Journal of Pharmaceutical Research International

23(4): 1-10, 2018; Article no.JPRI.35932
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Volatile Constituents, Antimicrobial and Antioxidant Activities of the Aerial Parts of *Origanum majorana* L. from Yemen

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JPRI/2018/35932
Editor(s):
(1) Vasudevan Mani, Universiti Teknologi MARA (UiTM), Selangor, Malaysia.
Reviewers:
(1) Alok Kumar Dash, V. B. S. P. University, India.
(2) Bruno dos S. Lima, Federal University of Sergipe, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/25967

Original Research Article

Received 13th June 2017
Accepted 21st August 2017
Published 21st August 2018

ABSTRACT

Aims: There is no report about the analysis of volatile composition of *Origanum majorana* L. (*Lamiaceae*) by static headspace method in combination with GC-MS. The present study reports for the first time the volatile composition, antimicrobial and antioxidant activities of *O. majorana* from aerial parts of plant collected in Yemen.
Methodology: The volatile composition was obtained by headspace vapour of plant aerial parts and determined by gas chromatography-mass spectrometry (GC-MS) analysis. Antioxidant activity of extracts was evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay and antimicrobial activity by agar diffusion.
Results: The major volatile compounds were found to be monoterpenes: *Trans*-sabinene hydrate (16.0 %), sabinene (14.1%), *cis*-sabinene hydrate (11.8 %), γ-terpinene (10.2%),*α*-terpinyl acetate (10.0%), α-terpinene (8.9%), terpinen-4-ol. In addition, five volatile non terpenoid compounds were identified for the first time: 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal and methyl 2-methylbutanoate. Furthermore, dichloromethane, methanol and aqueous extracts were tested for their antimicrobial activities against five bacteria and two fungi strains. The most sensitive microorganism was *Staphylococcus aureus*, reaching MIC values of 50, 100 μg/mL for methanol

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and dichloromethane extracts, respectively. The methanol extract showed strong radical scavenging activity in the DPPH assay followed by dichloromethane extract.
Conclusion: The *O. majorana* extracts showed potent antimicrobial and antioxidant activities mostly attributed to identified oxygenated monoterpenes. The obtaining of volatiles of the plant as vapour by headspace method showed more qualitative and accurate quantitative results than by hydrodistillation method. Therefore, the headspace analysis should be useful in analytical control of herbal crude drugs.

Keywords: Origanum majorana; headspace; volatiles; GC-MS; antimicrobial; antioxidant.

1. INTRODUCTION

The genus *Origanum* belongs to the family *Lamiaceae* (Labiatae) which includes 221 genera and 5600 species widespread all around the world [1]. In the flora of Yemen, *Lamiaceae* includes 11 genera and *Origanum* genus is represented by only by the single, cultivated species *Origanum majorana* L. (*Lamiaceae*, Labiatae) syn. *Majorana hortensis* Moensch (Fig. 1) [2]. It occurs in the higher mountains in of North Yemen in Sanaa, Ibb and Taiz [2,3]. The Yemeni local name of the plant is *Ozzab* or *Lizzab*. Its name in Islam Arab traditional medicine is (Bardaqush). In the Yemeni folk medicine, the fresh and dried herbs of *O. majorana* are used as powder or decoction to treat pain, headache, and cold diseases [2]. Many phytochemical studies on *O. majorana* essential oils EOs have reported variations in the volatile compositions according to geographical origins [4-12]. Monoterpenes have been reported as dominant content of *O. majorana* EOs while sesquiterpenes with low content [4-12].The essential oils obtained from *O. majorana* from different origins have been studied for their antimicrobial [4-8] and antioxidant activities [6-8]. These biological activities have been attributed mainly to sabinene hydrate and terpinen-4-ol [6-12]; which are the major constituents of the *O. majorana* EOs from different geographical origins [4-7].

