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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OILS FROM DIFFERENT AROMATIC PLANTS GROWN IN YEMEN

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Abstract

Chemical compositions and related total antioxidant capacities of nine spice essential oils were analyzed. To enable a comparison of their relative antioxidant potentials, essential oils were extracted by hydrodistillation from selected spice plants and their chemical compositions were determined by the GC-MS system. Antioxidant effectiveness was examined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Based on their antioxidant capacity, nine spice essential oils can be sorted in descending order: *Thymus laevigatus* > *Lavandula pubescens* (Taiz) >*Plectranthus barbatus* > *Lavandula pubescens* (Ibb) > *Nepeta deflersiana* (Taiz) > *Nepeta deflersiana* (Sana'a) > *Origanum majorana* > *Teucrium yemensis* > *Mentha x piperita* > *Stachys yemensis*.

Key words: Yemeni *Aromatic plants*, Essential Oil, Antioxidant, Chemical Composition.

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INTRODUCTION

The role of free radical in many diseases conditions have been established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenge by cellular constituents, they lead to disease conditions [1]. Essential oils from aromatic and medicinal plants have been known to possess biological activity, and antioxidant activities [2, 3]. The antioxidant activity of essential oils is a biological property of great interest because they may preserve foods from the toxic effects of oxidants [4]. Moreover, essential oils being also able of scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals [5,6].

Essential oils have a complex composition, containing from a dozen to several hundred components. The great majority of components identified in essential oils includes terpenes (oxygenated or not), with monoterpenes and sesquiterpenes prevailing. Nevertheless, allyl- and propenylphenols (phenylpropanoids) are also important components of some essential oils [7].

To the best of our knowledge, essential oil composition of *Nepeta deflersiana*, (Taiz and Sana'a) *Lavandula pubescens* (Taiz and Ibb) , *Teucriumyemensis, Plectranthusbarbatus, Mentha x piperita*, *Stachys yemensis, Origanum majorana* , and *Thymus laevigatus* have not been reported and no literature data is available for the oil composition of them. Antioxidant activities of the various extracts of these plant also have not been reported. The aim of this study is determining the chemical composition and antioxidant activities of the essential oils of various extracts of aromatic plants grown in Yemen.

MATERIALS AND METHODS

Plant material

Plant Collection and Extractions:

Nepeta deflersiana, (Taiz and Sana'a) *Lavandula pubescens* (Taiz and Ibb) , *Teucrium yemensis*, *Plectranthus barbatus*, *Mentha x piperita*, *Stachys yemensis*, *Origanum majorana* , and *Thymus laevigatus* were collected from Taiz, Sana'a, Almahweet, and Ibb, Cities, Republic of Yemen.

Extraction of the Essential oils

One hundred grams of plant material and 500 ml water were placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for 3 h. The obtained essential oils were separated, dried over anhydrous sodium sulphate, and stored under argon in a sealed vial, at - 20 °C before usage [3]. The chemicals and all applied solvents were of pro analysis purity and were purchased from Fluka Chemie, Buchs, Switzerland.

Gas Chromatography–Mass Spectrometry (GC-MS)

The analyses of the volatile compounds were run on a Agilent Technologies AutoSystem XL GC-MS system operating in the EI mode at 70 eV, equipped with a split/splitless injector (250°C).

Two columns were used: a HP 5MS (30m x 0.25 mm; film thickness 0.25 mm) and an HP Innowax (30m x 0.32mm i.d., film thickness 0.50 mm). Oven temperature was programmed as following: isothermal at 70 °C for 4 min, then increased to 180 °C, at a rate of 4 °C/min and subsequently held isothermal for 15 min (for Innowax column); isothermal at 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C/min and held isothermal for 15 min (for 5MS column). The carrier gas was helium (1.3 ml/min). The injection port temperature was 250 ºC and the detector temperature was 280 °C. Ionization of the sample components was performed in the EI mode (70 eV). Injected volume was 1 µl.

