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Research Article

In Vitro Antibacterial Efficacy of Essential Oils from Moroccan Plants Against Pathogenic Bacteria Isolated from Hospital Environment in Morocco

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ABSTRACT

Even if aromatic and medicinal plants have been used from ancient times as natural therapies and are considered as alternatives to synthetic drugs, scientific investigations to evaluate antimicrobial activity of Essential Oils (EOs) are needed. The aim of this study was to evaluate the antimicrobial activities of five EOs against pathogen bacteria as well as to compare its inhibitory effect versus commercial antibiotics. The essential oils has been obtained by hydrodistillation using a Clevenger apparatus and its has tested in vitro against bacteria isolated from surfaces in nephrology service at Ibn Al khatib Hospital in the Fez city, such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia liquefaciens* and *Pseudomonas aeruginosa* using the paper disk agar diffusion method. The essential oils of *Junniperus communis* and *Artemisia absinthium* showed stronger antibacterial effects than the other essential oils. However the essential oil of *Rosmarinus officinalis* which has the smallest inhibition zone. *Pseudomonas aeruginosa* and *Serratia liquefaciens* were found to be the most resistant species, *Staphylococcus aureus* was the most sensitive bacterial species to essential oils. Among the commercial antibiotics, Chloromphenicol had the widest coverage against all bacteria's, followed by Gentamicin, but the Penicillin antibiotic has showed poor activity against all bacteria's. Our results suggest that essential oils 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, Pathogenic bacteria, Essential oils, Antibiotics, Antibacterial effect, Fez-Morocco.

INTRODUCTION

The hospital environment, colonized by many micro-organisms, is composed of true ecological niches. Contamination by these microorganisms is diffuse. Cumbersome procedures that are complex and costly are needed to master such contamination[1]. As possible causes of infection, contamination of surfaces may be mentioned, even if cross contamination by hands is probably the greatest risk. In fact, hospital surfaces colonized by different types of microorganisms constitute special ecological niches that require cumbersome, complex and costly procedures that are necessary for better safety of the patient. Bacterial infectious diseases represent a serious risk to the world population once they have been responsible for high morbidity and mortality through the times. 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[2]. Emergence of MDR is a phenomenon occurring worldwide, due to the selective pressure exerted by extensive use of antibiotics and that has hindered the infectious illness therapy. Therefore, the search for new antimicrobial agents or new compounds able to potentiate the antimicrobial activity of old antibiotics against resistant microorganisms has become an important area of research[3,4]. In this perspective, medicinal plants have presented as an important source of bio-molecules actives against different microorganism groups[5]. The technologic prospection of medicinal plants (essential oils and extracts) and their pharmacological properties is of fundamental importance to the validation of its use in traditional medicine and development of bio-products of industrial interest as well as to its own conservation of the natural resource[6,7]. Traditional medicine has been an important source of products for developing countries in treating common infections.

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

Family	Species	Vernacular name	Medicinal uses in Morocco	Major compounds of plants from Morocco origins
Lamiaceae	*Rosmarinus officinalis*	Azir أزير	Intestinal parasites, rheumatism, Kidney[14]	Camphor (6.02%), 1.8-Cineole (5.25%), Camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%)[15].
Asteraceae	*Artemisia absinthium*	Chiba شيبة	Stomach pains, heart[14].	β-Thujone (35.6%), Chamazulene (3.1%) and Linalool (1.9%) [16].
Anacardiaceae	*Pistacia lentiscus*	Drou ضرو	Digestive diseases[14, 17]	α -pinene (16.5–38.5%), β -myrcene (10.2–11.5%) and limonene (6.7–9.8%) [18].
Rutaceae	*Citrus limon*	Lūmon ليمون	Cosmetic, Headache, Fever[14]	Limonene (49%), myrcene (15.3%), δ-3-carene (8%), sabinene (5%), linalool (1.8%), carvone (1.8%) and trans-carveol (1 .6%) [19].
Cupressaceae	*Juniperus communis*	Al araar العرعار	Diarrhea, Abdominal diseases, , Bronchitis[20, 21]	α-pinene (14.2-9.7%), sabinene (12.4-16.1%), γ-terpinene (5.9- 1.7%), terpinene 4-ol (14.1, 9.1%), (*Z,Z*)-farnesol (5.4- 6.6%) and manoyl oxide (4.1- 11.7%)[22].

