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Research Article

Antibacterial Efficacy of Essential Oils from Three Moroccan Plants (*Lavandula officinalis, Origanum majorana* and *Thymus vulgaris*) Against Clinical Isolates

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ABSTRACT
Traditional medicine is widespread in the world and it is the almost exclusive source of primary health care for 65% of the world's population. The essential oils possess antibacterial, antifungal, antiviral, antioxidant and wide spectrum of pharmacological activities. The aim of this study was to evaluate the antimicrobial activities of *Lavandula officinalis, Origanum majorana* and *Thymus vulgaris* essential oils against pathogen bacteria as well as to compare its inhibitory effect versus commercial antibiotics. No studies have approached this aspect in hospitals of Fez city. The essential oils has been obtained by hydrodistillation using a Clevenger apparatus and it has been tested in vitro against bacteria, isolated from surfaces in Hemodialysis service at El Ghassani Hospital in the Fez city, such as *Staphylococcus aureus*, *bacillus sp* and *Streptococcus sp* using the paper disk agar diffusion method and the macrodilution assay for determination of Minimum Inhibitory Concentrations (MIC). The essential oils of *Origanum majorana* and *Thymus vulgaris* showed stronger antibacterial effects than the *Lavandula officinalis* essential oil. Among the commercial antibiotics, Ofloxacin had the widest coverage against all bacterias but the Amoxicilin antibiotic had shown a poor activity against all bacterias. Our results suggest that essential oils of *Origanum majorana* and *Thymus vulgaris* could be used for the development of new types of antibacterial agents and may therefore be used as therapeutic or disinfection compounds against these bacteria.

Keywords: Hospital Environment, Surface, *Origanum majorana*, *Thymus vulgaris*, *Lavandula officinalis*, Antibacterial effect, Fez-Morocco.

INTRODUCTION

The hospital environment is usually colonized by many micro-organisms and is composed of true ecological niches. Contamination by these microorganisms is diffuse and the procedures necessary to control such contamination is complex and expensive[1]. The surfaces of some hospital services in the city of Fez were found recently contaminated by many microorganisms as: coagulase negative *Staphylococcus*, *Bacillus sp*, *Staphylococcus aureus*, *Aeromonas salmonicida*, *Echerichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas vesicularis*, *Serratia rubidaea*, *Acinetobacter baumannii* and *Klebsiella sp*[1-3]. Much less is known still about microorganisms reduced sensitivity to germicides such as surfaces disinfectants. There have been some studies indicating that imprudent and inappropriate uses of chemicals, especially synthetic disinfectants, can encourage the beginning stages of resistance[4]. However, this problem has been much more serious in the last years with the increase in the prevalence of infections caused by multi-drug-resistant (MDR) strains[5]. Emergence of MDR is a phenomenon occurring worldwide, due to the selective pressure exerted by extensive use of antibiotics and disinfectants and that has hindered the infectious illness therapy. Traditional medicine has been an important source of products for developing countries in treating common infectious diseases[6]. Essential oils from aromatic and medicinal plants are potentially useful as antimicrobial agents and their use as medicines has long been recognized[7]. Excellent review papers are available on this subject in the specialist literature[8,9]. Volatile compounds from leaves, stems, flowers and fruits of various plants

