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ORIGINAL ARTICLE

ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS OF PLANTS SPECIES FROM MOROCCO AGAINST SOME MICROBIAL STRAINS

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ABSTRACT

Background. Essential oils have important antibacterial activities and can successfully replace antibiotics, which show their inefficiency, especially against fungi and multi-resistant bacteria.

Objective. The main purpose of our research was to investigate the antibacterial and antifungal activity of essential oils from fifteen plants harvested in the Taroudant region.

Material and Methods. In this work, the essential oils were extracted by hydrodistillation using a Clevenger-type apparatus. The method of disc diffusion in agar medium (aromatogram) is the one used to evaluate the activity of these essential oils against four pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Bacillus* sp., and *Enterococcus cloacae*) and two yeasts (*Candida albicans* and *Cryptococcus neoformans*).

Results. Our findings, show that all of the plants' leaves yielded extremely aromatic essential oils that differed in look and color. Furthermore, the 93.33% of the fifteen essential oils that were evaluated proved to be effective against at least one kind of bacteria or fungus. This suggests that the proportion of essential oils with no antibacterial action was rather low, at around 7%. Our data also showed that the freshness or dryness of the plant at the time of harvest could affect the extraction rate of essential oils. This screening showed us that these essential oils present inhibitory activities towards the studied Gram+ bacteria, as well as a resistance against Gram-, in particular *Enterococcus cloacae*.

Conclusion. These essential oils can therefore be used in the prevention and treatment of certain infectious diseases and to fight against bacteria that are multi-resistant to the usual antibiotics.

Keywords: *antibacterial activity, antifungal activity, Bacillus, Cryptococcus, Candida, Staphylococcus, essential oils*

INTRODUCTION

Plants have long held significant significance in human daily existence [1]. Traditional medicinal plants, widely utilized due to their affordability, accessibility, and lack of evidence of resistance or inefficacy to whole plant extracts, have been integral to human healthcare practices [2]. This traditional medicinal wisdom has been consistently upheld within households and transmitted through generations over time. Moreover, Man has always been inspired by nature, using its resources, especially plants for his medical and food needs. This has led scientists, over the centuries, to develop their knowledge of medicinal plants. Indeed, the remedies of good reputation have prevailed in spite of the development of modern medicine, which has marginalized the recourse to natural medical techniques [3]. Medicinal and aromatic plants (MAPs) play a significant role in the food and cosmetic industries, but they are also crucial for pharmacological research and the development of drugs. Plant components are used as building blocks for the synthesis of medications and as therapeutic agents, serving as models for compounds with

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pharmacological activity [4]. Nowadays, the use of plants as a means of treatment is still very important for many rural and urban Moroccans citizens [5]. In developing countries, bacterial infections take a heavy toll, causing millions of deaths each year [6]. As well as fungi, they are the cause of many diseases in animals and plants. However, The effectiveness of medicines like antibiotics, considered the universal solution to dangerous infections decreases, due to increasing resistance of bacteria [7]. With its diverse geographical contrasts, Morocco provides a wide spectrum of bioclimates, facilitating the establishment of a diverse flora. Scientific studies estimate that there are about 4500 species and subspecies and a diversity of phylogenetic resources in MAPs [8]. In this context Morocco has long been a provider of MAPs to the global market. This practice involves taking use of both cultivated and wild species. For the food herb trade, a number of items (more than 70) are transported as dried plants. More than twenty species are utilized to produce aromatic extracts, or essential oils (EOs), mostly for the perfume and cosmetics industries. These extracts are also used to prepare sanitary goods and flavor formulations [9]. However, the area of southwest Morocco, especially the region of Taroudant, is known for its richness and diversity of flora including MAPs, which account for almost one-third of the total flora of the country [10]. Given that the region of Taroudant is a region of rural life and given its wealth of aromatic and medicinal plants, traditional medicine is much practiced and often uses aromatic and medicinal plants. These plants are not valued for their biological potential. Moreover, the discovery of antibiotics and synthetic antifungals has caused the decline of herbal medicine and relegated it to a secondary rank. In addition to their food and cosmetic properties, MAPs contain natural substances with antibacterial and antifungal properties.

