American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

ISSN: 2321-9114
AJEONP 2017; 5(2): 12-18

Received: 05-02-2017
Accepted: 07-03-2017

Khaled Hussein
Chemistry Department, Faculty of Science, Sana'a University Alzoberi Street, Building of old Sana'a University, Sana'a, Yemen

Ahlam H Ahmed
Chemistry Department, Faculty of Education, Amran University Amran, Amran, Yemen

Sahar Algabali
Chemistry Department, Faculty of Science, Sana'a University Alzoberi Street, Building of old Sana'a University, Sana'a, Yemen

Correspondence:
Khaled Hussein
Chemistry Department, Faculty of Science, Sana'a University Alzoberi Street, Building of old Sana'a University, Sana'a, Yemen

Antibacterial/radical scavenging activities, content, chemotaxonomy and chemical components of volatile oils of two *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae), grown in Yemen

Khaled Hussein, Ahlam H Ahmed and Sahar Algabali

Abstract
Evaluate the content, identify the chemotype and analyze the chemical components of volatile oils of the cultivated *Plectranthus amboinicus* (CPSU) and the wild *P. amboinicus* (WPRR), from Yemen, were the main targets of this work. Antibacterial activity of the investigated oils were tested against four standard bacterial strains. Radical scavenging activity (RSA) of oils were estimated as well, using spectrophotometric DPPH method. Volatile oils components were achieved by IR spectroscopy and GC/MS. The presence of phenolic and alcoholic compounds within the oils components were inferred by IR analysis. GC/MS analysis of CPSU and WPRR oils showed that the dominant component of both is thymol (36.90% and 79.20% correspondingly) and indicated that the examined oils have thymol chemotype. The evaluation of the oil's content show that the CPSU and WPRR possess 1.43% and 1.57% (v/w) oil content, respectively. Remarkable antibacterial activity and promising RSA of oils, were found to be correlated mainly to thymol and other alcoholic components. RSA results indicated that WPRR oil could be considered as a natural source of hydroxyl radical scavenger.

Keywords: *Plectranthus amboinicus,* volatile oil components, antibacterial activity, radical scavenging activity, thymol

1. Introduction

Plectranthus amboinicus (Lour.) Spreng, (synonyms include *Plectranthus aromaticus* Roxb. *Coleus aromaticus* Benth. and *Coleus amboinicus* Lour.) [4], is a medicinal aromatic perennial herbal plant, commonly called Cuban oregano, Spanish thyme, Indian borage, Mexican mint, English borage and in Yemen, it is locally called Mdan**.** Traditionally, *P. amboinicus* was used for the treatment of various disease [4-6]. Previously, several researches have also indicated that this plant extracts, chiefly the volatile oil extract, has many pharmacological properties, including antimicrobial, antibacterial, antiviral and antioxidant activities [2, 4, 7, 8, 9]. Moreover, it was found that *P. amboinicus* extracts have several effects against animal and insect bites and cardiovascular diseases [2, 4, 8]. Literature review on *P. amboinicus* have also documented that the economic interest of this plant extracts is for their horticultural uses and their uses as food, food additives, and as materials for general uses [2].

Certainly, there is a relationship between therapeutic action(s) of any *P. amboinicus* extract and its chemical constituents. This relatioship is based primarily on functional group(s) of chemical class (es) which are identified to be existing within the chemical constituents of this plant extract. This is in agreement with the concept that the structural feature required for a strong free radical scavenging and antimicrobial activities are those containing phenolic groups [10].

With respect to volatile oil extract of *P. amboinicus*, the presence of 76 volatile constituents in this extract, including oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes were recorded by several authors from different parts of the World [1, 2, 4]. In addition, it was found that *P. amboinicus* volatile oil is mainly rich in phenolic monoterpenes such as thymol, carvacrol, chvicol, eugenol and methyl eugenol, which showed confirmative biological properties [1, 2, 4, 9, 11].

Though many studies have carried out concerning the volatile oil chemistry of *Plectranthus amboinicus* in the world, none has been reported on volatile oil chemistry of Yemeni *Plectranthus amboinicus* (Lour.) Spreng., and its biological activities.

For this reason and because only few plants that are grown in Yemen have so far been analyzed as potential medicines, there is still a great opportunity of research in this field. Therefore, the objectives of the present study is to identify the chemotype, determine the content, and analyze the chemical components of the volatile oils of the two *P. amboinicus* taken from two different geographical locations in Yemen. RSA and antibacterial activity of these oils were estimated as well with a view to recommend their applications in food and in pharmaceutical industries as natural additives.