Morocco has a long history of traditional herbal medicine and its geographic position in the extreme north-west of Africa has favored a development of a rich flora and biodiversity[8]. Also, Moroccan medicinal plants are frequently used by the population to prevent or cure various disorders. Essential oils and their components are gaining increasing interest in the food and pharmaceutical industries as natural antioxidants, because of their relatively safe status and their wide acceptance by consumers[9]. According to Enrico et al.,[10] the essential oils, unlike antibiotics, are composed of many molecules so that bacteria cannot resist in mutant. Preventively and curatively, they are especially known for their potent antibacterial, antiviral, anti inflammatory[11], antioxidant, anti fungal[12], and larvicidal effects[13]. This work was carried out in order to demonstrate the in vitro antibacterial properties of five essential oils against five bacterial isolated from surfaces of nephrology service at Ibn Al Khatib Hospital in the Fez city strains as well as to compare its inhibitory effect versus commercial antibiotics.

MATERIALS AND METHODS

Plant material

The aerial parts (leaves, stems and wood) of *Rosmarinus officinalis (R. officinalis), Artemisia absinthium* (*A. absinthium*), *Pistacia lentiscus* (*P. lentiscus*), *Citrus limon* (*C. limon*) and *Juniperus communis* (*J. communis*) are collected in Taounate Province from north east of Morocco between April and June 2012 and they are air-dried at 40°C with forced ventilation for two days. 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 air-dried aerial parts plants were subjected to hydrodistillation for 2 hours using a Clevenger apparatus. The obtained Essential Oils (EOs) were dried over anhydrous sodium sulphate and, after filtration, stored at 4°C until realizing the test. 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 plants essential oils were tested against Gram positive bacterial strains *Staphylococcus aureus* (*S. aureus*) and Gram negative bacterial strains *Klebsiella pneumonia* (*K. pneumonia*), *Enterobacter cloacae* (*E. cloacae*), *Serratia liquefaciens* (*S. liquefaciens*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). These bacteria have been isolated from surfaces of nephrology service at Ibn Al Khatib 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 above mentioned Gram positive, Gram negative bacteria by disc diffusion method[23]. The susceptibility tests were performed on Muller–Hinton Agar, 10 µl of essential oil was diluted with two volumes

of 5% dimethylsulfoxide (DMSO) and impregnated on the filter paper discs and used for the study. Amoxicillin (10 µg/disc), Chloromphenicol (30 µg /disc) and Gentamicin (10 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 dishs 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)[24]. 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.

Statistical analysis

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

RESULTS AND DISCUSSION

The *in vitro* results of antibacterial activity of the EOs of *R. officinalis*, *A. absinthium*, *P. lentiscu, C. limon* and *J. communis* by the paper disk agar diffusion method against five (05) microorganisms of significant importance are summarized in Table 2.

Antimicrobial activity of Rosmarinus officinalis EO

Rosemary (*R. officinalis*) has been harvested for countless years for use in food[25]; also the essential oils (Eos) and water soluble extracts have been used for preserving food and as a treatment against many different kinds of illness (Table 1). On the species of *S. aureus* and *E. cloacae* (11 ± 3 and 9±0.6 mm respectively) tests showed an inhibition zone between 8 and 15 mm, but is very week against *K. pneumonia*, *S. liquefaciens* and *P. aeruginosa* (inhibition zone <8 mm). Several authors have described *S. aureus* and *E. coli* as being very resistant bacterial strains, which might explain the lack of any effect of rosemary EO in our study[26]. This affirmation underlines the influence of the chemical composition, or even of the different proportions of the oil components, on the efficacy of rosemary EOs against the bacterial strains under study[27- 31].

