République **A**lgérienne **D**émocratique et **P**opulaire

Ministère de l'**E**nseignement **S**upérieur et de la **R**echerche **S**cientifique

Université A. MIRA – **B**ejaia

جامعة بجاية
Tasdawit n Bgayet
Université de Béjaïa

Faculté des **S**ciences de la **N**ature et de la **V**ie

Département des **S**ciences **A**limentaire

Filière : **S**cience **A**limentaire

Option : **P**roduction et **T**ransformation **L**aitière

Réf:.....

Mémoire de Fin de Cycle

En vue de l'obtention du diplôme

MASTER

Thème

Activité antioxydante et anti-mammite de quelques huiles essentielles.

Presenter par

MOUNSI Adouda et SALHI Souhila

Soutenu le 14/07/2022

Devant le jury composer de :

- M^me^ MERZOUK.H — Encadreur.
- M^me^ HAMRI. — Présidente.
- M^me^ OULD SAADI.L — Examinatrice

Promotion: 2021/2022


بسم الله الرحمن الرحيم

REMERCIEMENTS

This work is the fruit of efforts and sacrifices made by several people that we could not forget to thank

Our thanks go first to GOD, creator of all things, for breath and all innumerable benefits.

Our work would not be as rich and would not have or to have the day without the help and the framing of Mrs. MERZOUK, we thank her for the quality of its exceptional framing, for its patience, its invaluable councils and its availability during our preparation of this memory.

Our warm thanks go to Mrs. HAMRI and Mrs. OULD SAADI to agree to examine our present work.

Our thanks also go to Ms. TOUATI, Ms. SAIDANI, Ms. OUAR, Mr. BOUJOUAN and Mr. NOURI for their practical help and their moral support and encouragement in spite of their academic and professional loads.

Finally, all the persons who have participated in the accomplishment of this work

DEDICATION

With the expression of my gratitude I dedicate this modest work to what, whatever the terms embraced I would never manage to express my sincere love to them.

To the man, my precious gift from God, who owes my life, my success and all my respect: my dear father

"NACER".

To the woman who suffered without letting me suffer, who never said no to my soul and spared no effort to make me happy: my lovely mother

"FATMA (ZAHIA)".

To my adorable sisters "DJAZIA" and "KENZA" who always knows how to bring joy and happiness to the whole family.

To my aunts and uncles exceptionally my uncle "Ferhat" that GOD give them a language and joyful life.

To my grandmother, may GOD have mercy on you, the most heart and the most precious thing I have lost

To the person who never stopped advising, encouraging and supporting me throughout my studies, may GOD protect him and offer him luck and happiness.

To my friends who have supported and encouraged me during these years of study exceptionally "FAIROUZ, KATIA,FADILA AND HALIM".

Without forgetting my partner "SOUHILA" for his moral support his patience and his understanding throughout this project.

ADOUDA

DEDICATION

With the expression of my gratitude, I dedicate this modest work to that which, whatever the terms embraced I would never manage to express my sincere love to them.

To the man, my precious gift from God, who owes my life, my success and all my respect: my dear father

"el hadi".

To the woman who suffered without letting me suffer, who never said no to my soul and spared no effort to make me happy: my lovely mother

"Farida".

To my lovely sisters and brothers-in-law

To my brothers and sisters-in-law

May GOD give them a language and joyful life.

To my nephews and nieces

May GOD give them a good life, and may GOD know how to bring joy and happiness to the whole family.

To my brother, may GOD have mercy on your smile that did not scare me from my memory GOD smiles without your paradise (zizi nadir)

To the person who never stopped advising, encouraging and supporting me throughout my studies, may GOD protect him and offer him luck and happiness.

To my friends who have supported and encouraged me during these years of study exceptionally "SARAH".

Without forgetting my partner "ADOUDA" for his moral support, his

patience and his understanding throughout this project.

SOUHILA

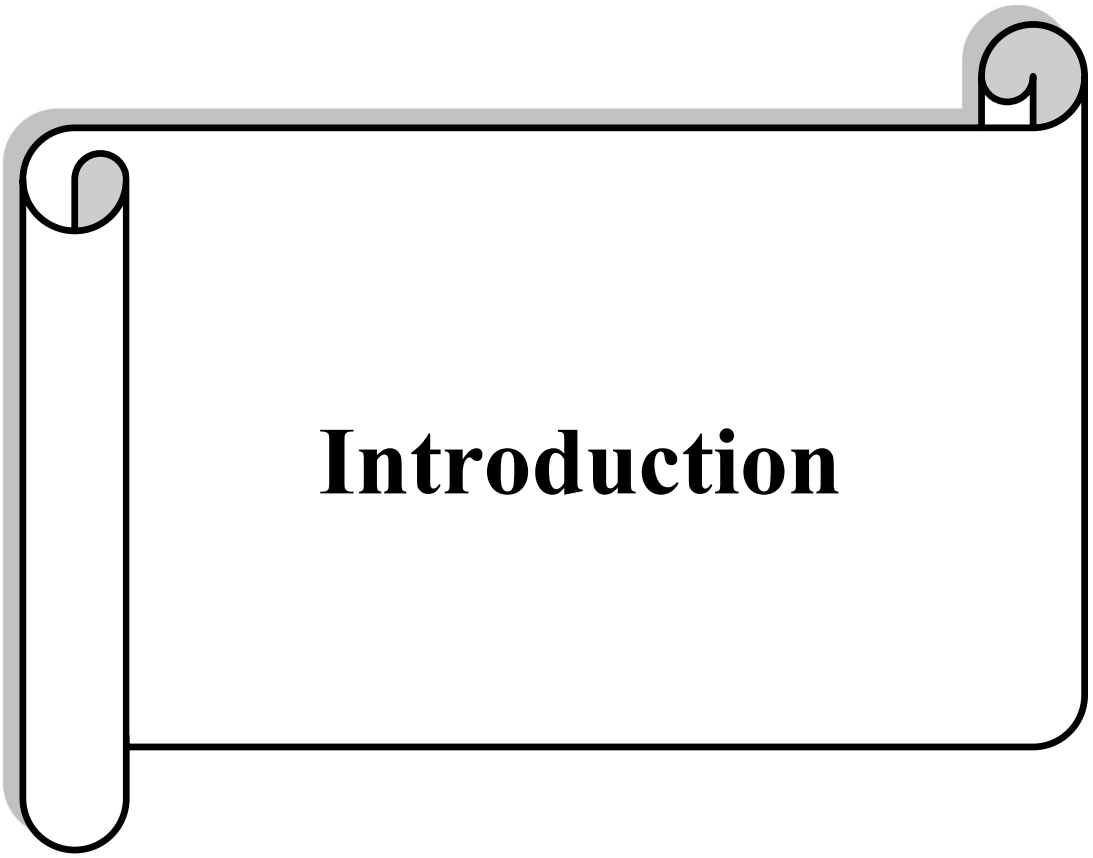
Table of contents

Figure list

Table list

List of abbreviations

ABTS	2, 2’-azinobis (3-ethyl benzothiazoline -6-sulfonie).
BD	biofilm development.
BHI	brain heat infusion.
CNS	coagula-négative staphyloccocci.
CRA	congo red agar.
CV	crystal violet.
DPPH	1, 1 diphenyl-2 pioryl hydrazyl.
EO	essential oils.
FRAP	ferric reducing antioxidant power.
IC50	inhibitory concentration.
OD	optical density.
OD	optical density of control.
PS	psyological saline.
S	strains.
TPTZ	ripyridyltriazine.



Introduction

Introduction

Medicinal and aromatic plants have been used by man since antiquity. Nowadays their use has taken a considerable rise in the perfume, cosmetic and pharmaceutical industries. Plants are the main source of biologically active compounds where at least 35,000 species are used worldwide (Mouas et al. 2017). Among the vastness of plant products, essential oils deserve particular attention. These are complex mixtures of hydrocarbons and oxygenated hydrocarbons arising from the isoprenoid pathways, mainly consisting in monoterpenes and sesquiterpenes (Sharifi-Rad et al. 2017).

The active components of essential oils (EOs) represent a source of antioxydant molecules with phenolic structures or aldehydes, such as thymol, carvacrol, eugenol, cinnamaldehyde and citraland are the subject of many studies for their possible use as an alternative for the protection of food against oxidation (Sanaa et al.2017).

The extensive use of antibiotics leading to the rapid spread of antibiotic resistance poses high health risks to humans and animals because of potential toxicological risks, which is why the use of EOs is more recommended (Talbi et al. 2015).

Essential oils possess an aromatherapy and act as pain relievers, antioxidant, anticancer and antimicrobial effects. This is why there is an increasing interest in the use of EOs for prevention and treatment of dairy animal mastitis. Mastitis is a common and costly disease that affects all milk producing animals. Mastitis is an infection of the udder caused predominantly by the ingress of bacteria. The infected udder produces less milk and milk of a lower quality (Mayssar et al. 2017).

This disease has important economic repercussions, mainly due to the costs of veterinary care and medication, production losses due to the death or slaughter of animals and the decline in the price of milk due to its poor quality. It is known that mastitis, whether clinical or subclinical, has a negative impact on udder tissues and productivity and milk components (Mayssaretal. 2017).

The overall aim of this work is to evaluate the antioxidant and anti-mastitis activities of twelve essential oils of local aromatic plantsby conducting several tests antimicrobiennes:

- Evaluation of antioxidant properties of these essential oils by DPPH, ABTS, FRAP methods;

- Evaluation of antimicrobial activity of these essential oils on 9 strains, first by detection biofilm production then by evaluating effects on biofilm establishment and eradication.

Bibliography

I. Antioxidant activity of essential oils

I.1. Essential oil

Essential oils (EOs) are liquid mixtures of volatile compounds obtained from different parts of aromatic plants most commonly by steam distillation. (Amorati et al., 2013). Chemically, essential oils are homogeneous mixtures composed of a range of fractions from the most volatile to the heaviest terpenoids, which are natural compounds that are usually produced as secondary metabolites and act as defense phytochemicals for plants (Machado et al.,2022).

EOs also called volatile or ethereal oils, are aromatic lipophilic liquids obtained fromplant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots). Theterm ‘essential oil’ is thought to derive from the name coined in the 16th century by the Swiss reformer of medicine, Paracelsus von Hohenheim, who named the effective component of adrug Quinta essential (Neheme et al., 2021).

As mentioned above, an estimated 3000 EOs are known, of which about 300 are commercially important destined chiefly for the flavors and fragrances market .It has long been recognized that some EOs have antimicrobial properties ,and these have been reviewed in the past,like antimicrobial properties of spices.Still, the relatively recent enhancement of interest in ‘green’ consumerism has renewed scientific interest in these compounds(Neheme et al., 2021).

They are rich sources ofbiologically active compounds. Thus, plants are a good source of biologically activecompounds known as phytochemicals. Phyto-constituentshave been found to work as antioxidants by scavengingfree radicals and many have curative potential for freeradical associated disorders and they also have antimicrobial activity (Hagos et al., 2022).Besides antibacterial properties,EOs or their components have been shown to exhibit antiviral, anti-mycotic,anti-toxigenic,antiparasitic, and insecticidal properties.They constitute what is called the “essence” of a plant and usually have pleasantly scented fragrances(Neheme et al., 2021).

They can be obtained by Water distillation, water and steam distillation, steam distillation, cohobation, maceration and enfleurage are the most traditional and commonly used methods. Maceration is adaptable when oil yield from distillation is poor. Distillation methods are good for powdered almonds, rose petals and rose blossoms, whereas solvent extraction is suitable for expensive, delicate and thermally unstable materials like jasmine, tuberose, and hyacinth. Water distillation is the most favored method of production of citronella oil from plant material (yadav et yadav.,2016).

I.1.1. General use of Eos

EOs are one of the constituents in the products related to perfumery, cosmetics, sprayers, deodorants, food products, beverages, soaps, fumigants, and detergents (Singh et al.,2022).

The applications of essential oils are diverse. Widely used in cosmetics and perfumes, they also have medicinal applications due to their therapeutic properties as well as agro-alimentary uses because of their antimicrobial and antioxidant effects (Selmiet al., 2022).

EOs of tea tree is effective in controlling the growth of pathogens because of which they are widely used in hand washes and antiseptics liquids.

Eucalyptus, thyme, and menthol are generally used in mouthwashes for providing refreshing fragrance as well as antiseptic properties.

EOs derived from Species of Ocimum, Eucalyptus, Cymbopogon are widely used as mosquito repellants.

There are many patents regarding these repellants containing EOs around the world. Chemical moieties mainly responsible for the repellant properties are monoterpenes and sesquiterpenes.

Cinnamomum camphora, Syzygium aromaticum L., Lavandula angustifolia, Cinnamomum zeylanicum are also known for their mosquito repellency properties.

Synergism among different constituents also plays an influential role in the properties of Eos.

Camphor, vanillin, limonene, thymol, citronellol, and alpha-pinene are some of the major components of EOs having insecticidal properties.

These EOs derived repellants have no side effects and are eco-friendly options of mosquito repellants (Singh et al., 2022).

I.1.2. Physico-chemical characteristics of essential oils

I.1.2.1. Physical properties

Physicochemical properties of oil like colour, odour, density, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value etc indirectly influence the quality of both essential and fixed oils. The commercial importance of oils mostly depends on these physicochemical properties, which provide baseline data to determine its suitability for consumption (Parthiban et al., 2011).

A typical physicochemical dataset consists of:

Organoleptic properties (colour, odour, taste if relevant)at room temperature, they are generally liquid, colorless or pale yellow, there are, however, some exceptions (EO with azulene of blue coloring.

- Solubility in water and relevant solvents, including receptor fluids (at 37 °C):They are soluble in alcohol, ether, chloroform, fixed oils, emulsifiers and in most organic solvents, and not very soluble in water to which, however, it communicates their odor.

- Physical properties depending on the physical state:boiling point(varies from 160° to 240°C), relative density (at 25 °C), pKa (at 100°C),viscosity (at 20 °C), vapour pressure (at 25 °C),refractive index (often high), density is generally lower than that of water, it varies from 0.75 to 0.99 (Abdelouahid et al., 2010):

- They are dextrorotatory or levorotatory, rarely inactive on polarized light.
- They dissolve fats, iodine, sulfur, phosphorus and reduce certain salts.
- They are perfumes, and are of limited conservation.
- They are very alterable and sensitive to oxidation but do not go rancid.)
- They are oily growth substances, more or less fluid, even retinoid.
- They are stimulating products, used inside and outside the body, sometimes pure, generally dissolved in alcohol or a suitable solvent.

I.1.2.2. Chemical composition

The chemical composition of species is complex and can vary depending on the organism, climatic factors, the nature of the soil, cultivation practices and the method of extraction (Selmi et al., 2022). In order to rationalize the mechanism of antioxidant activity expressed by essential oils it is necessary to briefly address their composition. Despite the observed large chemical diversity, the main components of common essential oils can be classified in two structural families with respect of hydrocarbon skeleton: terpenoids, formed by the combination of 2 (monoterpene), 3 (sesquiterpene), or 4 (diterpene) isoprene units, and phenylpropanoids. Both terpenoid and phenylpropanoid families comprise phenolic compounds, sometimes accounted among principal components of several Eos (Amorati et al., 2013).

The main constituents of essential oils are the following. (Abdelouahid et al., 2010)

I.1.2.2.A. Terpenoids

Terpenes have very diverse structures (acyclic, monocyclic, bicyclic, etc.) and contain most of the chemical functions of organic materials (Selmi et al., 2022).In the case of E0, only the most volatile terpenes will be included: mono and sesquiterpenes.

- **Monoterpenes**

The simplest constituents of the series, monoterpenes are derived from the coupling of two units, they can be acyclic (myrene, ocimene), monocyclic (α and γ, p-cymene) or bicyclic (pinene, camphene, ocimene). They sometimes constituting more than 90% of the EO (citrus...) (figure1). Structural variation justifies the existence of many molecules: alcohol, aldehydes, cetones, and ester (Abdelouahid et al., 2010).

