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Comprehensive Analysis of Phytochemical Constituents, Antioxidant Potential, and Antibacterial Activity of *Rosmarinus officinalis L.* Essential Oils from Mostaganem, Algeria

Research Article

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Abstract

This study is part of an effort to valorize the Algerian Mediterranean flora, focusing more specifically on the plant species *Rosmarinus officinalis L*. The main objective was to analyze the chemical composition of its essential oils as well as to evaluate their toxicity and biological properties, notably their antimicrobial and antioxidant activities. The rosemary essential oil was extracted from the leaves and flowers of the plant by hydrodistillation using a Clevenger apparatus, with a yield of approximately 0, 62%. The physical indices of this essential oil closely align with AFNOR standards, showing an acid index of 2.24 and an ester index of 325,10. Chemical analysis performed by gas chromatography (GC) revealed the presence of 15 different compounds, representing about 92, 56% of the total identified components. The antibacterial activity was assessed using the agar diffusion method, testing several bacterial strains. The most significant effect was observed against Staphylococcus, with an inhibition diameter of 21 mm, thus demonstrating a positive impact of this essential oil on the tested microorganisms.Furthermore, the antioxidant capacity was measured using a free radical scavenging test with DPPH (2,2-diphenyl-1-picrylhydrazyl). The results showed a moderate antioxidant potential, with an activity level of 64% for plants cultivated in the Mostaganem region. In summary, this essential oil of *Rosmarinus officinalis L*. exhibits interesting biological activities, making it a promising candidate for therapeutic applications in traditional medicine, aromatherapy, as well as in cosmetic and culinary fields. Indeed, it is rich in multiple health and wellness benefits.

Keywords

Rosmarinus officinalis L ; Essential oils ; Antioxidants ; DPPH ; Ascorbic acid

Introduction

Algeria, with its diversity of climates and soils, its geographical location, and its reliefs, presents a varietal

diversity of medicinal and aromatic plants, most of which exist in a spontaneous state [1]. In Algeria, particularly in the region of Mostaganem, the natural medicinal flora is relatively abundant and includes more than 3000 species used in traditional medicine. With its species belonging to several botanical families, of which 15% are endemic, the flora remains underutilized both phyto-chemically and pharmacologically [2]. In the context of promoting Algerian plant species, and considering the therapeutic virtues represented by the Lamiaceae (Labiatae), rosemary essential oil from the Mostaganem region is a natural

resource with varied therapeutic virtues, notably as a mild analgesic and antiinflammatory agent, used to treat headaches, muscle spasms, and sleep disorders. Various studies have highlighted the neuropharmacological properties of rosemary as the main theme. Rosemary possesses significant antimicrobial, antiinflammatory, antioxidant, anti-apoptotic, anti-tumorigenic, antinociceptive, and neuroprotective properties. Moreover, it shows crucial clinical effects on mood, learning, memory, pain, anxiety, and sleep [3]. Moreover, the essential oil of R. officinalis L. possesses antibacterial properties due to its active compounds such as monoterpene hydrocarbons (39, 32-40,70%)and oxygenated monoterpenes (36,08-39,47%). The active compounds in R. officinalis essential oil include 1,8-cineole, α-pinene, camphor, and trans-caryophyllene. These compounds can help combat various strains of pathogenic bacteria, making it a useful agent in the fight against bacterial infections [4]. The essential oil of R. officinalis can be used to treat dyspepsia and milder forms of spastic gastrointestinal disorders, circulatory anomalies, as a complement to the treatment of muscle or joint pain, and inflammation [5].

The Most aganem region in Algeria is characterised by rich plant biodiversity, but only a few studies have been conducted on local medicinal plants, particularly rosemary. However, climatic and pedological conditions can influence the chemical composition and quality of plants, which justifies a specific study on local samples. The concentration of bioactive compounds and the intensity of antioxidant activity can vary depending on geographical origin, harvest season, and extraction methods.

The objective of this study is to analyze the chemical composition, evaluate the antioxidant activity and determine the antibacterial efficacy of essential oils extracted from rosemary leaves harvested in the Mostaganem region (Algeria).

Materials and Methods

Study area presentation

The plant material used in this study was collected from the Mostaganem region (35°56’ N, 0°05’ E), located in the northwest of Algeria, along the coast of the Gulf of Mostaganem. This area is approximately 80,7 km east of Oran and 363 km west of Algiers. The choice of this region aims to highlight the influence of regional factors, particularly climate, on the studied parameters, especially the antibacterial activity.