2. Materials and methods

2.1. Plant material

2.1.1. Collective samples

The two varieties of *Plecteranthus amboinicus* (Lour.) Spreng. (Lamiaceae) were collected in March-April 2014 and March-April 2015, from Ribat Al-Nhari, Rimh (WPRR) and Sana'a University Garden, Sana'a (CPSU), Yemen. The samples were kindly identified by Al-Khulaidi A.A., Faculty of Applied Studies and Continuous Education, Saudi Arabia and clarified by Dr H. M. Ibrahim, the staff member of Plant Taxonomy unit, Biology Department, Faculty of Science, Sana'a University, Sana'a, Yemen, according to Flora of Yemen [3]. Voucher specimens were deposited under the number Bot. 719Kh and Bot. 720Kh respectively, in the herbarium of the Department of Biology, Faculty of Science, Sana'a University, Sana'a, Yemen.

2.1.2. Plant preparation for extraction

Fresh herb of plant parts were cut into small pieces and let to dry naturally on laboratory benches at room temperature (23-27 ºC) for two weeks until they were crispy. The dried plant materials were ground into fine powder using a mortar and pestle. Drying was carried out in natural way, in shade in a dry place.

2.2. Extraction of the volatile oils

The collected fine powder of the dried plant materials (432 g of CPSU and 439 g of WPRR) were subjected separately to hydro distillation for three hours using a modified Clevenger apparatus. The extracted volatile oils of both plant varieties were dried over anhydrous sodium sulfate and then filtered off through Whatman filter paper number 1. The produced volatile oil of both varieties were reserved away from light in dark color sealed glass vials and maintained in a refrigerator at 4 ºC for further analysis.

2.3. Volatile oils analysis

2.3.1. Infrared (FTIR) spectral analysis

The infrared spectra (FTIR) were recorded on a Shimadzu-FTIR-410 Spectrometer (Japan) in the range 500 to 4000 cm^{-1}. The spectra were obtained using sodium chloride (NaCl) plate technique. The spectra were plotted as intensity versus wave number.

2.3.2. GC/MS analysis

GC/MS electron impact ionization (EI) analysis of the studied volatile oils were achieved on Shimadzu gas chromatography. GC equipped with DB-5 wax cross–linked fused silica capillary column (30 m long × 0.25 mm internal diameter) coated with polydimethylsiloxane (0.5 µm film thickness). The oven temperature was programmed from 40ºC for three minutes with an increase of 4 ºC/minute to 250ºC and isothermally for 10 minutes at 250 ºC. Injections were performed with injector temperature of 200 ºC, ion source temperature 250ºC. The volume of the oil injected was 1µL. Helium was used as a carrier gas at a flow rate of 1 mL/minute. Mass spectra were taken at 70 eV, a scan interval of one second and a scan range of 40-650 m/z. Retention indices (RIs) were calculated using a homologous series of n-alkanes (C5-C30). Mass spectra was tested on GC-MSQP 1000EX., Schimadzu (Japan) (National research center Dokki, Cairo, Egypt).

2.4. Bioassays

2.4.1. Antibacterial assay

The antibacterial activity of the volatile oils of *Plecteranthus amboinicus* (CPSU and WPRR) was evaluated in vitro by traditional antibiotic susceptibility testing using agar disc diffusion method [12]. One drop of the concentrated sample (10 µL) was poured on the agar prepared as required. After incubation (18 hours at 37 °C), diameters of inhibition zones (in mm.) were measured. The evaluation of the antibacterial activities of the investigated volatile oils were carried out against four standard bacterial strains: *Staphylococcus aureus* ATCC 6538, *Streptococcus pyogens* ATCC 10541, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 25619. Gentamycin as a therapeutically important antibiotic in treating infections caused by these microorganisms was used as a comparative substance (as positive control). The tests were carried out in triplicate with the two volatile oil samples. Data of the investigated oil samples were expressed as means ± S.D.

2.4.2. Assay of radical scavenging activity

Radical scavenging activity (RSA) of the volatile oils of *P. amboinicus* (CPSU and WPRR) was estimated by the spectrophotometric DPPH method [13]. The stable radical 2.2`-diphenyl-1-picrylhydrazyl (DPPH) was used to estimate the electron donation ability of the investigated essential oils. The control solution (DPPH solution) and six samples of increasing concentrations of each volatile oil were prepared by diluting 0, 5, 10, 20, 40 and 60 µg with methanol to a total volume of 1 mL, and each sample was then mixed with 2 mL of 90 µM methanol solution of DPPH. After one hour incubation period at room temperature, the absorbance of investigated samples were read against the absorbance of the control solution at 517 nm. A parallel RSA assay on ascorbic acid with the same set of concentrations was also performed. Inhibition of DPPH free radical in percent (I %) was calculated as 100 (A° – A)/ A°, where A° is the absorbance of the control solution (DPPH solution) and A is the absorbance of the individual investigated samples. The tests were run in triplicate with the two volatile oil samples. Data of the investigated oil samples were expressed as means ± S.D.

