EVALUATION OF ANTIBACTERIAL ACTIVITY OF LEMON ESSENTIAL OIL

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Abstract: Citrus essential oils are a complex mixture of more than a hundred components of differing chemical natures. The study reported the antibacterial activity of Lemon (*Citrus limon*) essential oil (Rutaceae) against eight bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* spp, *Bacillus subtilis*, *Listeria monocytogenes* and *Staphylococcus aureus*) chosen for their high pathogenicity strong. The obtained results showed that the essential oil exhibited average to strong antimicrobial activity against the tested microorganisms.

Key words: Lemon, *Citrus limon*, essential oil, chemical composition, antibacterial activity.

ÉVALUATION DE L'ACTIVITÉ ANTIBACTÉRIENNE DE L'HUILE ESSENTIELLE DE CITRON

Résumé:

Les huiles essentielles d'agrumes sont un mélange complexe de plus d'une centaine de composants de natures chimiques différentes. L'étude a rapporté l'activité antibactérienne de l'huile essentielle de citron (*Citruslimon*) (Rutaceae) contre huit souches bactériennes (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella* spp, *Bacillus subtilis*, *Listeria monocytogenes* et *Staphylococcus aureus*) choisis pour leur haute pathogénicité.Les résultats obtenus ont montré que l'huile essentielle présentait une activité antimicrobienne moyenne à forte contre les micro-organismes testés.

Mots clés: Citron, *Citrus limon*, huile essentielle, composition chimique, activité antibactérienne.

Introduction:

Citrus is the main fruit tree crop in the world and therefore has a tremendous economical, social and cultural impact in our society [1].

Lemon (*Citrus limon*) is a flowing plant belonging to the Rutaceae family. Citrus plants constitute one of the main valuable sources of essential oil used in foods and medicinal purposes [2].

An essential oil is a concentrated hydrophobic liquid containing volatile (easily evaporated at normal temperatures) chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An essential oil is "essential" in the sense that it contains the "essence of" the plant's fragrance-the characteristic fragrance of the plant from which it is derive [3].

The application of essential oils as antimicrobial, anticancer, anti-inflammatory and anti-viral agents is due to their effective and efficient properties, inter alia [4].

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1. Materials and methods:

1.1. Plant material:

Lemon was collected at the mature stage from fruit orchards located in the region in Collo (Skikda city, North-East of Algeria). Plant harvesting was carried in March 2013.

1.2. Isolation of essential oil:

Lemon essential oil is cold-pressed from the rinds of the lemons.

Cold Pressing is a mechanical process in which the essential oil is extracted and separated from the epicarp without chemical intervention and without the addition of any substances, at a temperature that does not exceed 35°C.

1.3. Chromatography analysis:

The GC-MS analysis was performed using a Hewlett Packard 5973-6800 system operating in EI mode (70 eV) equipped with a split/splitless injector (250°C), a split ratio 1/50, using a fused silica HP-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 µm. The temperature program for the HP-5 MS column was from 60°C to 280°C at a rate of 2°C/min. Helium was used as a carrier gas at a flow rate of 0.5 ml/min. Injection volume of the sample was 0.2 µl.

The identification of the components was conducted in an IS system managing a library of spectrum wiley7n.l. The GC-MS analysis was performed at the Scientific and Technological Scientific Research Center on Physico-Chemical Analysis (CRAPC), Bab Ezzouar (Algiers, Algeria).

1.4. Microorganisms:

Microorganisms were obtained from the Bacteriology Laboratory, Faculty of Medicine and Pharmacy, HCU of Dorban in Annaba (Algeria). Five strains of gram-negative bacteria [*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 15380), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* spp. strains (Samples (date))] and three strains of gram-positive bacteria [*Bacillus subtilis* (MTCC 441), *Listeria monocytogenes* (ATCC 19112), *Staphylococcus aureus* (ATCC 25923)] were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

