International Journal of Global Science Research **ISSN: 2348-8344 (Online)**
Vol. 9, Issue. 2, October 2022, pp. 1831-1837 **DOI: 10.26540/ijgsr.v9.i2.2022.211**
***Available Online* at www.ijgsr.com**

Research Paper

In vitro antibacterial activity of essential oils of sweet orange

Guetarni Hassina [1,2*], Zaboun Abdeldjalil[1] and Akouchi Mohamed[1]
[1]University Bounaama Djilali of Khemis Miliana, Faculty of Nature, Life Sciences and Earth Sciences, Biology Department, 44225, Ain Defla, Algeria.
[2] Laboratory of Natural Substances Valorization, University Bounaama Djilali of Khemis Miliana, 44225, Ain Defla, Algeria.
*Corresponding author E-mail: kmhg2009@yahoo.fr

Received: 29/08/2022
Revised: 03/09/2022
Accepted: 18/09/2022

Abstract: This work focuses on the study of the antimicrobial activity of essential oils of orange. Several studies have shown that the essential oils of orange species *Citrus sinensis* have better inhibitory activity against Gram positive bacteria (*Staphylococcus aureus)* than Gram negative bacteria (*E. coli)*. However, the determination of the efficiency of essential oils on microbial agents remains difficult to achieve, because of some uncontrollable external parameters such as the composition of essential oils, which varies according to environmental conditions.

Keywords: Essential oils, Antibacterial Activity, *Citrus*, Sweet orange, *E. coli, S. aureus.*

Introduction:

Bacteria and fungi are the most dangerous microorganisms causing health problems. In the perpetual fight against microbial infections, antibiotics have been regarded as the ultimate weapon. But the phenomenon of antibiotic resistance of different genera and species and the side effects of synthetic drugs, underestimated, have brought herbal medicine back to the fore. Humans have used natural substances in a wide range of pharmaceutical, food and cosmetic industries (Taghigolmakani & Moayyedi, 2015).

Citrus fruits are of considerable economic importance to many countries. The same is true for Algeria where they constitute a source of employment and economic activity both in the agricultural sector and in various auxiliary branches (Farhat *et al.*, 2010).

Among the *Citrus* fruits, there is the sweet orange, cultivated in Algeria in all regions (https://www.sante-dz.com/conseils/2008/5/10/tout-sur-lorange-une-baie-particuliere).

Considerable interest has been generated in essential oils extracted from aromatic plants with antimicrobial activity against pathogenic microorganisms. Many studies have been carried out to estimate the antiseptic power of essential oils for a very long time (Chutia *et al.*, 2009 ; Jafari *et al* ., 2011).

The general principle of essential oils is that they can be used in all foods, and are generally considered safe (GRAS, Generally Recongnised As Safe). *Citrus* zest accounts for about 45% of the total fruit weight, available as a by-product of

International Journal of Global Science Research **ISSN: 2348-8344 (Online)**
Vol. 9, Issue. 2, October 2022, pp. 1831-1837 **DOI: 10.26540/ijgsr.v9.i2.2022.211**
***Available Online* at www.ijgsr.com**

citrus processing, which creates environmental problems (Ferhat *et al.*, 2011). Although *Citrus* fruits are mainly used for dessert, its essential oils have significant economic value due to its aromatic compounds (Minh *et al.*, 2002).

1. General information on essential oils

According to Durvelle (1893-1930), essences or, also known as volatile oils, perfumes, etc., are oily, volatile odorous substances, poorly soluble in water, more or less soluble in water, in alcohol and in ether, colorless or yellowish, flammable and which easily deteriorate in air and become resinous. They are liquid at ordinary temperature, some are solid or partly crystallized, they do not have the greasy and creamy feel of fixed oils, from which they are distinguished by their volatility. Their smell is more or less strong, sweet, pungent or unpleasant. They have the property of not leaving a lasting stain on the paper. Most of the essential oils are produced by the plant organism (Charabot *et al.*, 1899).

According to Naves (1976), none of the definitions of essential oils have the merit of being clear or precise. Essential oils are mixtures of various products from a plant species, these mixtures pass with a certain proportion of water during a distillation carried out in a stream of water vapor. This definition can be extended to essential oils obtained by cold expression of the peel or zest of *Citrus* fruits, because of the intervention of water in the mechanical processes to entrain the product released from the oleiferous cells (Naves, 1976).