3. Results & Discussion (Times New Roman, 12, Bold)

3.1. Volatile oil extraction and content

A hydro distillation method using Clevenger apparatus for 3–4 hours is commonly employed for extracting volatile oil. In the present study and in order to save time, effort, quantity and quality, a hydro distillation method using a modified Clevenger apparatus for 3.5 hours was employed for extracting *P. amboinicus* volatile oil. This method was in employment to prevent the direct contact between the water and plant material and to allow only water vapor to pass through the plant material. It is a method by which volatile oil and water extracts could be obtained in a good quality and quantity, without there being any interference in the quality or quantity of each extract. The investigated volatile oils of *P.*

amboinicus (Lour.) Spreng. (Lamiaceae) were colorless and then turned to light yellow by time. Out of 432 g and 439 g of the dried plant materials of CPSU and WPRR, ~ 6.2 mL and 6.9 mL light yellow oils were yielded, particularly 1.43% and 1.57%, respectively. The content of the volatile oils was expressed in the percentage of volume of volatile oil/ dry weight of plant sample (v/w %). A study on the effect of extraction technique on the volatile oil content of *P. amboinicus,* recorde 6.52% oil content using solvent (hexane) extraction technique in comparison to those obtained by using steam distillation (0.55%) and supercritical CO_2 (1.40%) techniques [14]. However, the volatile oil contents of CPSU (1.43%) and WPRR (1.57%) which reported here for the first time, were found to be the highest in comparison to the volatileoil contents previously extracted from the same species by a hydro distillation method (using a Clevenger type apparatus), steam distillation and supercritical CO_2. It was varied from 0.1-1.4% [14-18]. In the mean time, and on the basis of dry matter, previous published works documented that *Plectranthus* species are volatile-oil-rich and it was more than 0.5% v/w [1]. Although there are many known factors that have an influence on the volatile oil content, the high volatile oil contents of the investigated oil samples, could be due to the modified extraction technique used in this study.

3.2. Volatile oils analysis

3.2.1. Infrared (FTIR) spectral analysis

Although IR spectra of the volatile oils are extremely complex, the chemical profile of the investigated volatile oils was preliminarily evaluated by the IR spectroscopy. Regardless of the rest chemical structure of chemical compound, IR is a spectroscopic tool which is useful in estimating the presence of a functional group that give rise to a characteristic absorption band(s). In the present study, characteristic absorption bands were assigned in the IR spectrum of volatile oils of CPSU and WPRR by a simple check up. Table 1 represent the characteristic IR absorption bands of both volatile oils.

The IR spectral analysis results of the investigated volatile oils, reveal the presence of mainly oxygenated terpenes (mono- and/or sesquiterpene), with feature structures of phenolic and/or alcoholic as well as aryl alkyl ether.

Contrary to the way by which IR spectroscopy was used in this study, in some cases, a band by band correlation could be an excellent evidence in proving the chemical structure by matching the spectrum of volatile oil against that of an authentic sample of the main components.

By this way, IR spectrum is an advantage that could be used to confirm or deny the results of GC/MS analysis of the volatile oil components.

Table 1: IR spectral analysis results of volatile oils of two *P. ambonicus* (Lour.) Spreng. grown in Yemen

Absorption bands [frequency (cm^{-1}), intensity and shape]		Corresponding structural information
WPRR[a] volatile oil	**CPSU[b] volatile oil**	
3535-3278 (medium & slightly broad).	3424-3226 (medium & slightly broad).	Mainly Phenolic OH, as well as alcoholic OH.
2967-2865 (strong & sharp).	2980-2826 (strong & sharp).	Hydrogen bonded to *sp2* and hydrogen bonded to *sp3* carbons, respectively.
1638, 1610, 1551 and 1511 (medium & sharp).	1642, 1622, 1547 and 1453(medium & sharp).	Aromatic system
1375 and 1361 (medium & sharp).	1371 and 1354 (medium & sharp).	two equivalent bands attributed to isopropyl group of monoterpenes
1245 and 1053 (medium & sharp).	1235 and 1055 and 1o13 (medium & sharp).	C-O of Alkyl aryl ether

Note: [a] Wild *P. ambonicus* from Ribat Al-Nhari, Rimh, Yemen; [b] Cultivated *P. ambonicus* from Sana'a University Garden, Sana'a, Yemen.