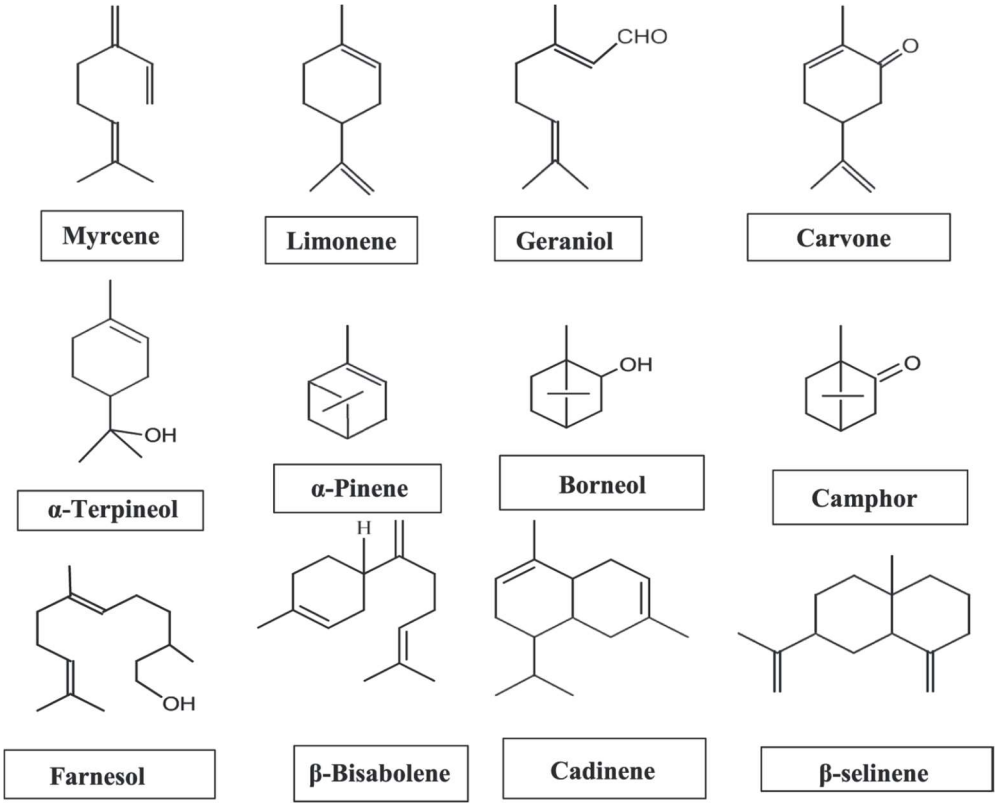


Figure 1: Examples of mono- and sesquiterpene structures (Selmi et al.,2022).

- **Sesquiterpenes**

A large number of sesquiterpenes (figure 1) are usual constituents of oils essentials of higher plants, it can intervene in the pharmacological properties attributed to these volatile fractions.

Biologically, many sesquiterpene structures are phytoalexins, others seem to act as growth regulators, and others attract insects or act against them as anti-nutritive factors.

I.1.2.2.B. Aromatic compounds

Phenylpropane derivatives (C_6-C_3) are much less frequent than the previous ones.They are very often allyl-and propenylphenols ,sometimes aldehydes.We can also meet in EO compoundsin (C_6-C_1)likevantilin or like anthranilate (figure 2) (Abdelouahid et al, 2010)

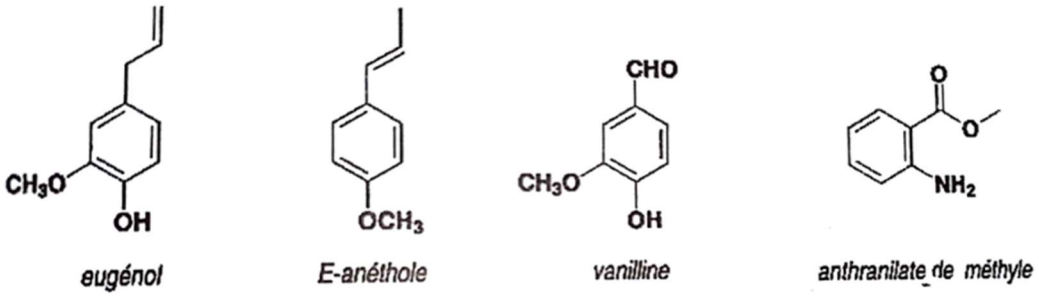


Figure 2: example of the structure of aromatic compounds found in essential oils (Abdelouahid et al., 2010).

I.1.3. Toxicity of Eos

Some EO can cause various toxicity, so it is important to seek advice from the pharmacist.

Many drugs based on terpenic derivatives from EO have been withdrawn from the market in 20110 after the reporting of many cases of convulsion and respiratory difficulties in children.

These three molecules can also present toxicity in adults.

I.1.3.1. Photosensitization

Photosensitization, or phototoxicity, occurs following exposure to the sun after skin application of one of the following substances: essences of bergamot, lemon, tangerine ... etc... this photo toxicity can manifest it self in the form of a burn of a pigmentation (Toubou,2021).

I.1.3.2. Convulsions

Convulsions can lead to a coma, even death. It also presents an abortive risk. it is provoked by EO of rosemary comphre, peppermint, cedar ... etc(Toubou, 2021).

I.1.3.3. Difficulty breathing

Some strong smelling EO (like mints) can cause asthma attacks, especially in patients with existing breathing difficulties (Toubou, 2021).

I.1.4. Renal toxicity

The renal toxicity, or nephro toxicity, concerns the EO of citrus or conifers in supra-therapeutic dose. It is however unlikely in the normal conditions of uses (Toubou, 2021)

I.2. Antioxidant activity

I.2.1. Generalities on antioxidants and oxidative stress

In general, an antioxydant is any compound that presents at low concentration compared to that of the oxidizable substrate significantly delays or prevents the oxidation of this substrate (Agbàdan et al., 2014). By definition, antioxidants are compounds capable of slowing down or retarding the oxidation of an oxidizable material, even when used in very modest amount (less than 1%, commonly 1-1000 mg/L) as compared to the amount of material they have to protect. Focusing on processes of relevance in biological systems or in food science, the materials to protect are most commonly lipids, proteins, carbohydrates, and to minor extent other organic molecules that compose animal or vegetal tissues (Amorati et al., 2013).

Generation of free radicals or reactive oxygen species (ROS) during metabolism and other activities beyond the antioxidant capacity of a biological system gives rise to oxidative stress (Hagos et al., 2022). Thus, antioxidants are capable of stabilizing or deactivating free radicals before they attack cells, so they are completely essential for maintaining optimal cellular and systemic health and well-being (Hagos et al., 2022).Focusing on processes of relevance inbiological systems or in food science, the materials to protect are most commonly lipids, proteins, carbohydrates, and to minor extent other organic molecules that compose animal or vegetal tissues (Amorati et al .2013). The Mechanisms, generalized in (figure 3), of formation and biological action of reactive oxygen species (ROS) in living systems are the main element of a number of physiological and pathophysiological processes. In fact, the organism's protection from ROS is a universal complex system of chemical and biochemical reactions realized at different biological levels and proceeds in accordance with different mechanisms, which involve various high and low molecular weight compounds, such as redox enzymes, polypeptides, and some vitamins, amino acids, polyphenols, etc.

As a rule, six basic mechanisms are described in the literature, which are shown in Figure 3, considering the action of AO in biological media (Ivanova et al.2020).

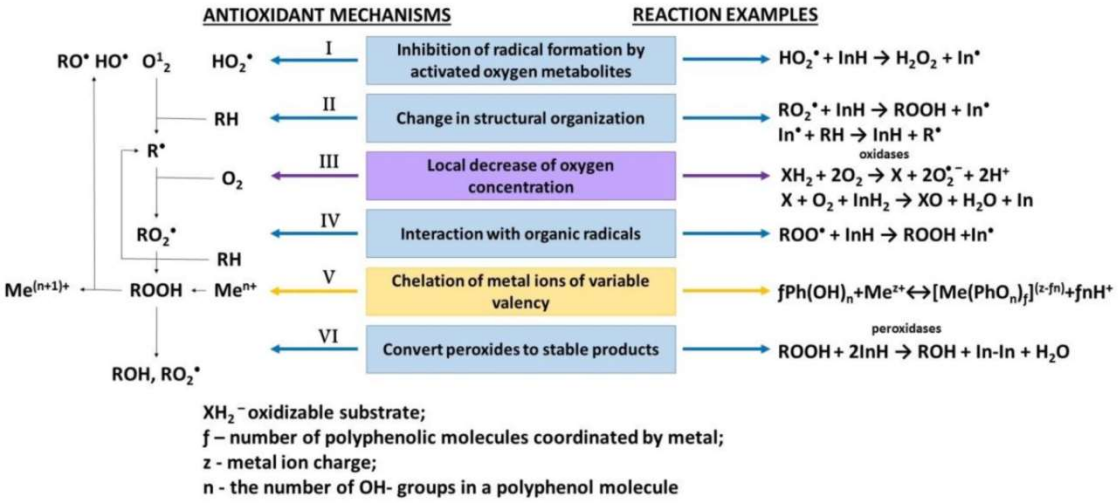


Figure 3: Mechanisms of biological action of antioxidants

I.2.1.1. Oxidative stress

In general, oxidative stress is defined as the result of an imbalance in the balance between oxidizing species and defense systems (antioxidants), resulting in the appearance of damage often irreversible for the cell (Dermier ,2016).Oxidative stress plays a role incardiovascular diseases, neurodegeneration, and cancer and inthe aging process (Hagos et al., 2017).

I .2.1.2 A free radical

A free radical is a species caracterized by instability and / or, a strong oxidizing power. It is differentiated by the presence of an unpaired electron on the outher most electronic layer (Toure, 2014; Hagos et al., 2017).

I.2.2. Essential oils as an antioxidant

EOs is a potential source of natural bioactive molecules and are the subject of numerous studies regarding their possible uses as antioxidants. Thus, essential oils, phenolic substances such as tocopherol (vitamin E and related compounds), various classes of flavonoids, phenolic acid, tannins, lignans, etc., are of special interest (Hagos et al., 2017).

These oxidation reactions lead to changes in taste, smell and color during the manufacturing process and storage, which leads to the loss of quality and safety of food (Fenandez, 2012).

I.2.3. Main techniques for determining the antioxidant activity of Eos

there is a great diversity of physical-chemical method to evaluate antioxidant natural extracts, the values of the antioxidant capacity of a compound differ from one test to another which makes it impossible to compare methods and, therefore a certain standardization (Fenandez, 2012) Most often, to have an indication as precise as possible on the antioxidant capacity of a sample it will be necessary to combine the answers of tests that can be carried out (Fenandez, 2012)

The antioxidant activity can be evaluated either by direct methods by measuring the products formed or by indirect methods through which the effectiveness of an antioxidant will be revealed by measuring the capacity of the latter to trap free radicals through the use of an intermediate probe (Fenandez, 2012).

Mechanisms of biological action, in turn, from the chemical point of view of (AO oxidation reaction) conversion are reduced to three main mechanisms (Ivanova et al., 2020):

1) electron transfer reactions from AO to the substrate AO ET-mechanism.
2) transfer reactions ofan hydrogen atom from AO to a substrate which, in aqueous media, can be considered as proton transfer accompanied by electron transfer AO HAT-mechanism.
3) transfer reactions of an or more electron pairs with the formation of the covalent bond by the donor-acceptor mechanism (the reaction of the complexation of AO with metal ions of variable valency) chelating-mechanism.

Main wide spread methods are presented in (Figure4).

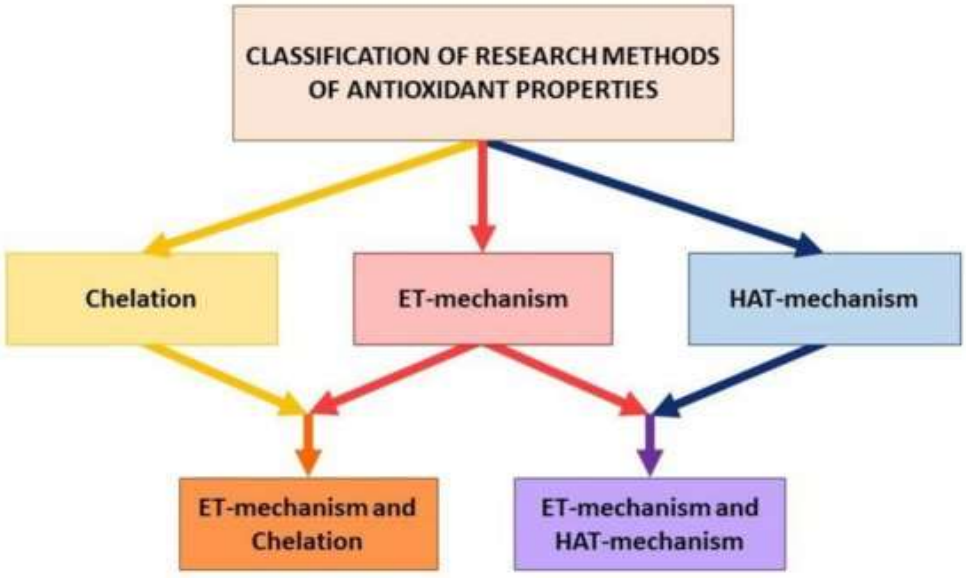


Figure 4: Classification of methods to evaluate the integrated antioxidant properties.

EOs obtained from different plants is a source of natural antioxidants. Most EOs have the virtue of being non-toxic. However, in high concentrations, they can exert toxicity, such as necrosis. Hence, it is a common practice in the study of EOs, although not entirely correct to identify natural antioxidants as “molecules able to react with radicals” or molecules that present the reducing power to counteract the oxidative stress caused by radicals.

Accordingly, there are several methods used to examine the antioxidant properties of EOs. The most common tests used to screen the antioxidant activity of EOs are based on the in vitro reaction of the phytochemicals with some colored persistent radicals (e.g., 2,2-diphenyl-1-picrylhydrazyl (DPPH) or 2,2 0-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) $ABTS^{+}$assay) (antiradical) or with some oxidizing nonradical species such as Fe^{3+}ions (e.g., ferric reducing antioxidant power (FRAP assay).From a mechanistic point of view, these methods are classified as electron transfer (ET)-based assays, contrary tohydrogen atom transfer (HAT)-based assays (figure 5), also widely used, which include oxygenradical absorbance capacity (ORAC) assay, radical-trapping antioxidant parameter (TRAP)assay, crocin bleaching assay using 2,2-azobis-2-methyl-propanimidamide dihydrochloride(AAPH) as a radical generator, and -carotene bleaching (BCB) assay (Nehme et al., 2021).

