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RESEARCH ARTICLE

CHEMICAL COMPOSITION, EVALUATION OF RADICAL SCAVENGING AND ANTIBACTERIAL ACTIVITIES OF ESSENTIAL OIL LEAVES OF *MENTHA LONGIFOLIA* (L.) HUDSON SUBSP. *SCHIMPERI* (BRIQ.) BRIQ. CULTIVATED IN ADEN GOVERNMENT, YEMEN

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Abstract

This is the first report on the chemical composition, radical scavenging and antibacterial activities of essential oil obtained from the leaves of cultivated *Mentha longifolia* (L.) Hudson, subs. *schimperi* (Briq.) Briq., in Aden-Yemen. Gas chromatography/mass spectrometry analysis of the hydro-distillated essential oil revealed eighteen compounds representing 100 % of total oil. The main components detected were pulegone (46.3624%), eucalyptol (10.5653%), and menthone (9.5726%). Determination of the total content of phenols and flavonoids revealed the presence of 11.653 mg GAE /g and 12.310 mg QE/g, respectively. The antioxidant activity performed using DDPH radical showed that the sample concentration necessary to inhibit 50% of DPPH radicals (IC_{50}) was 28.3837 µg/mL, indicating a strong antioxidant activity. Regarding the antibacterial activity, the essential oil showed moderate-to-strong broad-spectrum antibacterial activity against the four tested bacterial strains (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853). *S. aureus* was the most sensitive strain to the essential oil with the largest inhibition zone (25±1). Cultivated *Mentha longifolia* (L.) Hudson, subs. *schimperi* may be a valuable candidate for further studies for the development of antibiotics and food preservatives.

Keywords: *Mentha longifolia* subsp. *schimperi* leaves, Radical scavenging, Antibacterial activity.

1. Introduction

Medicinal plants represent a significant source of therapeutic remedies, being also the basis of traditional or indigenous healing systems, still widely used by the majority of populations in many countries. Recently, the ethnopharmacological potentials of these plant matrices have received important consideration by both scientists and the pharmaceutical industry towards complementing or even replacing conventional pharmacotherapies. Moreover, many of these plants have also been highlighted for their added-food value ability, providing a dual role, i.e., food flavor and bioactive compounds [1].

Mentha is a genus belonging to the family of Lamiaceae, whose plants are among the most aromatic and spread in diverse environments with distribution across Europe, Africa, Asia, Australia, and North America [2, 3], having simple characteristic leaves with pleasant scent. *Mentha* taxonomy is highly complicated and includes about 42 species and 15 hybrids, with hundreds of subspecies and cultivars [1]. *Mentha* leaves have traditionally been used as tea in the treatment of headache, fever, digestive disorders and various minor ailments. Furthermore, mint essential oils have been widely used in the treatment of mild-intensity fungal and bacterial infections of human skin, headache syndromes and postherpetic neuralgia [1]. Plants of this genus are used for essential oil production, mainly in USA, India, China, and Iran [4].

The interest in essential oils and their components is growing due to their friendly environment nature and human health, their broad acceptance by consumers, and their consumption for potential multi-purpose functional use [5, 6]. Fresh and dried plant materials of the mint and their essential oils are widely used in industry as part of confectionaries, flavor enhancing agents, pharmaceuticals, cosmetics, etc [7].

Mentha is represented in Yemen by one species, *Mentha longifolia* (L) Huds and two subspecies, *M. longifolia* subsp. *schimperi* Briq. [8, 9], and *M. longifolia* subsp. *typhoides* (Briq.) Harley [10]. *Mentha longifolia* extracts and essential oil are characterized by a great chemical diversity and were reported to contain a number of chemical compounds responsible for their pharmacological properties such as antioxidant, antibacterial, antifungal, antiviral and anicancer activities [1, 11-20].

The wildly grown *Mentha longifolia* subspecies, *schimperi* and *typhoides* were subjected to few studies. We found only one study performed in Yemen on the chemical composition, antibacterial and radical scavenging activities of the essential oil obtained from the aerial part (flowers, leaves, and stems) of wild *M. longifolia* subsp. *schimperi* grown in Wadi Bana area of Abb province [10]. In addition, *Mentha longifolia* subsp. *typhoides* and *Mentha longifolia* subsp. *schimperi* grown wild in Egypt were subjected to taxonomical studies of the leaf, petiole, stem and seeds [21, 22] and analysis of essential oils of the leaves and aerial parts followed by biological investigation (antimicrobial, antiquorum-sensing and burn wound healing activities) of the essential oils and different solvent fractions from the methanol extracts of the aerial parts [23, 24].

In this work, we performed a study- for the first time- on a cultivated *Mentha longifolia* subsp. *schimperi* in Aden. We conducted a comparative study on the composition of the essential oil extracted from the leaves of our cultiviated *Mentha longifolia* subsp. *schimperi* and evaluate its antibacterial and antioxidant activites versus the composition of the essential oils extracted from the leaves and aerial parts of the wildly grown *Mentha longifolia* subsp. *schimperi* and their corresponding activites. Our work has provided scientfic infromation on the cultviated *Mentha longifolia* subsp. *schimperi* that could be utilized in the documentation of this species as a new addition to the flora of Yemen.

