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Evaluation of essential oils for antimicrobial activity from some Moroccan aromatic plants medicinal

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Abstract

Biological properties associated to many medicinal and aromatic plants have recently gained a great popularity and scientific interest. The plants have been reported to have antimicrobial, antiviral, antiparasitic and antidermatophytic properties. The present study aims to evaluate the antimicrobial activity of four distilling essential oils from four Moroccan medicinal and aromatic plants *(S. aromaticum, E. rossi, R. officinalis and T. vulgaris*), Antimicrobial activity screening was conducted by the well diffusion method according to the Clinical and Laboratory Standard Institute guidelines using 7 gram positive,14 gram negative bacteria and 6 species of *Candida spp*.The results indicated that essential oils *of R. officinalis* and of *T. vulgaris* showed high inhibitory activity against all the investigated bacterial strains except essential oil of *Thymus vulgaris* was inactive for *P. mirabilis*. Also essential oil of *E. rossi* was active against 14 strains among 21 strains tested, while essential oil of *S. aromaticum* was inactive for most the investigated bacterial strains. MIC values of *S. aromaticum, R. officinalis* and *T. vulgaris* were between 6.25-12.5-25µg/ml and were bactericidal for most bacterial strains. While, MIC values of *E. rossi* were between 12.5-25µg/ml and bactericidal for all investigated bacterial strains. All essential oils showed that have the maximum antimycotic activity against all types of investigated candida strain. In conclusion, our results confirm that these oils plants which can be used to cure infections and can also have pharmaceutical and preservatives properties.

1. Introduction

Medicinal and aromatic plants have a great promising for producing new drugs of great benefit to humanity. There are many approaches to the search for new biologically active principles in higher plants [1]. Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds [2].

Further, scientific investigation and information of the therapeutic potential of the plant material is limited. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs [3].

Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created [4]. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal and aromatic plants [5].

In traditional medicine, medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases. Today, a great number of different medicinal tea and other plant products are available in market (including cosmetics and pharmaceuticals), which contains biologically active substances.

In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries because plant-derived drugs have been reported to be safe and without side-effects. A survey of literature reveals that there are many essential oils which possess antimicrobial activity [6].

In general, essential oils plant-derived are considered as non-phytotoxic compounds and potentially effective against several microorganisms including many bacterial and fungal pathogens [7,8].The aim of this study was to determine the efficacy of essential oils extracted from aromatic plants medicinal growing in morocco against 21 bacterial strain and 6 fungal strain

2. Material and Methods

2.1. Preparation of essential oils

The dried aerial plant of *S. aromaticum*, leaves and flowers of *R. officinalis, T. vulgaris* and only leaves of *E. rossi* were collected based on ethno pharmacological information from the villages around the Errachidia region located on the south east of Morocco.

These oils were hydro distilled in a Clevenger apparatus (Sigma Chemical Company) for 5hours accordance with the British Pharmacopoeia [9].The obtained essential oils were dried and, after filtration were stored in a sealed glass vial in a refrigerator at 4 ºC until required. These all oils of above plants were screened for their antimicrobial activity.

2.2. Microorganisms used

The test organisms used included: 7gram positive and 14gram negative bacteria strains namely : *R. equi, S.aureus, S. aureus MDR,S. agalactiae, E. faecalis, Listeria spp, L. monocytogenes, P.aeruginosa1,P. cepacia, P. Aeruginosa ATCC27853, A. baumanii, S.blockley, S.aequatoria, S. braenderup, P. rettgeri MDR, P.mirabilis, M. morganii, E. coli enteropathogenes, E.coli1, E.coli2, E.coli MDR* and 6 yeast strains (*C.tropicalis 2,C. famata 2 and C.glabrata* 2).

These strains were obtained from three different sources; the American Type Culture Collection (ATCC, USA),Bacteriology Laboratory of the National Institute of Hygiene (Morocco) and Institute of Agronomy and Veterinary (Morocco).All media used were manufactured by Oxoid Basingstoke, Hampshire, England).All bacteria and fungi were stored in BHI (Brain and Heart Infusion broth) containing 30 % (v/v) glycerol (Sigma-Aldrich) at-20°C.Prior to use bacterial strains were first grown in Muller Hinton agar (MHA) at 37°C for 24hours.

2.3. Culture Media and Antimicrobial Assay

Mueller-Hinton agar (MH) and Sabouraud Dextrose agar (SD) medium (Hi-Media, Bombay, India) were respectively used for bacteria and yeasts growth at 37 and 30°C were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10^5 CFU/mL.

To evaluate antimicrobial activity, an agar well diffusion method was used as described by Nongpanga et al., [10]. The organisms were spread on MH and SD agar plates by cotton swab. Wells of 6 mm diameter were punched into the agar medium and filled with 50 µL/mL of plants oils. The plates were incubated for 24 h at 37°C for bacteria and 48h at 30°C for yeast. Antimicrobial activity was evaluated by measuring the inhibition zone diameter against the test organism.

3. Results and discussion

The four aromatic medicinal plants investigated in present work *(S. aromaticum, R. officinalis, T. vulgaris, E.rossi*) have wide use in Moroccan traditional medicine, for treatment of gastric disease, pharyngitis, cough and other problem health.