Quantitative Analysis of Antioxidant Activity

The antioxidant activity of essential oils was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [8].A methanolic stock solution of each essential oil was prepared (concentration of stock solution was 10 µl / ml). Then, 50, 100, 200 and 400 µl of each stock solution were transferred to different test tubes and their volumes were adjusted to 1 ml with methanol (solvent). A positive control (ascorbic acid) was prepared in the same way as essential oils. Then, 2 ml of DPPH (6 x 10-5 mol / l) in methanol were added to each test tube (samples and positive control). Finally, a solution containing only 1 ml methanol and 2 ml of DPPH was prepared and used as a blank. All test tubes were incubated in a dark place at room temperature for 1 hour. All determinations were carried out in triplicates.

The spectrophotometer was set at 517 nm and the absorbance was adjusted at zero for methanol. The absorbance's of blank, positive control, and samples were recorded. The disappearance of DPPH was recorded and the percentage inhibition of the DPPH radical by samples, is calculated as follows:

$$\% \text{ Inhibition (Or \% Radical scavenging activity)} = [(A_b - A_s) / A_b] \times 100$$

Where A_b: is the absorbance of blank, A_s: is the absorbance of positive control or sample.

STATISTICAL ANALYSIS

The obtained antioxidant were stated in mean ± standard deviation of three replicates.

RESULTS AND DISCUSSION

Chemical composition of essential oil of various Aromatic plant grown in Yemen

The yield, physical properties, region, and aerial parts of essential oils extracted from different plants grown in Yemen were presented in Table 1.

Table 1: The yield and physical properties of essential oils extracted from different plants grown in Yemen

Plant(essential oil)	Weight	Color	Consistency	Yield of ml	Percentage	Region	aerial parts
Lavandullapubescens	100g	Pale yellow	Thin	1.0 ml	1%	Taiz	Leaves
Lavandullapubescens	100g	Pale yellow	Thin	1.0 ml	1%	Ibb	Leaves
Menthe x piperita	100g	Pale yellow	Thin	1.0 ml	1%	Sana'a	Leaves
Nepetadelflersina	100g	Dark yellow	Medium	0.7 ml	0.7%	Taiz	Leaves
Nepetadelflersina	100g	Pale yellow	Medium	0.1ml	0.1%	Sana'a	Leaves
Origanummajorana	100g	Pale green	Thin	2.0ml	1%	Almahweet	Leaves
Plectranthusbarbatus	100g	Pale yellow	Medium	2.0 ml	2%	Taiz	Leaves
Stachysyemensis	100g	Pale yellow	Thin	0.1 ml	0.1%	Taiz	Leaves
Tecriumyemensis	100g	Pale yellow	Thin	0.4 ml	0.4%	Taiz	Leaves
Thymus laevigatus	100g	Pale yellow	Medium	0.8 ml	0.8%	Sana'a	Leaves

All the composition of essential oils of Yemeni aromatic plants were presented in Table 2 to 10. The highest components determined in the essential oils of *Nepeta deflersiana* (Taiz and Sana'a), *Lavandula pubescens* (Taiz and Ibb) , *Teucrium yemensis*, *Plectranthus barbatus*, *Mentha x piperita*, *Stachys yemensis*, *Origanum majorana* , and *Thymus laevigatus* were Germacrene D (40.5 % and 77.7%), Carvacrol(20.6 % and 70%), (E)-Caryophyllene (19.1%) , Thymol (48.7%), Limonene (7.9%), Germacrene D (38.4%), Germacrene D (40.5%), Terpinen-4-ol (35.2%), and Thymol (52.46%), respectively.