Plant material

The plant *Rosmarinus officinalis L.* (Figure 1) was harvested fresh in the Mostaganem province in March 2024. The aerial part selected (the leaves) was carefully cleaned and then air dried away from direct light exposure. Once dried, the leaves were separated from the twigs and stored in clean, breathable paper bags for subsequent essential oil extraction.

Hydrodistillation by clevenger

For the extraction of essential oils from rosemary by hydrodistillation under optimal operating conditions, a quantity of 100 g of rosemary was added to 800 ml of distilled water in a 2-liter fask [6]. The set was placed in a balloon heater attached to a refrigerator to ensure condensation of essential oils for 3 hours. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The essential oil was collected, dried under anhydrous sodium sulphate, and stored in sealed vials in the dark, at 4°C, until used. Experiments were conducted twice for each condition.

Yield of essential oils

The yields of essential oil of rosemary were expressed in g relative to 100 g of dry vegetable matter; it was calculated according to Equation (Figure 2) [7]:

$$\text{Yield (\%)} = \frac{\text{Dry plant material mass (g)}}{\text{Amount of extracted oil (g)}} \times 100$$

Figure 1: Fresh leaves of *Rosmarinus officinalis L.*

Figure 2: Clevenger hydrodistillation apparatus used for the extraction of essential oils from *Rosmarinus officinalis L.*

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Chromatographic analyses of essential oils

The chemical composition of the rosemary essential oils extracted by hydrodistillation Clevenger (CH) method is performed by gas chromatography coupled with mass spectrometry (GC/MS). The GC analysis was performed using a chromatography equipped with a fame ionization detector (FID) and two capillary columns of diferent polarities OV type: 101 (25 m x 0,22 mm x 0,25 mm) and Carbowax 20 M (25 m x 0,22 mm x 0,25 µm). The carrier gas is helium with a fow rate of 0,8 ml/min and the oven programming temperature is between 50 and 200°C with a gradient of 5° C/min. CPG/MS coupling was performed on a DB1-type fused silica capillary column (25 m x 0,23 mm x 0,25µm) with helium as a carrier gas and temperature programming identical to that of the GC.

Study of antimicrobial activity

To evaluate the antimicrobial activity of rosemary essential oil, the disk diffusion method (aromatogram) was used [8]. Sterile Mueller-Hinton agar was poured into Petri dishes at a volume of 15 ml per plate and allowed to solidify. The bacterial inoculum, adjusted to 0,5 McFarland standard with a concentration of 107 CFU/ml, was evenly spread over the entire surface of the Mueller-Hinton agar in a back-and-forth streaking pattern [9] . Whatman No. 1 filter paper disks, 6 mm in diameter, were sterilized by autoclaving at 121°C for 15 minutes wrapped in aluminum foil. Using sterile forceps, disks impregnated with a quantity of crude essential oil previously dissolved in dimethyl sulfoxide (DMSO) were placed on the surface of the inoculated Petri dishes. The plates were then closed and left to diffuse at room temperature for 30 minutes, followed by incubation at 37°C for 24 hours. The antimicrobial activity was assessed by measuring the diameter of the inhibition zone around each disk in millimeters. Results are expressed as the diameter of the inhibition zone and can be symbolized using signs according to the sensitivity of the strains to the essential oil [Table 1].

Study of Antioxidant Activity

Table 1: Sensitivity of microbial strains according to inhibition zone diameters [10].

Sensitivity	Inhibition Zone Diameter
Not sensitive or resistant (-)	diametre < 8mm
Sensitive (+)	diameter between 9 and 14 mm
Very sensitive (++)	diameter between 15 à 19 mm
Extremely sensitive (+++)	diametre > 20 mm

The antioxidant activity was measured using the free radical DPPH (2,2-diphenyl-1 picrylhydrazyl) method, following the protocol described by Brand-Williams et al., (1995) [11] .A stock solution of 10 mg/mL was prepared in methanol, from which serial dilutions were made for the assay. Ascorbic acid was used as a standard, for which a stock solution of 1 mg/mL was prepared in methanol and similarly diluted for testing.100 µL of the test sample was added to 1200 µL of a methanolic DPPH solution (0,4%). After mixing, the mixture was left in the dark for 30 minutes, then the optical density was measured at 517 nm. The negative control contained only the DPPH solution, while the positive control was represented by ascorbic acid used as the standard. The free radical scavenging activity was expressed as the percentage of DPPH° reduction. The percentage of reduction (PR) was calculated using the following formula:

$$PR = (AC - AE) / AC \times 100$$

Where:

PR: Percentage of reduction (%)

AE: Absorbance of the DPPH° solution in the presence of the essential oil or ascorbic acid

AC: Absorbance of the DPPH° solution in the absence of the essential oil and ascorbic acid

The variation of the reduction power as a function of the concentration of the essential oil and ascorbic acid also allows the calculation of the EC_{50} parameter, which represents the "Effective Concentration." This is defined as the concentration of essential oil (or ascorbic acid) required to reduce 50% of the DPPH° activity. The mean EC_{50} values were determined by linear regression from three separate trials, where the x-axis corresponds to the concentration of the tested samples and the y-axis to the percentage of reduction power.

Results and Discussions

Yield extraction of essential oils

In this study, the essential oil was extracted from the aerial parts of *Rosmarinus officinalis L.* using the Clevenger hydrodistillation method. This technique yielded a pale yellow to almost white essential oil, with a yield of 0, 62%. This result is consistent with the findings of Derwich et al., (2011) [12], who reported a low extraction yield of 0, 54% from rosemary samples collected in Morocco. The extraction yield of essential oils is influenced by various factors, including the plant's origin, soil type, harvest period, irrigation regime, drying duration, extraction

method, genetic properties, climatic changes, and plant variety [13].

Chemical composition of essential oils

The results relating to the chemical composition of the essential oils of *Rosmarinus of cinalis L.* extracted by hydrodistillation Clevenger method are summarized in table 2. The chromatographic profles are illustrated in figures 3. These results made it possible to identify 15 compounds which represent a total of 99, 80%. The cineole has the major constituent with a slightly higher rate which

Table 2: Chemical composition of rosemary essential oils obtained by CH.

No	Compounds	Kovat's index	CH (%)
	Monoterpene hydrocarbons		37,19
1	α-Pinene	939	15,82
2	Camphene	954	9,77
3	β-Pinene	979	3,56
4	α-Terpinene	1017	2,44
5	para-Cymene	1025	4,79
6	Limonene	1028	0,81
	Oxygenated monoterpenes		61,76
7	Cineole	1030	31,2
8	β -myrcene	1048	3,75
9	Linalool	1097	1,49
10	Camphor	1146	16,54
11	Borneol	1169	1,47
12	α-Terpineol	1199	7,16
13	Verbenone	1205	0,15
	Sesquiterpene hydrocarbons		0,11
14	β-Caryophyllene	1419	0,08
15	α-Caryophyllene	1423	0,03
	Total oxygenated compounds		62,5
	Total nonoxygenated compounds		37,3

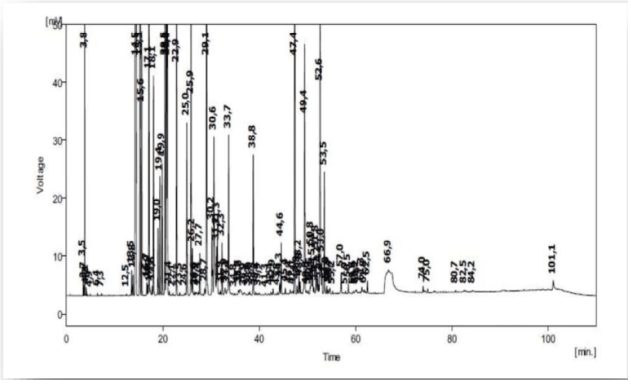

Figure 3: Chromatogram of essential oils of rosemary extracted by CH.

Table 3: Antibacterial effect of rosemary essential oils against *S. aureus* and *P. aeruginosa*,

Diameter of inhibition zones(mm)		
Essential oil concentration (%)	*S. aureuse*	*P .aeruginosa*
	T	
100	27	15
75	22	9
50	15	/
25	9	/

is 31, 20%. However, the percentages of camphor is 16, 54% and α-pinene is 15, 82%. A critical observation of the composition of the oils has revealed that the amounts of oxygenated compounds are substantially higher and the amounts of monoterpene hydrocarbons are lower .The largest proportion of oxygenates in CH extracted essential oils is probably due to the low water content in the system and the speed of the heating process . Thus, the thermal and hydrolytic degradations of oxygenated compounds are limited [14]. Oxygen compounds have a high dipole moment and can be extracted more easily unlike monoterpene hydrocarbons that have a weak dipole moment. Among other aspects, we compared the chemical composition of our essential oil with several studies conducted on rosemary essential oils harvested from different localities in Algeria. We observed that the chemical composition of rosemary essential oils varies according to the geographical origin. Indeed, in the Bordj-Bou-Arreridj region, the major compound is 1,8-cineole (52,4%), followed by camphor (12,6%) [15].