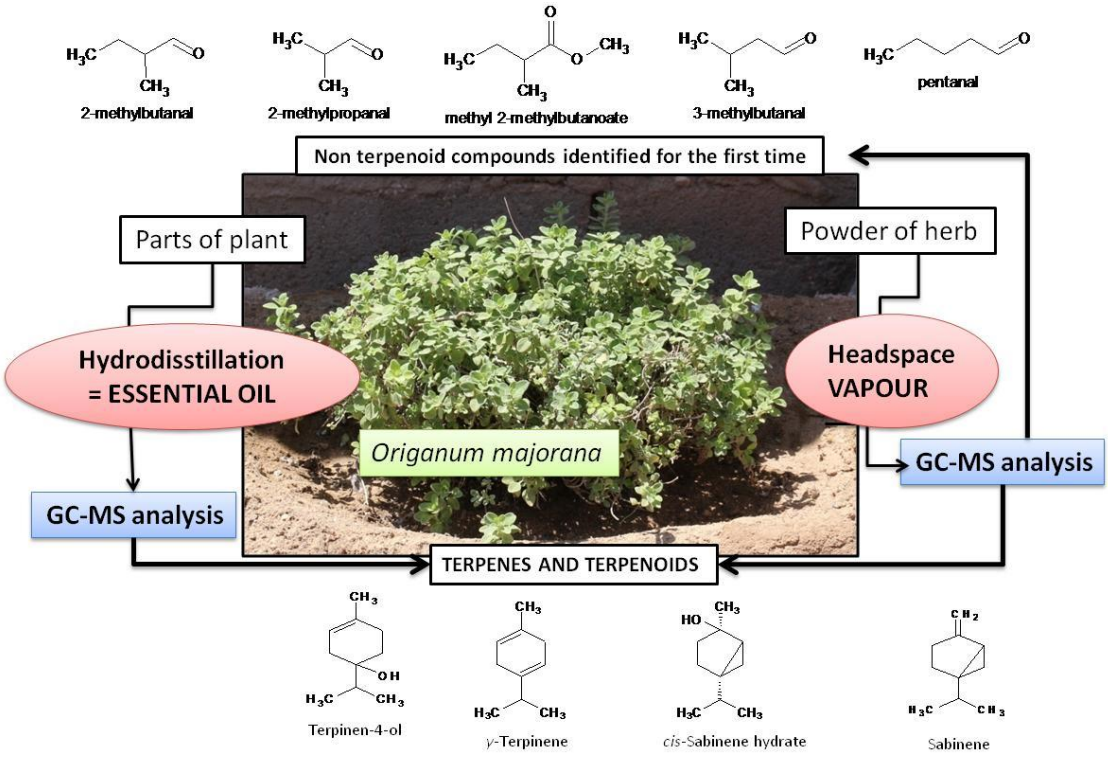

Fig. 1. *Origanum majorana* L. (Foto by author)

Volatile compositions, that have been reported for many *Origanum* species, including *O. majorana*, were obtained by extraction of essential oils (EOs) [4-22]. They have been collected and studied from different geographical world origins, except from Yemen. Nevertheless the volatile composition on headspace of plant powder have never been studied so far. Therefore, the aims of this study were to identify the volatile components obtained by headspace vapour of the *O. majorana* aerial parts from Yemen, using GC-MS analysis and investigate the antimicrobial and antioxidant properties of the plant extracts.

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts of *Origanum majorana* L. were collected from occurrences in Taiz, Yemen, in March 2010. The collected plant was taxonomically identified at the Pharmacognosy Department, Aden University, Aden, Yemen, where a voucher specimen (MAF-T-OR 012) of the plant material was deposited. The *O. majorana* aerial parts were allowed to air dry and afterwards pulverized in grinder.

2.2 Extraction of Plant for Biological Tests

Thirty grams of the fine pulverized materials were successively extracted with 300 ml of dichloromethane, 300 ml of methanol and 300 ml of water at room temperature for three times, each run for 8 h. After that the extracts were concentrated under reduced pressure at 40°C, freeze-dried and stored in an exsiccator.

2.3 Determination of Volatile Chemical Compositions

2.3.1 Static headspace method

The Static headspace procedure was connected to a gas chromatography to analyze the vapour phase above 5 g of dry powder of *O. majorana* aerial parts powder contained in a 20 ml vial. Static headspace conditions were as follows: split injection (split ratio 1:5); incubation temperature 40^{o}C; incubation time 30 min; syringe temperature 45^{o}C and injection volume 500 µl.