Based on the above, the aim of our research was to evaluate the antibacterial and antifungal activity of essential oils extracted from plants in the Taroudant region against selected microbial strains. We assessed the efficacy of these oils using two key parameters: minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

MATERIAL AND METHODS

Biological materials

Fifteen plants were collected manually in their natural habitat in the region of Taroudant, Morocco, and transported in paper and plastic bags. Some of these plants were collected in the region of Ouzioua (latitude: 30.7207°, longitude: -8.0349, altitude: 1352 meters); others were collected in the region of Ouled Berhil (latitude: 30.646397, longitude:-8.479927). The identification of the studied medicinal and aromatic plants is carried out according to Bellakhdar [11] and is reported in (Table 1). The pictures of the studied medicinal and aromatic plants are reported in (Figure 1).

Figure 1. Pictures of the 15 medicinal and aromatic plants harvested

Table 1. Common and scientific name of the plants studied

Common name	Plant specie
Sage	*Salvia officinalis*
Rosemary	*Rosmarinus officinalis* L.
Vermifuge Anserine	*Chenopodium ambrosioides* L.
Marjoram with shells	*Origanum majorana* L.
Round-leaved mint	*Mentha suaveolens*
Rose geranium	*Pelargonium graveolens*
Absinthe	*Artemisia absinthium*
Tooth-leaved lavender	*Lavandula dentata* L.
Thyme	*Thymus vulgaris*
Pennyroyal mint	*Mentha pulegium* L.
White mugwort	*Artemisia herba-alba*
Cypress	*Cupressus atlantica*
Green mint	*Mentha spicata*
Basil	*Ocimum basilicum*
Lemongrass	*Cymbopogon citratus*

Essential oil extraction

For each plant, the EOs extraction was made from a fresh sample and a dried sample. For drying, the plants were air-dried, protected from light and humidity, or even oven-dried at 45°C. The samples were finely ground to create small particles, which facilitated the extraction process. Each sample weighing 100 g underwent extraction through water distillation using a Clevenger apparatus for three hours, in accordance with the guidelines outlined in the European Pharmacopoeia [12]. The weight of the EOs collected post-sodium sulfate dehydration was meticulously measured, and the resultant EOs was stored in darkness at 4°C until required. The EOs yield was calculated based on weight percentage (w/w). This procedure was repeated three times for each plant. Subsequently, the average yield of EOs from three repetitions for each site was reported as the mean ± standard deviation. The essential oils were stored in dark-colored bottles in a refrigerator (+4°C) to shield them from light and heat.

Microorganisms and media

Concerning the evaluation of the biological activities of these plant extracts, Four bacterial and two fungal species were included in the antimicrobial test strains; these were obtained from different culture collections, such as the National Cultures Collection of Microorganisms at the Pasteur Institute in Paris, France; the Collection of Pasteur Institute (CIP); and the Fungi Culture Collection (FCC) of the former. *Candida albicans* CIP884.65 and *Cryptococcus neoformans* ATCC11576 were the strains of fungi, while *Bacillus* sp. CIP104717, *Escherichia coli* CIP54127, *Staphylococcus aureus* CIP209 (ATCC25923), and *Enterobacter cloacae* ATCC13047 were the strains of bacteria. These strains were cultured and maintained on Luria Bertani agar medium for bacteria and Sabouraud's agar medium for yeast [13].

Agar disc-diffusion assay

The antimicrobial activity screening of the essential oils utilized the agar disk-diffusion method [14], employing Muller Hinton Agar (MHA) medium [Difco] for antibacterial assessment and yeast morphological agar (YMA) medium [Difco] for antifungal evaluation. Bacterial and yeast inoculums were prepared by suspending colonies from 24-hour cultures on Luria Bertani and Sabouraud's agar medium, respectively, in Galerie API NaCl 0.85% medium (3 ml) from Biomerieux. The cell density was determined using a Biomerieux ATB 1550 densitometer and adjusted to 104 CFU/ml for yeast and 106 CFU/ml for bacteria. Each cellulose disk (6 mm in diameter) saturated with 20 μL of EOs was applied to the test media, which were previously inoculated with each test strain. The agar plates were kept at 4°C for at least 2 hours to allow the diffusion of essential oils, and then incubated at 37°C for bacteria or 28°C for fungus. The inhibition zones were measured after 24 h of incubation for bacteria and 48 h for fungus. Standard disks (30 μg) of rifampicin and chloramphenicol served as antibacterial positive controls, while Fluconazole and Econazole served as antifungal positive controls. All tests were performed in triplicate. The antimicrobial activity was determined in millimeters using a ruler measuring the diameter of the inhibition zone, and the result was the average of the three tests. The scale of antimicrobial activity estimation is given by Mutai et al. [15] they classified the inhibition zones into five classes (Table 2).