The results of antimicrobial activity of four essential oils are presented in **Table1**, have showed that the *R. officinalis* and of *T. vulgaris* oils has a good activity against all investigated bacteria strains (gram positive and

gram negative bacteria) except essential oil of *T. vulgaris* was inactive for *Proteus mirabilis* and the diameters of zone inhibition of this oils were between 8–26mm, with MDI (The mean diameter of inhibition) are 18.14 mm for gram positive and 15.14 mm for gram negative bacteria respectively, it was found to have maximum zone of inhibition against *R. equi* (26 mm) while the minimum zone of inhibition was against *E.coli 2* (8mm).

Table 1: Antibacterial activity of essential oils of some Moroccan plants medicinal (Diameters of zone inhibition (mm))

Bacterial	**Bacterial strains tested (N=21)**	**ESA**	**EER**	**ERO**	**ETV**
Gram +	*Rhodococcus equi*	24	22	26	16
	Staphylococcus aureus	0	10	12	15
	Staphylococcus aureus MDR	0	20	18	15
	Streptococcus agalactiae	0	0	12	10
	Enterococcus faecalis	15	12	16	10
	Listeria spp	10	18	20	20
	Listeria monocytogenes	8	0	20	12
	MDI (%)	**8.2**	**12**	**18**	**14**
Gram -	*Pseudomonas aeruginosa1*	0	0	10	12
	Pseudomonas cepacia	10	18	18	16
	Pseudomonas aeruginosa ATCC27853	0	0	15	10
	Acinetobacter baumanii	0	17	10	20
	Salmonella blockley	0	18	16	20
	Salmonella aequatoria	0	20	22	22
	Salmonella braenderup	0	10	12	15
	Proteus rettgeri MDR	9	0	17	12
	Proteus mirabilis	0	0	18	0
	Morganella morganii	0	20	18	16
	E. coli enteropathogenes	0	0	16	12
	Escherichia coli 1	0	0	15	10
	Escherichia coli 2	10	8	8	12
	Escherichia coli MDR	10	15	15	18
	MDI (%)	**3**	**9**	**15**	**14**

ESA: Essential oil of *S. aromaticum*, EER:Essential oil of *Eucalyptus rossi*,ERO:Essential oil of *R. officinalis*, ETV: Essential oil of *T. vulgaris*, MDI:Mean Diameter of Inhibition.

Essential oil of *E. rossi* was active against 14 strains from 21 strains tested except *S.agalactiae, L.monocytogenes, P.aeruginosa1, P.aeruginosa ATCC27853, P. rettgeri MDR, Proteus mirabilis, E. coli Enteropathogenes, E.coli1*.While essential oil of *S. aromaticum*was active against 8 from 21 investigated bacterial strains tested with MDI (The mean diameter of inhibition) 8.2mm for Gram positive and 3mm for Gram negative. It was found to have maximum zone of inhibition against *R. equi* (24mm) while the minimum zone of inhibition was against *L.monocytogenes* (8mm).

In our research of *T. vulgaris*, those results are in accordance with the strong toxic properties of ETV and its active compounds, such as thymol and carvacrol, against a large number of microorganisms described by Soliman et al., [11]. Also oils of *S. aromaticum*, the major compound were *eugenol*. Eugenol is phenol and it is one of the aromatic compounds containing many free phenol groups and the antimicrobial activity increased by increasing these groups, this finding is in agreement with Al-Zubaydi and Al-Hmdany [12] who had found that the biological effects of the essential oils depends on the type and quantity of active compounds.

The chemical compositions of the leaf oils of various *Eucalyptus* species had been reported with Singh et al, [13]. The major component was 1,8-cineole, but a main contributor for the bioactivity was assumed to be *α-terpineol*, which showed eight-fold higher activity than 1,8-cineole against *S. aureus 1, 8-Cineole* had not been reported as an active principle in other eucalyptus oils (Inouye et al.,[14]. Our results are in accordance with other studies who had found that antibacterial activity of leaf essential oils of *E. globulus* and *E. camaldulensis* [15].

The major compounds in the essential oil were α-pinene, borneol, camphene, camphor, and verbenone and bornylacetate. An inhibitory effect on fungal growth, especially toward *F. graminearum*, was observed. Essential oils of *rosemary* exhibited an intermediate antifungal activity (MIC=1.10 mg/mL) against *C.albicans* [16].

MICand MBC values of *S. aromaticum, R. officinalis* and *T. vulgaris* were between 6.25-12.5-25µg/ml and were bactericidal except *E. faecalis, L.monocytogenes* and *S.aureus MDR* (**Tables 2, 3, 4 and 5**).It is bacteriostatic, and while MIC values of *E.rossi* were between12.5-25µg/ml and were bactericidal for all investigated bacterial strains except *S.aureus*. It is bacteriostatic, confirming our previously published results [17].