2.3.2 Chromatographic analysis

Volatile compounds identification was based on gas chromatography-mass spectrometry GC-MS measurements, which were performed on a QP-2010 Ultra (Shimadzu Corporation, Kyoto, Japan). Mass spectrometry conditions were: electron impact ionization (EI) mode system; electron energy 70 eV; detected mass range *m/z* 50-500 and ion source temperature 200 ^{o}C. The Gas chromatography conditions were set as the follow: column, ZB-5MS (Phenomenex, 30 m x 0.25 mm, 0.25 µm film thickness); injector temperature 220^{o}C; interface temperature 300^{o}C; carrier gas helium; column flow 1.02 ml/min; constant flow mode and column temperature program: 40^{o}C for 1 min, then raised to 300^{o}C at a rate of 10^{o}C min^{-1} and then hold on 300^{o}C for 5 min.

2.4 Determination of Antimicrobial Activities

2.4.1 Microorganisms

The following bacterial strains were employed in the screening: *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6059), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Micrococcus flavus* (SBUG 16). As fungal strains *Candida maltose* (SBUG 17), *Candida albicans* (ATCC 90028), were used. The SBUG strains were obtained from the strain collection of the Institute of Microbiology (SBUG) of the Ernst-Moritz-Arndt-University Greifswald, Germany.

2.4.2 Antimicrobial assays

Modified agar diffusion method [23] was used to determine antibacterial and antifungal activities. The bacterial cell suspension was prepared from a 24 h culture and adjusted to an inoculation of 1×106 colony forming units per ml. Sterile nutrient agar (Immunpräparate, Berlin, Germany, 26 g agar/l distilled water) was inoculated with bacterial cells (200 µL of bacterial cell suspension in 20 mL medium) and poured into dishes. Yeasts and hyphomycetes (1×105 colony forming units per mL) were inoculated into sterile Mueller-Hinton-agar (Becton Dickinson, Heidelberg) according to DIN E 58940-3 [24] for the agar disc-diffusion assay. Forty microliters of test material (equivalent to 2 mg of the dried extract), dissolved in the same solvent which was used to prepare the tested extract, were applied on sterile paper discs (6 mm diameter, Schleicher and Schuell, D, ref. no. 321860).

Ampicillin, gentamicin and nystatin were used as positive control, and the solvents dichloromethane and methanol as negative control. The solvents were allowed to evaporate in a stream of air. The discs were deposited on the surface of inoculated agar plates. Plates were kept for 3 h in the refrigerator to enable prediffusion of the substances into the agar. Plates with bacteria were incubated for 24 h at 37°C, plates with yeasts for 48 h at 36°C and plates with hyphomycetes for 72 h at 30°C. Inhibition zone (IZ) diameters around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured and recorded at the end of the incubation time. An average zone of inhibition was calculated for the three replicates.

Minimal inhibitory concentrations (MICs) were determined by the agar diffusion technique as described by [25]. The highest concentration of extract tested during the experiment was 1 mg/ml. The MIC corresponds to the lowest concentration of the test extract able to inhibit any visible microbial growth.

2.5 Determination of Radical Scavenging Activity

The free radical scavenging activity was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay described as Brand and Sievers [26,27]. The reaction mixture contained 500 µL of test extract and 125 µL of DPPH in ethanol. Different concentrations of test samples (10, 50, 100, 500 and 1000 µg/mL) were prepared while the concentration of DPPH was 1mM in the reaction mixture. These reaction mixtures were taken into Eppendorf tubes and incubated at 37°C for 30 min, the absorbance was measured at 517 nm. Percent radical scavenging activity by sample treatment was determined by comparison with ethanol treated control group. Ascorbic acid was used as positive control. The DPPH radical concentration was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})}{\text{Absorbance}_{\text{control}}} \times 100$$

3. RESULTS

3.1 Identification of Volatile Components by Static Headspace GC-MS Analysis

Static headspace GC-MS analysis was used to examine the volatile organic compounds released by powder of *O. majorana* aerial parts collected from Yemen. The compounds were identified on the basis of their electron impact ionization (EI) mass spectra compared with the corresponding data in the NIST 11, the FFNSC2 library (Shimadzu) and the MassFinder 4.0 software which also consider the Kovats retention indices.