It seems that there were no similarities among chemical compositions of the studied essential oils. Some oils have very simple chemical composition. For example, the (*Menthax piperita*), *(Lavandulla pubescens.*(Ibb)), (*Origanum majorana*), *(Thymus. Laevegatus*), (*Stachys yemensis), (Teucrium yemense*), and *(Lavandula pubescens* (Taiz)) essential oils were composed of only two, four, five, six and seven compounds, respectively. On the other side, some oils were very complex. The *Nepeta deflersiana* (Taiz and Sana'a) , and, *Plectranthus barbatus* (Taiz). essential oils were composed of 19, and 41 compounds, respectively. In some of the essential oils, the main constituents accounted for more than 50 % of total oil, e.g., *Nepeta deflersiana*, (Sana'a) (Germacrene D 77.7 %), *Lavandula pubescens* (Ibb) (Carvacrol 70 %) and *Thymus laevigatus* (Thymol 52.46 %). In *Nepeta deflersiana*, (Taiz) essential oil, the content of Germacrene D was 40.5 %; in *Teucrium yemensis*, the content of Thymol was 48.7 %, in *Stachys yemensis* the content of Germacrene D was 38.4%, in *Origanum majorana* the content of Terpinen-4-ol was 35.2% , and in *Lavandula pubescens* (Taiz) essential oil, the content of Carvacrol was 20.6 %.

In other essential oils, the main compounds accounted for less than 20 % of total oil. The main compounds of these last ones were the following: (E)-Caryophyllene (19.1 %) and Limonene (7.9 %) in *Teucrium yemense* and *Menthax piperita* oils, respectively. The composition of the essential oils of a particular species of plant may differ depending upon the harvesting season, extraction method, and geographical
location of the plant materials. [9].

Our result on *Nepeta deflersiana*, (Sana'a) agree with [9], but *Nepeta deflersiana* from Taiz, represents the first GC-MS analysis of *N. deflersiana*. In previous studies [10-15], the chemical composition of the essential oils of different *Nepeta* species e.g. *N. menthoides*, *N. cataria*, *N. racemosa*, *N. crispa*, *N. kotschyi* , *N. nuda* and *N. meyeri*, was investigated.

Different species of *Thymus* genus were reported for their extracts and EOs constituents, from those, with presence of phenolics monoterpenes thymol and/or carvacrol as main or minor constituents. Most reported species EOs contained thymol beside its isomer carvacrol with average of (3.3-66.3%) for thymol and (3-58.9%) for carvarol, from those were *T. persicus*, *T. eriocalyx* [16], *T. tosevii*, *T. caramanicus*,*T. vulgaris*, *T. pallescens* and *T. pulegioides* [17-21].Our result for *T. laevigatus* agreed with [22] ,from their main constituents.

In total, 17 different compounds were identified in the volatile fractions from *P. barbatus,* by [23]**,** but our results indicate to 41 compounds from *Plectranthusbarbatus* (Taiz).
Hydrodistillation of the leaves of *T. yemense from Taiz* identified six compounds, in contrast to same species from Sana'a [24], identified twelve compounds, but the major compounds were d-cadinene , caryophyllene, and a-humulene, common in all different species of *Teucrium* [25-29].
Terpinen-4-ol was detected as the major compound (35.2 %), in *Origanummajorana,*
this result agree with [30] from Tunisia.

Table 2. Chemical composition of *Nepetadeflersiana* essential oil Sana'a

Compound	% Composition	
	Taiz	Sana'a
(*E*)-β-Ocimene	0.4	1.1
Linalool	0.4	2.4
4aα,7α,7aα-Nepetalactone	3.0	**77.7**
β-Bourbonene	3.4	1.3
4aα,7α,7aβ-Nepetalactone	19.2	0.7
β-Elemene	2.6	0.8
4aα,7β,7aα-Nepetalactone	4.6	0.2
β-Ylangene	1.3	tr
GermacreneD	**40.5**	6.0
Germacrene A	1.2	0.1
γ-Cadinene	1.5	0.1
δ-Cadinene	1.9	0.4
τ-Muurolol	0.6	0.4
α-Muurolol (= Torreyol)	0.9	0.2
Unidentified	1.3	---
α-Cadinol	1.9	1.3
Unidentified	1.2	---
Germacra-4(15),5,10(14)-trien-1α-ol	1.2	---
Unidentified	1.1	---

Table 3. Chemical composition of *Lavandulapubescens* (Taiz) essential oil.