Table 2: Determination of MIC and MBC of essential oil of *S. aromaticum*

Bacterial strains	Bacterial strains tested	MIC (mg/ml)	MBC (mg/ml)
Gram+	*Rhodococcus equi*	6.25	6.25
	Enterococcus faecalis	12.5	ND
	Listeria monocytogenes	12.5	ND
	Listeria spp	25	25
Gram-	*Pseudomonas cepacia*	25	25
	Proteus rettgeri MDR	25	25
	Escherichia coli 1	12.5	12.5
	Escherichia coli 2	25	25
	Escherichia coli MDR	25	25

MIC =Minimum Inhibition Concentration,MBC = Minimum Bactericidal Concentration, ND= No Detected.

Table 3: Determination of MIC and MBC of essential oil of *E.rossi*

Bacterial strains	Bacterial strains tested	MIC (mg/ml)	MBC (mg/ml)
Gram+	*Rhodococcus equi*	25	25
	Staphylococcus aureus SARM	12.5	-
	Enterococcus faecalis	25	25
	Listeria spp	25	25
Gram-	*Pseudomonas cepacia*	25	25
	Acinetobacter baumanii	25	25
	Salmonella aequatoria	25	25
	Salmonella braenderup	25	25
	Salmonella blockley	12.5	12.5
	Morganella morganii	12.5	12.5
	Escherichia coli 1	12.5	12.5
	Escherichia coli MDR	25	25

Table 4: Determination of MIC and MBC of essential oil of *R. officinalis*

Bacterial strains	Bacterial strains tested	MIC (mg/ml)	MBC (mg/ml)
Gram+	*Rhodococcus equi*	25	25
	Staphylococcus aureus MDR.	25	25
	Enterococcus faecalis	25	25
	Listeria monocytogenes	25	25
	Listeria spp	25	25
Gram-	*Pseudomonas aeruginosa1*	25	25
	Pseudomonas cepacia	12.5	12.5
	Acinetobacter baumanii	25	25
	Salmonella braenderup	25	25
	Salmonella aequatoria	6.25	6.25
	Morganella morganii	12.5	12.5

Table 5: Determination of MIC and MBC of essential oil of *T. vulgaris*

Bacterial strains	Bacterial strains tested	MIC (mg/ml)	MBC (mg/ml)
Gram+	*Rhodococcus equi*	6.25	6.25
	Staphylococcus aureus MDR.	25	25
	Streptococcus agalactiae	25	25
	Enterococcus faecalis	25	25
	Listeria spp	12.5	12.5
Gram-	*Pseudomonas aeruginosa1*	12.5	12.5
	Pseudomonas cepacia	12.5	12.5
	Acinetobacterbaumanii	12.5	12.5
	Salmonella blockley	12.5	12.5
	Salmonella braenderup	25	25
	Salmonella aequatoria	12.5	12.5
	Morganella morganii	25	25
	Escherichia coli1	25	25
	Escherichia coli2	12.5	12.5

Our results as antimycotic activity showed that all investigated essential oils of *T. vulgaris, S. aromaticum, R. officinalis and E. rossi* had antifungal activity against all tested fungi (**table 6**).

The highest degree of antifungal activity showed by essential oil of *T. vulgaris* followed by essential oil of *S. aromaticum, R. officinalis and E.rossi*, with a mean of inhibition zone are 32 mm, 30 mm, 29mm and 27 mm respectively. These results are in accordance with reports on the antifungal activity of medicinal species belonging to the Myrtaceae family include the herbal food clove *S. Aromaticum* (Merrand Perry) [18] and extracts of *E. globules Labill*. This significantly inhibited the growth of the fungus *T.mentagrophytes* [19] and Lamiaceae family include *R. officinalis and T. vulgaris* [20].

Table 6: Antifungal activity of essential oils of some Moroccan plants medicinal.Diameters of zone inhibition (mm) of EO of (MAP)

Fungal strains	Fungal strains tested	ESA	EER	ERO	ETV
1	CT1	28	24	24	34
2	CT2	32	30	30	30
3	CF1	30	30	24	30
4	CF2	30	24	35	35
5	CG1	30	30	35	30
6	CG2	30	22	24	30
Total of inhibition (%)		180	160	172	189
MDI (%)		30	27	29	32

CT1:*C. tropicalis1*,CT2:*C. tropicalis2*,CF1:*C. famata1*,CF2:*C. famata2*,CG1:*C. glabrata1*,CG2:*C. glabrata2*, ESA: Essential oil of *S. aromaticum*, EER: Essential oil of*E. rossi*,ERO: Essential oil of *R. officinalis*,ETV: Essential oil of *T. vulgaris*, MAP: Medicinal and Aromatic Plants.

Conclusion

In recent years a large number of essential oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi. There is vast diversity among aromatic and medicinal plants. In our study demonstrates that the essential oils which has shown the best antimicrobial activity in well diffusion methods were attributed to *R. officinalis* and *T. vulgaris* and according to the results mentioned, it is confirm that these oils plants can be used to cure infections and can also have pharmaceutical and preservatives properties.

Author contribution

Mohammed EL MORHIT carried out the data analysis and drafted the manuscript and provided the conceptual guidance and polished the manuscript. This author read and approved the final manuscript.

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