A classification are observed according to the retention time and increasing molecular weights of compounds: in the first 5 minutes, the volatile non-terpene hydrocarbons were identified; followed by the terpene hydrocarbons in order to their chemical structures: Monocyclic and bicyclic monoterpene hydrocarbons followed by oxygenated monoterpenes (alcohols than esters) and after 14 minutes end by sesquiterpene hydrocarbons.

32 volatile compounds were identified in the headspace gas of *O. majorana* (Table 1). The identified volatile compounds can be divided in two main groups: a) 5 volatile non-terpene organic compounds including four aldehydes and one ester revealed low content (1.1%) and b) 27 volatile terpenes with high content (98.1%) (Table 1). The volatile terpenes were 25 monoterpenes (97.5%) and two sesquiterpene hydrocarbons (0.6%). The monoterpenes were 12 monoterpene hydrocarbons (48.8%) and 13 oxygenated monterpenes (48.7%). The contents of the oxygenated monoterpenes and monoterpene hydrocarbons were equal. The volatile content of the plant was dominated by monoterpenes which accounted for 97.5% of the volatile gas (Table 1). The major monoterpenes were *trans*-sabinene (16.0%), sabinene (14.1%), *cis*-sabinene hydrate (11.8%), γ-terpinene (10.2%), *α*-terpinylacetate (10.0%), α-terpinene (8.9%) and terpinen-4-ol (5.8%) (Table 1). Other monoterpenes present in fairly good amount were p-cymene (3.8%), α-thujene (2.8%), β-phellandrene (2.6%), α-pinene (1.7%), terpinolene (1.5%), β-myrcene (1.2%), α-terpineol (1.1%) and bornyl acetate (1.0%). (Table 1). The sesquiterpenes fraction contained caryophyllene and bicyclogermacrene less than 1% (Table 1). In addition, five non terpenoid volatile compounds were identified in *O. majorana* for the first time: 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal and methyl 2-methylbutanoate (1.1%) (Table 1).

3.2 Antimicrobial Activities of the Extracts

The results of the antibacterial and antifungal screening of dichloromethane, methanol and

Table 1. Relative compositions of the volatile components of the powder of *O. majorana* aerial parts determined by headspace-GC-MS