3.2.2. GC/MS analysis

The volatile oil samples extract (obtained by hydro distillation method), were analyzed by GC and CG/MS. CG/MS analysis results of the volatile oils of cultivated *P. amboinicus* from Sana'a University garden (CPSU), Sana'a and wild *P. amboinicus* from Ribat Al-Nhari, Rimh (WPRR), Yemen, revealed the presence of twenty four and twelve chemical components constituting 91.39% and 99.91% of the total composition of volatile oils, respectively. The chemical components of both oil samples with their peaks numbers, composition percentage (%), and retention index (RI) are presented in Table 2. Analysis results showed that the dominant component of both volatile oils is thymol (36.90 and 79.20% correspondingly).

The chemical components were quantified by GC and identified by matching their MS fragmentation patterns with those of GC/MS computer database, using the main library (NIST), and Wiley library, and by a visual comparison of the fragmentation patterns with those reported in the literature, as well as comparing their calculated retention index with those of literature values measured on columns with identical polarities [19]. In addition to the chemical components illustrated in Table 2, the presence of a trace amounts of monoterpenes, sesquiterpenes hydrocarbons and oxygenated sesquiterpenes were also detected within the chemical components of CPSU volatile oil (Composition percentage for each one is less than 0.5%). However, it is important to record that the presence of 3-tert-butyl-4-methoxyphenol and 1-tert-butyl-2-(methylthio)benzene within the main chemical components of WPRR volatile oil are reported here for the first time.

Compared with the results of previously published works relevant to the subject of our study, many published works from different parts of the world, indicate that thymol and/or carvacrol are the common major constituent(s) of *P. amboinicus* volatile oil. As a major constituent, thymol composition percentage was varied, starting from the lowest (41.3%) [9], passing by many medium (64.3% - 88%) [20, 21] and right up to the highest one 94.3% [4, 22], whereas the presence of carvacrol as a major component, record variation in its composition percentage, from 40.4% - 77.16% [4, 23] up to the highest one 90.55 - 98.03% [24].

Table 2: Chemical components of volatile oils of two *P. ambonicus* (Lour.) Spreng. grown in Yemen.

Chemical components[a]	**PN**[b]		**(%)**[c]		**RI**[d]	
	WPO[e]	CPO[f]	**WPO**	CP**O**	**CRI**[g]	**LRI**[h]
3-Carene	1	---	0.05	---	1008	1011
o-Cymene	2	---	0.56	---	1026	1025
γ-Terpinene (*p-Mentha-1,4-diene*)	3	---	0.08	---	1059	1062
1,8-Cineole (*Eucalyptol*)	---	1	---	3.34	1032	1033
γ-Terpinene (*p-Menth-1,4-diene*)	---	2	---	0.53	1062	1062
Linalool	---	3	---	13.24	1098	1098
α-Terpineol (*p-Menth-1-en-8-ol*)	---	4	---	0.64	1189	1189
p-Cymen-7-ol (Cumin alcohol)	4	---	0.09		1284	1287
p-Menth-8-en-2-ol	---	5	---	0.82	1226	1226
Hexyl-2-methylbutanoate	---	6	---	0.87	1234	1234
Thymoquinone (*p-Cymene-2,5-dione*)	---	7	---	0.68	1262	1262
Thymol	5	8	79.20	36.90	1288	1290
1-tert-Butyl-2-(methylthio) benzene	6	---	3.15	---	1487	---
trans, trans-2,4-Decadienal	---	9	---	4.14	1314	1314
3-tert-butyl-4-methoxyphenol	7	---	16.63	---	1495	---
cis-Isoeugenol	---	10	---	4.22	1402	1402
γ-Elemene	---	11	---	1.11	1433	1433
Alloaromadendrene	---	12	---	0.63	1461	1461
γ -Curcumene	---	13	---	2.50	1480	1480
α-Selinene	---	14	---	0.50	1494	1494
β -Curcumene	---	15	---	2.0	1512	1512
δ-Cadinene (*Cadina-1(10),4-diene*)	8	16	0.04	4.66	1521	1524
trans- γ -Bisabolene	---	17	---	0.93	1530	1533
8,14-Cedranoxide	---	18	---	0.61	1538	1540
α -Cadinene	---	19	---	2.38	1540	1538
Germacrene B	---	20	---	0.81	1556	1556
Ledol	---	21	---	4.40	1561	1565
β-Caryophyllene oxide	**9**	**22**	**0.03**	**0.59**	**1579**	**1581**
Viridiflorol	---	23	---	1.14	1586	1590
Epi-α-Cadinol (*tau*-Cadinol)	10	24	0.02	3.75	1643	1640
α-Cadinol (*Cadin-4-en-10-ol*)	11	---	0.02	---	1650	1653
8-Cedren-13-ol	12	---	0.04	---	1690	1688