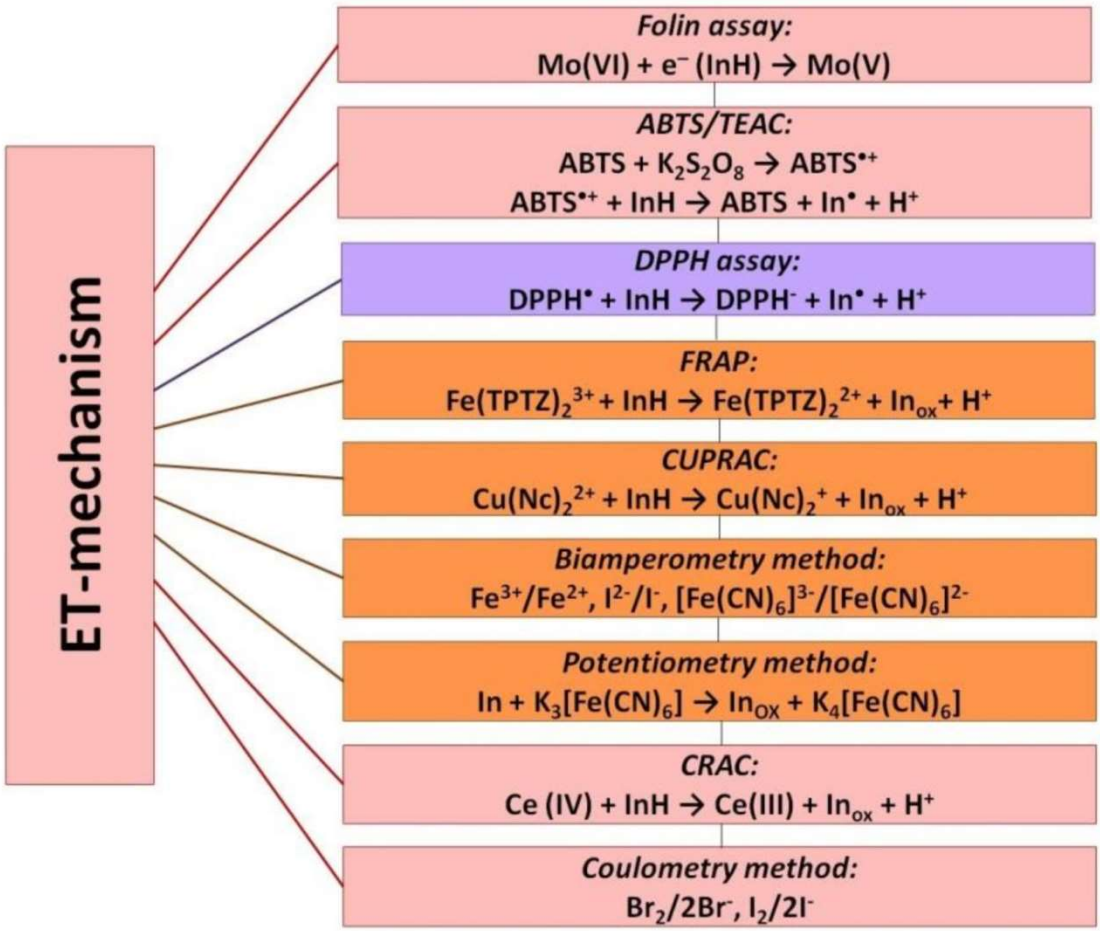


Figure 5: Electron-transfer-based assays.

I.2.4. Antioxidant of some EOs

Many studies exploring the bioactivity of EOs have mainly attributed their antioxidant capacity to terpenoids with phenolic groups such as carvacrol, methyl chavicol, thymol, andeugenol, as they can donate hydrogen atoms to free radicals and transform them into more stable products For example, about 80% of oregano EOs are constituted by carvacrol and thymol, mainly responsible for its antioxidant activity.Similarly, EOs from other aromatic plant species, like lemon balm, basil, thyme, and sage,have also been established as rich sources of antioxidants (Nehme et al., 2021).

The study conducted byViuda-Martos and co-workers demonstrated the ability of EOs from oregano (*Origanumvulgare*), rosemary (*Rosmarinusofficinalis*), and sage (*Salvia officinalis*) to chelate Fe^{2+}, withrosemary EO displaying the highest effect (76.06%). Moreover, oregano EO showed themost increased antioxidant activity in the Rancimat.Tunisian Thymus capitatusEO's antioxidant activity, mainly composed of carvacrol, p-cymene, and -terpinene, wascompared with butylatedhydroxyanisole (BHA) and Butylatedhydroxytoluene (BHT)by DPPH and thiobarbituric acid-reactive species (TBARS) methods, evidencing betterantioxidant properties (Nehme et al., 2021).

In line with these findings, in vitro experiments dealing with the DPPH scavengingactivity of *Nigella sativa* EO revealed that its potent antioxidant activity (The half maximalinhibitory concentration (IC50) = 19 g/mL) could be attributed to the presence of oxy-Antioxidants . genatedmonoterpenes such as thymol and thymoquinone(Nehme et al.,2021).

II. Anti-mastitis bacterial activity

Bovine mastitis is an inflammatory response of the udder tissue in the mammary gland caused due to physical trauma or microorganism infections. It is considered the most common disease leading to economic loss in dairy industries due to reduced yield and poor quality of milk (Cheng et Han, 2020).Clinical mastitis infections are those with symptoms like udder swelling or redness that are visible to the naked eye. On the other hand, subclinical mastitis infections don't cause any visible changes in milk or udder appearance, making it difficult to detect. This can be demonstrated a posteriori by counting individual somatic cells or those in the udder quarter (Remy et al., 2010).

II.1.Udder

Is very large organ, present about 50kg (includes blood and milk).Given the fact that it can sometimes reach a weight of 100kg; it is capital that the udder is very well attached to the skeleton to the muscles. The median ligaments are composed of elastic fibrous tissue, while the lateral ligaments are formed of connective tissue less elastic. If the ligaments weaken.

If the ligaments weaken, the udder will no longer be suitable for mechanical milking since the teats will spread outwards. The quarters are covered with more or less long hair (Remy et al., 2010).

II.2. Anatomy of the udder

The udder of the cow is a very large organ, weighing about 50 kg (including blood and milk). Since it can sometimes reach a weight of 100 kg, it is very important that the udder is very well attached to the skeleton and muscles. The median ligaments are made of elastic fibrous tissue, while the lateral ligaments are made of less elastic connective tissue. If the ligaments weaken, the udder will no longer be suitable for mechanical milking because the teats will spread outwards. The quarters are covered with hair of varying length (Remy et al., 2010).

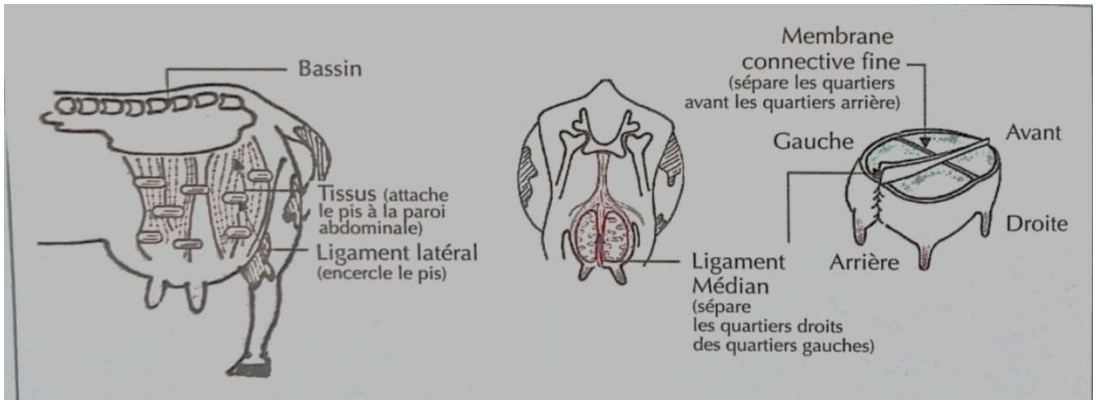


Figure 6: different tissues that support the udder (Remy et al., 2010).

The udder of the dairy cow is made up of four separate quarters, each with a teat. They contain glandular cells or mammary acini, which synthesize milk. These alveoli are surrounded by parenchymatous tissue and are connected to the cistern of the gland, which has an average volume of 400 ml, via the tubules and the galactophore ducts. The milk secreted in one of the glands cannot pass through another gland. The quarters are physically separated by different structures, including the median ligaments, and when a germ enters the teat canal, it infects only one quarter. This gland cistern is separated from the teat cistern by an annular fold (Remy et al., 2010).

When a cow produces 60 liters of milk per day, it means a lymphatic system that transports the waste outside the gland. Sometimes, at the time of a first calving, the heifers can suffer from oedema due, in part, to the presence of milk in the udder which compresses the various vessels and blocks the lymph in the organ (Remy et al., 2010).

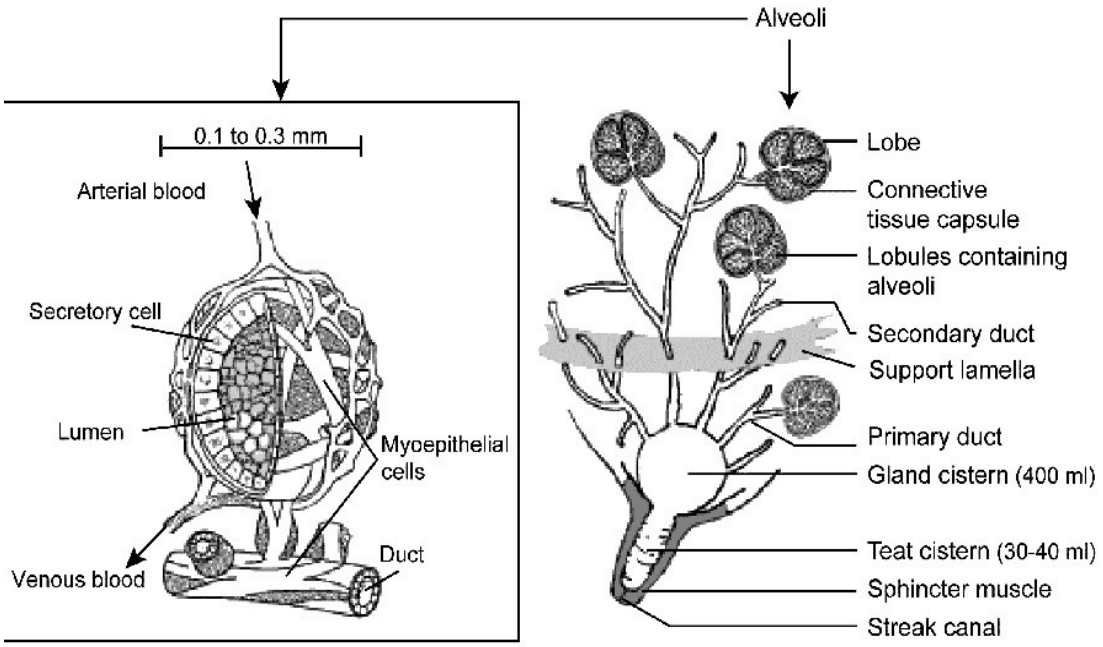


Figure 7: Different internal structures of the udder (Remy et al., 2010).

II.3. Mastitis

Mastitis is an inflammation of the udder whose most common origin is the penetration of a bacterium in a quarter through the teat canal (Remy et al., 2010).

Is the most common and costly disease of dairy cows in theentire world. Although stress and physical injury can be other causes of udder inflammation, infections with bacteria or other microorganisms are the main cause. The most common etiological agents are Staphylococcus *aureus, variousstreptococci (Streptococcus dysgalactiae, Streptococcusuberis)* and coliforms *(Escherichia coli*), which may be contracted fromotherinfectedcowsordirectlyfrom the environment. In response to bacterial infiltration in dairy cows, there are two major forms of mammary gland inflammation: clinical mastitis and subclinical mastitis (Taga et al., 2012).

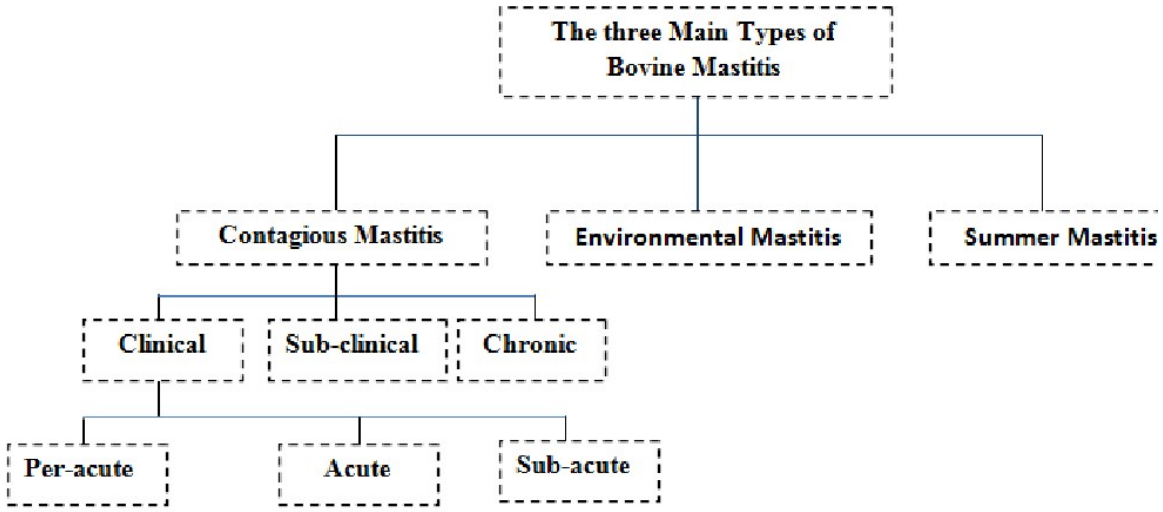


Figure 8: the three main types of bovine mastitis

II.4. Origin of mastitis

There are several risk factors known to be associated with the incidence of bovine mastitis that play significant role, including pathogen, host, and environmental factors.
Mastitis may occur with or without infection butare predominantly caused by bacteria that colonize the skin. Exceptionally, it can be due to fungus or parasites, mastitis of chemical or traumatic origin is rare and is most often complicated by a mammary infection.