Peak no.	Rt[a]	Compound[b]	Class[c]	MF[d]/MW[e]	Rc[f] (%)
Non-terpenoid hydrocarbons (5 with 1.1%)					
1	2.33	2-Methylpropanal	Aldehyde	C_4H_8O/ 72.1057	0.2
2	2.95	3-Methylbutanal	Aldehyde	$C_5H_{10}O$/ 86.1323	0.3
3	3.03	2-Methylbutanal	Aldehyde	$C_5H_{10}O$/ 86.1323	0.3
4	3.34	Pentanal	Aldehyde	$C_5H_{10}O$/ 86.1323	0.2
5	4.32	Methyl 2-methylbutanoate	Ester	$C_6H_{12}O_2$/116.1583	0.1
Monoterpene hydrocarbons (12 with 48.8%)					
6	6.65	*α*-Thujene	Bicyclic	$C_{10}H_{16}$/136.2340	2.8
7	6.78	*α*-Pinene	Bicyclic	$C_{10}H_{16}$/136.2340	1.7
8	7.04	Camphene	Bicyclic	$C_{10}H_{16}$/136.2340	0.8
9	7.42	Sabinene	Bicyclic	$C_{10}H_{16}$/136.2340	14.1
9	7.50	β-Pinene	Bicyclic	$C_{10}H_{16}$/136.2340	0.9
11	7.66	*β*-Myrcene	Acyclic	$C_{10}H_{16}$/136.2340	1.2
12	7.93	*α*- Phellandrene	Monocyclic	$C_{10}H_{16}$/136.2340	0.3
13	8.12	*α*-Terpinene	Monocyclic	$C_{10}H_{16}$/136.2340	8.9
14	8.25	p-Cymene	Monocyclic	$C_{10}H_{14}$/134.2182	3.8
15	8.33	*β*-Phellandrene	Monocyclic	$C_{10}H_{16}$/136.2340	2.6
16	8.80	*γ*-Terpinene	Monocyclic	$C_{10}H_{16}$/136.2340	10.2
18	8.96	Terpinolene	Monocyclic	$C_{10}H_{16}$/136.2340	1.5
Oxygenated monoterpenes (13 with 48.7%)					
17	9.29	*cis*-Sabinene hydrate	Bicyclic, Alcohol	$C_{10}H_{18}O$/154.2493	11.8
19	9.42	Linalool	Acyclic, Alcohol	$C_{10}H_{18}O$/154.2493	0.6
20	9.47	*trans*-Sabinene hydrate	Bicyclic, Alcohol	$C_{10}H_{18}O$/154.2493	16.0
21	9.83	*cis*-p-Menth-2-en-1-ol	Monocyclic, Alcohol	$C_{10}H_{18}O$/154.2493	0.8
22	10.12	*trans*-p-Menth-2-en-1-ol	Monocyclic, Alcohol	$C_{10}H_{18}O$/154.2493	0.3
23	10.56	Borneol	Bicyclic, Alcohol	$C_{10}H_{18}O$/154.2493	0.2
24	10.71	Terpinen-4-ol	Monocyclic, Alcohol	$C_{10}H_{18}O$/154.2493	5.8
25	10.91	*α*-Terpineol	Monocyclic, Alcohol	$C_{10}H_{18}O$/154.2493	1.1
26	10.98	Piperitol	Monocyclic, Alcohol	$C_{10}H_{18}O$/154.2493	0.2
27	11.30	*β* -Terpinyl acetate	Monocyclic, Ester	$C_{12}H_{20}O_2$/196.2860	0.4
28	11.79	*α*-Terpinyl acetate	Monocyclic, Ester	$C_{12}H_{20}O_2$/196.2860	10.0
29	12.30	Bornyl acetate	Bicyclic, Ester	$C_{12}H_{20}O_2$/196.2860	1.0
30	12.48	Terpin-1-en-4-ol acetate	Monocyclic, Ester	$C_{12}H_{20}O_2$/196.2860	0.5
Sesquiterpene hydrocarbons (2 with 0.6%)					
31	14.23	*β*-Caryophyllene	Bicyclic	$C_{15}H_{24}$/ 204.3511	0.5
32	15.21	Bicyclogermacrene	Bicyclic	$C_{15}H_{24}$/ 204.3511	0.1
		Total			**99.2**

Rt[a], Retention time (as min); Compounds[b], listed in order of retention time; Class [c], classification of compounds according to chemical structures; MF[d], Molecular formula; MW[e], Molecular Weight in g/mol; Rc[f], Relative compositions in% from the dried sample

water extracts of the *O. majorana* aerial parts against five bacteria strains and against two fungi species are summarized in Table 2 (inhibition zones in the agar diffusion assay) and Table 3 (MIC values). An inhibition zone > 15 mm in the agar diffusion assay was considered as a high antimicrobial activity. The controls utilized to evaluate the antimicrobial activities of plant extracts are standard antibiotics. Both dichloromethane and methanol extracts showed broad antibacterial activities against Gram-Positive and Gram-negative bacteria strains and

antifungal activities against two *Candida* species. Dichloromethane and methanol extracts showed stronger antibacterial activity against *S. aureus* (IZ: 30, 35 mm) (MIC: 100, 50 µg/mL), successively (Table 2,3). While methanol extract exhibited high antibacterial activity against *M. flavus* and *P. aeruginosa*, dichloromethane extract exhibited same strength activity against *B. subtilis* and *E. coli* (IZ: 20 mm) (250 µg/mL) (Tables 2,3). The methanol and dichloromethane extracts showed also good antifungal activities against *C. albicans* and *C. maltose* with inhibition zone (18-20 mm) (Table 2).