The Funk and Wagnalls Encyclopedia (2004) describes essential oils as volatile liquids, mostly insoluble in water, but free in alcohol, ether, and vegetable and mineral oils. They are usually non-oily on contact with the skin. Their components can be grouped into six classes according to their chemical structure:

- Hydrocarbons, such as limonene in lemon oil;
- Alcohols, such as borneol in the *Borneo camphor* tree;
- Esters, such as methyl salicylate in wintergreen oil;
- Aldehydes, such as benzoic aldehyde in bitter almond oil;
- Ketones, such as menthone in peppermint oil;
- Lactones and oxides, such as coumarin from tonka beans (Funk & Wagnalls, 2004).

AFNOR (2000) has defined essential oils as products obtained either from natural raw materials by water or steam distillation, or from fruits of *Citrus* by mechanical methods and which are separated from the aqueous phase by physical methods. Bruneton (Bruneton, 1999) defines the terms most commonly used in this field, such as:

- Floral ointment: Perfumed fatty substance obtained from flowers, either by cold enfleurage or by hot enfleurage;
- Resinoid: Extract with a characteristic odor, obtained from a dry raw material of natural origin, by extraction with a non-aqueous solvent;
- Concrete: Extract with a characteristic odor obtained from a fresh raw material of plant origin, by extraction with a non-aqueous solvent, followed by the removal of this solvent by physical processes;
- Absolute: Product having a characteristic odor, obtained from a concrete, a floral ointment or a resinoid by extraction with ethanol at room temperature, the ethanolic solution obtained is generally cooled and filtered in order to remove waxes;
- Spices: Natural plant products or mixtures thereof, without foreign matter, which are used to impart flavor and aroma and to season foods, the term applies to both the whole product and the product in powder.

2. Antibacterial activity

Antibacterial activity is determined by the aromatogram method. The principle of the method is based on the inhibition of microbial growth in the Petri dish after a

International Journal of Global Science Research **ISSN: 2348-8344 (Online)**
Vol. 9, Issue. 2, October 2022, pp. 1831-1837 **DOI: 10.26540/ijgsr.v9.i2.2022.211**
***Available Online* at www.ijgsr.com**

certain time of contact between the product and the target microorganism. The effect of the antibacterial product on the target is assessed by measuring a zone of inhibition. Depending on the diameter of inhibition, the strain will be qualified as resistant (-) for diameters less than 8 mm; sensitive (+) for diameters from 8 to 14 mm; very sensitive (++) for diameters of 15 to 19 mm and extremely sensitive (+++) for diameters greater than 20 mm (Himed *et al.*, 2014).

An Algerian study (Himed *et al.*, 2014) on the evaluation of the power of the essential oil of *Citrus limon*, extracted by hydrodistillation of the fresh lemon zest harvested in the region of Takariet wilaya of Bejaia, on bacterial strains involved in contamination and spoilage of foodstuffs (Gram-positive bacteria: *Staphylococcus aureus* and Gram-negative bacteria: *E. coli)* showed that the bacterial strains tested are sensitive to the essential oil studied, they have also found that Gram positive strains (*Staphylococcus aureus*) are the most sensitive strains compared to Gram negative strains *(E. coli*).

The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration make it possible to determine the bactericidal and bacteriostatic power of the essential oil studied. They found that all the strains were totally inhibited at 1000 $\mu g.ml^{-1}$. Gram positive bacteria (*Staphylococcus aureus*) are the most sensitive to the essential oil studied compared to Gram negative bacteria with MIC and MBC of 240 µg / ml and 300µg / ml respectively. According to their results, the MBC/ MIC ratio is less than 4, therefore the essential oil studied has bactericidal power against the bacterial strains tested (Canillac & Mourey, 2001).

Another Algerian study conducted by Boughendjioua & Djeddi (2017) concluded that essential oils(cold extraction of the upper part of the pericarp or fresh lemon zest from the Collo wilaya region of Skikda) shows no zone of inhibition against the strain studied (*Bacillus subtilis*). These bacteria have a very high resistance potential against the antibacterial action of the essential oil of *Citrus*. It is well known that Gram-positive bacteria are more sensitive to essential oils than Gram-negative bacteria, but the mechanisms of action of essential oils and their selectivity towards certain bacteria remain poorly understood to date. It should also be noted that no significant correlation was observed between the content of the chemical components and the antibacterial activity of the essential oil of *Citrus* tested for the strains. In fact, the major component limonene of essential oil(61.65%) demonstrated lower antimicrobial efficacy. From these observations, it is evident that limonene did not exert any influence on the antibacterial potential of the essential oil tested.

