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Chemical Composition and Antibacterial Activity of the Essential oil from *Aloysia citriodora* Leaves (Verbenaceae) cultivated In Morocco

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Abstract

Objective: The objective of the present study was to determine the chemical constituents, the antibacterial activity of essential oils isolated by hydrodistillation of *Aloysia citriodora* against bacterial strains.

Methods: The phytochemical characterization of essential oil was evaluated using gas chromatography-flame ionization detector and gas chromatography-mass spectrometer analysis. Antibacterial activity of the oil was tested against four bacterial strains responsible for nosocomial infections: *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Citrobacter koseri* using disc diffusion method.

Results: Thirty six components were identified representing 91.44 % of the total oil composition. The yield of essential oil was 0.19 % and the predominant components were : limonene (23.40%), geranial (10.42 %), neral (7.30%), cineol (7.17%), α -zingiberene (4.76%), β-caryophyllene (5.19%), geranyl acetate (4.67%), arcurcuminne (3.64%), + trans-β-ocemene (3.60%), methyl-6-heptene- 5-one-2 (3.39%), sabinene (2.73%). Antibacterial activity of the oil showed the higher activity against all bacterial strains tested.

Conclusions: The essential oil extracted from lemon balm can be used to clean the environment of reanimation polyvalent and anesthesia service.

Keywords: *Aloysia citriodora*, verbena, essential oils, chemical composition, Morocco, antibacterial activity.

INTRODUCTION

The fragrant verbena, *Aloysia citriodora* (formerly *Lippia citriodora*) belongs to the Verbenaceae family. It is native to Chile and was introduced to Europe around 1784.

The genus *Aloysia* belongs to the family of Verbenaceae and consists of around 200 species of herbs, shrubs and small trees, often aromatic [1]. The species *Aloysia citriodora* or *Lippia citriodora* is commonly called Lemon Verbena, Verbena grass Louise, Arabic tea and lemongrass. This plant grows spontaneously in South America, especially in Argentina and Chile. In Morocco, this species has been cultivated for more than a century and has been used in folk medicine as herbal tea preparations, for its antispasmodic, digestive, stomach, sedative and antipyretic properties. The essential oil extracted from the dried leaves of *A. Citriodora* is indicated for anxiety, stress, insomnia, certain depressions, nervous fatigue, multiple sclerosis, psoriasis, tachycardia, rheumatism, enterocolitis, Crohn's disease, anorexia, dyspepsia, intestinal parasites (amoebiasis and amoebic cysts) and prevention of asthma attacks [2**,** 3]. The wide range of biological activities in essential oils could be generally correlated with chemical composition. Consequently, this biological difference can be explained in part by the variation in their chemical composition [4]. It is well established that sesquiterpenoids and their derivatives are credited with many biological activities such as anti-inflammatory, antibacterial, antiasthmatic and antifungal properties [5]. Thus, structure-activity relationships describe broad classes of activities for the different chemical groups of molecules present in essential oils.

Several authors have reported the therapeutic properties of lemon verbena and its essential oil. According to [6], verbena acts against anxiety and insomnia. It also offers antioxidant activities thanks to the presence in the composition of certain phenols [7, 8]. The leaves have a febrifuge effect, nerve painkillers, anti-anemia and are carminative. Verbena is also tonicardiac, hypoglycemic and anti-migraine [9].

In the present study, we evaluated the antibacterial activities and the chemical composition of essential oil. *A. citriodora*. distilled from the leaves dried in the shade harvested during the summer season (May-2016) in the regions from Fez in morocco.

Use of its plant in traditional Moroccan medicine

Morocco is one of the most producing countries of verbena. Native to the Andes and the South American cordillera, this shrub has been used for more than four centuries as an ornamental, aromatic and medicinal plant on the edge of the Mediterranean basin.

In Morocco, it is mainly cultivated around the towns and villages of the Marrakech region. The most common use of this aromatic plant was the preparation of infusions.

the families of plants most used in Morocco in the treatment of the anxiety problem identified during a survey [10], are the Lamiaceae with a frequency of use of 99 and represented by 13 species is 16, 88% of the species mentioned, Asteraceae with 37 as frequency of use, followed by Verbenaceae (34). The analysis of the results obtained by this ethnobotanical survey made it possible to identify around twenty plants used mainly in Morocco to treat manifestations of anxiety, in particular veirveine or *Aloysia citrodora*. the results of another study show that this plant is rich in polyphenols, flavonoids, flavones and flavonols [8].