3.3 Antioxidant Activity of the Extracts

The methanol extract *O. majorana* showed an antioxidant activity (IC_{50}102.6 µg/mL) in the DPPH assay, stronger than the DCM extract (IC_{50} 127.0 µg/mL). The aqueous extract was only weakly active compared with ascorbic acid (Table 4).

4. DISCUSSION

The volatile constituents of a plant can be obtained as essential oil (EO) by extraction or as gas by headspace (HS) of the plant part. The content of EO is formed from two main groups of volatile components: phenylpropanoids and/or terpenes, that can be identified by GC-MS [28], while the other chemical groups of volatile constituents cannot be separated in the EO. In contrast, the headspace gas of the dried plant parts can separate all volatile compounds to identify be GC-MS analysis.

Previous studies investigated the volatile constituent obtained by essential oils extraction from *O. majorana* [4-12], but never investigated its headspace vapour.

Our results obtained by headspace of plant aerial parts powder (Table 1) support the previous observations, that *O. majorana* EOs from different geographical origins in the world contain terpenes with absence of phenylpropanoids [4-12]. The main terpenes of the Plant EOs were mostly reported as *trans*-sabinene hydrate, *cis*-sabinene hydrate and terpinen-4-ol [4-12], that were investigated in the present study in the headspace of plant powder with remarkable different in their amounts.

Table 2. Antimicrobial activity of the extracts of *O. majorana* aerial parts, investigated with agar diffusion test

Microorganisms	Diameter of inhibition zone (mm)[a]					
	Extracts			Standards		
Bacterial strains	**D**	**M**	**W**	**Nys.**	**Amp.**	**Gen.**
Bacillus subtilis	20	15	10	NT	28	NT
Escherichia coli	20	15	10	NT	NT	15
Micrococcus flavus	15	20	10	NT	31	NT
Pseudomonas aeruginosa	15	20	10	NT	NT	18
Staphylococcus aureus	30	35	15	NT	26	NT
Fungal strains						
Candida albicans	20	25	8	26	NT	NT
Candida maltosa	18	20	8	25	NT	NT

[a]*Values are inhibition zone diameter (mm); D, dichloromethane; M, methanol; W, water; (conc, 2 mg/disc); Amp, ampicillin (10 µg/disc); Gen, gentamicin (10 mg/disc); Nys, nystatin (100 µg/disc); NT: not tested; negative controls did not show any activity*

Table 3. MIC values of the antibacterial activity of the extracts of *O. majorana* aerial parts

Bacteria strains	MIC in µg/mL		
	Plant extracts		
Gram-positive	**D**	**M**	**W**
Staphylococcus aureus	100	50	500
Bacillus subtilis	250	1000	>1000
Micrococcus flavus	1000	250	>1000
Gram-negative			
Escherichia coli	250	1000	1000
Pseudomonas aeruginosa	1000	250	>1000

D, dichloromethane; M, methanol; W, water

To the best of our knowledge, the headspace of the powder *O. majorana* from Yemen showed specific different in the quality and quantity of the volatile compounds of the plant. Five volatile non terpenoid compounds were identified in the plant for the first time: 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, pentanal and methyl 2-methylbutanoate (Table 1).These aldehyde and ester compounds (Table 1) are responsible for the aroma of certain natural products [29,30] besides the mono and sesquiterpenes which are known as potent odorants and flavoring agents of the plants [28]. In addition, a new chemotype feature for the plant was added by identification of these five volatile organic compounds in the plant powder.

Table 4. Free radical scavenging activity in the DPPH assay for the extracts of *O. majorana* aerial parts

Extracts/standard	Free radical scavenging activity IC_{50} (μg/mL)
Dichloromethane	127.0
Methanol	102.6
Water	650.5
Ascorbic acid	14.7

Moreover, four monoterpene esters have been identified in our study for the first time as acetate of borneol, terpinenol, *β*–terpinyl and *α*-terpinyl. The last compound was found for the first time as a major component (10.0%) in *O. majorana* (Table 1); it has been not reported except in poor amount (0.1%) [9]. Camphene was found in good quality while it has been not reported except in very low content [8].