2. Materials and Methods

2.1. Plant material

Mentha longifolia subsp. *schimperi* was cultivated in the Faculty of Pharmacy (1st January 2023) and collected in flowering stage, April 2023. The plant identification was clarified by Prof. Abdul Nasser Al-Gifri, Biology Department, University of Aden, Yemen, and has the voucher specimens (No:- 6144, AL-Hawshbi, collection from the garden of the Faculty of Medicine, Aden University, at 11/12/2021).

2.2. Sample preparation

Fresh leaves of the plant were cut into small pieces and left to dry naturally at room temperature (23-27ºC) in the shade for 3 – 4 weeks. The dried plant materials were ground into fine powder using a grinder, just before submitting to hydro-distillation.

2.3. Essential oil preparation

According to the European pharmacopoeia method, the fine powder of the leaves of *M. longifolia* subsp. *schimperi*. (50 g), was hydro distilled for 3.5 hours using modified Clevenger-type apparatus to give about 1 mL essential oil. The oil was dried over anhydrous sodium sulfate and stored in sealed dark colored vial at 4 °C before analysis [10].

2.4. Essential oil analysis

2.4.1. TLC analysis

A pure essential oil was applied on the TLC plates as spot, and developed according to Wagner and Bladt (1996) method for essential oils [25], using toluene: ethyl formate: formic acid (8:4:0.1) as a mobile phase and further sprayed with anisaldehyde sulphuric acid reagent. Retention factors (Rf) values were calculated for the developed spots.

2.4.2. Gas chromatography analysis

GC/MS analysis was carried out using an Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector. Capillary column (HP-5MS Capillary; 30.0 m × 0.25 mm ID × 0.25 µm film thickness) was used; the carrier gas was helium at a pressure of 7.65 psi with 1 µl injection (diluted with CH_2Cl_2). The sample was analyzed with the column held initially for 3 min at 50°C after injection, then the temperature was increased to 300°C with a 20°C/min heating ramp, with a 2.0 min hold. Injection was carried out in split mode with a ratio (100:1) at 300°C. MS scan range was (*m*/*z*): 40–550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV) and solvent delay 3.0 minutes.

2.5. Quantitative estimation

2.5.1. Estimation of total phenolic contents

The total phenolic content in the essential oil was determined according to the Folin- Ciocalteu reagent and external calibration with gallic acid as the standard. Briefly; about 0.2 mL of essential oil and 0.2 mL of Folin-Ciocalteu reagent were added and the contents mixed vigorously. After shaking 4 min, 1 mL of 15% Na_2CO_3 was added, and finally the mixture was allowed to stand for 1 hour at room temperature. The absorbance was measured at 760 nm using Perkin Elmer, Lambda 25, Germany. The total phenolic content was estimated as mg of gallic acid equivalent (GAE) per g (mg GAE /g) by using an equation obtained from gallic acid calibration curve. The quantification of total phenolic content was carried out in duplicate. The mean value of the two determinations was calculated as the mean ± Standard deviation (SD) [26].

2.5.2. Estimation of total flavonoids content

The total flavonoid content was estimated by using the aluminum chloride colorimetric method as described by Willet [27], with some modifications. Essential oil (0.5 mL) mixed with 10% aluminum chloride (0.1mL), 1M potassium acetate (0.1mL) and distilled water (4.3 mL). The mixture was incubated at room temperature for 30 min. The absorbance was measured at 415 nm using Perkin Elmer, Lambda 25, Germany. Quercetin was used as the standard to make the calibration curve and the total flavonoid content is estimated as mg quercetin equivalent (QE) per g weight (mg QE/g). The determination of total flavonoids in the essential oil was carried out in duplicate and the mean value was calculated as the mean ± Standard deviation (SD) [26].

2.6. Biological activities of essential oil

2.6.1. Antioxidant assay

The antioxidant activity of the essential oil was measured by using 2.2`-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color which is typical for free DPPH radical decays, and the change in absorbency at 517nm is followed spectrophotometrically. Six samples of increasing concentrations of the essential oil were prepared by diluting each of the 10, 20, 40, 60, 80 and 100 µl of essential oil with methanol to a volume of 1mL. To each sample, 2 mL of methanolic solution of DPPH radical (concentration of 0.04mg/ml) was added and, after shaking, the reaction mixture was left in the dark for one hour at room temperature.

The absorbance of each sample was recorded against the absorbance of the control solution (2mL of DPPH methanolic solution (0.04mg/ml concentration) / 1mL methanol) at 517 nm. A parallel DPPH assay on ascorbic acid with the same set of concentrations was also performed. Inhibition percent of DPPH radical (I %) was calculated as follow: I % = 100 (A° ─ A)/A° Where A° is the absorbance of the control solution and A is the absorbance of individual investigated samples. The test was carried out in triplicate and their mean value was calculated as the mean ± Standard deviation (SD) [10, 15].