Table 2. Estimation scale of antimicrobial activity [15]

Diameter (D) of inhibitory zone	Activity
D ≥30 mm	Very strongly inhibitory
21 mm≤ D ≤29 mm	Strongly inhibitory
16 mm≤ D ≤20 mm	Moderately inhibitory
11 mm≤ D ≤16 mm	Slightly inhibitory
D <10 mm	Non-inhibitory

RESULTS AND DISCUSSION

Medicinal plants are an important part of traditional medicine in many parts of the world, including our study region, where people have traditionally relied on plant extracts to treat acute illnesses such as bacterial and fungal infections. For our study, we chose plants that are locally cultivated and have been used for generations by the community to treat infections.

Given the points mentioned above, the fifteen medicinal and aromatic plants studied belong to six families. Nine species, *Salvia officinalis, Rosmarinus officinalis* L., *Origanum majorana* L., *Mentha Suaveolens, Lavandula dentata* L., *Thymus vulgaris, Mentha pulegium* L., *Mentha spicata* and *Ocimum basilicum* belong to the *Lamiaceae* family; two species, *Artemisia absinthium* and *Artemisia herba-alba,* belong to the *Asteraceae* family; and only one species belongs to each of the families of *Amaranthaceae, Geraniaceae, Cupressaceae,* and *Poaceae*, which are respectively *Chenopodium ambrosioides* L., *Pelargonium graveolens, Cupressus atlantica,* and *Cymbopogon citrates.*

The EOs from the leaves of the fifteen plants collected by hydrodistillation all have strong and persistent smells, but their color and appearance are variable among species. The results of the EOs yields of the plants studied according to the fresh or dry phase of the plant are summarized in (Table 3). We observed that the EOs yields of the plants in our study ranged from 0.1% to 3.73%. *Thymus vulgaris* presents the highest yield (3.73%), followed by *Arthemisia herba-alba* (3.59%). *Cupressus atlantica* has the lowest yield.

The results of the assay show that yield depends on the physiological state of the plant. For the same parameters, *Chenopodium ambrosioides* L. yields more essential oil when fresh, while *Rosmarinus officinalis* L. and *Mentha suaveolens* yield more when dried. In order to give more value to our work, a comparison of the yields of essential oils obtained in our study with those obtained in previous research works showed that in the case of *Rosmarinus officinalis* L. (yield = 0.5%), whereas Wang et al. [16] obtained a yield of 0.76%. This difference (of 0.26%) may be due to the various factors that come into play; among these factors we can cite: the nature of the soil, the harvest period, and the drying time. For *Cupressus atlantica,* the yield of essential oils that we obtained by hydrodistillation is 0.1%. The latter is significantly lower than that obtained by steam distillation (0.41%) in the study of Amara and Boughérara [17]. However, the findings of Mouden et al. [18], suggest that this difference in yield is due to the extraction technique. The distillation time influences not only the yield but also the composition of the extract [19]. The comparison of the yield of *Salvia officinalis* (0.15%) with that of other research works showed that this value is lower than the results obtained by Benkherara et al. [20] on the same species, whose yields of EOs obtained are between 1 and 2.5%. However, the yield of *Thymus vulgaris* (3.73%) is higher than the yield found by El-Akhal [21] (1%) of the same species harvested during the period March–June 2010 from different stations in central Morocco. This variation may be due to environmental and climatic factors and the extraction techniques used. The outcomes of the antibacterial and antifungal activities of the EOs against pathogenic bacteria and fungi are outlined in Tables 4 and 5, respectively. In addition, the Antibacterial and antifungal tests showed that of the 15 essential oils tested, 14 essential oils exhibited antimicrobial activity against at least one target bacteria or fungus. Either (93.33%) of the essential oils tested showed antibacterial and antifungal activity, while only (6.66%) of the essential oils tested had no