Secondary metabolites compounds classes	Number of components and their composition percentage	
	WPO[e]	WPO[e]
Monoterpenes		
Hydrocarbon	3 Components, representing 0.69% of TC	1 Component, representing 0.53% of TC[i]
Oxygenated	1 Component, representing 0.09% of TC	5 Components, representing 18.72% of TC
Phenolic	2 Components, representing 95.83% of TC	1 Component, representing 36.90% of TC
Sulfurous	1 Component, representing 3.15% of TC	--------
Aromatic compounds (Shikimates)		
Phenylpropanoid	--------	1 Component, representing 4.22% of TC
Sesquiterpenes		
Hydrocarbon	1 Component, representing 0.04% of TC	9 Component, representing 15.52% of TC
Oxygenated	4 Component, representing 0.11% of TC	5 Component, representing 10.49% of TC
Others		
Ester	--------	1 Component, representing 0.87% of TC
Aldehyde	--------	1 Component,, representing 4.14%
TIC[j]	12 Component, representing 99.91% of TC	24 Component, representing 91.39% of TC

Notes: [a] Components are listed in order of their elution from a DB-5 column; [b] Peaks numbers; [c] Composition percentage; [d] Retention index;[e] Wild *P. ambonicus* volatile oil; [f] Cultivated *P. ambonicus* volatile oil; [g] Calculated retention index; [h] Literature retention index [19]; [i] Total composition; [j] Total identified components..

In two separate published studies, the existence of carvacrol (28.65% - 29.25%) beside thymol (21.66%) [25, 26] and the presence of carvacrol (23.0 - 41.3%) alongside camphor (22.2% - 39.0%) as major components of *P. amboinicus*, were also reported [27, 28]. In another study, GC/MS analysis showed that the major component of the volatile oil of Malaysian *P. amboinicus* is 3-carene (20.78%) [7]. Likewise, volatile oil of *P. amboinicus* leaves were previously studied and showed (by GC/MS) that the volatile oil extracted with head space solid phase micro extraction (HS-SPME) contain linalool as main component, whereas the volatile oil which were extracted by a solid phase micro extraction (SPME) method contain eucalyptol as a major component [4].

This qualitative and quantitative variation in chemical components of volatile oils of *P. amboinicus,* lead us to suggest a classification system based on the classification pattern proposed by Lawrence and Grayer [29, 30], which is based on the major component of volatile constituents, explicitly the components major than 20%. In this regard, *P. amboinicus* species from different countries around the world could be classified into seven chemotypes including carvacrol-rich, thymol-rich, carvacrol, thymol, 3-carene, linalool and eucalyptol (1,8-cineol) chemotypes, as well as two sub-chemotype comprise carvacrol/thymol and carvacrol/camphor. It should be noted that chemotype-rich means that the chemical composition of volatile extract include more than 80% of a particular component. This is an easy way to identify as well as to evaluate different samples through their volatile oils GC/MS analysis results. Similarly, by the same way and based on this classification system as well as our GC/MS analysis results, volatile oils of CPSU and WPRR, Yemen, should belong to a thymol chemotype.

On the other hand, if we compare the chemotype of the investigated volatile oils, with those of volatile oils of *P. amboinicus* from other countries, we would find that the volatile oil of WPRR, resembles closely the chemotype of volatile oil of the Pakistani *P. amboinicus* containing 79.6% thymol [31], whereas the chemotype of the volatile oil of CPSU, resembles to some extent chemotype of volatile oil of the Indian *P. amboinicus* which comprise slightly high concentration of thymol (41.3%) [9].

Finally, as expected and known in advance, the reason for these variations, is due to a number of factors: specifically, geographical location, age of the plant, harvested season, collection time, collected part, method of drying, volatile oil extraction method, in addition to the type of plant, (wild or cultivated) [1, 2, 4, 7, 8].