2.6.2. Antibacterial assay

The evaluation of the antibacterial activity was carried out at Supreme Board of Drugs & Medical Appliances, against four standard bacterial strains: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853, by using the agar well diffusion method [28-30]. The tested bacterial strains were supplied from Médecins Sans Frontières. All bacterial cultures were first grown on MHI plates incubated at 37 °C for 18-24 h prior to inoculation onto the nutrient agar. One or several colonies of similar morphology of the respective bacteria were transferred into Normal Saline medium and adjusted to 0.5 McFarland turbidity standard. The inoculums of the respective bacteria were streaked onto MHI agar plates using a sterile swab. A pure essential oil (10 µl) was poured in the agar well as required. The treated Petri dishes were placed at 4 °C for 1-2 h and then incubated at 37 °C for 18-24 h. Antimicrobial activity was evaluated by measuring the zone of bacterial growth inhibition around the essential oil well and the antibiotic discs after 24 h of incubation at 37 °C. The standard discs (6 mm diameter) of the antibiotics Gentamycin, Ampicillin\ Sulbactam, Cefotaxime and Ciprofloxacin were used as comparative substances (positive control). The diameter of the zone of inhibition around the essential oil well and each of the positive control discs were taken as measure of the antimicrobial activity. The evaluation of the antibacterial activity was carried out in triplicate and their mean value was calculated as the mean ± Standard deviation (SD).

3. RESULTS AND DISSCUSION

3.1 The yield of essential oil

The leaves of our sample, cultivated *M. longifolia* subsp. *schimperi*, yielded 2 % v/w of essential oil of pale-yellow color that changed to gold color especially after a long period of time. The yield of essential oil from our cultivated sample is greater than that obtained from the leaves of the wild grown Egyptian *M. longifolia* subsp. *schimperi* (0.98%) [21, 22]. It has been reported that the essential oil yield of different plants including the Lamiaceae herbs not only varies due to genetic factors, nevertheless, environmental and geographical variables that influence genetic expression and plant tissue development stages, also affect the yield and chemical composition of oils [14].

3.2. The contents of total phenols and flavonoids in essential oil

The estimated total phenolic and flavonoids contents of the essential oil of our cultivated sample were 11.653 mg GAE /g and 12.310 mg QE /g, respectively (Table 1). These results were obtained by the calculation from the regression equation of the calibration curves of the standard gallic acid [y=0.0049x+ 0.1498, R^2= 0.9933] and the standard quercetin [y=0.0074x-0.011, R^2=0.9981], respectively (Figures 1 and 2). The content of bioactive constituents, including phenolics by plant correlates with ecological growth conditions. Their amounts mostly not only depend on abiotic conditions, such as climate, meteorology (temperature, humidity),

and soil composition, but also on geographical factors (ecology) and their vegetation stage at harvest time [31].

3.3. Essential oil analysis

3.3.1. Thin layer chromatography of essential oil

A preliminary analysis of the essential oil terpene compounds was performed using TLC. The essential oil was applied as a spot onto a TLC plate (silica gel G 60 F 254 with layer thickness 0.25 mm Merck – Germany) and developed with the mobile phase (toluene: ethyl formate: formic acid 8:4:0.1) over a path of 9 cm. The developed spots were initially observed with day light and under UV light (254 nm) and UV light (365 nm), and then the plate was sprayed with the reagent (anisaldehyde sulphuric acid) and heated in the oven at 105 °C for 5 minutes. The R*f* values of the developed spots were calculated and the color of the spots that appear under the UV light (365 nm) and after reaction with the spray reagent were recorded (Table 2, Figure 3). The color reactions of the spots with the reagent indicated the presence of terpene compounds in the essential oil, which were detected later by the GC-MS analysis.

3.3.2. GC/MS analysis

The results of the GC/MS analysis of the essential oil chemical composition are presented in Table 3, Figure 4. Eighteen compounds representing 100 % of total oil composition were identified in the *M. longifolia* subsp. *schimperi* essential oil. Monoterpenes and sesquiterpenes, were the main constituents, accounting for 91.2787 % and 7.5871 %, respectively. The main compounds were the oxygenated monoterpenes pulegone (46.3624%), eucalyptol (10.5653%), and menthone (9.5726%). The composition percentage of the rest of the identified volatile components (14 components), comprising three oxygenated monoterpenes (15.2077%); one bicyclic oxygenated monoterpene (0.4239%); two bicyclic monoterpenes (2.8011%); three sesquiterpene hydrocarbons (3.4159%) and three oxygenated sesquiterpenes (4.1712%), one oxygenated diterpene (0.4704%) and phthalic acid ester (0.6638 %).The essential oil from the leaves of the wild grown Egyptian *M. longifolia* subsp. *schimperi* was found to contain pulegone (41.03%) and eucalyptol (17.96%) as the main compounds followed by piperitenone oxide (8.66%) [21, 22]. In addition, the essential oil from the aerial parts of the wild grown Yemeni *M. longifolia* subsp. *schimperi* was reported to contain the main constituent, trans-piperitone oxide (97.71%) [10], while in the essential oil from the aerial parts of the wild grown Egyptian *M. longifolia* subsp. *schimperi*, pulegone (56.493%) and menthone (26.593%) were dominant [23].

3.4. Essential oil bioactivities

The antimicrobial activity along with the antioxidant effectiveness of essential oils is one of the most examined features, important for both food preservation and control of human and animal diseases of microbial origin [16].