The last cause of mastitis is trauma: a violent shock can lead to an intra-mammary hematoma but, more often; it is trauma or aggression of the skin of the quarter or the teat that causes mastitis.Finally, the teat canal can become stenotic, either as a result of damage caused by a malfunctioning milking machine or by another obstacle such as a papilloma. In the end, the result of these attacks is a secondary infection

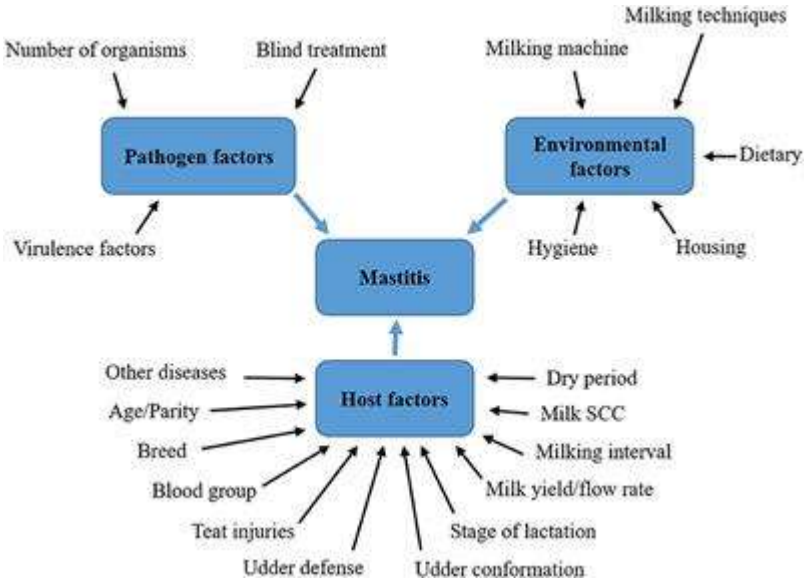


Figure 9: Possible sources or transmission causes of mastitis

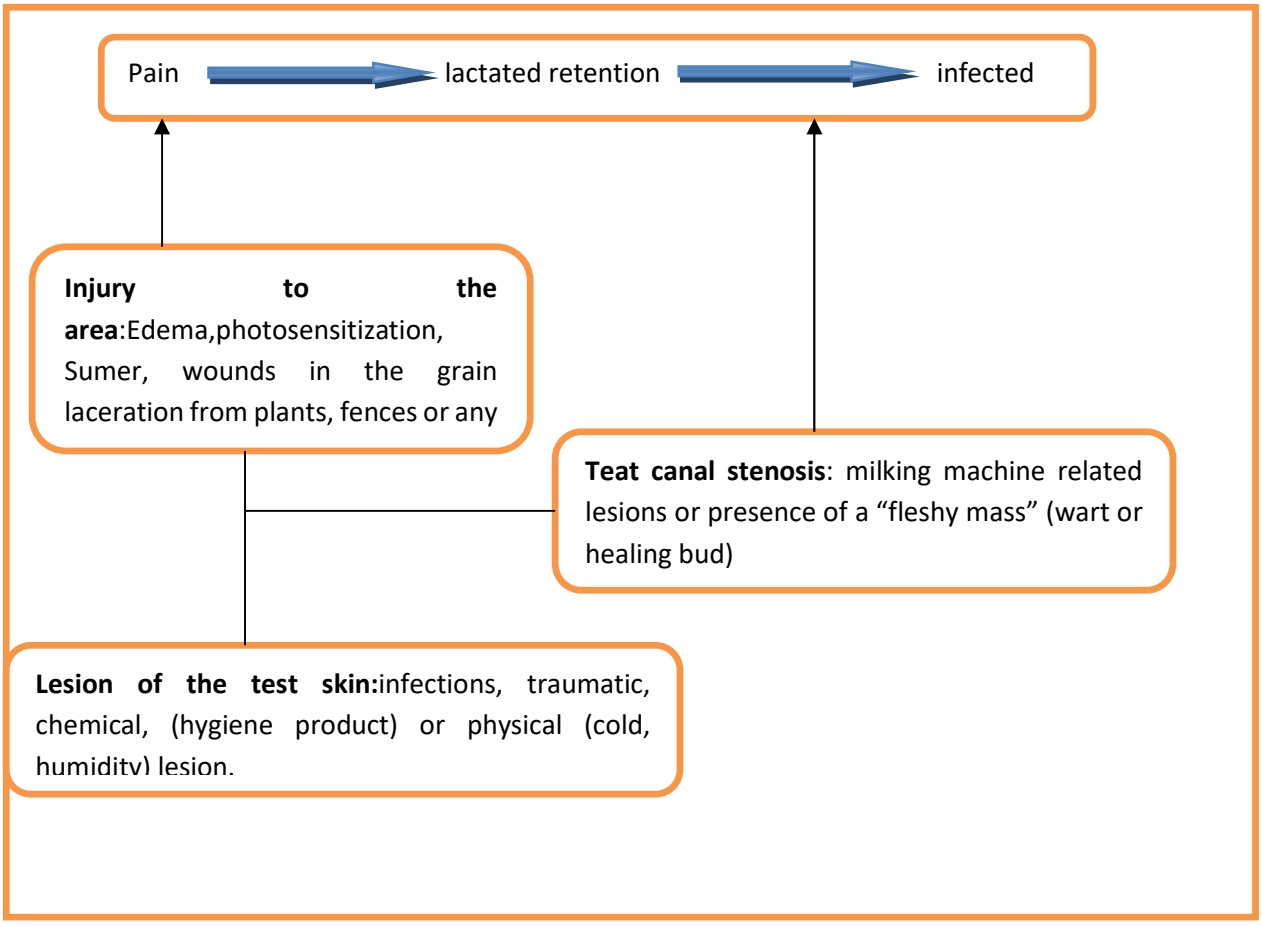


Figure 10: different causes of mastitis.

II.5. Types of mastitis

Table1: Characteristics of different types of mastitis (Hansen, 2009).

Types of mastitis	Typical symptoms
Acute clinic	Inflammation of the udder, fever of more than 39°C, weak and depressed subject, lack of appetite, milk yield decreases drastically, often follows calving and in a less serious way, the drying up
Superacuteclinic	Hot red painful swollen quarter, the milk passes with difficulty, fever of more than 41°C, the cow has no appetite, shivers and loses weight quickly, the lactation is often interrupted.
Sub-acute clinic	No apparent change in the pind, presence of clots in the milk especially in the first spurts, healthy subject
Subclinical	No symptoms 15-40 cases per clinical case milk is normal in appearance, the only change is the detection of the pathogen on analysis and the increase of the somatic compound mainly caused by *staphylococcus aureus*
Chronic	Repeated but weak chemical attacks, usually without fever, lumpy milk, swollen quarters, sometimes the quarters can become hard (fibrous indurations) antibiotic treatment often does not work
Gangrenous	The affected quarter is blue and cold to the touch, the discoloration progresses from the bottom to the top, the necrotic parts fall off the body, the cow dies
Contagious	Mastitis caused by bacteria such as *Staphylosoccusaureus* and *agalactiae* of which infested cows are the main source
Environmental	Mastitis by bacteria such as coliform (*E.coli*) whose main source is a contaminated environment most often by fumes

II.6. Pathogens involved in mastitis

Mastitis is caused by several classes of pathogens (Theran et al.,2010).

II.6.1. Usual pathogens

Responsible for mastitis according to the intensity of the cellular response they can induce in the udder and their potential impact in clinical mastitis.

We distinguish between major and minor pathogens.

II.6.1.1. Major pathogens:

sterptococcusuberis, staphylococcus aureus, E,Coli, staphylococcus dysgalactiae, streptococcus agalactiae, enterococci, pseudomonas sp, arconobacteriumpyogens.

II.6.1.2. Minor pathogens:

coagulopathic staphylococci (SCN) such as *staphylococcus chromogens*.

II.6.2. Unusual pathogens

- Rare bacterial agent (*mycoplosmabovis*).

- Agents normally responsible for mastitis (*nocardiasp, salmonella ssp, A.pyogens, mycobacterium sp, baccillus sp.*

- Non-bacterial agents (*protothciaspp, candida ssp*)(Theron et al., 2010).

II.7. Antibacterial action of essential oils:

Several studies have tried to explain the mechanism of action of EO towards bacteria.

The complexity of this mechanism is related to the chemical composition of EO, which presents a great diversity of molecules (each molecule acts on a specific target) (Nehme, 2021).

The mode of action of essential oils depends mainly on the type and characteristics of the active components, including their hydrophobic property that allows them to penetrate the phospholipid double layer of the bacterial cell membrane (Jalila et al .,2014).

This causes destabilization of the structure and increase in membrane permeability (Sikkemaet *al*., 1994).

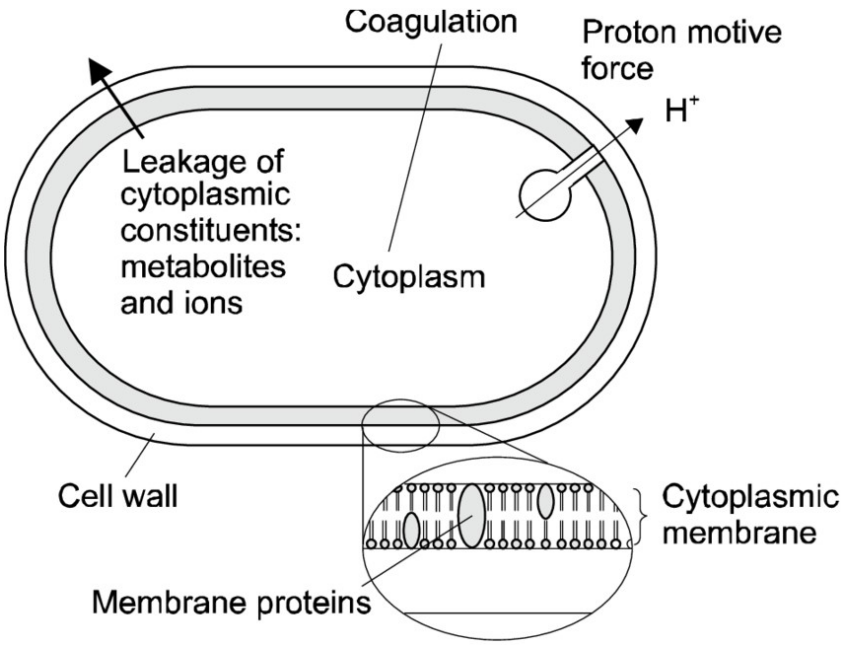


Figure 11: essential oils and their constituents on bacterial cell (Burt, 2004)

The antimicrobial activity is mainly a function of their chemical composition, and in particular the nature of their major volatile compounds. They act by preventing the multiplication of bacteria, their sporulation and the synthesis of their toxins. For yeasts, they act on the biomass and the production of pseudomycelium while they inhibit spore germination, mycelium elongation, sporulation and toxin production in molds. (Caillet et al., 2007)

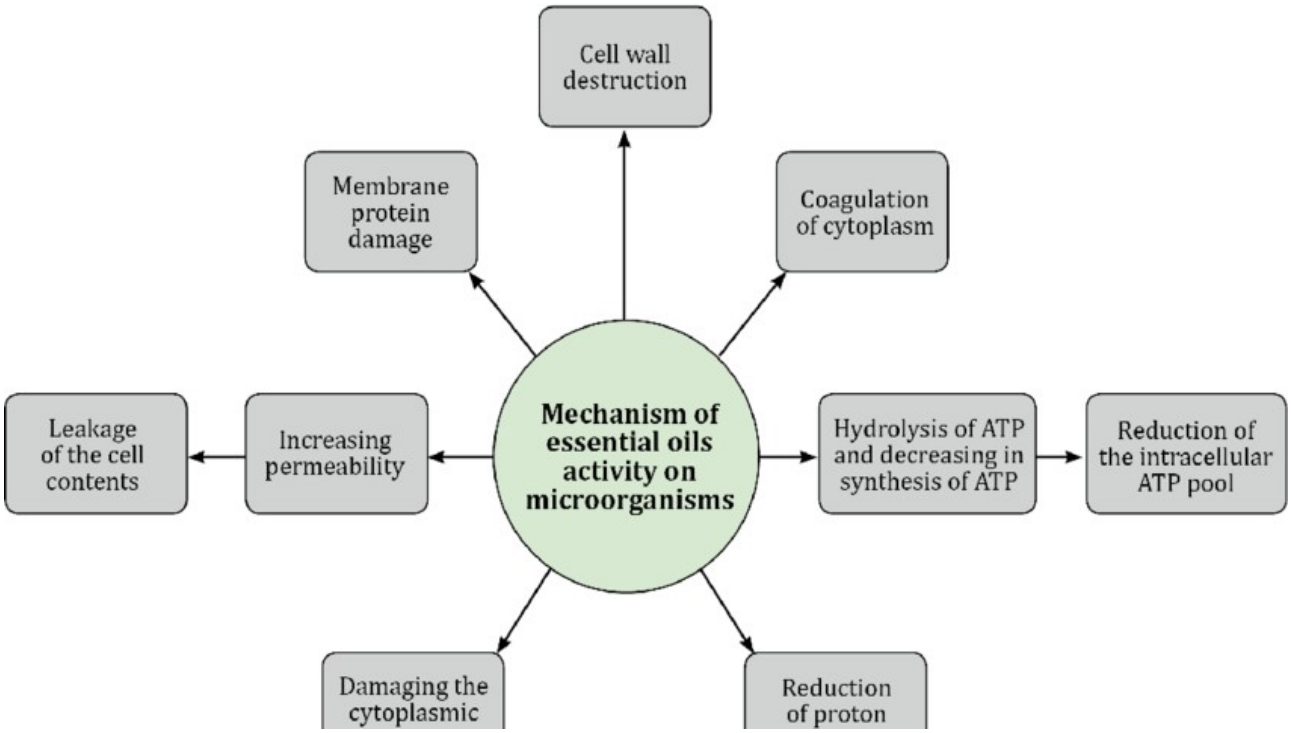


Figure 12: Mechanisms of antimicrobial activity of essential oils (Khorshidian et al., 2018).

Essential oils have a very broad spectrum of action as they inhibit the growth of bacteria as well as molds and yeasts. Generally, characterized by a high content of phenolic compounds, such as carvacrol, eugenol and thymol, exert significant antibacterial activity (Dhifi et al., 2016).

Material and methods

I. Material and methods

I.1. Biological material

In the current work, ten essential oils were used; seven of them hail from aromatic plants in collected from different regions of Algeria *Lavandula stoechas* (lavender), *Eucalyptus radiate* (eucalyptus),*Mentha piperita* (peppermint), *Rosmarinus officinalis* (Rosemary), *Thymus vulgaris* (thyme), *Pistacia lentiscus L* (lentisk), *origanum vulgare* (oregano) , *Syzygium aromaticum* (clove), Thymol, Eugenol, one was derived from *C. lemonand*(lemon) controls were from university .All samples were purchased from a local producer and transferred as ready-to-use samples in the laboratory and analyzed within three months of recovery. Essential oils were isolated using only typical distillation procedures.

I.2. Physical and chemical properties

The physicochemical properties of tested essential oils such as density at 25°C and Refractive index at 20°C were carried out, in order to determine the quality of essential oils.

I.2.1. Relative density determination

International Standard specifies the reference method for the determination of the relative density of essential oils at 20 °C

I.2.2 Refractive Index determination

The refractive index of the oil samples was determined with the help of Abbe refractometer.Sample should be free from moisture and any other residual matters and record the ambient temperature. Then, observe through the eyepiece and turn the dispersion correction compensator knob until the colored indistinct boundary seen between the light and darkfield becomes a sharp line. After cleanningrefractometer with soft cotton and calibration with pure water, two drops of essential oil were placed on the lower part of the prism and the refractometer was firmly closed by tightening the screw head. The apparatus was allowed to stand for sometime, Read the refractive index from the magnifier in the pointer and record the reading.(Ibrar Barkatullah Muhammad et al.,2012).

I.3. Determination of antioxidant activity

I.3.1. DPPH free radical-scavenging assay

The free radical scavenging potential against DPPH (1, 1-diphenyl-2-picryl-hydrazyl) was determined spectrophotometrically using the method described by Sánchez-Moreno et al. (1998) and slightly modified by González-Peña et al. (2013).

The 1.1-diphenyl-2-picrylhydrazyl (proton scavenger) is a free radical that comes from the different free radical scavenger antioxidants (ArOH) that are in the reaction medium according to the reaction mechanics below:

$$DPPH^{+} + ArOH \longrightarrow DPPH\bullet + ArO^{-} + H^{+}.$$

The reduction reaction of DPPH- causes the decrease of the color intensity which is measured with the spectrophotometer at 517

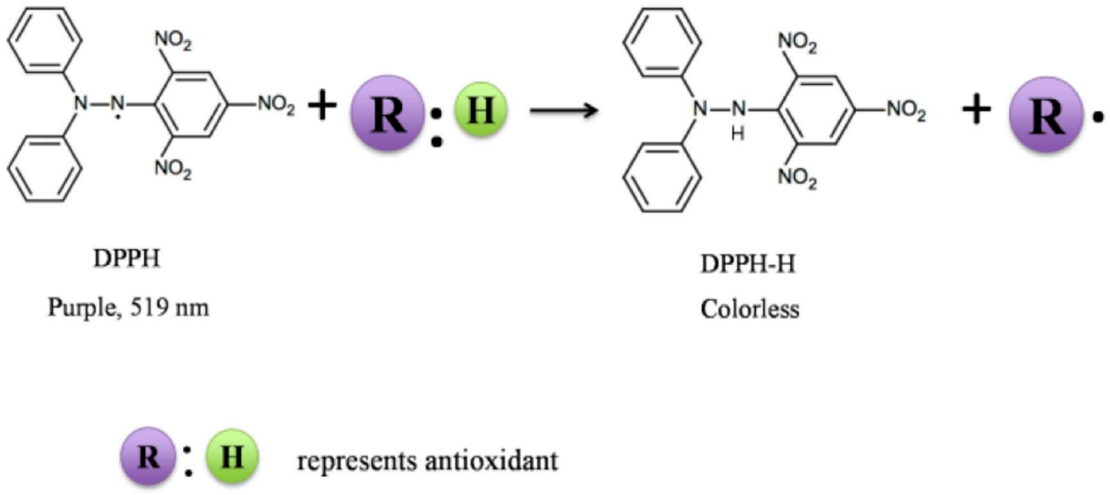


Figure 13: Reduction reaction of DPPH- assay with antioxidant. R: H = antioxidant radical (Liang et al. 2014)

Preparation of essential oil solutions

For antioxidant tests, the samples were prepared by dissolving in ethanol for all essential oils prepared solutions in ethanol at a rate of 160mg/ml. These solutions called stock solution will then undergo dilutions to have different concentrations described as mg /ml.