3.3. Antibacterial activity

It is known that the antibacterial activity of the volatile oil plant extract is directly linked to the presence of bioactive chemical component(s) [with structural feature containing phenolic and/or hydroxyl groups] within the components of this extract [10]. The presence of thymol (79.20% and 36.90%), 3-tert-butyl-4-methoxyphenol (16.63% and 0.0%) and *cis*-Isoeugenol (0.0% and 4.22%) in volatile oil composition of WPRR and CPSU respectively, and in addition to the use of volatile oil of *P. amboinicus* from different parts of the world in folk medicine for treatment of infections caused by bacteria [2, 4], encourage us to evaluate the antibacterial activity (experimentally in vitro) of the investigated volatile oils in order to find out the actual reasons for these traditional uses and to verify whether the traditional uses of this plant is actually useful for treatment this infections and supported by pharmacological properties. So, the first step of our study towards this target is studying the bacterial activity of the investigated volatile oils against four standard clinical pathogens bacteria like *Staphylococcus aureus* ATCC 6538, *Streptococcus pyogens* ATCC 10541, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 25619. The antibacterial activities results of the investigated volatile oils are summarized in Table 3.

Table 3: Inhibition zone of volatile oil of two *P. ambonicus* (Lour.) Spreng. grown in Yemen

Organism	**Inhibition zone** (mm.)		
	VO[a]		**PC**[b] **(mm.)**
	CPSU[d]	**WPRR**[c]	**Gentamycin**
Staphylococcus aureus ATCC 6538	21.6 ± 0.85	26.1 ± 0.28	23 ± 1.15
Streptococus pyogens ATCC 10541	13.20 ± 0.35	28.3 ± 0.45	12 ± 0.25
Escherichia coli ATCC 8739	22.7 ± 0.74	3.8 ± 0.17	20 ± 1.21
Pseudomonas aeruginosa ATCC 25619	36.35 ± 1.21	15.4 ± 0.32	14 ± 0.42

Notes: [a] Inhibition zone of volatile oil (in millimeter); [b] Inhibition zone of positive control (in millimeter); [c] Wild *P. amboinicus* from Ribat Al-Nhari, Rimh, Yemen; [d] Cultivated *P. amboinicus* from Sana'a University garden, Sana'a, Yemen.

As seen in Table 3, the obtained results allow us to record that the volatile oil of *P. amboinicus* (WPRR) was able to inhibit the growth of tested bacteria and the most highest important antibacterial activity of this oil, was spoken against *Streptococcus pyogens* and followed by *Staphylococcus aureus* whereas, the antibacterial activity against *Pseudomonas aeruginosa* was observed to be slightly higher (Approximate rate of 110%) in comparison with that of positive control, *Escherichia coli* had the lowest sensitivity to WPRR oil, in contrast with the sensitivity of the rest tested bacteria strains.

Noteworthy, that *Pseudomonas aeruginosa* and *Escherichia coli*, were the most sensitive organism to CPSU volatile oil, followed by *Streptococcus pyogens* which exhibited slightly high sensitivity with rate 110% in comparison with its sensitivity towards the positive control. In association with that of the positive control, CPSU volatile oil exhibit slightly lower inhibition zone against *Staphylococcus aureus*.

Beside to the known relationship between the variation in chemical components of the investigated volatile oils and their activities against tested bacteria strains, our results reveals that Gram – positive is selectively sensitive to the volatile oil containing high percentage of thymol. In the present study, we also record that the investigated CPSU volatile oil have synergistic effect on *Pseudomonas aeruginosa* as well as *Escherichia coli* and generally on Gram – negative bacteria which could be selectively sensitive towards volatile oils containing convergent chemical components of combination of mono-, sesquiterpene hydrocarbons; oxygenated mono- and sesquiterpenes (alcohol type and phenolic compounds). Similarly, it was found that *Escherichia coli*, as Gram – negative bacteria strain, is the most sensitive towards Malaysian and Indian *P. amboinicus* volatile oil containing the same combination of chemical components described above [7, 32]. On the other hand, the observed activity of the investigated WPRR volatile oil towards *Streptococcus pyogens* and *Staphylococcus aureus*, are consistence with earlier research works which documented that the volatile oils having high concentration of thymol and carvocrol, inhibit Gram – positive more than Gram – negative pathogenic bacteria [32, 33].

3.4. Radical scavenging activities (RSA)

RSA is a biological test, usually used to assess electron donation ability (antioxidant activity) of the chemical components of any botanical extract. In any plant extract, electron donation ability is linked to the existence of chemical components with structural feature containing reactive oxygen species like hydroxyl group (functional group in alcoholic and phenolic or phenolic derivatives compounds). The presence of chemical components with these structural specifications within volatile oil extracts of WPRR and CPSU, give us a strong motivation to achieve RSA of these plant extracts. For this reason, the investigated volatile oils were subjected to screening for their possible RSA by using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Stable radical DPPH was used to estimate the electron donation ability of the investigated volatile oils by measuring their ability to reduce DPPH radicals (deep purple) into the neutral non-radical form (pale yellow). The obtained RSA results of the investigated oils are reported in Table 4.