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have been reported to have antimicrobial activity[10-12]. *Origanum* is an important multipurpose medicinal plant which belongs to the family Lamiaceae, tribe Mentheae and comprises of 42 species and 18 hybrids widely distributed in Eurasia and North Africa. Several essential oils of the gender of Origanum, especially *Origanum majorana*, have proved use in medical and industrial applications[13-15]. The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants all over the world. In Morocco, the thyme has been used traditional medicine for the treatment of diarrhoea, fever, cough, infected areas and wounds. It was also used as a tonic and stimulant[16,17] and, generally, for its anti-inflammatory properties after topical or oral administration[18,19]. *Thymus vulgaris* L. (thyme), is known for its many antimicrobial activities[20,21]. Lavender, also known as medicinal lavender, true lavender, or common lavender (*Lavandula angustifolia*, *Lavandula officinalis*, *Lavandula vera*), is an evergreen perennial plant. Lavender is native to the Mediterranean region (France, Spain, Andorra, and Italy)[22], but is grown in many other countries of the world, including Morocco. *Lavandula* species are employed in perfumery and cosmetics[23]. *L. officinalis* is used as bactericide to disinfect the hospitals and sick rooms in ancient Persia, Greece and Rome[24]. This work was carried out in order to evaluate the in vitro antibacterial properties of *Lavandula officinalis*, *Origanum majorana* and *Thymus vulgaris* essential oils against three bacterial isolated from surfaces of Hemodialysis service at El Ghassani Hospital in the Fez city strains as well as to compare its inhibitory effect against commercial antibiotics. No studies have approached this aspect in services of hospitals of Fez city.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves, stems and wood) of *Thymus vulgaris (T. vulgaris), Origanum majorana* (*O. majorana*) and *Lavandula officinalis* (*L. officinalis*) were collected in Taounate Province from north east of Morocco between April and June 2014. The botanical identification and authenticated voucher specimens have been deposited in the Herbarium of the National Institute of Medicinal and Aromatic Plants, Sidi Mohamed Ben Abdellah University, Fez, Morocco.

Extraction of the essential oils

Samples of 100g of the fresh aerial parts of *O. majorana*, *T. vugaris* and *L. officinalis* were subjected to hydrodistillation for 2 hours using a Clevenger apparatus; the obtained Essential Oil (EO) was stored at 4°C so that it can be used in the upcoming experiments. Our bibliographical investigations of vernacular names, medicinal uses and major's compounds of Moroccan plants are summarized in Table 1.

Antimicrobial activity

Microorganisms

The antimicrobial activity of essential oils of the plants was tested against Gram- Positive bacterial strains *Staphylococcus aureus* (*S. aureus*), *Bacillus sp* and *Streptococcus sp.* These bacteria have been isolated from surfaces at Hemodialysis service at El Ghassani Hospital in the Fez city. They have been identified and confirmed by classical biochemical gallery and the API (bioMérieux, France) in Microbiology Unit at the Regional Diagnostic Laboratory Epidemiological and Environmental Hygiene (RDLEH) falling within Regional Health Directorate of Fez. This laboratory follows the requirements of the NM ISO 17025 since 2008.

Disc diffusion assay

Antimicrobial susceptibility test of the essential oils was tested against the mentioned Gram-positive right above, Gram negative bacteria by disc diffusion method[8]. The susceptibility tests were performed on Muller–Hinton Agar, 10 µl of essential oil was diluted in two volumes of 5% dimethylsulfoxide (DMSO) and impregnated on the filter paper discs and used for the study. Amoxicillin (10 µg/disc) and Ofloxacin (5 µg /disc), were used as positive reference standards to determine the sensitivity of the tested strains and 5% DMSO was used as blind control. Finally, the Petri dishes inoculated, were incubated at 37°C for 24 h and the inhibition zones were observed including the diameter of the disc (6 mm) according to the guidelines of the Antibiogram Committee of the "Société Française de Microbiologie" (CA-SFM)[30]. If the inhibition zone exceeds 15mm in diameter, we consider the antimicrobial activity as very good. If the diameter is between 15mm and 8mm, the antibacterial activity is average. For diameters below 8mm, the antibacterial activity is weak. All experiments were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimal Inhibition Concentration (MIC) values were evaluated according to published procedures[31-33]. The tested oil was dissolved in 5% DMSO to obtain 1000 µl/ml stock solution. 0.5 ml of stock solution was incorporated into 0.5 ml of Mueller Hinton broth to get the concentration of 500µl/ml and serially diluted to achieve 1.56, 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml. Fifty microliters of standardized suspension of the test organism was transferred onto each tube. The control tube contained only organisms and not the essential oil and 5% DMSO was used as blind control. The culture tubes were incubated at 37 °C for 24 hours. The lowest concentrations, which did not show any growth of tested organisms after macroscopic evaluation was determined as MIC.