Scientific name	Taxonomy	Vernacular name	traditional use	Picture
Aloysia citrodora	Ordre: *Lamiales* Famille:*Verbenaceae* Genre: *Alyosia* Espéce: ***Aloysia citrodora***	Lwiza	Antispasmodic Anxiolytic Antidiarrheal Antioxydant Anti-Inflammatory	

Table 1: Taxonomy and traditional use of *alyosia citriodora*

MATERIAL AND METHODS

- ***Plants material and isolation of the essential oils***

The plant material was collected in Morocco in May 2016 (***Table :1***). A voucher specimen was deposited in the laboratory of the University of sciences from Fés. Batches of 200 g of fresh leaves were submitted to hydrodistillation for 2h using a Clevenger-type apparatus; according to the European Pharmacopoeia [11]. after decantation, the oils were dried with anhydrous sodium sulfate and stored at 4 °C until used. The essential oil yield was determined relative to the dry matter, evaluated from a 30 g sample dried 48 hours in an oven at 60 ° C.

- ***Gas chromatography analysis***

The isolated oil was diluted with hexane (dilution ratio10:100), and 1mL was sampled for the gas chromatographic analysis. Trace gas chromatograph (GC) (ULTRA S/N20062969, Thermo Fischer), gas chromatograph equipped withHP-5MS non polar fused silica capillary column(60 m × 0.32 mm, film thickness 0.25mm) was used. Operating conditions: oven temperature program from 50°C (2 min) to280°C at 5 C/min and the final temperature kept for 10 min; 2"split mode"ratio 1:20; carrier gas Azoth (N),flow rate 1 mL/min; temperature of injector and detector (flame ionization de-tector) werefixed at 250°C and 280°C, respectively.

- ***Gas chromatography–mass spectrometry (GC–MS)***

The analysis of the volatile constituents was run on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729), using an HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, 0.25mm film thickness). The operating condition of GC oven temperature was maintained as: initial temperature 40°C for 2 min, programmed rate 2°C/min up to final temperature 260°C with isotherm for 10 min; injector temperature 250°C. The carrier gas was helium, flow rate 1 mL/min. Samples were run in hexane with a dilution ratio of 10:100.The volume of injected specimen was 1mL of diluted oil, splitless injection technique; ionization energy 70 eV, in the electronic ionization mode; ion source temperature 200°C, scan mass range of m/z40–650 and interface line temperature 300°C. Components identification was made by determination of their retention indices (KI) relative to those of a homologous series of n-alkanes (C8–C20) (Fluka, Buchs/sg, Switzerland) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0) and the bibliography[12].

- ***Antimicrobial activity assessment***

Microorganisms included *Pseudomonas aeruginosa* (P. aeruginosa), *Klebsiella pneumonia* (K.pneumonia), *Staphylococcus aureus* (S. aureus) and *Citrobacter koseri* (C. koseri). These bacteria were isolated in hospital environment from clinical patients in reanimation service (CHU, Morocco).

For the bacteria sensitivity testing test experiments, we used the agar disc diffusion method as previously mentioned [13,14]**.** Each stock of microorganisms was suspended in Mueller-Hinton broth (MH) and then incubated at 37° C for 18-24 hours. The overnight cultures were diluted and adjusted to obtain a density of 108 CFU / mL (0.5 McFarland turbidity standard). They were inoculated by flooding on the surface of MH agar and 6 mm in diameter, and sterile filter discs of Whatman No. 3 paper were impregnated with 15 mg / disc of the main part of the milk powder delivered in the inoculated agar (HD). The plates were incubated for 18 h at 35 ° C. The antimicrobial activity was evaluated by measuring the zone of inhibition relative to the microorganisms tested. The antibiotic disks of imipenem (IMP), cefaclor (CEF), oxaciline (OXA), vancomycin (VAN) are the standard antibiotics for comparison. The tests were carried out in duplicate. The ANOVA test was used to determine if there are significant differences between all of the inhibition tests.

RESULTATS ET DISCUSSIONS

- ***The yield and the organoleptic properties essential oils of verbena***

The organoleptic properties noted of the essential oil of *Aloysia citrodora* extracted by hydrodistillation are summarized in (***Table:2***). These characteristics are in accordance with those reported by [15] who analyzed the essential oils of *Lippia citrodora*.

The average yield provided by extraction of the HE from our sample is enlarged lower than that. recorded by [16] by studying *Lippia grandis* from the Brazilian Amazon, which is around 2.1%.

The essential oils of scented verbena *Aloysia citrodora* are sought after by the cosmetic and pharmaceutical sectors, for the various therapeutic and aromatic virtues reported by many authors such as [17,18].