Table 3. Essential oil yields of the plants studied

Plants	Yield (%)	
	Fresh leaves	Dry leaves
Salvia officinalis	0.15	-
Rosmarinus officinalis L.	0.32	0.5
Chenopodium ambrosioides L.	0.5	0.26
Origanum majorana	-	2
Mentha suaveolens	0.8	0.9
Pelargonium graveolens	-	0.33
Artemisia absinthiul L.	0.6	-
Lavandula dentata L.	-	2
Thymus vulgaris	-	3.73
Mentha pulegium L.	2	-
Arthemisia herba-alba	-	2.4
Cupressus atlantica	0.1	-
Mentha spicata	-	0.6
Ocimum basilicum	0.3	-
Cymbopogon citratus	0.6	-

Table 4. *In vitro* antimicrobial activity of essential oils

Essential oil	Diameter of the inhibition zones in mm			
	Gram positive bacteria		Gram negative bacteria	
	Bacillus sp. *CIP104717*	*Staphylococcus aureus* CIP209	*Enterobacter cloacae* ATCC13047	*Escherichia coli* CIP54127
Salvia officinalis	0	9	9.66	12
Rosmarinus officinalis L.	0	10	0	8
Chenopodium ambrosioides L.	0	0	0	0
Origanum majorana	0	0	0	0
Mentha suaveolens	12.66	15.5	13.33	11.66
Pelargonium graveolens	13	0	0	15.5
Artemisia absinthiul L.	0	0	0	0
Lavandula dentata L.	0	7	11	12
Thymus vulgaris	0	27	27	37
Mentha pulegium L.	7	0	0	8.33
Arthemisia herba-alba	0	0	0	0
Cupressus atlantica	0	0	0	0
Mentha spicata	0	0	0	0
Ocimum basilicum	8	17.5	15	15.5
Cymbopogon citratus	26.5	19	0	13.33
Rifampicin (30 mg)	41.66	33	13,33	41.66
Chloramphénicol (30 mg)	18.66	30.66	13	38

effect on the microbial strains studied. This is the EOs extracted from *Arthemisia herba-alba*. In addition, the essential oils extracted from seven plants, i.e. 33.33% (*Mentha suaveolens, Artemisia absinthiul* L., *Lavandula dentata* L., *Thymus vulgaris, Mentha pulegium* L., *Ocimum basilicum* and *Cymbopogon citratus*) are active both against the bacteria and yeasts studied. Among these essential oils, only three (*Mentha suaveolens, Lavandula dentata* L. and *Ocimum basilicum*) have the ability to inhibit all the strains studied. While the essential oils of the other plants tested (*Salvia officinalis, Rosmarinus officinalis* L., *Chenopodium ambrosioides* L., *Origanum majorana, Pelargonium graveolens, Cupressus atlantica* and *Mentha spicata*) only inhibited the bacteria. In this context, several studies have shown that essential oil from *Salvia officinalis* can exhibit activity against multi-resistant bacteria; the work of Benkherara et al. [20] showed that *Salvia officinalis* exhibits inhibitory activity even against the most resistant strains, such as *Pseudomonas aeruginosa*.

The analysis of the findings presented in Table 5 show that *Enterococcus cloacae* is resistant to most essential oils, a remarkable sensitivity of *Staphylococcus aureus* has been observed. The same results are obtained by Benkherara et al. [20], which confirms the sensitivity of *Staphylococcus aureus* and the resistance of *Enterococcus cloacae*. The findings of Bounihi's study [22] show that EOS have activity against all bacterial strains. Indeed, *Staphylococcus aureus* is the most sensitive, while *Pseudomonas aeruginosa* and *Esherichia coli* are the most resistant compared to the oils tested. Our results are in agreement with the literature, according to which gram-positive bacteria show the greatest sensitivity towards essential oils. Gram-positive bacteria are less protected against antibacterial agents because they lack a layer of peptidoglycan, which prevents the passage of essential oils through the cell wall. In this context, Multiple studies have demonstrated the antibacterial properties of EOs, particularly against bacteria that are multi-resistant [23, 24]. Terpenes, aromatic molecules, and the oxygenated compounds generated from them, such as terpenic alcohols, aldehydes, and ketones, are the active ingredients in essential oils [24, 25, 26]. The breakdown of bacterial membranes and walls by the phenolic chemicals in EOs is probably the source of its antibacterial effect.