3.4.1. Radical scavenging activity

The increasing interest in the utilization of plant-based natural antioxidants is due to their safer nature and medicinal benefits compared to synthetic formulations. In this regard, extracts and essential oils from many medicinal and food herbs have been investigated as promising source of effective antioxidant agents [7, 32].

The free radical DPPH scavenging activity of the essential oil of our sample is presented in Table 4, Figure 5. The essential oil showed antioxidant activity in inhibiting DPPH., reaching up to 50% at a concentration of 28.3837 µg/mL. It has been reported that according to the parameters, the IC_{50}value category is very strong if the IC_{50}value <10µg/ml, strong if the IC_{50}value is between 10 and 50 µg/ml, mild if the IC_{50}value is between 50 and 100 µg/ml, weak if the IC_{50}value is between 100 and 250 µg/ml and not active if IC_{50} is above 250 µg/ml [33]. Accordingly, we can conclude that our essential oil has a strong antioxidant activity.

The antioxidant activity of our sample can be attributed to the presence of phenolic compounds (phenols and flavonoids) and oxygenated monoterpenes. The antioxidant activity of essential oil containing phenol components is reported to be due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [16], while the flavonoids exert their antioxidant efficacy by neutralizing reactive oxygen species through hydrogen atom and electron donation, augmenting endogenous antioxidant enzyme activity, and chelating transition metal ions [34]. In addition, pulegone (46.3624%), eucalyptol (10.5653%), and menthone (9.5726%) the main constituents of our sample could play an important role in the antioxidant activity. It has been reported that the essential oil components such as (+)-pulegone and (-)-menthone showed much closer relationships with antioxidant activity by using multiple linear regression analysis and partial least square analysis [35]. The Eucalyptol (1,8-cineole) has also been found to demonstrate potent free radical scavenging activity in a variety of in vitro assays [36].

3.4.2. Antibacterial activity

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. They not only are more severe and require longer and more complex treatments, but they are also significantly more expensive to diagnose and to treat [37]. Therefore, there is a need to explore for some safer alternative that might prevent the development of this drug resistant. One of the primary benefits of utilizing phytochemicals for antimicrobial purpose is their ability to interact with

several factors or their molecular promiscuity. This muti-target affinity makes it difficult to generate possible resistance mechanisms in bacteria [38].

The results of the antibacterial activity of the essential oil are presented in Table 5 and Figure 6. The inhibition zone values (IZV) (25±1-15±1mm) varies against tested standard bacterial stains. *S. aureus* (25±1) is the most sensitive, followed by *E. coli* (20.67±0.57), *P. aeruginosa* (17.33±0.57) and then *E. faecalis* (15±1) (Table 5). The antibacterial activity of the essential oil exceeding those of the positive controls (standard antibiotics) Ampicillin\ Sulbactam (AS), Cefotaxime (CF), and Gentamycin (GM) against *S. aureus*. It also exceeding that of (AS) against *E. coli* and *P. aeruginosa*. In addition, the antibacterial activity of the essential oil approaching those of the positive control (AS) against *E. faecalis* and (GM) against *E. coli* (Table 5). It was noteworthy to mention that the essential oil (IZV: 20.67±0.57) was far more active against *E. coli* comparing the standard antibiotic Ampicillin\ Sulbactam (AS) with IZV: 8.33±7.23. This may be attributed to the resistance of *E. coli* against the antibiotic Ampicillin\ Sulbactam. Comparing the antibacterial activity of our sample with the antibacterial activity (tested against Gentamycin as positive control) of essential oil of the aerial parts of Yemeni wild grown *M. longifolia* subsp. *schimperi*, showed that our sample was more active against *S. aureus* (IZV: 25±1 exceeding that of the Gentamycin with IZV of 23.33±0.57) than their sample (IZV: 17.25 ± 0.34 less active than Gentamycin with IZV of 25.10 against *S. aureus*) [10]. In addition, our sample was more active against *E. coli* (IZV: 20.67±0.57), approaching the IZV of the Gentamycin (IZV: 21.66±6.80)), than their sample (IZV: 12.00±0.56 less active than Gentamycin with IZV of 25.10 against *E. coli*) [10]. However, our essential oil was less active (IZV of 17.33 ± 0.57) than Gentamycin with IZV of 23.33±2.08 against *P. aeruginosa* comparing to the essential oil of the Yemeni wild grown plant, which was found to be (IZV= 15.50 ± 0.47) exceeding that of Gentamycin with IZV of 10.00 against *P. aeruginosa*. [10]. Consequently, we can conclude that the essential oil of our sample showed broad spectrum of antibacterial activity with inhibition zone values exceeding those of the positive controls against *S. aureus*, *P. aeruginosa*, and *E. coli* and approaching that of *E. faecalis*. The antimicrobial activities of *Mentha* essential oils have mainly been attributed to volatile bio-actives such as oxygenated monoterpenoids along with monoterpene hydrocarbons and sesquiterpene hydrocarbons [1, 39]. Pulegone, menthone among other constituents were found responsible for the antibacterial activity of *M. pulegium* [40]. Flavonoids were also found to be active against Gram-positive and Gram-negative, impeding bacterial growth and causing cell death [34]. The hydroxyl group in phenol compounds was supposed to have a significant role in the antimicrobial activity of essential oils; treatment of *E. coli* cells by phenolic compounds caused surface blebbing and inhibition of RNA and protein synthesis [39]. Thus, the antibacterial activity of our sample may be mainly due to its content of oxygenated monoterpenes (pulegone (46.3624%), eucalyptol (10.5653%), and menthone (9.5726%) and phenolic compounds.