The results of a Tunisian study (Ben Hsouna *et al.*, 2017) of the antimicrobial power of the essential oil of *Citrus limon* (extraction by hydrodistillation of the flowers of *Citrus limon* collected in the Cap Bon region of Tunisia) on bacteria Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*) that contaminate food have shown that Gram positive bacteria are more sensitive with a range of 0.078 to 0.625 mg / ml.

The antibacterial power of *Citrus medica* L. *var. sarcodactylis* (Extraction by hydrodistillation) studied by a Chinese team on these same strains (Ze-Hua *et al.*, 2019) showed that lemon essential oil is a bacterial inhibitor and a bactericide. The diameter of the growth inhibition zone was measured. Ciprofloxacin was used in this experiment as a positive control.

The results of the majority of these studies showed that *Citrus* essential oil had some antibacterial activity against all pathogens tested. This activity is associated with the phytochemical components of essential oils or with the monoterpene or

ISSN: 2348-8344 (Online)
DOI: 10.26540/ijgsr.v9.i2.2022.211

sesquiterpene hydrocarbon and their oxygenated derivatives which are the main components of essential oils exhibiting antimicrobial activities (Cakir *et al*., 2004). In addition, the essential oil showed better activity against Gram-positive bacteria than Gram-negative bacteria. This could be attributed to the structure of the bacterial membrane for Gram-negative bacteria which possess a membrane rich in liposaccharides which provide a hydrophilic surface (Shakeri *et al*., 2014), and therefore the latter acts as a penetration barrier which blocks macromolecules from entering the cell (Kong *et al*., 2014). As a result, Gram negative bacteria are relatively resistant to hydrophobic antibiotics.

The essential oils most studied for their antibacterial properties belong to Labiatae: oregano, thyme, sage, rosemary, clove are all aromatic plants with essential oils rich in phenolic compounds such as eugenol, thymol and carvacrol. These compounds have strong antibacterial activity. Carvacrol is the most active of all, known to be non-toxic, it is used as a preservative and food flavoring in drinks, sweets and other preparations. Thymol and eugenol are used in cosmetics and food. These compounds have an antimicrobial effect against a broad spectrum of bacteria: *E. coli, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Clostridium* spp and *Helicobacter pylori* (Pauli, 2001).

Belleti *et al.* (2004) and Fisher *et al.* (2007), demonstrated that the essential oil of *Citrus* are effective against pathogenic bacteria, bacterial spores, but also on certain bacteria responsible for food poisoning such as: *Mycobacterium jejuni, Listeria monocytogenes, E. coli* O157: H7, *Staphylococcus aureus, Salmonella thyphimurium,* and *Acrobacter butzleri.*

3. Mechanisms of antibacterial action

The mechanisms by which essential oils exert their antibacterial activity are not well understood. Due to the complexity of their chemical composition, it is difficult to give a precise idea of the mode of action of essential oils. It is likely that their antibacterial activity is not attributable to a single mechanism, but to several sites of action at the cellular level (Dorman & Deans, 2000).

Burt (2004a) argued that the important characteristic of essential oils is attributed to the hydrophobicity of some of these components which allows them to easily cross the phospholipid bilayer of the cell membrane altering its permeability and resulting in abnormal ion losses, or even macromolecules. Oussalah *et al.* (2006), suggests that the action of essential oils on microbial proliferation is through alteration of the membrane permeability of bacteria by disrupting ion transport systems, electron transport and energy production.

The way essential oils work depends on the type of microorganism. In general, Gram negative bacteria are more resistant than Gram positive bacteria due to the structure of their outer membrane. Thus, the outer membrane of Gram-negative is richer in lipopolysaccharides (LPS) making it more hydrophilic, which prevents hydrophobic terpenes from adhering to it (Cristiani *et al.*, 2007).

4. Methods for determining antibacterial activity

A review of the bibliographic data reveals the diversity of methodologies used to demonstrate the antibacterial activity of essential oils. The choice of method is conditioned by the insolubility of essential oils in aqueous media, their volatility, and the need to test them at low concentrations.