Statistical analysis

The antimicrobial results are expressed as mean ± SD. One-way analysis of variance (ANOVA).

RESULTS

Disc diffusion study

The *in vitro* results of antibacterial activity of the EOs of *O. majorana, L. stoechas* and *T. vulgaris* by the paper disk agar diffusion method against three (03) microorganisms of significant importance are summarized in Table 2. The EOs of *O. majorana* and *T. vulagris* inhibited the growth of bacterial strains, producing a zone diameter of inhibition (ZI) from 31.9±5 to 51.75±6.5 mm. The essential oils of *O. majorana* and *T. vulgaris* exhibited notable antibacterial activity against all the bacteria species tested.

Table 1: List of selected essential oils and their properties in Morocco.

Species	Vernacular name	Medicinal uses in Morocco	Major compounds of plants from Morocco origins
Origanum majorana	El-مرددوشmerdedouch	asthma, headache, and rheumatism Fever, cough, flatulence[25]	4-terpinene (28.96%), γ-terpinene (18.57%), α -terpinene(12.72%) and sabinene(8.02%)[26]
Thymus vulgaris	Zaatar- الزعتر-	diarrhoea, fever, cough, anti-inflammatory[16-19] expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties[27]	Thymol (41,4 %,), γ-terpinène 22,25%, p-cymène 15,59%, α -terpinene (3,25)[28]
Lavandula officinalis	El-الخزامةkhozama	Renal diseases, Rheumatism, system digestive[25]	linalyl acetate (44. 96%), linalool (44.64%), Caryophyllene oxide (3.15%) Camphor (2.66) Borneol (2.51)[29]

Table 2: Antimicrobials activities of essentials oils and commercial antibiotics determined by the agar diffusion method.

Microorganisms	Inhibition zone diameter (mm)				
	Essential oils			Antibiotics	
	Lavandula officinalis	*Origanum majorana*	*Thymus vulgaris*	Ampicillin (10µg/Disc)	Ofloxacin (5µg/Disc)
Staphylococcus aureus	14.25±3.4	47.08±8	39.5±3.6	NI	20.4±3.2
Bacillus sp	16.33±4.1	51.75±6.5	34.5±7.6	8.2±0.8	25.4±3.2
Streptococcus sp	10.33±2.8	31.9±5	30±4.2	7±0.6	21.6±2.2

- Values represent averages ± standard deviations for triplicate.
- Inhibition zone including disc diameter (6 mm).
- NI: No Inhibition

Table 3: Antimicrobial activity of three EOs using macro- dilution method.

Microorganisms	MIC (mg/ml)		
	L. officinalis	*O. majorana*	*T. vulgaris*
Staphylococcus aureus	ND	25	25
Bacillus spp	ND	25	3.125
Streptococcus spp	ND	100	100

-ND: Not Determined

In our investigations the essential oil of *L. officinalis* from north east of Morocco exhibited the highest antimicrobial activity against *Bacillus spp* with diameter inhibition value equal to 16.33±4.1mm, and showed moderate activities against *S. aureus* (Figure 1(A)) and *Streptococcus sp* by inhibition zone of 14.25±3.4 and 10.33±2.8 mm respectively. The inhibition zone of Ofloxacin against *S. aureus* (Figure 1(B)), *Bacilus sp* and *Streptococcus sp* showed between 20.4±3.2 and 25.4±3.2 mm. The bacterium *S. aureus* had a resistance for Ampicillin, *Bacillus sp* and *Streptococcus sp* bacteria's had a very low sensitivity for Ampicillin (8.2±0.8 and 7±0.6 respectively).