RSA results reveal that the investigated volatile oils exhibited significant inhibition in DPPH free radicals and this inhibition is due to the ability of investigated volatile oils to form reactive oxygen species during this bioassay. GC/MS analysis results above indicate that these reactive oxygen species are in the form of hydroxyl radicals (phenolic and/or alcoholic). These results reveal that the producing hydroxyl radicals have reducing power towards DPPH free radicals and subsequently towards such reactive molecules. However, our findings are in agreement with previous published works, which revealed that the aqueous, ethanol and volatile oil extracts of *P. amboinicus* containing phenolic compounds such as carvacrol and thymol, possess significant antioxidant activities [4].

Table 4: Radical scavenging activity of volatile oils of two *P. ambonicus* (Lour.) Spreng. grown in Yemen.

SC[a] (*μg/ml*)	Absorbance and Radical scavenging activity[b]						
	The investigated oils					Ascorbic acid	
	A[c] at 517 *nm*		RSA[d] %		VRSA[g] %	A at 517 *nm*	RSA %
	WPRR[e]	CPSU[f]	WPRR	CPSU			
0	0.4263	0.4263	0.00	0.00	0.00	0.4263	0.00
10	0.2135 ±0.0011	0.2511±0.0015	49.91 ±0.26	41.09 ±0.36	08.82	0.0386	90.95
20	0.1950 ±0.0018	0.2347 ±0.0031	54.26 ±0.43	44.94 ±0.73	09.32	0.0380	91.08
40	0.1451 ±0.0014	0.2222 ±0.0040	65.96 ±0.33	47.88 ±0.94	18.08	0.0377	91.16
60	0.1130 ±0.0022	0.2013 ±0.0019	73.49 ±0.51	52.78 ±0.45	20.71	0.0350	91.79
80	0.0663 ±0.0019	0.1768 ±0.0025	84.45 ±0.45	58.53 ±0.58	25.92	0.0341	92.00
100	0.0453 ±0.0032	0.1559 ±0.0016	89.37 ±0.75	63.43 ±0.37	25.94	0.0336	92.12

Notes: [a] Sample concentration (*volatile oils & ascorbic acid*); [b] Data were expressed as means ± S.D; [c] Absorbance; [d] Radical scavenging activity; [e] Wild *P. amboinicus* from Ribat Al-Nhari, Rimh, Yemen; [f] Cultivated *P. amboinicus* from Sana'a University garden, Sana'a, Yemen; [g] Variation in Radical scavenging activity of both oils.

On the other hand, among the natural origin antioxidants, it was found that the volatile oils with high content of the total phenolic components, including those extracted from *P. amboinicus*, are preferable. This is because they are cheap, as well as without any side effects [34].

Finally, RSA results indicate that the volatile oil of WPRR has a promising antioxidant activity more than CPSU volatile oil. It has been found that the average variation in the RSA between the two studied oils (at the different concentrations) equivalent to 18.13%. The differences in the RSA of the investigated volatile oils were found to be correlated mainly to the variation in content of thymol and other alcoholic components. For this reason, volatile oil of WPRR could be considered as a potential source of safe radical scavenger. Therefore, it could serve as safe natural additives in foods, cosmetics and pharmaceuticals.

4. Conclusions

This study could be considered the first study that provides information that was not previously available on content, chemotype and chemistry of the volatile oils of two of *P. amboinicus* grown in Yemen. This work also provides an initial survey on some of potential biological activities of these oils, like radical scavenging and antibacterial activities and through which, this study has provided additional information about the promising RSA and the significant anti-bacterial activities of these volatile oils. It should be noted here that one of the investigated volatile oils was found to possess synergistic effect towards Gram – negative bacteria. This synergism could be due to the presence of the combination of terpenes and phenolic compounds within the components of this oil.

Acknowledgments

This work was supported by Sana'a University, Faculty of Science. F.A. Author is indebted to Alsaeed Foundation for Science and Culture for financial support. Authors are grateful to Dr. Fatima Al-yusufy for reviewing the manuscript.

Supplementary material

Supplementary material relating to this paper, include GC/MS and IR Figures (Figures S1─S4).

Conflict of interest

The authors declare that this article content has no conflict of interest.