4.1. Aromatogram: The aromatogram is based on a technique used in medical bacteriology called antibiogram or disc method or method by diffusion in agar medium. The technique consists of using paper discs impregnated with the different substances to be tested, then deposited on the surface of an agar plate uniformly inoculated with a suspension of the bacteria to be studied. After incubation, colonies grow on the surface of the agar, leaving blank areas around the discs called the zone of inhibition : more the diameter of inhibition zone is larger, more the strain is sensitive to the test substance, more the bacterium is smaller and larger more it is resistant. The diameter of these zones of inhibition is proportional to the bacteriostatic activity of the essential oil on the germ tested. This activity can be expressed either by indicating directly the diameter of the zone of inhibition in millimeters, or by translating the degree of activity as a cross (Guerin & Carret, 1999).

4.2. Well diffusion method: Method proposed by Cooper in 1946 and taken up by Shroeder and Messing in 1949. It provides radial diffusion of the essential oil from a well giving a clear, easily measurable zone of inhibition. The method involves cutting a circular hole in the agar and pouring a solution of the essential oil of known strength into it. The essential oil diffuses radially, giving a circular zone of inhibition on the surface of the agar previously seeded with the bacterial suspension (Khabat Noori *et al.*, 2020).

4.3. Dilution method: The essential oils to be tested can also be directly mixed in a known concentration with the culture medium, whether solid or liquid (requires homogeneous dispersion by an emulsifier). The medium is then inoculated with a determined rate of microorganisms, after incubation, the presence or absence of culture is noted. The reading can be visual or using a spectrophotometer, the degree of inhibition is related to the turbidity of the medium (Robert- Demuet, 1995).

4.4. Micro-atmosphere method: This technique consists in cultivating the microorganisms to be tested in the Petri dishes on an appropriate culture medium. The difference lies mainly in the position of the disc impregnated with essential oil which is placed in the center of the lid of the Petri dish, inverted after fixing the essential oil on the disc. It is therefore not in contact with the agar medium. The oil evaporates in the atmosphere of the tin, it can exert its inhibitory effect on the microorganisms tested (Pibiri *et al.,* 2003 ; Khabat Noori *et al.*, 2020).

Conclusion:

Citrus essential oils represent an important class of chemicals secreted by plants, with several benefits for human health.

It is a concentrate of oily substances, generally strong odor and flavors, extracted from the different parts of certain aromatic plants, from flower stems, roots or leaves. They are used as ingredients in perfumery or as a flavoring agent in food. They are widely used in the pharmaceutical and cosmetic industry.

Sweet orange oils have not shown any antibacterial effect on *E. coli*, according to studies, this is due to the resistance potential that this bacterium has against the antagonistic effect of essential oils. In contrast, *S. aureus* exhibited moderate susceptibility to essential oils.

Gram-positive bacterial strains are the most sensitive to orange essential oils, this is due to the difference in the structure of the cell wall of bacteria.

Conflict of interest :

No conflict

References :

Taghi-Golmakani M. and Moayyedi M. (2015) Comparaison of heat and mass transfer of different microwave assisted extraction method of essential oil from *Citrus limon* (*Lisbon variety*) peel. Food Science and Nutrition, 3(6), 506-518.

Ferhat M. A. , Meklati B. Y. and Chemat F. (2010) *Citrus* d'Algérie : les huiles essentielles et leurs procédés d'extraction. In : OPU(ed). Alger, 5130 ;157 pp. https://www.sante-dz.com/conseils/2008/5/10/tout-sur-lorange-une-baie-particuliere.

International Journal of Global Science Research **ISSN: 2348-8344 (Online)**
Vol. 9, Issue. 2, October 2022, pp. 1831-1837 **DOI: 10.26540/ijgsr.v9.i2.2022.211**
***Available Online* at www.ijgsr.com**