From the stock solutions of essential oils of 160mg/ml, the dilution were prepared by dissolving Eos in ethanol, then to each concentration a volume of DPPH solution was added.

An aliquot of 100 µL of each EO on radical is mixed with 2900 µL of 100 µM DPPH• in ethanol. The mixtures are vortexed and incubated in the dark at room temperature for 30min. The absorbance is measured using a spectrophotometer at 517nm.

Where blank is the absorbance of DPPH with sample replaced by ethanol and sample" refers to the absorbance of DPPH mixed with essential oil or control. Eugenol and thymol were used as positive controls with the same concentrations.

Results are also expressed as the concentration of EO that inhibits DPPH radical formation by 50% (IC50). The IC50 for each sample was calculated graphically by elaborating a curve where is represented the concentration (mg/ml) and the percentage of inhibition using the following formula:

I % = (At-Ae)/At ×100.

At: absorbance of control.

Ae: absorbance of the sample

I.3.2. 2, 2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic Acid Radical Cation ($ABTS^{\bullet+}$) scavenging capacity essay:

A blue/green radical cation ABTS is generated by the oxidation of ABTS with potassium persulphate. The anti-$ABTS^{\bullet+}$ activity was estimated by the method described by Re et al (1999). The ABTS cation radical ($ABTS^{\bullet+}$) is produced by reacting ABTS with 2.45 mM potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use.

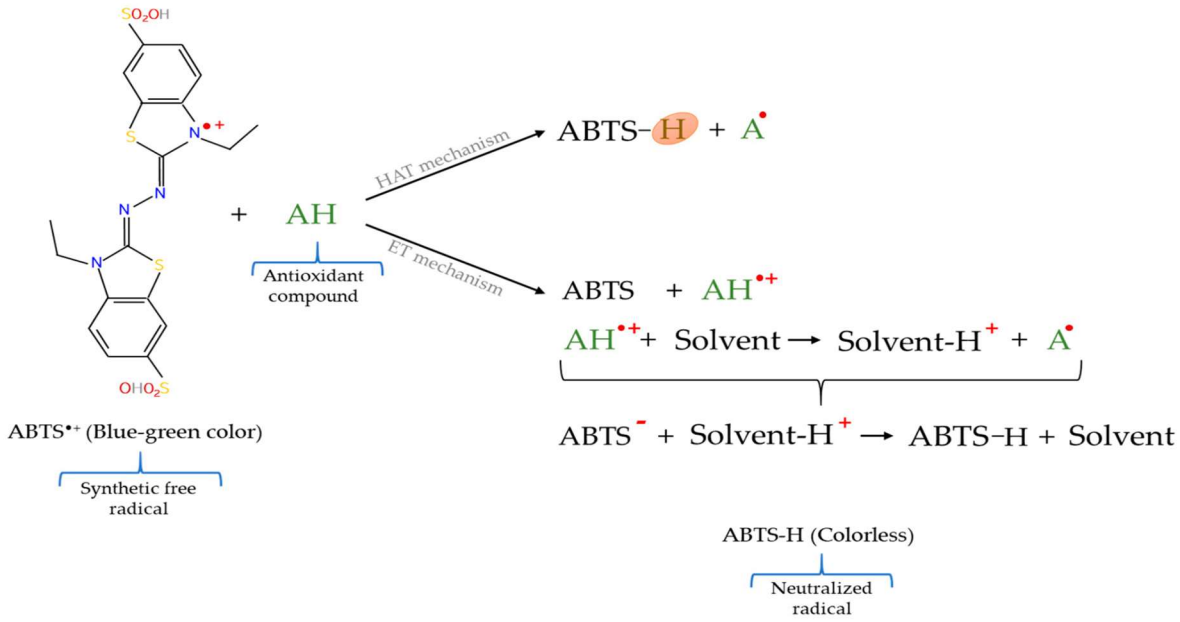


Figure 14: Reactions involved in ABTS+ assay.

The $ABTS^{\bullet+}$ solution (stable for two days) is diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Next, 5µL of each EO is incorporated into a glasse tube and 1495 µL of 7 mM $ABTS^{\bullet+}$ is added, thoroughly mixed. After 20 min in the dark at 30 °C, the absorbance is measured at 734 nm. All samples were run in triplicate.

The percent inhibition was also calculated by using the following formula:

I % = (At-Ae)/At ×100.

At: absorbance of control.Ae: absorbance of the sample.

I.3.3. FRAP (ferric reducing antioxidant power) assay

Ferric reducing ability of plasma (FRAP) assay is based on the principle of reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) with ferric chloride hexahydrate complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by the antioxidants of a sample at low Ph.

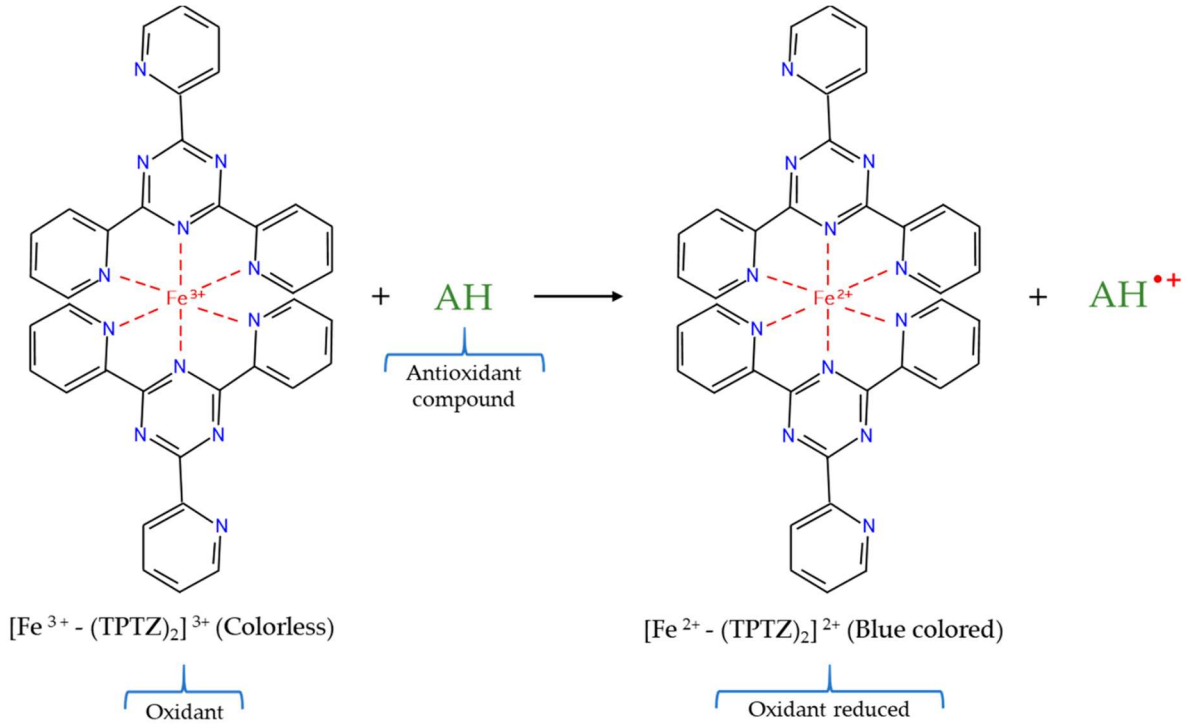


Figure 15: Reduction reaction of FRAP assay.

I.3.4 Procedure

FRAP solution is prepared by mixing 10ml of 0.3M acetate buffer (pH 3.6), with 1ml of TPTZ (2, 4, 6-tris-2, 4, 6-tripyridyl-2-triazine)) in 40 mMHCl and 20 mM FeCl3 with 10:1:1 (v/v/v) respectively. The assay is carried out in tubes, adding 50µl of each extract and 1450µl of FRAP reagent after 4 min of shaking in the dark at 37°C, the absorbance is measured at 593nm.

I.4. Antibiofilm activity

Among the 9 strains tested, there were 4 presumptive *E. coli* (S3, S6, S7, S8), 1 *Staphylococcus aureus* (S9), isolated from cows with clinical mastitis, 1 SARM (S4) and *E. coli (S5)* isolated from hospital, *Klebsiella* (S2), and *Pseudomonas* (S1).

The capabilities of biofilm formation by the bacterial isolates were observed by the way of adherence to the walls of culture glass tubes and in red Congo agar medium.

Concentrations under the minimum inhibitory concentration were used to determine the antibiofilm activity of all essential oils against the different stains.

Tableau 2 : The different strains test

Strain	Reference
E. coli 5	ATCC 25922
E. coli 1	Laboratory strain
E. coli 2	Laboratory strain
E. coli 3	Laboratory strain
E. coli 4	Laboratory strain
K. pneumonia	*E47
P. aeruginosa	ATCC 6633
SARM	ATCC 43300
S. aureus	Laboratorystrain

ATCC: American Type Culture Collection.

MRSA: methicillin-resistant Staphylococcus aureus.

*Quinolone resistant.

I.4.1. Strains and growth conditions

Test strains were stored at -80°C in 15% (v/v) glycerol. All the strains were grown at 37°C for 24 h in the appropriate media before use, then cultured and resuspended in sterile saline solution to the appropriate OD at 600 nm for testing.

I.4.2. Evaluation of the Biofilm Formation with Rouge Congo redox indicator

Congo red agar (CRA) method that is a qualitative assay for detection of biofilm producer microorganism, as a result of color change of colonies inoculated on CRA medium, is described by Freeman et al. The CRA medium is constructed by mixing 0.8 g of Congo red and 36 g of sucrose to 37 g/L of Brain heart infusion (BHI) agar. Congo red was prepared as a concentrated aqueous solution and autoclaved at 121°C for 15 minutes separately from other medium constituents and was added when the agar was cooled to 55°C. After incubation period that was 24 h at 37°C, morphology of colonies that undergone to different colors is differentiated as biofilm producers or not. Isolates were considered as strongly positive when there was the presence of black colonies with a dry crystalline consistency. A darkening of the colonies with the absence of a dry crystalline colonial morphology indicated a moderately positive biofilm producer, whereas colonies retained pink are non-biofilm producers. (Freeman et al., 1989).

I.4.3. Evaluation of the Biofilm Formation with Crystal Violet Assay

Concentrations below the minimum inhibitory concentrations were used to determine the antibiofilm activity of all essential oils against *S. aureus, E. coli 5, E. coli1, E. coli 2, E. coli 3, E.coli 4,SARM*,*Klebsiella* and *Pseudomonas* as described in the work of. So, all these strains were tested for their ability to form biofilms on the glass test tubes containing tryptic soy broth supplemented with 1% glucose (TSBG) under shaking conditions.

Inoculum was prepared using 2ml of TSBG. After 24 h of incubation at 28°C, turbidity was adjusted to 0.5 McFarland standards. For biofilm experiment, 200 µl of inoculum was transferred into 2 ml of nutrient broth in 5 ml test tubes the cultures were incubated with 50ug/ml of EO at 37°C for 48 h. All the test tubes were kept in shaker at 95 rpm speed for 24-48 h. After incubation, culture broth containing the cells was discarded. The tubes were washed with sterile pure water. About 3 ml of 0.5% crystal violet (CV) solution was added and allowed for 30 min. All the tubes were washed with sterile pure water and allowed to dry, and the tubes were visually observed for the presence of biofilms on the inner walls of the test tubes. All the tubes were added with 2ml of pure ethanol and mixed gently. Optical density value was measured at 570 nm. The OD values of the test samples were compared with sterile phsiological saline on (PS, 0.9% NaCl in distilled water, pH 7.0) solution a control sample was also prepared in addition to the positive controls (eugenol and thymol).

To interpret results, categorization can be done as no biofilm production (0), weak (+ or 1), moderate (++ or 2), and strong biofilm production (+++ or 3) by the calculation of cutoff value (ODc) shown below (Stepanović et al. 2007).

OD ≤ ODc no biofilm production.

ODc< OD ≤ 2 × ODc weak biofilm production.

2 × ODc< OD ≤ 4 × ODc moderate biofilm production.

4 × ODc< OD strong biofilm production.

I.4.4. Evaluation of the eradication of preformed biofilm

To establish biofilms, 100 µL of standardised(OD = 0.5) strain cell suspensions prepared as described above in sterile saline solution, were placed in 5mL glass tubes, containing 2ml of TSB and incubated at 37°C. After 48 h of biofilm formation, a concentration of EO above MIC (160mg/ml) was added to the media culture. Positive biofilm controls (cells + TSB) were included. Tubes were then placed at 37°C for 24h; then the medium was gently aspirated and the tubes were rinsed twice with PS and fixed by drying for 30min. CV staining was performed as described previously. Repeated spectrophotometric readings were taken for each strain (Das *et al.* 2016; Kırmusaoğlu, 2019). Results are expressed as reported by Stepanović et al., (2007).

II. Statistical Analysis

Each experiment was performed in triplicate to gain statistical confidence. Data values of experimental results were recorded as the mean ± standard deviation.

Results and discussion

I. Results and discussion

The quality of essential oils can vary widely. It depends on many factors, such as plant part, harvest-time, and extraction-method, type of cultivar, geographic origin and storage conditions. The purity and quality of essential oils affects their therapeutic value, aroma, color and flavor in the food and beverage industries (Turek and Stintzing, 2013;Yadav, 2022).

I.1.physical and chemical properties:

The physicochemical properties are significant to assess the quality of the studied oils in order to enhance its value. The obtained results were compared with those found in literature from other places to highlight the quality of the purchased EOs.

Tableau 3 : Refractive index and density of essential oils

Essential oils	Refractive index (20°C).	Density (25°C).g/cm3
Clove	1.525	1.0019
Thyme	1.510	0.9185
Lentisk	1.475	0.8466
Eucalyptus	1.470	0.8997
Tea tree	1.4765	0.9240
Oregano	1.507	0.9013
Lavander	1.4685	0.9124
Peppermint	1.462	0.834
Rosemary	1.4635	0.8845
Lemon	1.471	0.858
Thymol	1.536	0.960
Eugenol	1.380	1.06

Data presented in Table 3 showed the physico-chemical properties of all essential oils. It is obvious from the results; it is evident from the results that only eugenol and clove oil have a density greater than 1 in the order of 1.06 and 1.0019 respectively. Clove oil density is reported to be higher than the density of water.

The density values of the essential oils are almost identical, which indicates that the physical properties of these oils are quite similar (mantel et al., 1995).

At 20°C, the Refractive Index values of EOs are very close to each other including clove, thyme, oregano and thymol followed by Lentisk, eucalyptus, tea tree and lemon oil than lavander, rosemary, and peppermint, eugenol recorded the lowest refractive index.

A refractive index varying essentially with the content of content of monoterpenes and oxygenated derivatives. A high content of monoterpene content will give a high index(Kanko et al., 2004). The low refractive index of the HE indicates its low refraction of light which could favor its use in cosmetic products. (Mantel et al., 1995).