Conclusion

This is the first report of the chemical composition, radical scavenging antioxidant activity and antibacterial activity of in Aden cultivated *M. longifolia* subsp. *schimperi* leaves essential oil. In conclusion, the essential oil was found to be rich in oxygenated monoterpenes, pulegone (46.3624%), eucalyptol (10.5653%), and menthone (9.5726%) and contained phenolic compounds as well. These constituents could be responsible for its strong antioxidant and broad-spectrum antibacterial activities. Therefore, we suggest the essential oil of our sample as a good candidate for further studies for the development of antibiotics and food preservatives. However, further studies on the potential toxicity of the essential oil to humans are needed for safety purposes.

Table 1: Total phenolic content **(**TPC) and total falvonoids content (TF) of essential oil (EO) of cultivated *Mentha longifolia* subsp. *schimperi* leaves.

TPC at 720 nm		TFC at 430 nm	
Abs	Conc of GA in µg\ml	Abs	Conc of Q in µg\ml
0.1467±0.0106	6.25	0.0191±0.0013	3.125
0.20025±0.032	12.5	0.0426±0.005	6.25
0.3054±0.013	25	0.07045±0.022	12.5
0.4144±0.039	50	0.1823±0.059	25
0.5288±0.036	75	0.3377±0.013	50
0.6243±0.020	100	0.5541±0.035	75
0.76855±0.041	125	0.7354±0.072	100
0.8747±0.010	150	-	-
0.2069±0.039	At 200 µl of EO	0.0801±0.0052	At 500 µl of EO
TPC=11.315±0.335 mg\g		TFC =12.102±0.207 mg\g	