Antimicrobial activity of Artemisia absinthium EO

A. absinthium, species of the family of asteraceae, known well for her medicinal virtues, possesses multiple aromatic properties. The chemical analysis of its essential oil showed that it is rich in thujone (Table 1). Used in the diseases of the stomach, to provoke rules, in the fight against the laziness, against the seasickness and her nausea, it is also used as a vermifuge, an insecticide, in the treatment of chronic fevers and for the inflammation of the liver, as an antispasmodic and antiseptic[32]. Recently the antifeedant, antiparasitic and antioxidant effects of *A. absinthium* L. were evaluated by Azucena Gonzalez-Colomaa et al[33]**.** The essential oil of *A. absinthium* indicated significant activity against *Staphylococcus aureus* (24.7±4.9 mm) and moderate inhibitory activity against *K. pneumonia* and *E. cloacae* (14.3±2.4 and 13.3±2.4 mm respectively). This oil has been reported to be weakly inhibitory against *S. liquefaciens* and *P. aeruginosa* (8 and 7.3±0.4 mm respectively). In agreement with our results, the EO of *A. absinthium* in Canada has a high activity against *S. aureus* with a diameter of inhibition equal to 25 ± 1.4 mm[34]. Essential oil of Kairouan from Tunisia has the same activity against *S. aureus* strain with a diameter of inhibition equal to 25 ±1.13 mm[35]. Morever the EO from western Anatolia in Turkey is less active against *S. aureus* and *E. cloacae* with inhibition zone of 18 and 8 mm respectively and characterized by no inhibition against *P. aeruginosa*[36]. Although it is a more widely held point of view that the action is due to a synergistic effect between various components, whether major or minors compounds[23].

Antimicrobial activity of Pistacia lentiscus EO

P. lentiscus, which belongs to the important Family Anacardiaceae, is a dense bush with a strong characteristic aromatic odour and green leaves and is found in all the Mediterranean countries[37]. In Morocco, *P. lentiscus* L. occurs and grows wild in various regions, particularly in the mid-west of the Rif Mountains, and it used by Moroccan population for treatment of digestive diseases and its characterized by richness of α-pinene (16.5–38.5%), β-myrcene (10.2–11.5%) and limonene (6.7–9.8%) compounds (Table 1). In this study *P. lentiscus* oil showed significant bactericidal power against *S. aureus* with a diameter of inhibition equal 23±3.3 mm and did not show inhibition against *K. pneumonia.* This oil has been reported to be weakly inhibitory against *E. cloacae, P. aeruginosa* and *S. liquefaciens* (9.3±0.4, 7±0.6 and 6 mm respectively). A study realized by Prokopios M et al[38] reported that antimicrobial activity of the leaves and resin essential oils of *P. lentiscus* against *S. aureus, K. pneumonia, E. cloacae* and *P. aeruginosa* between 9 and 10 mm for leaves EO and between 18 and 28 mm for resin EO. The gram positive bacterium is more susceptible to the antimicrobial properties of essential oil than gram negative bacteria and it is considered to be due to its outer membrane[39, 40]. In fact, the diameter of the growth inhibition zone can be affected by the major compounds and the solubility of the essential oil and the diffusion range in the agar.

Antimicrobial activity of Citrus limon EO

The lemon (*C. limon*) is a flowing plant belongs to the family Rutaceae. The genus citrus comprises of about 140 genera and 1300 species[35]. The predominant active compounds with high percentage were identified in the Moroccan oil were Limonene, myrcene, δ-3-carene, sabinene, linalool, carvone and trans-carveol (Table 1). The Lemon oil is characterized by moderate inhibitory activity against *P. aeruginosa, E. cloacae* and *S. aureus* by inhibition zone between 8.3±0.4 to 12±2.6 mm and did not show inhibition against *K. pneumonia* and *S. liquefaciens*. An antibacterial study realized by Najwa N and Mohammad A[41] at different concentrations of lemon essential oils obtained from imported Turkish and Indian lemon fruits samples against four pathogenic bacteria.