Generally, the yield of essential oils differs from one botanical family to another, from one species to another and even in plants of the same species. In addition, this difference in HE content can be linked to several factors such as: The geographic collection area, the climate, the time of collection, the extraction method [19].

- ***Chemical composition of Verbena essential oil by CPG-MS:***

The chemical analysis of the essential oil allowed us to identify 36 compounds with a percentage of 91.44 % (***Table 3***).

The results obtained have enabled us to identify the majority compounds of the essential oil of Lippia Citriodora which are: limonene (23.40%), geranial (10.42 %), neral (7.30%) (***Figure 1***), cineol (7.17%), α - zingiberene (4.76%), β-caryophyllene (5.19%), geranyl acetate (4.67%), arcurcuminne (3.64%), + trans-β-ocemene (3.60%), methyl-6-heptene- 5-one-2 (3.39%), sabinene (2.73%).

These results compared to those of *Aloysia citriodora* in Argentina shows qualitative and quantitative differences in the composition of HE). The main components were limonene (7.00%), geranial (22.70%), carvone (0.80%) and caryophyllene oxide (0.90%) [20].

In Portugal, [21]; highlighted variations in the chemical composition of verbena HE depending on the distilled organ. Geranial (26.8-38.3%), mineral (20.8-29.6%) and limonene (5.7-20.6%) are present in the leaves and flowers. 1-octene, 1-octene-3-ol, p-cymene, (Z) -β-ocimene and trans-carveol, are only identified in the essential oil extracted from flowers. While β-citronellene, β-pinene, neryl acetate and trans-calamene are found only in the essential oil from the leaves.

In Turkey, [22]; noted that the verbena HE from the leaves contains 14.8% limonene and 17.9% citrals while that from leafy branches contains 18.6% limonene and 27.9% citrals.

In Egypt, [23]; found in the essential oil of verbena scented cultivated in Egypt: d-limonene (6.3-16.2%), 1.8 cineole (4.7% - 7.3%) and citral (19.9% -28.8%).

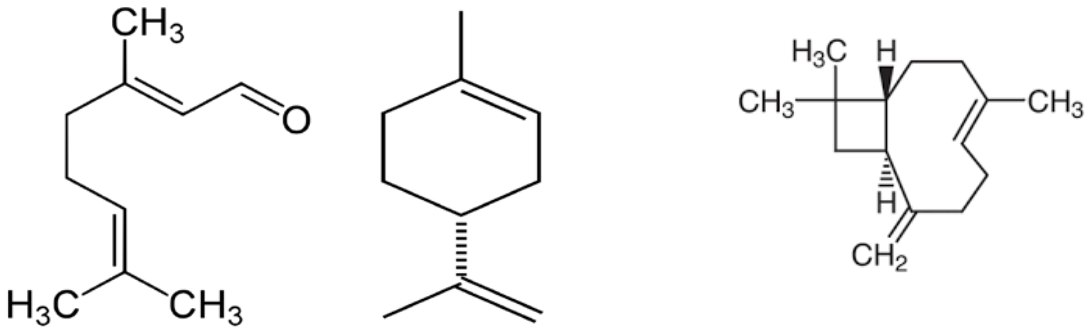
[24]; found in HE of verbena cultivated in Jordan limonene (17.7%), geranial (10.1%) and mineral (9.8%). These latest results are close to ours, especially with regard to limonene and citrals.

Table 2 : Organoleptic characteristics of essential oil of Lippia citriodora.

Essential Oil From	Aspect	Color	Odour	Flavor	Yield
Aloysia citriodora	Mobile liquid	Yellow	Pleasant lemon	Sweet	(0,195 ± 0.0007) %

Table 3: Chemical composition of the essential oil from leaves of *A.citriodora*

Compound	%	Compound	%
α-Pinene+ α-Thujene	1.19	β- Caryophyllene	5.19
β - Pinene	0.13	β - Cedrene	0.24
Sabinene	2.73	Alloaromadendrene	0.58
Myrcene	0.40	Neral	7.30
Limonene	23.40	α-Terpineol	0.98
Cineole	7.17	Zingiberine + Germacrene D	4.76
Cis β - Ocimene	0.19	Geranial	10.42
Trans, B-Ocimene + γ-Terpinene	3.60	Geranyl Acetate	4.67
Para Cymene	0.09	Bicyclogermacrene+B-Curcumene	1.07
6-Methyl-5-Hepten-2-One	3.39	Cadinene	0.28
Rosefurane	0.13	Arcurcumene	3.64
Trans-4-Thujanol	0.36	Nerol	1.50
Citronnellol	0.37	Geraniol	1.82
Cis,Cis-Photocitral	0.30	Isocaryophyllene Oxide	0.27
α- Copaene	0.61	Caryophyllene Oxide	1.42
β - Bourbonene	0.12	Trans-Nerolidol	0.53
Linalol	1.10	Spathulenol	1.02
Terpinene-4-ol	0.19	Cadinol	0.28
Total Identified			**91.44 %**



Geraniol **Limonene** **β- Caryophyllene**

Figure 1: Some examples of chemical compounds in verbena essential oils

Table 4 : Antibacterial effect of EO on bacterial strains tested by the microdilution method.