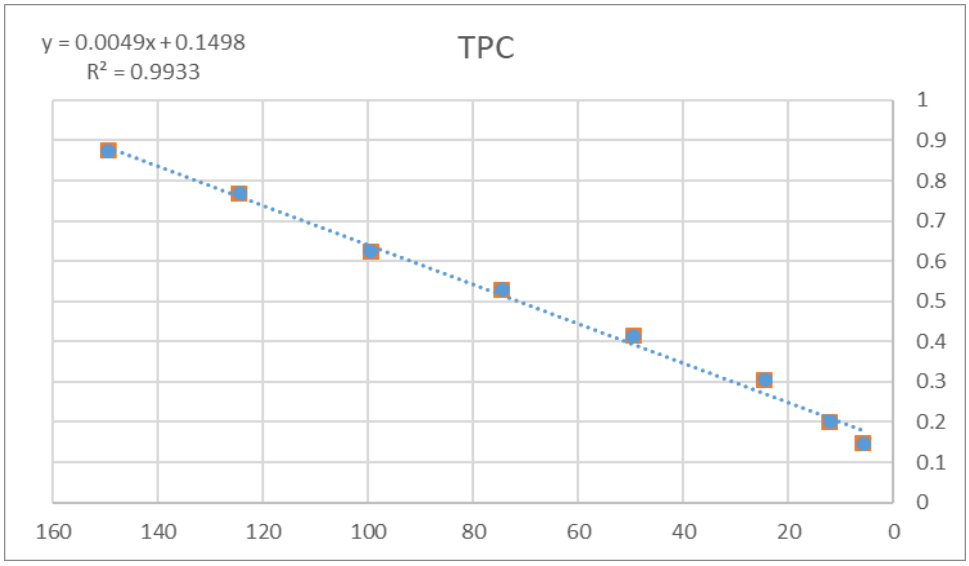


Fig. 1: Calibration curve of gallic acid concentarions

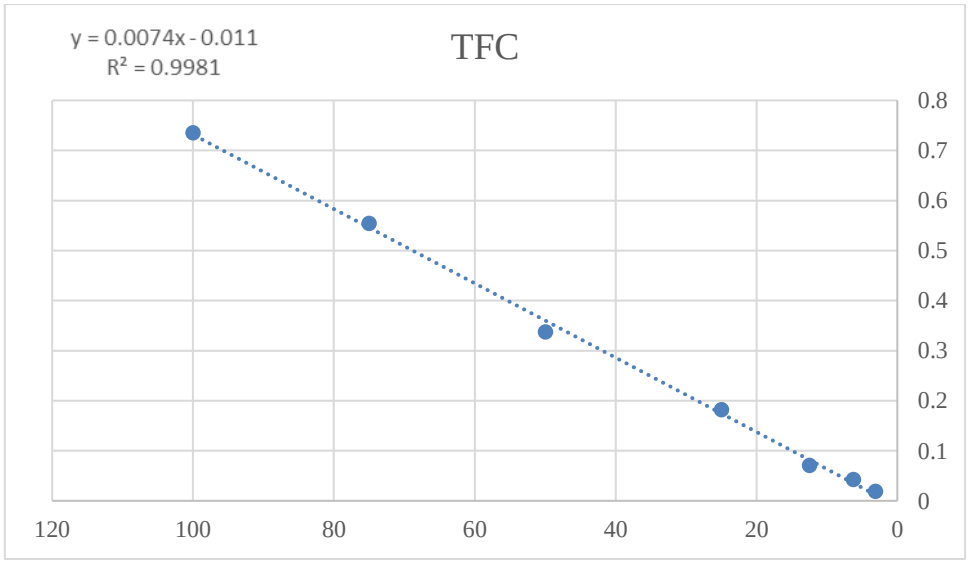


Fig. 2: Calibration curve of quercetine concentarions

Table 2: TLC of essential oil of cultivated Mentha longifolia subsp schimperi leaves.

UV 365 nm			Anisaldehyde sulphuric acid reagent		
No. of spots	Rf value	Spots color	No. of spots	Rf value	Spots color
11	0.071	Pale sky blue	11	0.071	Light purple
	0.14	Pale yellowish orange		0.14	Light yellow
	0.21	Sky blue		0.21	Gray purple
	0.28	Pale yellowish orange		0.28	Grey
	0.35	Pale red		0.35	Gray purple
	0.42	Pale orange		0.42	Light yellow
	0.5	pale orange		0.5	Light purple
	0.57	Pale yellowish sky blue		0.57	Grey
	0.64	Yellowish blue		0.64	Light yellow
	0.71	Pale red		0.71	Light purple
	0.78	Shiny orange		0.78	maroon

under UV 365 nm | with Anisaldehyde sulphuric acid reagent

Fig. 3: Colors of the spots of essential oil of cultivated Mentha longifolia subsp schimperi leaves on TLC plate

Table 3: Composition of essential oil from cultivated *Mentha longifolia* subsp. *schimperi* leaves.

Peak No.	RT	Composition %	Library/ID
1	4.9348	0.7863	Alpha.-Pinene
2	5.5177	2.0148	L-β-Pinene
3	6.1996	**10.5653**	Eucalyptol
4	7.3887	0.4239	L-Camphor
5	7.51105	**9.5726**	l-Menthone
6	7.7034	5.8717	Transe- isopulegone
7	7.8491	3.7759	δ - Terpineol
8	8.0764	6.3457	L-.Alpha.-Terpineol
9	8.4359	**46.3624**	Pulegone
10	9.0965	5.5601	Piperitenone
11	9.3937	2.1674	Caryophyllene
12	9.6269	0.4524	Humulene
13	9.9999	0.7961	γ -Cadinene
14	10.4895	1.6178	Caryophyllene oxide
15	10.6585	0.5253	Epicubenol
16	10.8334	2.0281	tau.-Cadinol
17	13.4563	0.4704	Isoincensole
18	14.8785	0.6638	Bis(2-ethylhexyl) phthalate
		Total 100	

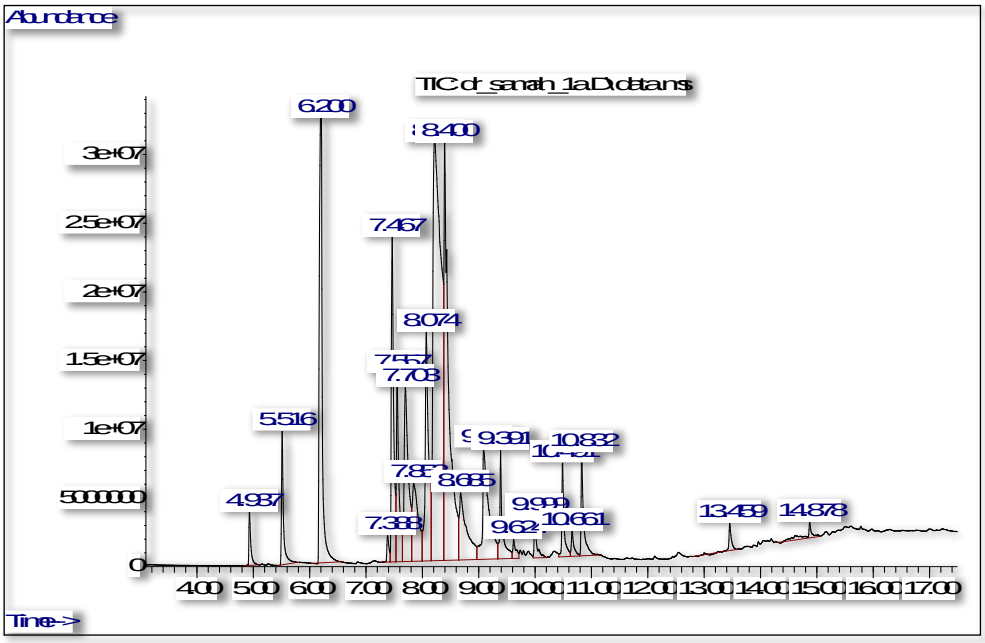


Fig. 4: GC chromatogram of essential oil of cultivated Mentha longifolia subsp. schimperi leaves

Table 4: Free radical DPPH scavenging activity (% inhibition) of essential oil of cultivated Mentha longfolia subsp schimperi leaves.