Determination of Minimum Inhibitory Concentration (MIC)

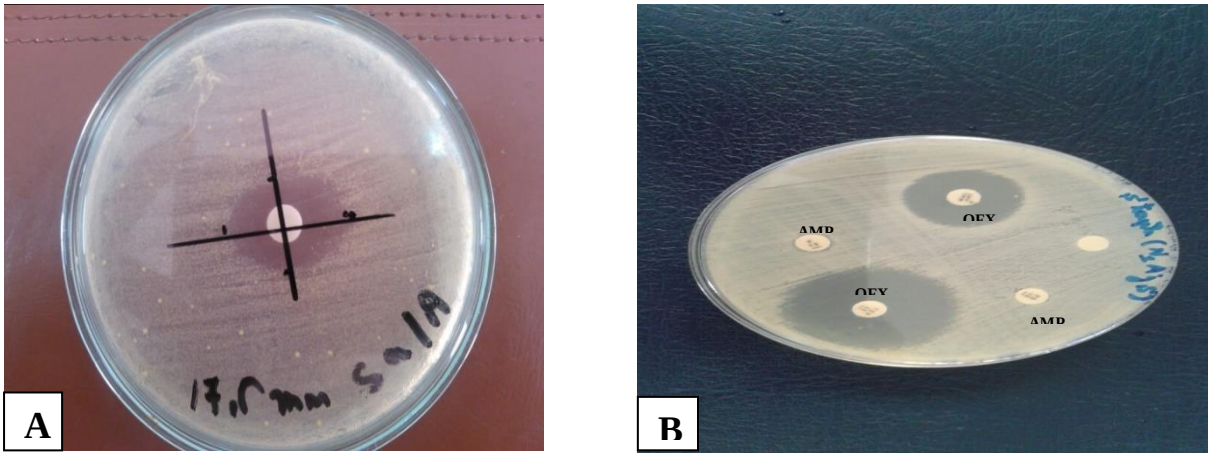
From Table 3, it can be observed that all the tested microorganisms were susceptible to the action of *O. majorana* and *T. vulgaris* essential oils, with a range of MIC values from 3.125 to 100 mg/mL. *O. majorana* EO exhibited antimicrobial activity against all tested strains. Inhibition values of MIC=25 mg/ml, 25 mg/ml and 100 mg/ml for *S. aureus*, *bacillus spp* and *Streptococcus spp* respectively.

DISCUSSION

In a first set of experiments, antimicrobial activity was evaluated by the disc diffusion method. EOs of *O. majorana* and *T. vulgaris* also found to be more effective (between 30±4.2 and 51.75±6.5 mm inhibition diameter) than Ampicillin and Ofloxacin antibiotics (between 7±0.6 and 25.4±3.2 mm) to inhibit *S. aureus*, *Bacillus spp* and *Streptococcus spp*. About *L. officinalis*, antimicrobial activity of this oil against *Bacillus spp* with diameter inhibition value equal to 16.33±4.1mm, and showed moderate activities against *S. aureus* and *Streptococcus sp* by inhibition zone between 10.33±2.8 and 14.25±3.4 mm. Reported the essential oils with the greatest spectrum of activity against all bacteria's as following order: *Origanum majorana* > *Thymus vulgaris*> Ofloxacin> *Lavandula offcinalis*> Ampicillin (Table 2).

Antimicrobial activity of Origanum majorana

In Morocco, *O. majorana* is commonly known by the name of "Merdedouch" or marjoram and is frequently used for culinary and medicinal purpose (Table 1). It is cultivated for its aromatic leaves, which have commonly been used in fresh and dried forms as a spice or condiment in various cuisines[34]. The EO was noted to be active against all microbial strains but in different degrees. The data indicated that the ZI from 31.9±5 mm 51.75±6.5 mm with MIC from 25mg/mL to 100 mg/mL for bacterial strains. Our results were greater than that reported by



(A): Antibacterial activity of *L. officinalis* essential oil against *S. aureus* (ZI=17.5mm)

(B): Antibiogramme of Ampicillin (AMP) and Ofloxacin (OFX) (22.1 and 19.5 mm) against *S. aureus*

Figure 1: Disc diffusion study on *S. aureus.*

Hajlaoui et al[34] with ZI from 8±0 mm to 18.33±0.57 mm. The antimicrobial properties of EO have purportedly been associated with the high proportion of oxygenated monoterpenes and especially to their major constituents, such as 4-terpinene, γ terpinene and α-terpinene. Other compounds such as β-caryophyllene, and sabinene are also known to have efficient antimicrobial properties[8,35]. Terpenes are thought to be inducing alterations in cell permeability by entering between the fatty acyl chains that make up the membrane lipid bilayers, thus disrupting lipid packing and causing changes to membrane properties and functions[36,37].