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

Microorganisms	Inhibition zone diameter (mm)							
	R. officinalis (10 µl/disc)	*A. absinthium* (10 µl/disc)	*P. lentiscus* (10 µl/disc)	*C. limon* (10 µl/disc)	*J. communis* (10 µl/disc)	Amoxicillin (10µl/disc)	Chloramphenicol (30 µl/disc)	Gentamycin (10 µl/disc)
Staphylococcus aureus (Gram +)	11±3	24.7±4.9	23±3.3	12±2.6	24.6±1.1	9±0.4	17.5±1.2	17.2±2.2
Klebsiella pneumonia (Gram-)	7.6±0.4	14.3±2.4	NI	NI	11.7±2.9	NI	21±2.2	14.4±1.4
Serratia liquefaciens (Gram-)	7.3±0.4	8	6	NI	14±2.6	13.4±1.8	23.2±3.6	16.4±2.8
Pseudomonas aeruginosa (Gram-)	6.3±0.4	7.3±0.4	7±0.6	8.3±0.4	NI	8±1.5	14.4±2.2	13±1.4
Enterobacter cloacae (Gram-)	9±0.6	13.3±2.4	9.3±0.4	11.3±0.5	13.3±0.9	12± 1.4	21.2±2.6	18±1.6

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

Turkish oil gave the inhibition against *P. aeruginosa* with the range of 0-7 mm. and *S. aureus* gave inhibition with the diameter zone of 6 mm. However, Indian lemon oil gave the inhibition against the pathogenic bacteria such as *S. aureus* and *P. aeruginosa* with inhibition zone between 6 and 70 mm. Almost similar antimicrobial results were obtained from both essential oils of lemon samples by other reported results[42-44].

Antimicrobial activity of Juniperus communis EO

The oil of *J. communis* has been used for centauries as diuretic, based on its terpinan-4-ol content[45]**.** The plant is also used in folk medicine in Morocco for diarrhea, abdominal pain and Bronchitis (Table 1). In our investigations the essential oil of *J. communis* from north east of morocco exhibited the highest antimicrobial activity against *S. aureus* with diameter inhibition value equal to 24.6±1.1 mm, and showed moderate activities against *S. liquefaciens, E. cloacae* and *K. pneumonia* with inhibition of 14±2.6, 13.3±0.9 and 11.7±2.9 mm respectively. This essential oil doesn't show any activity against the *P. aeruginosa*. Arben H et al[46] found that oil of *J. Communis* exhibited the highest antimicrobial activity against *S. aureus* with inhibition zone of 29 mm, and confirmed our results against *P. aeruginosa* with show any activity. The previous data shows that the antimicrobial products of *Juniperus communis* have an inhibitory effect which is limited by its chemical content, concentration and by the taxonomical properties of the microorganisms[47,48].

Antibiotics activities against pathogenic bacteria

The antibiotic susceptibility to eliminated bacteria was determined using a disk diffusion assay. Different classes of antibiotics were tested: Amoxicillin (penicillin family), Gentamicin (Aminoglycoside) and chloramphenicol (Amphenicol family). The inhibition zone of Chloramphenicol against all bacteria's showed between 14.4±2.2 and 23.2±3.6 mm, and the maximum inhibition of Gram-negative bacteria was caused by chloramphenicol. The bacterium *K. pneumonia* had a resistance for Amoxicillin and all species average sensitivity for Amoxicillin with inhibition zones between 8±1.5 and 13.4±1.8 mm. The obtained results, in accordance with the literary works, showed that the gram – bacteria developed a resistance against commercial antibiotics especially against Penicillin family[49].

CONCLUSION

The essential oils of *J. communis* and *A. absinthium* showed stronger antibacterial effects than the other essential oils. However the essential oil of *R. officinalis* which has the smallest inhibition zone compared to the others essential oils and commercial antibiotics. *P. aeruginosa* and *S. liquefaciens* were found to be the most resistant species, *S. aureus* was the most sensitive bacterial species to essential oils. Among the antibiotic, Chlorompheniсol had the widest coverage against all bacteria's, followed by Gentamicin, but the Penicillin antibiotic has showed poor activity against all bacteria's. Due to the increasing problem of antibiotic resistance in bacteria, using these essential oils as natural and new antimicrobial substances can be useful. Moreover, theses plant essential oils should be investigated in vivo for better understanding of their safety, efficacy and properties.

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