CONC in µg\ml	Essential oil		Ascorbic acid	
	Abs at 517 nm	%inhibition	Abs at 517 nm	% inhibition
10	0.5321±0.005	34.268±0.20	0.4022±0.008	50.31±0.42
20	0.402±0.012	50.3397±0.16	0.3089±0.003	61.84±0.35
40	0.3351±0.023	58.604±0.33	0.2011±0.034	68.04±0.17
60	0.2204±0.035	66.1538±0.26	0.1255±0.027	84.49±0.23
80	0.1512±0.019	81.321±0.10	0.0734±0.012	90.93±0.19
100	0.0575±0.030	92.8968±0.36	0.032±0.020	96.04±0.33
Control solution (negative)	0.8095	0	0.8095	0
	IC50= 28.3837 ±0.20 µg\ml		IC50= 1.648 ±0.090 µg\ml	

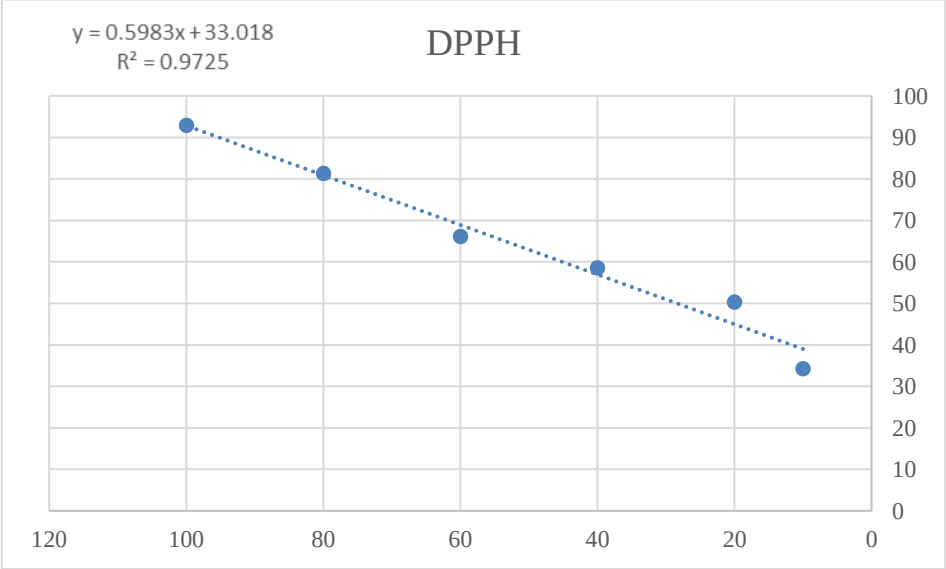
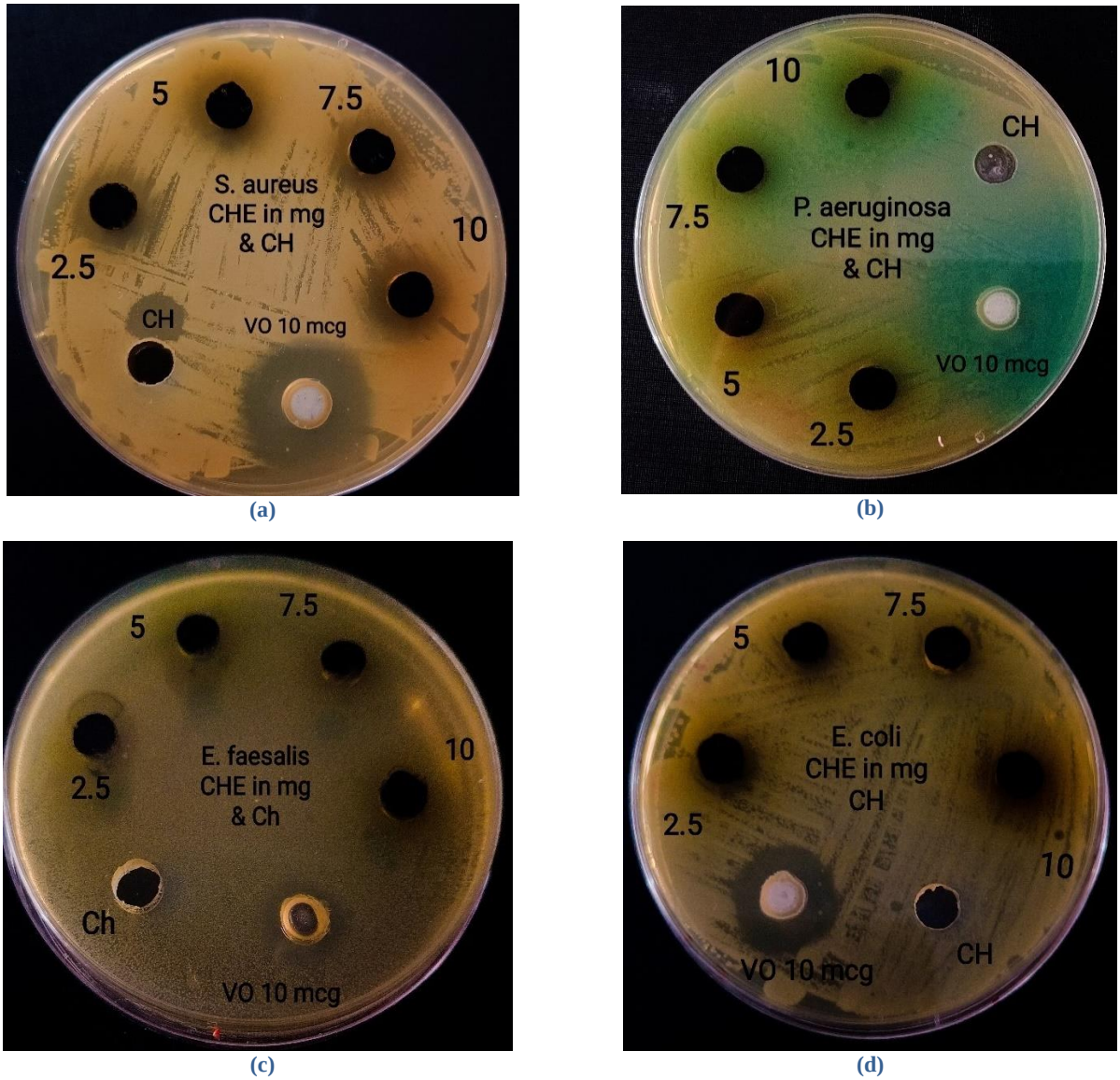