In the present study, volatile phenols were not found in the headspace of plant powder. This finding is consistent with the previous studies on the EOs of *O. majorana* from different geographical origins which reported the absence of volatile phenols [4,5,7-12], except in *O. majorana* EO from Mexico that was reported to contain thymol 16.3% [6]. In contrast, in some other species of *Origanum*, the presence of carvacrol was reported as component in their EOs, such as in EOs of *O. dictamnus* [15,17]*, O. libanoticum* [15]*, O. onites* [18], *O. microphyllum* [6,8], *O. minutiflorum* [19], *O. scabrum* [13] and *O. vulgare* subsp. *virens* [14] while thymol was reported as main content of *O. vulgare* subspecies EO [14,9]. Generally, terpenes have been reported as general chemotype for the species of *Origanum*, based on volatile content of essential oils [4-19].

There are no reports of antimicrobial activity and composition neither for the extracts nor for the essential oil of *O. majorana* from Yemen. However, *O. majorana* plants collected from other world localities have been studied for the antimicrobial activities based on the essential oils [4-6,10,31]. But there are few reports about the antimicrobial activities of the extracts, such n-hexane [14] and methanol extract [32,33].

The cyclic monoterpene alcohols α-terpineol, terpinen-4-ol and borneol [20] and alcohol acyclic monoterpenoid linalool [34] are known as strong antibacterial agents. Most of the identified oxygenated terpenes (terpenoids) were monoterpene alcohols (36.8%) (Table 1). In addition, the most terpene hydrocarbons that have been identified in our study are known for their antimicrobial activities such as *β*-pinene, *α*-pinene, α-terpinene, sabinene, γ-terpinene, terpinolene, bornyl acetate, myrcene, *β*-caryophyllene, α-phellandrene and *p*-cymene [20]. These results explain the strong antimicrobial activity of the DCM and MeOH extracts.

On the other hand, γ-terpinene, α-pinene and p-cymene have been reported as strong antifungal monoterpene hydrocarbons [35,36]. Therefore the strong antifungal activities of the non polar extracts may be attributed to the major proportion of these identified compounds. The antimicrobial activity of the dichloromethane and methanol extracts may be explained by the presence of flavonoids and phenolic acids [11,37-39], beside the presence of the monoterpene alcoholic compounds (Table 1), which may possess synergistic effect on the antimicrobial activities of the extracts.

The antioxidant data were supported by the headspace GC-MS analysis, indicating that the antioxidant potential of the DCM and methanol extracts was closely related to the high content of volatile alcoholic terpenoids (Table 1) and polyphenolic compounds which have been reported [23,39-42] as constituents of the polar and non polar extracts *O. majorana* [11,37,39,40]. There were some reports about the antioxidant activities of *O. majorana* based on the EO samples [18-20,42] but few reports based on its extracts [37,38,41,42].

5. CONCLUSION

The GC-MS analysis results of volatile composition obtaining by headspace gas of *O. majorana* were found to differ from previous studies that used only hydrodistillation method. The new chemotype data obtained by the headspace analysis should be useful in analytical control for *O. majorana* variants from different geographical origins. Therefore, the volatile compositions of a crude medicinal plant can be evaluated exactly by headspace GC-MS analysis. The potent antimicrobial and antioxidant activities of *O. majorana* extracts are attributed mostly to the presence of alcohol terpenes, *trans*-sabinene hydrate, *cis*-sabinene hydrate and terpinen-4-ol. These results confirm the traditional use of the plant as powder or extract in the Yemeni folk medicine. Further research is required to determine more pharmacological properties of the plant extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

I thank Ludger Wessjohann and Norbert Arnold from Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany, and Ulrike Lindequist, Institute for Pharmacy, Ernst-Moritz-Arndt University Greifswald, Germany, for giving me the opportunity to work in their laboratories. Besides I thank Jürgen Schmidt and Christine Kuhnt from the Leibniz Institute of Plant Biochemistry, Halle, for their support in static headspace GC/MS analysis.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
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