The refractive index is the degree of the deflection of a beam of light that occurs when it passes from one transparent medium to the other. It increases with the length of chains and with the number of carbon atoms present. Therefore, the refractive index determines evidences that the sample might be unsaturated long carbon chain(Pearson, 1976).

I.2.Antioxydant activity

I.2.1.free radical –scavenging activity

DPPH scavenging activity assay is widely used to evaluate the ability of compounds to scavenge-free radicals or donate hydrogen/electron, and determine the antioxidant activity in foods (Bidchol et al., 2011). So, in present work, DPPH is used as a reagent to evaluate the free radical scavenging activity of all EOs tested which can reduce the stable 2, 20-diphenyl-1-picrylhydrazyl radical of purple color to diphenyl-picrylhydrozine of yellow flow (cotelle et al., 1996).

Figure 16:photography of antioxidant activity test by the reduction of the DPPH radical.

The DPPH radical scavenging activity was quantified in terms of the inhibition percentage of the free radical by EOs and the IC50. It was evaluated by spectrometry by following the reduction of free radical measurable at 517 nm and then calculating for each concentration the corresponding percentage of inhibition.

According to the recorded results, the essential oils are endowed with a difference to yield the proton to neutralize the radical DPPH.

We can classify them as follows:

Eugenol> clove >thym>origan>thymol> peppermint >rosemary> lavender >tea tree>lemon>lentisk>eucalyptus.

The results of inhibition percentages are represented graphically (figure17):

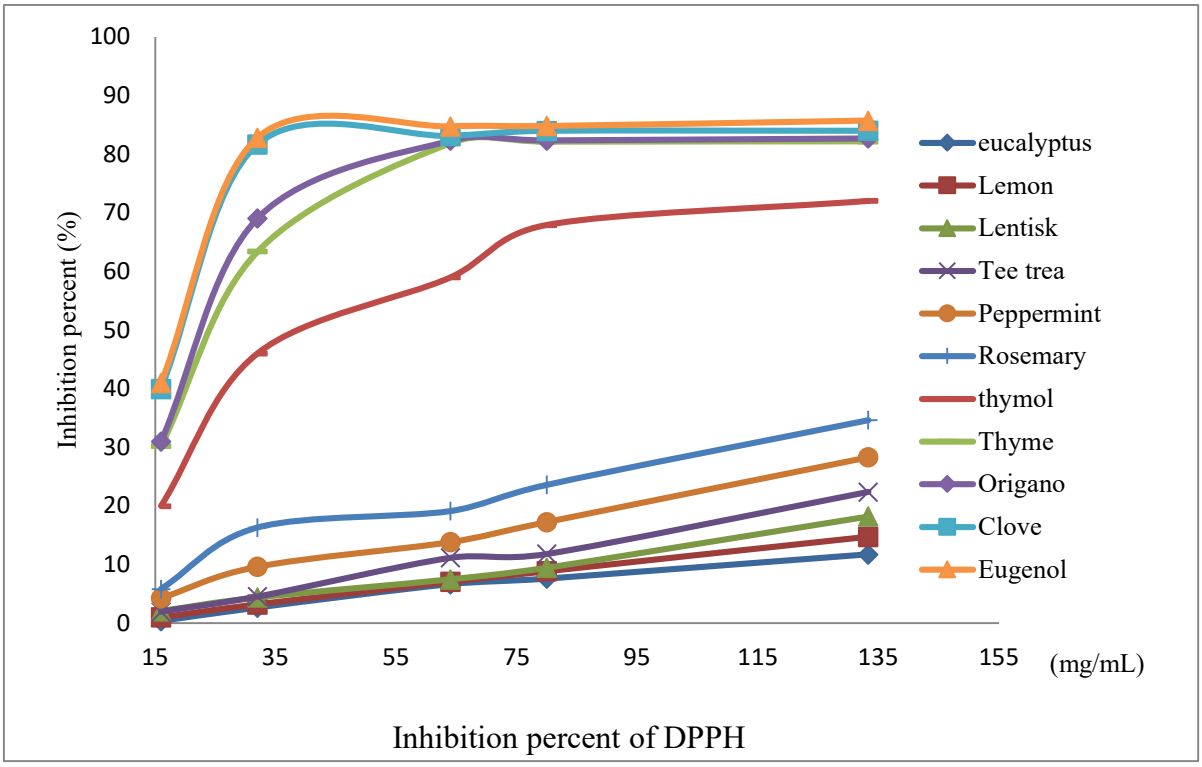


Figure 17: Results of percent of inhibition.

It can be seen that the percentage of inhibition increases with the increase of the concentration and lower absorbance values of reaction mixture indicate higher free-radical-scavenging activity. Different EOs showed different antiradical activity due to the types and contents of their compounds and other phytochemical constituents. Also, hydroxyl group position, the presence of other functional groups such as double bonds and the composition of the hydroxyl groups and ketones, alcohols, aldehydes, esters, phenols plays an important role in antioxidant activity (Memon et al., 2007).

Clove shows a DPPH radical activity which has a significant anti free radical capacity with a percentage of inhibition (85%), oregano (82%) compared to the control eugenol which has a percentage of inhibition of (85%), as well as thyme 80%) and thymol (70%) which is the control

of thyme. Amari, et al., (2011) showed from their studies on thyme; that phenolic compounds due to their redox property act as reducing agents, hydrogen and singular oxygen donors.

Thymol which is 6-isopropyl-2-methyphenol and 2-methoxy-4-(prop-2-enyl)-phenol and eugenol are phenolic compounds which have, according to several authors, a remarkable antioxidant potential.Thymol, which is predominant in thyme oil and clove oil respectively, are also responsible for the antioxidant activity of several other essential oils, such as Menthalongifolia and Thymus serpyllus (Dhifi et al., 2016). Eugenol is the most abundant ingredient in clove oil and is thought to be responsible for its aromatic as well as both beneficial and harmful effects (Hu, and al., 2018). These results suggest that eugenol and steam distilled clove extract, which have shown strong free radical scavenging activity, may be useful as potential antioxidants. The same authors reported that clove oil and its main effective composition eugenol show beneficial advantages on antibacterial and antifungal activity, aromaticity, and safety. Therefore, the essential oils studied contain a significant proportion of these compounds in their chemical composition.

By contrast the other oils present a low percentage of inhibition (peppermint; rosemary; eucalyptus; etc).

Mata et al., (2007) showed that the lack of antioxidant activity of terpenic hydrocarbon would be due to their low capacity to donate a hydrogen atom and also the low solubility of essential oils in solvents used such as methanol or ethanol. Athamena et al (2010) found that the values expressing antioxidant activity differed depending on the test used. The different antioxidant activities among these essential oils may be due to the variability in the composition and concentration of sulfur compounds. They can also be attributed to the presence and synergy of different minor compounds (DimaMnayer, 2014).

According to Bozin et al., (2008) in their study on essential oils, they found that thecompounds responsible for the reduction of the DPPH radical are oxygenated mono-terpenesand mixtures of hydrocarbon mono-sesquiterpenes. Essential oils rich in oxygenated compounds have more marked anti-radical activity than those with hydrocarbon terpenes (Miladi et al., 2013).

The antioxidant potential of an essential oil depends on its composition. The factors that determine essential oil composition are numerous. In some cases, it is difficult to isolate these factors from each other as they are interrelated and influence each other. These parameters include the seasonal variations, plant organ, and degree of maturity of the plant, geographic origin, and genetics (Dhifi et al., 2016).

EC_{50} values of actif EOs and standards were determined in order to compare them more accurately in terms of ability to inhibit free radicals DPPH. EC_{50} values denote the concentration of sample which is required to scavenge 50% of initial DPPH free radicals. So, lower EC_{50} value indicates higher antioxidant activity of samples. The difference in EC_{50} values is due to the difference amounts and or compounds in oils. As indicated in figure18, the eugenol show higher EC_{50} (19 mg/mL), whereas there was no significant difference between EC_{50} of clove oil (20 mg/mL) and origano (22, 5 mg/mL), thyme (24 mg/mL) and thymol (40 mg/mL).

Therefore, rosemary, lavender, tea tree, lemon, mastic and eucalyptus EOs have a lower free radical scavenging activity than the above EOs and require the use of higher concentrations to estimate their IC50.

I.2.2. ABTS radical scavenger activity

This assay involves the direct production of the blue/green ABTS + chromophore.

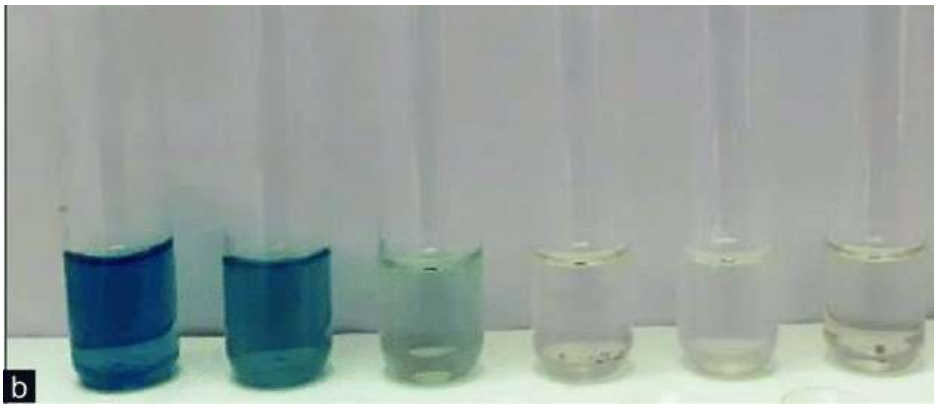


Figure 18:photography of antioxidant activity test by the reduction of the ABTS radical.

The addition of antioxidants to the preformed cation radical reducesABTS, it depending on the antioxidant activity of each EO and their concentration. Figure19:

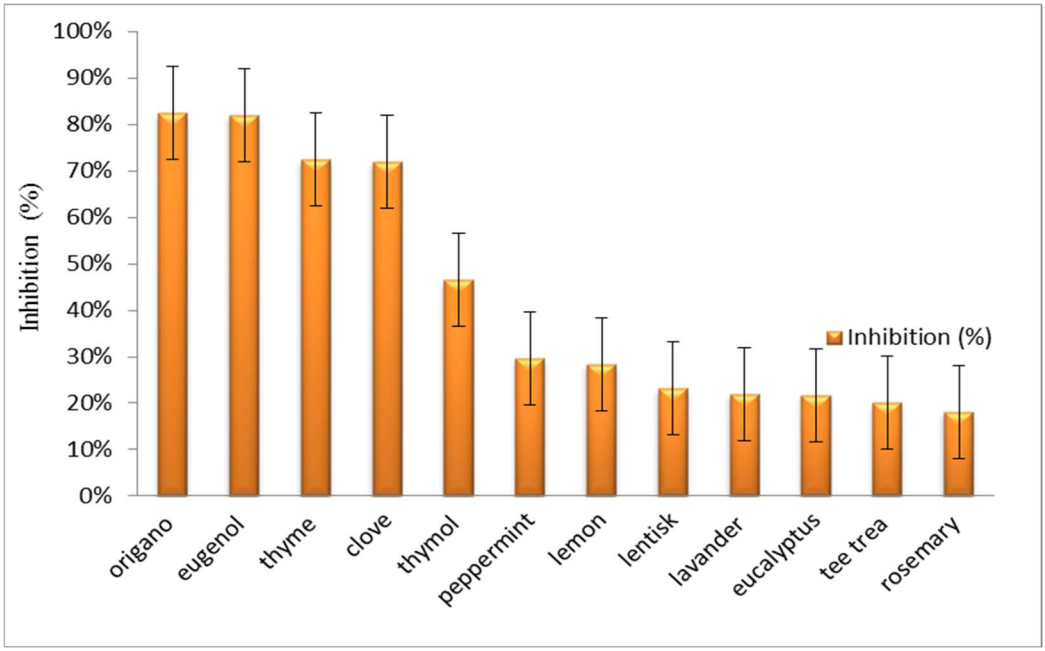


Figure 19: Scavenging activity of the ABTS$^{\cdot}$ +radicalof essential oils.

The figure shows that the oregano and eugenoloils showed a better anti-free radical activity of (80%)in comparison with the tea tree and rosemary oilswhich show a low activity of(10%).

The results were in agreement with previous studies on thyme oil which reported a significant correlation between concentrations of plant EOs and percentage inhibition:Peltoketo et al. (2001), found in the study of the antioxidant activity of essential oils of Lamiaceae family, the antioxidant activity of monotepenes such athymol, can be influenced by the extraction process.

They can beclassified in the following descending order:

Oregano (82,42%) >eugenol (82,01%) > thyme (72,42%) > clove (72,01%) > lavender (67,73%) >thymol(46 ,49%)> peppermint (29,68%) >lemon (28,44%) >lentisk (23,19%) > eucalyptus (21,70%) >tea tree(20,05%) >rosemary (18,15%).

ABTS is oxidized by antioxidants to its $ABTS^{\cdot+}$ radical with an intense color, the antiradical activity is reflected in the discoloration of the solution ,which indicate the ability of compounds to directly decrease the color of ABTS radical (Gulain,2010).

These differences can be influenced by the chemical composition of the Eos (Emani et al., 2007). However, it can also be explained by a weak interaction between the ABTS cation, the nature and the speed of complexassions (Javonovic et al., 1986).

Doman et al.,(2003) have shown that in most of the analysis of EO, when these have a high level of phenolic compounds, it is translated by a strong antioxidant activity, but also it would depend on the nature and the chemical structure, In addition, Montoro et al.,(2005) deduced that the anti-ABTS activity depends on the polarities, the most polar samples presented the best antioxidant capacities, this can be justified by the presence of polar compounds endowed with a great antioxidant potential that act as donors of $O_2$and electron .

I.3. Ferric reducing antioxidant power assay

Fe^{+3} reducing power of different EO samples are shown in figure 20.

FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.
This assay was based on the reduction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ by the sample and the subsequent formation of an intense blue colour at acidic pH (Gupta, 20

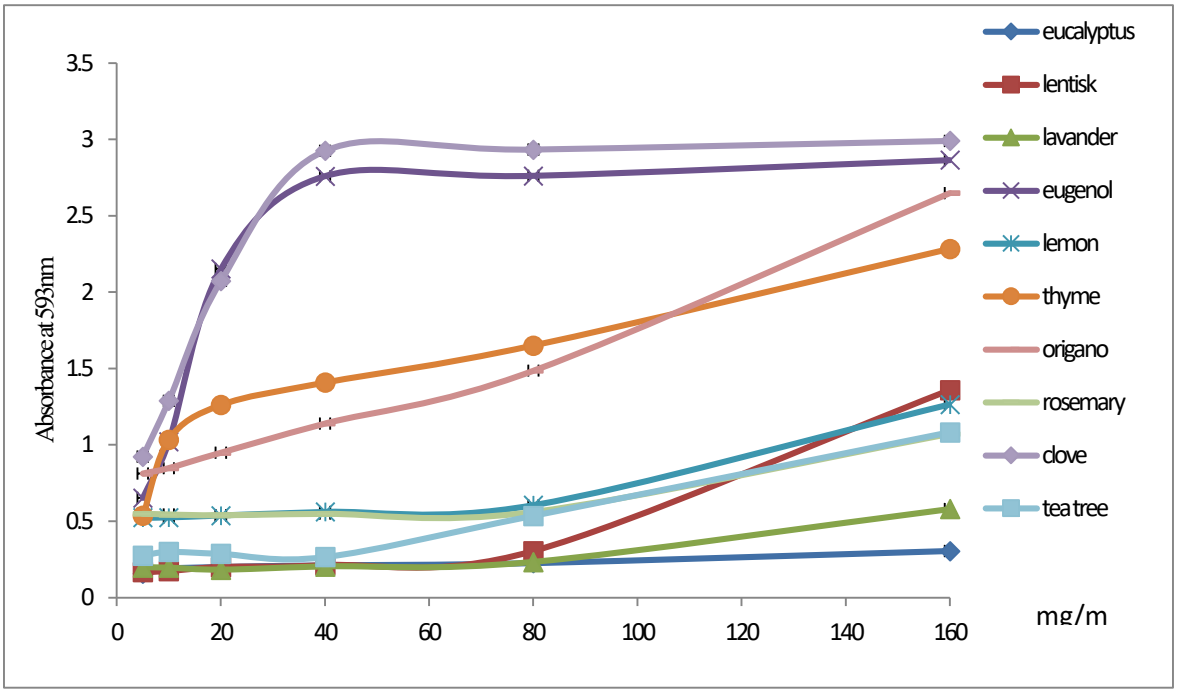


Figure 20:Ferric reducing antioxidant power assay of essential oil

Generally, the results proved that the type and concentration of the Eos had a significant effect on reducing power such as DPPH assay. The change in absorbance is therefore, directly related to thecombined or "total" reducing power of the electron donating antioxidants present in the reaction mixture. Thus, samples with higher reducing power are more able to donate electron and hydrogen atom (Gupta, 2015).