Compound	%
Terpinolene	6.0
p-Cymen-8-ol	11.8
Carvacrol methyl ether	7.2
Carvacrol	**20.6**
(*E*)-Caryophyllene	10.7
β-Bisabolene	12.0
(*E*)-Caryophyllene oxide	15.3

Table 4. Main Components of essential oil *Lavandullapubescens*.(Ibb)

Compounds	%
Carvacrol	**70.00**
Caryophyllene	3.74
α-Bisabolene	2.52

Table 5. Chemical composition of *Teucriumyemense* essential oil.

RI	Compound	%
942	α-Pinene	6.6
1419	**(*E*)-Caryophyllene**	**19.1**
1453	α-Humulene	6.4
1526	δ-Cadinene	6.5
1581	Caryophyllene oxide	4.3
1657	α-Cadinol	9.5

Table 6 .Essential oil composition of *Plectranthusbarbatus* (Taiz).

RI	Compound	%
936	α-Thujene	0.8
941	α-Pinene	0.3
954	Camphene	tr
976	Sabinene	tr
979	β-Pinene	0.1
993	Myrcene	1.4
1005	α-Phellandrene	0.3
1010	δ-3-Carene	tr
1016	α-Terpinene	2.8
1025	*p*-Cymene	9.3
1028	Limonene	0.6
1031	1,8-Cineole	tr
1039	(*Z*)-β-Ocimene	tr
1049	(*E*)-β-Ocimene	0.1
1059	γ-Terpinene	20.0
1067	*cis*-Sabinene hydrate	0.2
1088	Terpinolene	0.2
1098	*trans*-Sabinene hydrate	tr
1101	Linalool	0.2
1121	*cis*-*p*-Menth-2-en-1-ol	0.1
1165	Borneol	0.1
1177	Terpinen-4-ol	1.0
1186	*p*-Cymen-8-ol	tr
1191	α-Terpineol	0.1
1292	**Thymol**	**48.7**
1303	Carvacrol	1.1
1354	Thymol acetate	0.1
1375	α-Copaene	0.1

1419	(*E*)-Caryophyllene	6.4
1436	α-*trans*-Bergamotene	1.1
1453	α-Humulene	0.5
1478	γ-Muurolene	0.1
1482	Germacrene D	0.1
1486	β-Selinene	2.1
1495	δ-Selinene	0.4
1501	α-Muurolene	0.1
1514	γ-Cadinene	0.1
1524	δ-Cadinene	0.3
1583	Caryophyllene oxide	0.9
1610	Humulene epoxide II	tr
1642	τ-Cadinol	0.1
	Total Identified	100.0

Table 7. Essential oil composition of *Menthax piperita* from Sana′a.

RI	Compound	%
1029	**Limonene**	**7.9**
1031	1,8-Cineole	4.8

Table 8. Essential oil composition *Stachysyemensis*

Compound	%
β-Pinene	4.8
Germacrene D	**38.4**
δ-Cadinene	4.6
α-Cadinol	4.2
(*E*)-Caryophyllene	7.2

Table 9. Chemical composition of *Origanummajorana* essential oil from Al-Mahweet.

Compound	%
Terpinen-4-ol	**35.2**
p-Cymene	9.8
γ-Terpinene	7.7
trans-Sabinene hydrate	6.8

Table 10. Main Components of essential oil of *Thymus. laevegatus.*

Compounds	%
o-Cymene	8.97
Thymol	**52.46**
Cavacrol	4.96
Thymol acetate	8.83

Antioxidant activity of Essential Oils

Among the identified compounds in essential oils of aromatic plants has earlier been reported to be present as the major components of the various essential oils with potential antioxidant properties [10-13].

Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them [14]. The DPPH method has been widely applied for estimating the antioxidant activity of various natural products in recent years [15].

DPPH-radical scavenging capacity of essential oil of aromatic plants grown in Yemen were compared to L-ascorbic acid (ASA). Of all samples studied, the essential oil extract had the free radical-scavenging activity (Table 11).

Table 11. The antioxidant activity of different levels of essential oils extracted from different aromatic plants grown in Yemen using DPPH method.