Results of antibacterial activity

According to the results of the antibacterial effect of essential oils from *Rosmarinus officinalis* collected in

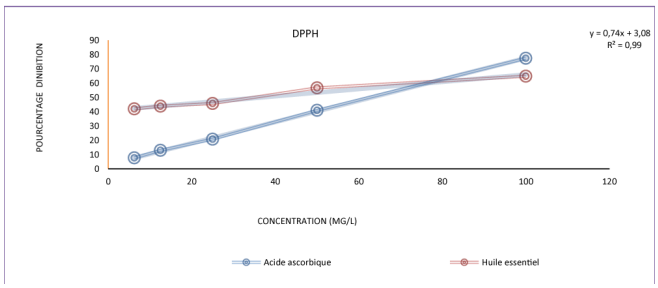

Figure 4: DPPH radical scavenging activity of rosemary essential oil.

the Mostaganem region on Staphylococcus aureus and Pseudomonas aeruginosa (Table 3), the oils exhibited antibacterial activity against both studied bacteria. Inhibition zones of 27 mm were recorded with pure essential oils against Staphylococcus aureus, whereas zones of 15 mm were observed against Pseudomonas aeruginosa. These findings indicate a stronger inhibitory effect of rosemary essential oil on Staphylococcus aureus compared to Pseudomonas aeruginosa, consistent with previous studies showing differential susceptibility of these bacterial strains to *Rosmarinus officinalis* essential oils. The variation in inhibition zone diameters reflects the varying sensitivity of the tested microorganisms to the essential oil. According to the work of Mouas et al., (2017) [16] , the essential oil of rosemary from the Blida and Djelfa regions in Algeria demonstrated notable efficacy against most of the tested bacteria, with inhibition zones measuring 23,75 mm and 22,75 mm (Blida), and 16,75 mm and 14,25 mm (Djelfa), respectively.

Results of antioxidant activity

The results show that the percentage of inhibition strongly depends on the substrate concentration, particularly for the essential oil. More specifically, increasing the sample concentration leads to a higher percentage of free radical inhibition and consequently an increase in the antioxidant activity (Figure 4).

The comparison of the DPPH radical scavenging activity between rosemary essential oil and the standard (ascorbic acid) demonstrates a concentration-dependent antioxidant activity (Figure 4). Each increase in concentration corresponds to an increase in the percentage of inhibition. At the lowest concentration tested (5 mg/mL), the inhibition percentage reached approximately 42% for the essential oil (Figure 4). It is noteworthy that even at low concentrations, the oil exhibits a significant inhibition percentage, suggesting that the phenolic compounds contained in the essential oil of R. officinalis are highly effective antioxidants. The antioxidant capacity of an essential oil largely depends on its phenolic constituents. Numerous studies reported in the literature have explored the structure-activity relationship of certain natural compounds. It has been proposed that antioxidant activity is related to the number and position of hydroxyl groups on the flavonoid nucleus [17]. According to Shen et al., (2022) [18], the strong antioxidant activity of the essential oil could be explained by the presence of polyphenols. The choice of an appropriate solvent system remains one of the most critical steps in optimizing the extraction of polyphenols, flavonoids, and other antioxidant compounds. Consequently, the results of antioxidant activity assays can be influenced by the nature of the plant organ studied.

Conclusions

Conventional hydrodistillation yields an essential oil with a higher content of oxygenated compounds while offering substantial energy savings. It can be concluded that the Clevenger hydrodistillation method is a suitable and efficient alternative for extracting rosemary essential oils. The results of this study on antibacterial activity demonstrate that the essential oils extracted from the leaves of *Rosmarinus officinalis* collected in the Mostaganem region (Algeria) exhibit antibacterial effects against the tested bacterial strains. Further studies are necessary to explore the potential of these essential oils in treating other bacterial pathogens and to evaluate their safety and toxicity profiles.

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None.

Conflict of Interest

Authors are declare that there are no conflict of interest.

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