Antimicrobial activity of Thymus vulgaris

Several studies have shown that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities[38]. The antifungal and antibacterial activity exhibited by *Thymus* genus essential oil has been demonstrated by several researchers[39-43]. *Thymus vulgaris* L. (thyme) is known as one of the most important species, and is used in the food, cosmetic and pharmaceutical industries[44], with a large number of studies providing evidence for its antimicrobial (especially antibacterial) effects under in vitro conditions[45,46]. In Morocco, the thyme is represented by many species of which certain are endemic. *Thymus vulgaris* L. (thyme), locally known "zaatar" or "zaitra', is widely used in Morocco folk medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties[27]. The authors also studied the chemical composition of this essential oil. Due to the fact that thymol was the most abundant substance (48.1%), followed by p-cymene (15.6%) and γ -terpinene (15.4%), they concluded that the registered antibacterial activities could be associated with the presence of thymol[47]. Later, Sienkiewicz et al[48] showed that *T. vulgaris* essential oil exhibited extremely strong activity against 120 strains of *Staphylococcus*, *Enterococcus*, *Escherichia*, and *Pseudomonas* genera, isolated from patients with infections of the oral cavity, respiratory, genitourinary tracts, and from the hospital environment and clinical staff. The antibacterial activity was tested using the agar diffusion method. *Staphylococcus* strains were found to be the most sensitive, while *Pseudomonas* bacteria were found the most resistant.

Antimicrobial activity of Lavandula officinalis

L. officinalis is used as bactericide to disinfect the hospitals and sick rooms in ancient Persia, Greece and Rome[24]. We have not found many works regarding the antimicrobial effects of *L. officinalis* against bacteria's. Among these works, *L. officinalis* also showed higher activity against *Citrobacter*. In contrast, *S. aureus* showed resistance to both essential oils[49]. Lis Balchin and Deans[50] showed that Lavandin, Frensh lavaneder and Bulgarian lavender essential oils all have activity against a large number of bacteria and fungi. Essential oils from plants of the genus *Lavandula* exhibit a broad spectrum of biological activities[51]. The essential oil of *Lavandula dentate* has an inhibitory effect on the growth of bacteria, including *Salmonella*, *Enterobacter*, *Klebsiella*, *E. coli*, *S. aureus*, and *L. monocytogenes*. In turn, the essential oil of *L. bipinnata* exhibits antibacterial properties (against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*) and antifungal properties (against *A. niger*, *P. notatum*, *C. albicans*) at concentrations of 0.5–2.0μg/ml for bacteria and 2.0–4.0 μg/ml for fungi[52]. In fact, the diameter of the growth inhibition zone can be affected by the major compounds, the solubility of the essential oil and the diffusion range in the agar[53].

CONCLUSION

The antimicrobial activity, *in vitro,* of the essential oils of *L. officinalis*, *O. majorana* and *T. vulgaris* against bacterial isolated from surfaces at Hemodialysis service at El Ghassani Hospital was determined. However, the essential oils of *L. officinalis* which has the smallest inhibition zone compared to the other essential oils and Ofloxacin antibiotic. Penicillin antibiotic has showed poor activity against all bacteria's. So, the application of essential oils in the treatment pathogens, which is suggested by traditional medicine, may be an interesting alternative to

synthetic drugs that show many side effects. Moreover, the essential oils of these plants should be investigated in vivo for better understanding of their safety, efficacy and properties.

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