345	1690	Tr
Acetovanillone	1516	-	0.9

IRa: Index of retention in non-polar chromatographic column HP-1
IRb: Index of retention in the polar chromatographic column HP-20, tr: trace

Table 3. Percentage and chemical composition of the HE part of the leaf of *T. maroccanus*

11.4 In vitro effect of essential oils of *T. broussonetii* and *T. maroccanus*

As has been reported in the literature, we considered that an essential oil has bacteriostatic action if the diameter of inhibition is greater than 12 mm (Baudoux, 2001; Sağdac ° 2003) or 15mm (Rossi, 2003). The diameters of inhibition zones obtained with the essential oils of *T. broussonetii* and *T. maroccanus*, from the region of Marrakech (Morocco), are 19 mm and 23 mm respectively. The essential oils acted actively on the growth of *Salmonella sp* responsible for salmonellosis. They have an inhibitory effect on the growth of Salmonella sp.

	Inhibition Zone (mm)			
	Essential oil		Standard antibiotics	
	T.b.	*T.m.*	Gentamicine	Tetracycline
Salmonella sp.	19±0.9	23±0.4	25±0.1	15±0.3

Table 4**.** Antibacterial activity of the essential oils of *Thymus broussonetii* (T.b.) and *Thymus maroccanus* (T.m.) and from antibiotic expressed by diameter of inhibition zone.

These values are reported as means ± standard deviations of three separate determinations. Disc diameter. 6mm (Romane et al, 2010).
The major activity of essential oils of *Thyme broussonetii* and *Thyme maroccanus* is due to their richness in phenolic compounds (thymol and carvacrol). Most antimicrobial compounds are phenols (carvacrol, thymol, eugenol), followed by alcohol (cineole, linalool ...) and to a lesser extent alkenes (p-cymene, pinene, terpinene ...) (Burt, 2004, Ultee et al., 2002). Indeed, several studies have shown that high antimicrobial power of essential oils of several species of thyme is attributed to their high phenolic compounds (carvacrol and thymol) (Marino et al. 1999; Mirmostafa and Rasool, 2002; Dafer and Ziogas , 2000; Baranauskiene et al. 2003; Di Pasqua et al. 2005; Di Pasqua et al. 2007; Cristan et al., 2007).
Most of the work that had for its object the study of the mechanism of action of phenolic compounds suggests that their main site of action is the bacterial plasma membrane

(Shunying et al., 2005). They are able to disintegrate the bacterial cell membrane (Ultee et al., 1999). The membrane loses its structure and becomes more permeable to ions (Lambert et al., 2001). Damages to the cell membrane may also allow the dissipation of pH gradient and decreased membrane potential (Ultee et al., 1999).

11.5 The antibacterial activity of antibiotics

The study by (Imelouane & ElBachiri, 2010) of the antibiotic susceptibility shows that the strain used is relatively sensitive to Florenfinicol, Spectinomycin, Enrofloxacin, Cotrimoxazole, Flumequine Tiafen, Tetracycline and Gentamycin. This sensitivity varies from one antibiotic to another and from one strain to another

	Inhibition Zone (mm) antibiotics				
	KF_{30}	AMP_{10}	AML_{25}	TPC_{30}	E_{15}
Salmonella sp	0	0	0	30	0

	Inhibition Zone (mm) antibiotics					
	UB_{30}	SXT_{25}	MY	ENR_5	SH	FFC
Salmonella sp	28	25	0	32	18	34

Kf: Cephalothin; AMP: Ampiciline; AML:Amoxycilline; TPC:Tiafen; E:Erythromycine
UB: Fluméquine ,SXT: Cotrimoxazole; MY:Lyncomycine ; ENR:Enrofloxacine ; SH : Spectinomycine;
FFC: Florenfinicol

Table 5. Inhibitory effect of different antibiotic discs: Diameter of inhibition zones in mm among Salmonella sp.

The study of antibiotic susceptibility shows that the strain used is sensitive to Florenfinicol, Spectinomycin, Enrofloxacin, Flumequine, Tetracycline and Gentamycin. This sensitivity varies from one antibiotic to another (Table), while the strain is resistant to Cephalothin, Ampicillin, Amoxicillin and Lyncomycine.

Quantitative comparison of the results of EO to antibiotics is difficult because the nature of the activity and composition of the molecules are not comparable. Unlike EO, complex mixtures of volatile compounds that evaporate, but also that diffuse into the agar at different speeds, antibiotics are large non-volatile molecules. There Dissemination takes place probably at the surface and / or volume in the mass of the agar.

	Inhibition Zone (mm)
microorganisms	Thymus pubescens
Salmonella sp	08

Table 6. Antibioaromatogramme of the essential oil of *Thymus pubenscens*. Diameter of inhibition zone of *Salmonella sp* determined using the diffusion method on solid medium.

12. Conclusion

Recently, Salmonella strains resistant to a range of antibiotics, including major therapeutic agents in human medicine have emerged and are threatening to cause serious public health problems.

This resistance results from the use of antibiotics; this situation is getting worse year by year due to misuse or inappropriate use of antibiotics to treat any disease;, hence the importance of direct research into plants that have always been a source of inspiration for new drugs. Thus, the essential oils have a lot of interest as a potential source of bioactive natural molecules which extracts have a strong antimicrobial potency.

The use of essential oils is a serious substitute to treatment with antibiotics in infectious diseases because of their interference with bacteria vital functions. The alteration of the cell membrane including permeability may result in abnormal losses of ions or macromolecules.

Several authors have put the relationship between the antibacterial activity of essential oils with their chemical composition; specially

phenolic and terpenic compounds but some studies have shown that the antimicrobial activity, antiviral, insecticidal, larvicidal and ovicidal essential oils are superior to those of its majority compounds tested separately.

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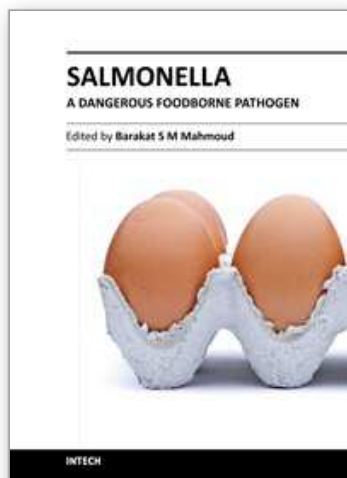
Salmonella - A Dangerous Foodborne Pathogen

Edited by Dr. Dr. Barakat S M Mahmoud

More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at $2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

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