Fig. 5: Free radical scavenging effect of standard ascorbic acid by DPPH assay

Table 5: Antibacterial activity of essential oil of cultivated Mentha longifolia subsp schimperi leaves.

Standard bacterial	Inhibition zone in mm	AS 20 µg	CF 30 µg	CP 5 µg	GM 10 µg
S. aureus	25±1	17.66±0.57	19.66±1.52	30±4.35	23.33±0.57
P. aeruginosa	17.33±0.57	14.33±4.93	19.33±5.13	34.66±2.51	23.33±2.08
E. faecalis	15±1	16±4	19.33±0.57	24±4.35	19.66±0.57
E. coli	20.67±0.57	8.33±7.23	23.33±5.13	32.33±6.80	21.66±6.80



(a) **(b)**

(c) **(d)**

Fig. 6: (a , b, c, d) Antimicrobial activity of essential oil of cultivated *Mentha longifolia* subsp s*chimperi* leaves against bacterial (*S. aureus, P. aeruginosa, E. faecalis, E. coli*)

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مقالة بحثية

التركيب الكيميائي وتقييم فعالية إزالة الجذور الحرة والأنشطة المضادة للبكتيريا للزيت العطري لأوراق نبات النعناع *MENTHA .SUBSP. SCHIMPERI* (BRIQ.) BRIQ (L.) HUDSON *LONGIFOLIA*، المزروع في محافظة عدن، اليمن

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المُلخّص

شمل هذا البحث دراسة التركيب الكيميائي والأنشطة المنظفة للجذور الحرة والمضادة للبكتيريا في الزيت العطري المستخرج من أوراق نبات النعناع (*Mentha longifolia* (Briq.) Briq (L.) Hudson subsp. *schimperi*.) المزروع في محافظة عدن- اليمن. كشف تحليل الغاز الكروماتوغرافيا/مطياف الكتلة للزيت العطري المستخلص بالتقطير المائي عن ثمانية عشر مركبًا تمثل 100% من إجمالي الزيت، وكانت المكونات الرئيسية المكتشفة هي بوليجون (46.3624%) وأوكاليبتول (10.5653%) ومنثون (9.5726%). أشار تحديد إجمالي الفينولات والفلافونويدات إلى محتوى 11.653 مجم / GAE جم و 12.310 مجم / QE جم على التوالي. أظهر النشاط المضاد للأكسدة الذي تم إجراؤه باستخدام جذور DDPH بأن تركيز العينة اللازم لتثبيط 50% من جذور DPPH كان 28.3837 ميكروجرام / مل= IC_{50}، ممَّ يشير إلى نشاط قوي مضاد للأكسدة. فيما يتعلق بالنشاط المضاد للبكتيريا، فقد أظهر الزيت العطري نشاطًا واسع النطاق مضادًا للبكتيريا يتراوح بين المتوسط إلى القوي ضد أربع سلالات بكتيرية تم اختبارها هي: *Staphylococcus aureus* ATCC 29213 و *Enterococcus faecalis* ATCC 29212 و *Escherichia coli* ATCC 25922 و *Pseudomonas aeruginosa* ATCC 27853. كانت *Staphylococcus. aureus*هي السلالة الأكثر حساسية للزيت العطري ولها أكبر منطقة تثبيط (25 ± 1). يمكن القول أن النوع المزروع من نبات *Mentha longifolia* (L.) Hudson, subsp. *Schimperi*، يحتاج لمزيد من الدراسات لتطوير المضادات الحيوية والمواد الحافظة الغذائية.

الكلمات المفتاحية: أوراق **Mentha longifolia subsp. schimperi**، الانشطة المنظفة للجذور الحرة، المضاد للبكتيريا.

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