I.4. screening antiobiofilm activity

Biofilm producer microorganisms cause recurrent infections. Biofilm formation begins with attachment of bacteria to biotic surface such as host cell. After attachment, aggregation of bacteria is started by cell-cell adhesion. Aggregation continues with the maturation of biofilm. Bacteria embedded in biofilm (sessile form) are more resistant to antimicrobials than planktonic bacteria. So it is hard to treat biofilm-embedded bacteria than planktonic forms. For this reason, it is important to detect microbial biofilms and to perform the efficacy of antimicrobial agents against biofilm-producing microorganisms (Kırmusaoğlu, 2019).

I.4.1. Evaluation of biofilm formation with Rouge Congo redox indicator

The technique of Congo red used for the detection of biofilm formation has the advantage of being fast and inexpensive, but it presents some problem of reproducibility. In

fact the appearance of the colonies can change according to some factor giving aspects not usual or difficult to interpret (Mathur et al., 2006)

The Congo Red Agar (CRA) method, which is a qualitative test for the detection of biofilm-producing microorganisms based on the color change of colonies inoculated onto CRA medium, is described by Freeman et al., (1989).

Colonies of non-slime-producing strains were pink in color, while slime-producing strains are slightly black to dark black in color (Arciola et al., 2006; Liberto et al., 2009).

After 48 hours of incubation at 37°C, it is observed that 4 strains among the 9 are slime producens*staphylococcus aureus* (+++),*k.aeruginosa* (++),*SARM* (++) and *P.aetaginosa* (+)represent in the same order shown in figure21

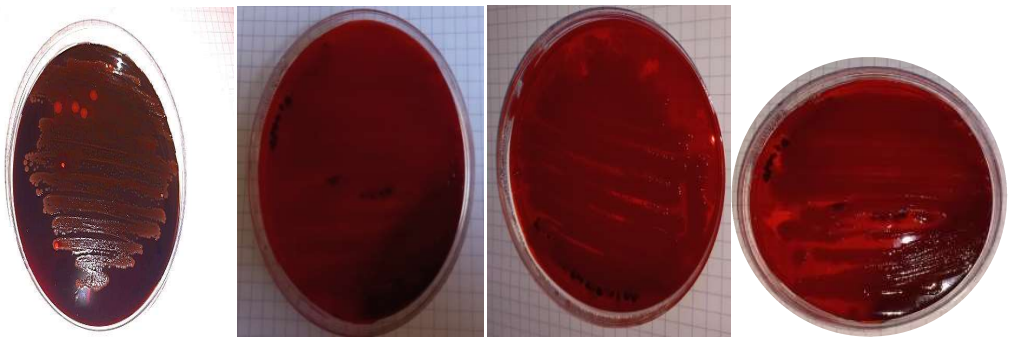


Figure 21:photography of biofilm detection by Congo red agar method, strong and moderately slime production.

I.4.2. Evaluation of biofilm formation with Crystal Violet Assay

Eugenol is a major volatile constituent up to70-80% of clove essential oil obtained through hydrodistillation. *In vitro*, eugenol has been shown to have antibacterial, antifungal, antioxidant and antineoplastic activity (Santin et al., 2011).

The results summarized in the table above 4:

Tableau 4 : control visual biofilm results.

	Control	Oregano	Thyme	Clove	Eugenol	Thymol
1. P.aetaginosa	+	+	+	+	+	+
2.k.aeruginosa	+	+	-	+	+	+
3. E coli1	+	+	+	+	-	+
4. SARM	+	+	+	+	-	+
5. Ecoli 5	+	-	-	-	-	-
6. E coli 2	-	-	-	+	+	-
7. E coli 3	+	+	+	+	+	-
8. E coli 4	+	-	-	-	-	-
9. Staphylococcus aureus.	+++	+++	+	+	+	-

- Or 0: no biofilm formation.
+ Or 1: weak biofilm formation.
+++ Or 2: Moderate biofilm formation.

After staining with crystal violet (the cv is a small molecule which diffuses through the membrane to negatively charged molecules), an observation with the naked eye is made for the tubes. We can see the formation of a film on the surface or at the bottom of the tubes, so we can visually determine the formation of biofilm attributed to each strain.

An indirect method which relates to the optical density (OD) of colored biofilm is determined by spectrometry at 600nm the results are summarized in the following table

Tableau 5 : Optical density results compared to the control

	Oils				
	Oregano	Thyme	Clove	Eugenol	Thymol
1. P.aetaginosa	1	1	1	1	1
2.k.aeruginosa	1	0	1	1	1
3. E coli1	1	1	1	0	1
4. SARM	1	1	1	0	1
5. E coli 5	0	0	0	0	0
6. E coli 2	0	0	1	1	0
7. E coli 3	1	1	1	1	0
8. E coli 4	0	0	0	0	0
9. Staphylococcus aureus.	2	1	1	1	0

The experiments carried out in our study allowed us to measure the rate of adhesion and the subsequent formation of biofilm of the bacteria tested. The tube test showed results significant result in staphylococcus aureus tube.

According to the results of the table, it can be seen that all the essential oils inhibits the adhesion of bacterial cells biofilm and the formation of certain such as *E.coli* 4and 5 strains in comparison with the control.

In our analysis we note on the one hand that Thymol inhibits the growth of certain bacteria such as *staphylococcus aureus,E.coli4*; 3; 2.and on the other hand it is noted that eugenol inhibits the formation of biofilm of *E. coli* 1; B; 4and *SARM.*

In general, essential oils characterized by a high content of compoundsPhenolics, such as eugenol and thymol, exert significant antibacterial activity (Dhifi et al., 2016).

Bacteria belonging to the species *E. coli* constitute the major part of the aerobic microbial flora of the digestive tract of man and many animals. Certain strains are capable of triggering specifically in humans or in certain animal spaces spontaneous infections of the digestive or urinary tracts or even neonatal meningitis. Other strains belonging to the commensal flora may be responsible for various opportunistic infections, especially in subjects with weakened immune defenses (Patrick et alq., 1988).

Dorman et al., (2000)demonstrated that thymol is the compound with the broadest spectrum of antibacterial activity against 25 genera of bacteria tested, is a mono terpene isolated from thyme, shown to have an anti-inflammatory effect on the mammary gland **(**Wei Nee Cheng etal.,2020). Studies carried out by the World Health Organization (WHO, 1999) have also shown that this constituent has strong antifungal and antibacterial activity against many species including Aspergillus sp. S. aureus and E. coli. And Lambert et al., (2001) ;Juven et al.,(1994) explained this phenomenon by the fact that thymol binds to membrane proteins and increases the permeability of the bacterial cell membrane. Other works have also suggested that this volatile compound is responsible for the inactivation of enzymes.

Numerous reports show that clove oil and its main active component eugenol commonly used in dentistry have beneficial effects on common food source Gram-negative bacteria as *Escherichia coli, Salmonella, Pseudomonas aeruginosa*, and so on, and Gram-positive bacteria as *Staphylococcus, Streptococcus, Listeria*, and so on. Based on its inhibiting effects on the migration, adhesion, virulence factors expression and biofilm formation(Hu et al.,2018).

1.4.2.1. Preformed biofilm inhibition :

The 3rd method is the method of detachment of the biofilm formation after 48 hours of incubation with the addition of the inoculum and the culture medium (TSB). The results of the optical density are shown in tables.

Tableau 6 : Detachment of biofilm formation result.

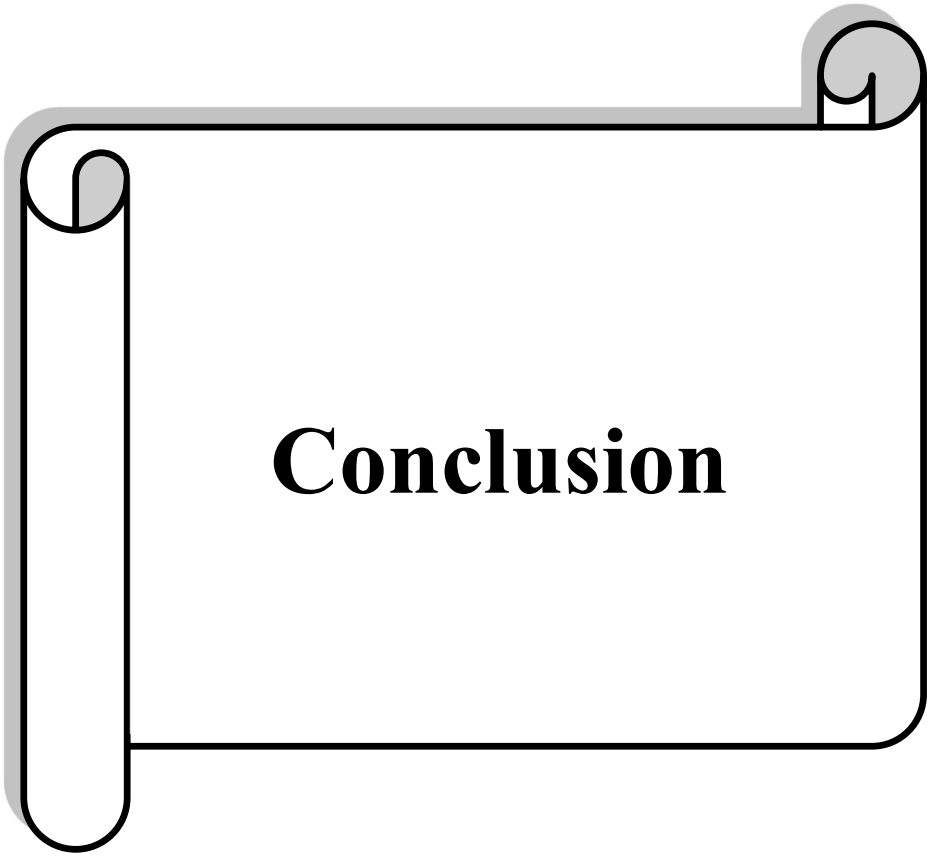
Strains	*P.aetaginosa*	*k.aeruginosa*	*E.coli 1*	*SARM*	*E.coli 5*	*E.coli 2*	*E.coli 3*	*E.coli 4*	*S.aureus*
OD	1	0	1	0	1	1	0	1	0

From the table note a detachment of biofilm; example, of the strain *staphylococcus aureus* which a moderate biofilm in the witness, after the addition of oils in finding that clove has destroyed the bacterial film formed for this it is considered as an effective substance for the antibacterial activity in particular for the mastitis.

Grosserode and Wenzel (1991).Shown that the key step in the pathogenesis of infections associated with staphylococcal foreign bodies is colonization and the formation of a stable biofilm on the surface of the foreign body. Colonized bacteria embedded in amorphous extracellular material (slime) are called biofilm (Heilmann et al., 1997). It has been hypothesized that biofilm formation assays may be a useful marker for the pathogenicity of staphylococci, especially coagula-negative species.

Bacteria having the capacity to form biofilms therefore constitute a serious health problem for both humans and animals, as for example in the case of bovine mastitis (MB). BD is an inflammation of the mammary gland of the cow leading to damage to the udder tissues and therefore a reduction in milk production as well as an alteration of the animal's well-being. It is therefore important to develop new prophylactic and therapeutic strategies against BD.Preliminary results obtained in our laboratory had demonstrated the capacity of certain strains of coagula-negative staphylococci (CNS) producing little or no biofilm to inhibit the formation of biofilm by other bacteria responsible for MB. (Goetz, 2019).

This is how he shows that the medicinal properties of these plants can be useful in a production center; the combination of essential oils has been tested with good results forgeneral health (Katiyare et al., 2010) and the mastitis (deryabin, 1991). It was applied in gentle massage for 5-7 minutes on the mammary gland 3 times a day in cows. Values of 95% efficiency were achieved compared to cows treated conventionally (Deryabin,1991).


Conclusion

Conclusion

The search for natural antioxidants with the virtue of being non-toxic has given rise to a large number of studies on the antioxidant potential of plant extracts. The evaluation of the antioxidant performance of EOs is a crucial issue. Several studies purported beneficial properties, such as antiproliferative, antioxidant and anti-inflammatory activity. Furthermore, the essential oils of many aromatic plant species show antimicrobial activity, so they can be used as substitutes to antibiotics in bovine mastitis treatment.

The evaluation of the antioxidant performance of EOs is however crucial issues since many commonly used “tests” are inappropriate and give contradictory results that may mislead future research. The chemistry explaining EOs antioxidant activity is discussed along with an analysis of the potential in food protection. Three methods to assess EOs’ antioxidant of the different Eos performance are used in this work:FRAP assay,ABTS test and DPPH free radical reduction method.

According to the results obtained in the current work, *Thymus vulgaris*, *Syzygium aromaticum*,*origanum vulgar* oil’s ,thymol and eugenol, showed a highest antioxidant capacity in a different *in vitro*antiradical tests(DPPH and ABTS) including the reducing power investigated by FRAP methodwith a inhibition percent more than 50% and a low IC50.

The antimicrobial activity of essential oils was carried out on reference, mastitis and clinical strains.The antimicrobial activity against biofilm-forming pathogens *Pseudomonas aeruginosa, Candida albicans klebsiella,E. Coli* 1, 2, 3, 4,*E. Coli* (ATCC 25922),MRS Aand *Staphylococcus aureus* according to Cristal Violet method indicate that the five EOs which are mentioned above from *Thymus vulgaris* , *Syzygium aromaticum*,*origanumvulgare*,thymol and eugenol have a remarkable antimicrobial activity. These essential oils appear to represent, at least *in vitro*, a valid tool against bovine mastitis pathogens.

It is therefore necessary, to search further about several bioactive compounds of this EOs such as:

- Characterization by GPC or LCMS;

- see if there is synergy between two or more;

- test the EOs alone or in mixtures on dairy animals with mastitis.

Bibliographic references

Bibliographic references

A

- Abdelouahid .D,Bekhechi.CH.,(2010).les huiles essetielles.N° 1 04.5145 ;Alger,17-21p.

- Agbodan, K. A., et al. (2015) .Effects of a strong acid on the reaction of DPPH with two essential oils from two aromatic plants acclimatized in Togo. International Journal of Biological and Chemical Sciences 9.6:2932-2939.

- Amarti, Fatiha, et al. (201)1.Activité antioxydante et composition chimique des huiles essentielles de quatre espèces de thym du Maroc. Acta botanicagallica 158. : 513-523.

- Amorati, Mario C. Foti, and Valgimigl .L. (2013).Antioxidant Activity of Essential Oils Riccardo i. Journal of Agricultural and Food Chemistry 61 (46), 10835-10847.DOI: 10.1021/jf403496k.

- Aricola C.R., Commpaoccia D., Baldassari L., Donati M.E., Gamberini S., Montanaro L(2005). Detection of biofilm formation in Staphylococcus epidermidis from implant infections Comparisons of a PCR method that recognize the presence of icagenes with two classic phenotypic methods.