Antibiotics / **Bacteria**	**IMP**	**CEF**	**OFX**	**VAN**	**Verveine**
K. Pneumoniae (gram-)	22mm	24.5mm	27mm	0mm	**10 ± 1.2mm**
P. aeruginosa (gram-)	27mm	7mm	12.5mm	11.5mm	**17± 0.24 mm**
S. aureus(gram+)	40mm	12mm	19mm	14.5mm	**25± 0.35mm**
C. koseri (gram-)	12mm	0mm	9.5mm	11mm	**12.4 ± 1.3mm**
E.coli(gram-)	31mm	16mm	0mm	7.5mm	**24.6± 0.07mm**

In other studies, the level of citrals reaches high levels. This is particularly the case in Iran, with a geranial rate of 30.67 to 36.87% and a general rate of between 21.71 and 28.33% [25].

Also note that citronellol, which represents a percentage of 0.37% in our study, reaches, in another study, the percentage of 8.87% [26], and a percentage of 0.47% [27].

The chemical variability of oils has so far been discussed with regard to the geographical origin of *A. Citriodora* [28], but the composition of essential oils from aromatic plants is also linked to their stage of development. Previous work has reported the presence of the same main components (limonene, neral, geranial) found in this work, but with significant differences in their compositions [26, 27,29,30].

Antibacterial activity

Analyzing the results in (***Table 4***), it is observed that all the bacteria exhibit resistance against HE and also against control antibiotics. The two bacteria *K. pneumoniae* (10.0 ± 1.2) and *C. koseri* (12.4 ± 1.3 mm) seem to be the most resistant to EO from Verbena. *P.aeruginosa* and *E. coli* show resistance similar to the palatial EO with a value of (17 ± 0.24) in a study, *A. citriodora* oils showed no activity against *P. aeruginosa* and have shown that this bacterial strain is naturally resistant [31], [32] for the oil fraction was inactive against *E.coli* bacteria. A value (24.6 ± 0.07 mm) against EO of verbena. While the bacterium *S. aureus* (***Figure 2***) has a low resistance against EO of the plant with a value of (25 ± 0.35mm),to [33] have found some traces.

In the agar disc diffusion method [34] the microorganisms *P.aeruginosa*, E.coli and *S. aureus* showed significant sensitivity (diameter of the inhibition zone> 10 mm) with *A. citriodora*.

Similar results were also found by the tests of the majority of the antibiotics used in particular, Ceftizoxime (CEF), Ofloxacin (OFX), Vancomycin (VAN) and Imipinine (IMP). *K. pneumoniae* which seems resistant to all antibiotics except (VAN). *P.aeruginosa* which has a strong resistance against CEF (7mm) while it is sensitive to other antibiotics. *S. aureus* which is sensitive to all antibiotics. *C.koseri* seems very sensitive to (CEF) and less sensitive to other antibiotics. *E. coli* seems very sensitive to OFX and less sensitive to other antibiotics. However, we can say that only Imipenem reveals a very important antibacterial activity against all bacterial strains.

Figure 2: Antibacterial effect of essential verbena oils on the bacterium *Staphylococcus aureus*

CONCLUSION

Finally, this study highlighted the in vitro antibacterial activity of A. essential oils. Citriodora. Based on these results, this natural species has antimicrobial activities which depend on the resistant strain. These species seem likely to be potential candidates for anti-infective therapies. However, in vivostudies are necessary in order to assess all their antibacterial capabilities. Very rich in terpene aldehydes (geranial and general for 17.72%), various terpene and sesquiterpene hydrocarbons, including limonene, trans β-ocimene, β-caryophyllene, germacrene D and arcurcumene, as well as a large number of oxygenated compounds and a wide range of alcohols (linalool, citonnellol, nerol, etc.) and some esters. This study allowed us to prove the effectiveness of the essential oil of this plant (A.citriodora) whose active ingredients can be used in several fields such as the food and pharmaceutical industries.

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