Essential oil	Region	(% Inhibition)			
		2mg/ml	1mg/ml	0.5mg/ml	0.25mg/ml
Nepeta deflersiana	**Sana'a**	**0.3781± 0.10**	**0.3579**	**0.2848**	**0.256**
Lavandula pubescens	**Taiz**	**0.1732**	**0.1049**	**0.0806**	**0.09**
Teucriumyemensis	**Taiz**	**0.5532**	**0.5456**	**0.4956**	**0.212**
Plectranthusbarbatus	**Taiz**	**0.2640**	**0.1086**	**0.0628**	**0.062**
Mentha x piperita	**Sana'a**	**0.5266**	**0.4361**	**0.2953**	**0.144**
Stachys yemensis	**Taiz**	**0.3918**	**0.3714**	**0.3026**	**0.117**
Nepetadeflersiana	**Taiz**	**0.3273**	**0.3678**	**0.3534**	**0.029**
Origanum majorana	**Almahweet**	**0.3386**	**0.3035**	**0.2984**	**0.223**
Lavandula pubescens	**Ibb**	**0.2523**	**0.2483**	**0.1917**	**0.0568**
Thymus laevigatus	**Sana'a**	**0.1362**	**0.08997**	**0.0767**	**0.0616**
Control					
Vitamin C		**0.0525**	**0.0378**	**0.0352**	**0.0183**

The strongest free radical scavenging activity were exhibited by *Thymus laevigatus*, *Lavandula pubescens* (Taiz), *Plectranthus barbatus*, *Lavandula pubescens* (Ibb), and *Nepeta deflersiana* essential oils (0.08997% ± 1.51, 0.0767 % ± 1.11 , and 0.0616% ± 1.23 at 1.00, 0.5, and 0.25 mg mL^{-1}), (0.0806% ± 1.62, and 0.09 % ±1.17 at 0.5, and 0.25 mg mL^{-1}), (0.0628± 1.01, and 0.062% ±0.099 at 0.5, and 0.25 mg mL^{-1}), (0.0568 % ±1.32 at 0.25 mg mL^{-1}), and (0.029 % ± 1.46 at 0.25 mg mL^{-1}), respectively.

The antioxidant activity of *Thymus laevigatus*, *Lavandula pubescens* (Taiz),*Plectranthus barbatus*, *Lavandula pubescens* (Ibb), and *Nepeta deflersiana* (Sana'a) essential oils are mainly due to the high content of thymol (52.46%), Carvacrol (20.6%), thymol (48.7%) , Carvacrol (70%), and 4aα,7α,7aα-Nepetalactone (77.7%), respectively.

Essential oils from other examined spices *Stachys yemensis*, *Mentha x piperita*, *Teucrium yemensis*, *Origanum majorana*, *and Nepeta deflersiana* (Taiz) showed less antioxidant capacities as the content of Germacrene D (38.4%), Limonene(7.9%), (E)-Caryophyllene (19.1%), Terpinen-4-ol (35.2%), and Germacrene D (40.5%),respectively.

Essential oils rich in phenolic compounds such as carvacrol, thymol, spathulenol and eugenol are widely reported to possess high levels of antioxidant activity [17-19].

As clearly indicated by [31], radical-scavenging capacity is directly related to the hydrogen atom donating ability of a compound and not correlated to the redox potentials alone, as observed by this researcher when studying the antioxidant capacity of phenothiazine and other related compounds.

To the best of our knowledge, antioxidant activities of the plant species presented here have not previously been reported elsewhere. Therefore, data presented here could be assumed as the first

report. We believe that this work will contribute to the discovery of new plant species having antioxidant properties.

CONCLUSION

The study showed that antioxidant activity was related to the chemical composition of the nine essential oils from spice plants commonly consumed in diet. The analysis of different aromatic plants grown in Yemen essential oils chemical composition, and antioxidant activities is the first work described in the literature for those species. According to the results of this study, all the essential oils exhibited antioxidant properties, may be suggested as a new potential sources of natural antioxidant microbial for food industry**.**

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