Chutia M., Bhuyanp D., Pathakm G., Sarmat C. and Boruah P. (2009) Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from NorthEast India. Food Science and Technology, 42,777–780.
Jafari S., Esfahani S., Fazelim R., Jamalifar H., Samadi M. and Najarian-Toosi A. (2011) Antimicrobiala ctivity of lime essential oil against food-borne pathogens isolated from cream-filled cakes and pastries. International Journal of Biological Chemistry, 5,258–265.
Minh Tu N. T., Thank L. X., Une A., Ukeda H. and Swaamura M. (2002) Volatile constituents of *Vietnamese pummelo*, orange, tangerine and limepeel oils. Flavour and Fragrance Journal,17, 169-174.
Charabot E., Dupont J. and Pillet L. (1899) Les huiles essentielles et leurs principaux constituants, Ed. CH. Beranger, Paris. 2022, S N Books World.
Naves Y. R. (1976) Parfums, Cosmétiques, savons. 58, 105pp. In Huiles essentielles. Techniques de l'ingénieur ; K345 : pp. 1-45.
Funk and Wagnalls (2004) Encyclopédie britannique Funk and Wagnalls. URL : http://www.Funkandwagnalls.com
AFNOR (2000) Huiles essentielles. Ed. PARA Graphic. Tome1 – Echantillonnage et méthode d'analyse. 471pp.
Bruneton J. (1999) Pharmacognosie Phytochimie Plantes médicinales. 3ème édition, Paris ; Tec et Doc. 1504 pp.
Himed L., Merniz S. and Bnbraham M. (2014) Evaluation des activités antioxydante et antibactérienne de l'huile essentielle de *Citrus limon* (variété Lisbon) extraite par hydrodistillation. Algerian Journal of Natural Products, 4, 1, 252-260.
Canillac N. and Mourey A. (2001) Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. Food Microbiol, 18, 261-268.
Boughendjioua H. and Djeddi S. (2017) Organoleptic and physicochemical properties of algerian lemon essential oil. World Journal of Applied Chemistry, 2 (5), 96-100.
Ben Hasouna A., Ben Halima N., Smaoui S. and Hamdi N. (2017) *Citrus lemon* essential oil : chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. Lipids in health and disease, 16, 146.
Ze-Hua L., Ming C., Yuna-Shuai L., Pei-Long S. and Shao-Lei L. (2019) Antibacterial Activity and Mechanisms of Essential Oil from *Citrus medica* L. *var. sarcodactylis*. Molecules, 22, 24(8),1577.
Cakir A., Kordali S., Zengin H., Izumi S. and Hirata T. (2004) Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. Flav Frag J, 19, 62–8.
Shakeri A., Khakdan F., Soheili V., Sahebkar A., Rassam G. and Asili J. (2014). Chemical composition, antibacterial activity, and cytotoxicity of essential oil from *Nepeta ucrainica* L. *spp*. kopetdaghensis. Ind. Crop. Prod, 58, 315–321.
Kong M., Chen X G., Liu C S., Liu C G., Meng X H. and Yu L J. (2014) Antibacterial mechanism of chitosan microspheres in a solid dispersing system against *E. coli*. Colloid Surf. B-Biointerfaces, 65, 197–202.
Pauli A. (2001) Antimicrobial properties of essential oil constituants. International Journal of Aromatherapy, 11, 126-133.
Belletti N., Nidagimana M., Sisto C., Guerzoni M. E., Lanciotti R. and Gardini F. (2004) Evaluation of the antimicrobial activity of *Citrus* essences on *Saccharomyces cerevisiae*. Journal Agricutural Food Chemistry, 52 (23), 6932-6938.
Dorman H. J. D. and Deans S. G. (2000) Antimicrobial agents from plants : antibacterial activity of plant volatile oils.

ISSN: 2348-8344 (Online)
DOI: 10.26540/ijgsr.v9.i2.2022.211

Journal of Applied Microbiology, 88 (2), 308-316.

Burt S. A. (2004) Essential oils their antibacterial properties and potentiel applications in foods. International Journal of Food Microbiology, 94 (3), 22-25.

Oussalah M., Caillet S., Saucier L. and Lacroix M. (2006) Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Journal of Food Protection, 69 (5), 1046-1055.

Cristiani M., D'Arrigo M., Mandalari G., Castelli F., Sarpietro M.G. and Micieli D. (2007) Interaction of four monoterpenes contained in essential oils with model membranes : Implications for their antibacterial activity. Journal of Agricultural and Food Chemistry, 55, 6300-6308.

Guerin-Faublee V. and Carret G. (1999) L'antibiogramme, principes, méthodologie, interêt et limites. Journées Nationales GTV- INRA ; pp. 5-12.

Pibiri M.C., Seignez C. and Roulet A. (2003) Study of the effect of the essential oils on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Healthy Buildings, Singapore.

Khabat Noori H., Timea M., Richard P., Adrienne T., Emna A., Laszlo F., Istvan D. and Gabriella K. (2020) In vitro antimicrobial activity of plant active components against *Pseudomonas lundensis* and *Listeria monocytogenes*. Progress in Agricultural Engineering Sciences, 16 S2, 163–172

Robert-Demuet S. (1995) Méthodes de dilutions. In Antibiotiques et antibiogrammes, Montréal-Canada ; pp.131-137.