- Athamena, S., Chalghem, I., Kassah-Laouar, A., Laroui, S., Khebri, S. (2010). Activité antioxydante et antimicrobienne d'extraits de Cuminumcyminum L. Lebanese Science Journal, 11(1): 69-81.

B

- Burt, Sara.(2004). Essential oils: their antibacterial properties and potential applicationsin foods—a review. International journal of food microbiology 94.3: 223-253.

- Bozin, Biljana, et al. (2008). Phenolics as antioxidants in garlic Allium sativum L., Alliacea). Food chemistry 111.4: 925-929.

C

- Caillet, Stephane, et al. (2007).Fenton reaction applied for screening natural antioxidants.Food Chemistry 100.2 : 542-552.

- Cheng, Wei Nee, and Sung GuHan(2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments-A review.Asian-Australasian journal of animal sciences 33.11: 1699-1713.

- Cortez, David.(2019). "Replication-coupled DNA repair." Molecular cell 74.5 : 866-876.

- Cotelle N., Bernier J.L., Catteau J.P., Pommery J., Wallet J.C. &Gaydou E.M., (1996).- Antioxidant properties of hydroxy-flavones. Free Radical Biology and Medecine. 20: 35 - 43.

D

- Das, M., Sandhu, P., Gupta, P. et al.(2016). Attenuation of Pseudomonas aeruginosa biofilm formation by Vitexin: A combinatorial study with azithromycin and gentamicin. Sci Rep **6,** 23347.

- Deryabin, V. I. (1991).Rotation of insecticides to overcome resistance." ZashchitaRasteniĭ (Moskva) 3 : 45-46.

- Desmier, Thomas.(2016). Les antioxydants de nos jours: définition et applications. Diss.

- Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines (Basel). 2016 Sep 22; 3(4):25. doi: 10.3390/medicines3040025. PMID: 28930135; PMCID: PMC5456241.

- Dima Mnayer.(2014). Eco-Extraction des huiles essentielles et des arômes alimentaires en vue d'une application comme agents

antioxydants et antimicrobiens. Autre. Université d'Avignon. Français.

- Dorman, HJ–Deans, and Stanley G. Deans (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of appliedmicrobiology 88.2: 308-316.

- Dorman, H. J. D., Peltoketo, A., Hiltunen, R., &Tikkanen, M. J. (2003). Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food chemistry, 83(2), 255-262.

E

- Emami, S. A., Javadi, B., &Hassanzadeh, M. K. (2007). AntioxidantActivity of the Essential Oils of Different Parts of Juniperuscommunis. subsp. hemisphaerica. andJuniperusoblonga. Pharmaceutical Biology, 45(10), 769-776.

F

- Fernandez Chémat, F., and. (2012). La Chimie des Huiles Essentielles: Tradition et Innovation. Vuibert: Paris, France.

- Freeman J, Falkiner FR, Keane CT(1989).. New method for detecting slime production by coagulase negative staphylococci.Journal of ClinicalPathology.;42:872-874.

G

- González-Peña D, Colina-Coca C, Vega E, de Ancos B, Sánchez-Moreno C.(2013). Novel approach for the determination of volatile compounds in processed onion by headspace gas chromatography–mass spectrometry (HS GC–MS). Talanta. Jan 15;103:137-44.

- Gupta, D. (2015). Methods for determination of antioxidant capacity: A review. International Journal of Pharmaceutical Sciences and Research, 6(2), 546.

- Gülçin, İ. (2010). Antioxidant properties of resveratrol: A structure–activity (1989).insight. Innovative food science & emerging technologies, 11(1), 210-218.

- Grosserode, M. H., and R. P. Wenzel.(1991). "The continuing importance of staphylococci as major hospital pathogens. Journal of Hospital Infection : 3-17.

H

- Hagos et al., (2022). Chemical Composition and Physicochemical Properties of Essential Oil from Myrtuscommunis. International Journal of Pharmaceutical and Clinical Research ; 9(6): 439-443.

- Hanzen, C., Theron, L., Vogin, N., Bayrou, C., Rollin, F., Maillard, R., &Navetat, H. (2009). Describing an emerging disease: a case of thrombocytopenic syndrome of the newborn calf.

- Haraguchi, Hiroyuki, et al.(1998). "Mode of antibacterial action of retrochalcones from Glycyrrhizainflata." Phytochemistry 48.1 :125-129.

- Heilmann, Christine, et al(1997). Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. Molecularmicrobiology 24.5 : 1013-1024.

- Hu, Q., Zhou, M., & Wei, S. (2018). Progress on the antimicrobial activity research of clove oil and eugenol in the food antisepsis field. Journal of food science, 83(6), 1476-1483.

I

- Ibrar, Muhammad, et al. (2012).Antinociceptive and anticonvulsant activities of essential oils of Zanthoxylumarmatum.Phytopharmacology 3.1 : 191-198.

- IvanovaAlla.(2020) Study of Antioxidant Properties of Agents from the Perspective of Their Action Mechanisms., Elena Gerasimova and Elena Gazizullina Molecules, 25, 4251 .

J

- JALILA, E., ELBADAOUI, K., TOURIA, Z., BOUHARB, H., CHAKIR, S., & ALAOUI, T. L. (2014). Étude de l'activité antibactérienne des huiles essentielles de TeucriumcapitatiumL et l'extrait de Silénevulgaris sur différentes souches testées. Journal of Applied Biosciences, 82, 7481-7492.

- Jovanovic, S. V., Steenken, S., Simic, M. Y., & Hara, Y. (1998). Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoid radicals.

- Juven, B. J., et al. "Factors that interact with the antibacterial action of thyme essential oil and its active constituents." Journal of applied bacteriology 76.6 (1994): 626-631.

K

- KANKO C, SAWALIHO BE, KONE S, KOUKOUA G, N'GUESSAN YT.(2004)Étude des propriétés physico-chimiques des huiles essentielles de Lippiamultiflora, Cymbopogoncitratus, Cymbopogonnardus, Cymbopogongiganteus. Comptes rendus Chimie 7 1039–1042.

- Katiyar, Atul Singh Dhananjay, and B. N. Mishra. (2010).Essential oil: production for health care in current scenario. Annals of BiologicalResearch 1.3: 200-209.

- Khorshidian, Nasim, et al. (2018).Potential application of essential oils as antimicrobial preservatives in cheese. Innovative Food Science & Emerging Technologies 45 : 62-72.

- Kırmusaoğlu, S. (2019). The methods for detection of biofilm and screening antibiofilm activity of agents. Antimicrobials, antibiotic resistance, antibiofilm strategies and activity methods, 1-17.

L

- Lambert, Michael J., and Dean E. Barley.(2001).Research summary on the therapeutic relationship and psychotherapy outcome. Psychotherapy: Theory, research, practice, training 38.4: 357.

- Liang, Ningjian, and David D. Kitts. (2014). Antioxidant Property of Coffee Components: Assessment of Methods that Define Mechanisms of Action. *Molecules* 19, no. 11: 19180-19208. https://doi.org/10.3390/molecules191119180

- Liberto, Maria Carla, et al.(200).Phenotypic and genotypic evaluation of slime production by conventional and molecular microbiological techniques." Microbiologicalresearch 164.5 : 522-528.

M

- (Machado et al (2022) Steam Distillation for Essential Oil Extraction: An Evaluation of TechnologicalAdvancesBased on an Analysis of Patent Documents.

- Mantel, Gerhard. Cello.(1995). technique principles and forms of movement. Indiana University Press.

- Mathur, T., et al.(2006). Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian journal of medicalmicrobiology 24.1 : 25-29.

- Mayassar.O ,Alkish1,Zuhair.B,ismail1,mofteh.S ,Awawdeh2et Shatanin3.S.(2017). effet de l'infusion intra mammaires d'huiles essetielle de sauge (salviaofficinalis) sue nombre de cellules somatiques du lait ,les paramètres de composition du lait et certain paramètres hématologiques et biochimiques du sérum chez les brebis awssi souffrants de mammite subcliniques .mode vétérinaire :10,8,895-900p .

- Memon, GhulamRasool. (2007).Education in Pakistan: The key issues, problems and the new challenges. Journal of Management and Social Sciences 3.1 : 47-55.

- Meta, Adriano, Peter Hoogeboom, and Leo P. Ligthart.(2007). Signal processing for FMCW SAR." IEEE Transactions on Geoscience and Remote Sensing 45.11 : 3519-3532.

- Miladi, H., Ben Slama, R., Mili, D., Zouari, S., Bakhrouf, A., Ammar, E. (2013).Chemical composition and cytotoxic and antioxidant activities of Saturejamontana L. essential oil and its antibacterial potential against Salmonella Spp. strains. J. Chem., 2013: 9-18.

- Montoro, E., Lemus, D., Echemendia, M., Martin, A., Portaels, F., &Palomino, J. C. (2005). Comparative evaluation of the nitrate reduction assay, the MTT test, and the resazurinmicrotitre assay for drug susceptibility testing of clinical isolates of Mycobacterium tuberculosis. Journal of AntimicrobialChemotherapy, 55(4), 500-505.

- Mouas.Y ,Benrebiha.T,Chaouia .Ch. évaluation de l'activité anti bactérienne de huile essentielset de l'extrait méthanolique du remarinRomaeinusofficinalL.revueagrobiologia ,2017,7,363-370p.

N

- Nehme, R.; Andrés, S.; Pereira, R.B.; Ben Jemaa, M.; Bouhallab, S.; Ceciliani, F.; López, S.; Rahali, F.Z.; Ksouri, R.; Pereira, D.M.(2021).Essential Oils in Livestock: From Health to Food Quality. Antioxidants: 10, 330. https:// doi.org.

P

- Parthiban, K.T., P. Selvan, M. Paramathma, S.U. Kanna, P. Kumar, V. Subbulakshmi and S. Vennila, 2011. Physico-chemical characterization of seed oil from Jatrophacurcas L. genetic resources. Journal of Economic and Natural Environment, 3(5): 163-167.

- Patric.H .Helvey, J. D., and J. H. Patric. "Research on interception losses and soil moisture relationships.Forest hydrology and ecology at Coweeta. Springer, New York, NY:.129-137.

- Pearson, D., (1976). The Chemical Analysis of Foods, 7 Edn. Churchill Livingstone, pp: 6-14.(1988).

- Peltoketo, A., Dorman, H. J. D., Ytjonen, T., Laakso, I., Vuorela, H., &Hiltunen, R. (2001). Antioxidant properties of volatile oils and aqueous fractions of selected aromatic and medicinal plants. In 3rd international Conference on Phytomedicine, Munich, Germany, Phytomedicine, Suppl p. 11-75.

R

- Re, Roberta, et al(1999).Antioxidant activity applying an improved ABTS radical cationdecolorizationassay.Free radical biology and medicine 26.9-10 : 1231-1237.

- Remy,D.,(2010).lesmammites.ISDN :978-2-85557-171-3.groupeFrance agricole,amazone France ,P1-19.

S

- Sanaa.L,moustais.Kh ,Ismail.R .(2017).etude de l'activité antioxydantes des huiles essentielles de planttes aromatique et médécinales marocaines .européanscientific journal , ,323p.

- SantínPérez.,Alemán, A ,E., Bordenave-Juchereau, S., Arnaudin, I., Gómez-Guillén, M. C., &Montero, P. (2011). Squid gelatin hydrolysates with antihypertensive, anticancer and antioxidant activity. Food Research International, 44(4), 1044-1051.

- Selmi, Slimen, et al.(2022).Essential Oil, Chemical Compositions, and Therapeutic Potential." Essential Oils: Advances in Extractions and Biological Applications: 163.

- Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, Tundis R, Sharifi-Rad M, Loizzo MR, Ademiluyi AO, Sharifi-Rad R, Ayatollahi SA, Iriti M.(2017).Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Molecules.Jan1;22(1):70. doi: 10.3390/molecules22010070.

- Sikkema, Jan, Jan A. de Bont, and Bert Poolman.(1994). Interactions of cyclic hydrocarbons with biological membranes. Journal of biological Chemistry 269.11: 8022-8028.

- Singh, S. R., & Taylor, A. M. (2022). Longer-run economic consequences of pandemics. Review of Economics and Statistics, 104(1), 166-175.

- Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić S, Ćirković I, et al(2007). Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS ;115(8):891-899.

T

- Taga,I.,Cheistoph ,Q .,Lan et Illimraltosaar.(2012).les huiles essentiles végétales et maladie de la mammite :leurs effets inhibiteur potentiel sur la production de cytokine pro-inflammatoire en réponse à une inflammation lié a une bactérie.NPC Naturel product communication ,7,5,P675-682.

- Talbi, S, et al(2015). Drought tolerance in a Saharian plant Oudneyaafricana: role of antioxidant defences."Environmental and Experimental Botany 111 114-126.

- Toubou, H Hasegawa, K., Tsukahara, T., &Nomiyama, T. (2021). Short-Term Associations of Ambient Fine Particulate Matter (PM2. 5) with All-Cause Hospital Admissions and Total Charges in 12 Japanese Cities. International journal of environmentalresearch and public health, 18(8), 4116.

- ToureDaouda.ETUDESCHIMIQUEETBIOLOGIQUEDESHUILES ESSENTIELLESDEQUATREPLANTENTAROMATIQUESMEDI CINALESDECÔTED'IVOIRE.Chimieorganique.UniversitéFelixHo uphoeutBoigny,Côted'Ivoire,2015.Français.NNT:29/2014.

- Turek, C. and Stintzing, F.C. (2022), Stability of Essential Oils: A Review. COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY, 12: 40-53.

Y

- Yadav R, and Yadav N .(2016). Review on Extraction Methods of Natural Essential Oils". International Journal of Pharmaceutics and Drug Analysis, vol. 4, no. 6, June , pp. 266-73.

Abstract

The present study deals with the evaluation of the antioxidant activity and the antimicrobial activity of 12 essential oils of local aromatic plants which are traditionally used in Algeria against 9 microbial strains responsible for udder infections of dairy cows in order to select the most interesting oils. Antioxidant activity of the extracts was evaluated by three methods: reducing power by TPTZ method, DPPH and ABTS free radical scavenging.

The antimicrobial activity of the tested oils was evaluated by biofilm formation inhibition using crystal violet assay.

The essential oils of thymus *vulgaricus, origanum vulgare, syzygium aromaticicum*, as well as eugenol and thymol showed significant antioxidant activity compared to other oils tested. Clove oil *(syzygium aromaticicum)* showed the best antibiofilm activity, especially that formed by *Staphylocuccus aureus.*

Key words: antioxidant activity, antimicrobial activity, essential oils, medicinal plants, udder infection

Résumé

La présente étude porte sur l'évaluation de l'activité antioxydante et de l'activité antimicrobienne de 12 huiles essentielles de plantes aromatiques locales qui sont utilisées traditionnellement en Algérie contre 9 souches microbiennes responsables des infections de la mamelle des vaches laitières afin de sélectionner les huiles les plus intéressantes. L'activité antioxydante des extraits a été évaluée par trois méthodes : le pouvoir réducteur par la méthode TPTZ, le piégeage des radicaux libres DPPH et ABTS.

L'activité antimicrobienne des huiles testées a été évaluée par l'inhibition de la formation de biofilm par le cristal violet.

Les huiles essentielles de *thymus vulgaricus, origanum vulgare, syzygium aromaticicum*, ainsi que l'eugénol et thymol ont révélé une activité antioxydante importante par rapport aux autres huiles étudiées. L'huile du clou *(syzygium aromaticicum)* de girofle présente la meilleure activité antibiofilm notamment celui formé par *Staphylocuccus aureus.*

Mots clés : activité antioxydante, activité antimicrobienne, huiles essentielles, plantes médicinales, infection de la mamelle