The EOs of *Mentha suaveolens* and *Ocimum basilicum* are active against the four bacterial strains. *Thymus vulgaris* and *Cymbopogon citratus* show significant inhibition diameters in all three strains. According to the scale of estimation of antimicrobial activity given by Mutai et al. [15] *Mentha suaveolens* is slightly inhibitory of all strains, while *Ocimum basilicum* is slightly inhibitory of Gram-negative bacteria and exhibits moderate inhibition against *S. aureaus* and considered non-inhibitory against

Table 5. *In vitro* antifungal activity of essential oils

Essential oil	Diameter of the inhibition zones in mm	
	Candida albicans CIP884.65	*Cryptococcus neoformans* ATCC11576
Mentha suaveolens	30	45
Thymus vulgaris	37	24
Ocimum basilicum	16	14.67
Cymbopogon citratus	35	13.5
Fluconazole 30 μg	30	18.5
Econazole 30 μg	30	23.33

Bacillus sp. According to the same scale, *Thymus vulgaris* is highly inhibitory against *S. aureus* and *En. cloacae* and shows very strong inhibition against *E. coli*. The strong activity of this essential oil is probably due to its richness in phenolic compounds such as eugenol, thymol, and carvacrol, which possess strong antibacterial activity due to the acidity of their hydroxyl substituents [23]. The broad spectrum of EOs inhibition of different Gram+, Gram-, and even multi-resistant bacteria is due to the biodiversity of chemical compounds in essential oils. In this sense, a study of *Klebsiella pneumoniae*, which is resistant to all standard antibiotics with the exception of amoxicillin-clavulanate, was shown to be sensitive to the majority of the essential oils studied. These results confirm our hypothesis that essential oils can be used as an antibacterial and antifungal alternative for strains resistant to standard antibiotics. Furthermore, the antifungal activity was evaluated in vitro by measuring the diameters of the zones of inhibition. The results are shown in the Table 5.

Based on the results of this table, we find that *Candida albicans* and *Cryptococcus neoformans* are sensitive to the four essential oils. We can also point out the high sensitivity of *Candida albicans* compared to that of *Cryptococus neoformans*. Based on the scale given by Mutai [15]. EOs from *Mentha suaveolens*, *Thymus vulgaris,* and *Cymbopogon citratus* exert a very strong inhibition against *Candida albicans*. On the other hand, *Ocimum basilicum* presents moderate inhibition. Concerning *Cryptococcus neoformans*, it is very strongly inhibited by *Mentha suaveolens* with a diameter of 45 mm (more than the controls). Indeed, the effectiveness of these essential oils against the fungi studied is directly linked to their chemical compositions as oxygenated terpenes. These results are consistent with data published in 2020 by Ben Salha [27] indicating that alcohols and polar terpene compounds act as potent antifungal agents and delay the biodegradation process.

CONCLUSION

In this work, the cold percolation technique was used to assess the antibacterial activities of fifteen traditional medicinal herbs from the Taroudant region. Among the plants studied, the results showed potential antibacterial properties. First, we sampled fifteen plants, and for each of them, we extracted by hydrodistillation essential oils that have different yields and vary between 0.1% and 3.73%, whose aim is to evaluate the activity of these essential oils against pathogenic agents. This screening has shown us that these essential oils have inhibitory activities against the Gram+ bacteria studied, as well as resistance against Gram-, in particular *Enterococcus cloacae*. These essential oils can therefore be used in the prevention and treatment of certain infectious diseases and to fight against bacteria that are multi-resistant to the usual antibiotics. In addition, these essential oils can be used as biopesticides, especially in the post-harvest treatment of fruits intended for export.

Disclosure conflict of interest

The authors declare that they have no conflicts of interest concerning this article.

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