1.5. Antibacterial test:

The antimicrobial activities were carried out by the disc diffusion method of Müller-Hinton on solid medium; the strains were reactivated using an 20 h culture growth at 37°C and adjusted to 10^8 CFU/ml. Petri boxes (9 cm in diameter) were filled with 10 mL of the medium Muller Hinton. The bacterial strains was sowed on the surface of the agar plates in radial spots form by means of swab and suspensions of young bacterial cultures prepared according to the committee for laboratory standards institute (CLSI) [5]. The application is made by sterile filters paper discs (6 diameter) which were placed on the inoculated agar surfaces and impregnated with 10 µl of each essential oil. A gentle downward pressure should be applied to each disc before incubation to ensure complete contact between the disc and the agar surface. Petri box were incubated at 37°C during 24 h to 48 h [6, 7] and the reading of the results was made by the measurement of the inhibition diameter around the disc, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. Each experiment was carried out in triplicate.

2. Results and discussion:

2.1. Chemical composition:

The qualitative and quantitative analysis by gas chromatography– mass spectrometry (GC / MS) of the essential oil allowed to identify 53 compounds which represent: 99.938%, the main ones being: Limonene (61.647%), β.-Pinene (13.852%), γ.-Terpinene (9.959%) followed by other low-molecules: α.-Pinene (2.279%), Myrcene (1.888%), α.-Citral (1.702%), β.-Citral (1.046%), β.-Bisabolene (1.026%) totaling approximately: 93.399%.

2.2.Antibacterial activity:

All microbiological results obtained during the study shows that all the products tested have a significant antibacterial activity. According to the disk diffusion test results, Lemon essential oil inhibited the growth of all the bacteria tested (**Table 1)**.

Many reports claim that Limonene is the major compound in Lemon essential oil. However, the inhibitory activity of Lemon essential oil stems from the presence of several constituents, mainly limonene, beta-Pinene, gama-terpinene, and Myrcene [8, 9]. As a result of lipophilicity, terpenes accumulate in the lipid structure of cell walls that causes proteins to denature and the loss of cell membrane integrity leading to membrane damage and finally bacterial death. Synergistic effects against pathogens might have resulted from the mixture of chemically different terpenes [10, 11].

Aibinu *et al*., (2007) [12] observed that each bacterial strain demonstrated a significant degree of sensitivity to Lemon essential oil, and extensive activity against Gram-positive bacteria, producing a clear zone of inhibition against the majority of the strains tested. In a previous study, the highest inhibitory zone was observed against *Bacillus subtilis* followed by *Staphylococcus aureus* [13].

The antimicrobial activity of Lemon essential oil could stem from the inhibition of cell membrane synthesis, specifically because of their hydrophobic nature. The inactivation mechanism of limonene was mediated by the tri-carboxylic acid cycle that eventually promotes hydroxyl radical formation, leading to oxidative DNA damage, as is observed in bactericidal drugs. The production of hydroxyl radicals arises from the Fenton reaction in which ferrous iron transfers electrons to hydrogen peroxide [14].

Limonene exhibited higher activity against Gram-positive strains than Gram-negative strains, and several studies report similar results against pathogenic bacteria [13, 15].

Table 1. Results of the antibacterial activity of *Citrus limon* essential oil.

Microorganisms	Sensitivity*
Escherichia coli	+++
Klebsiella pneumoniae	+++
Proteus vulgaris	+++
Pseudomonas aeruginosa	+++
***Salmonella* spp**	+++
Bacillus subtilis	++
Listeria monocytogenes	++
Staphylococcus aureus	++

Each value represents the mean of two replicates ± standard deviation

*The sensitivity to the different strains was classified by the diameter of the inhibition zone as follows [16]:
-: diameter less than 8 mm, not sensitive;
+: sensitive, diameter 9-14 mm;
++: very sensitive, diameter 15-19 mm;
+++: extremely sensitive for diameter larger than 20 mm.

Conclusion:

Lemon (*Citrus limon*) has received special attention over the past few years both for its antibacterial properties, mainly attributed to the presence of limonene. The findings of the present study highlight the promising role of Lemon essential oil as good candidates for further research to develop a new alternative antibacterial drug against pathogenic bacteria.

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