References

1. Abdel-Mogib M, Albar HA, Batterjee SM. Chemistry of the Genus *Plectranthus.* Molecules. 2002; 7(2):271-301.
2. Lukhoba CW, Simmonds MSJ, Paton AJ. *Plectranthus*: A review of ethnobotanical uses. J. Ethnopharmacol. 2006; 103(1):1-24.
3. Al-Khulaidi AA. Flora of Yemen (Checklist of flora). The Sustainable Natural Resource Management Project II, funded by EPA and UNDP, Republic of Yemen. 2013, 156.
4. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: Botanical, Phytochemical, Pharmacological and Nutritional Significance. Molecules. 2016; 21(4):369-394.
5. Yuthistran R, Balakrishnan C, Buddhan R. Antibiotic Effect of Leaf Extract from *Plectranthus amboinicus* (Lour) Spreng in Asthma. Int. J. Adv. Res. Innov. 2015; 3(2):430-432.
6. Rosidah, Poppy Anjelisa ZH. Cytotoxic Effect of n-Hexane, Ethylacetate and Ethanol Extracts of *Plectranthus amboinicus*, (Lour.) Spreng.) on HeLa and Vero Cells Lines. Int. J. Pharm Tech Res. 2014; 6(6):1806-1809.
7. Erny Sabrina MN, Razali M, Mirfat AHS, Mohd Shukri MA. Antimicrobial activity and bioactive evaluation of *Plectranthus amboinicus* essential oil. American Journal of Research Communication. 2014; 2(12):121-127.
8. Roshan P, Naveen M, Manjul PS, Gulzar A, Anita S, Sudarshan S. *Plectranthus amboinicus* (Lour) Spreng: An Overview. The Pharma Research (T. Ph. Res.). 2010; 4:01-15.
9. Selvakumar P, Edhaya naveena B, Prakash S. Studies on the antidandruff activity on the essential oil of *Coleus amboinicus* and *Eucalyptus globulus*. Asian Pacific

Journal of Tropical Disease. 2012; 2(2):S715-S719.

10. Ezoubeiri A, Gadhi CA, Fdil N, Benharref A, Jana M, Vanhaelen M. Isolation and antimicrobial activity of two phenolic compounds from *Pulicaria odora* (L.). J. Ethnopharmacol. 2005; 99(2): 287-292.
11. Khare RS, Banerjee S, Kundu KK. *Coleus aromaticus* Benth. A nutritive medicinal plant of potential therapeutic value. Int. J. Pharma. Bio. Sci. 2011; 2(3):B488-B500.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45(4):493-496.
13. Đorđević S, Dobrić S, Petrović S, Milenković M, Vučićević D, Žižić S *et al*. Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of *Carlina acanthifolia* root essential oil. J Ethnopharmacol. 2007; 109(3):458-463.
14. Pino JA, Garcia J, Martinez MA. Comparative chemical composition of the volatiles of Coleus aromaticus produced by steam distillation, solvent extraction and supercritical carbon dioxide extraction. J. Essent. Oil Res. 1996; 8(4):373-375.
15. Muthukumarana R, Dharmadasa RM. Pharmacognostical investigation of *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger. and *Plectranthus amboinicus* (Lour.) Spreng. World Journal of Agricultural Research. 2014; 2(5):240-246. DOI: 10.12691/wjar-2-5-7.
16. Khalid AK, El-Gohary AE. Effect of seasonal variations on essential oil production and composition of *Plectranthus amboinicus* (Lour.) grow in Egypt. International Food Research Journal (IFRJ). 2014; 21(5):1859-1862.
17. Dos Santos NO, Mariane B, Lago JHG, Sartorelli P, Rosa W, Soares MG *et al*. Assessing the Chemical Composition and Antimicrobial Activity of Essential Oils from Brazilian Plants—*Eremanthus erythropappus* (Asteraceae), *Plectrantuns barbatus*, and *P. amboinicus* (Lamiaceae). Molecules. 2015; 20(5):8440-8452. doi:10.3390/molecules20058440
18. Abdelrazik TM, Sabra AS, Astatkie T, Hegazy MH, Grulova D, Said-Al Ahl HAH. Response of Growth, Essential Oil Content and Its Constituent's of *Plectranthus amboinicus* to Iron and/or Urea Foliar Application Under Saline Irrigation. Int J Pharm Pharm Sci. 2016; 8(8):223-231.
19. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 2nd. edn. Allured Publishing Corporation, Carol Stream, IL. 1995.
20. Da Costa JG, Pereira CK, Rodrigues FF, de Lima SG. Chemical Composition, Antibacterial and Fungicidal Activities of Leaf Oil of *Plectranthus amboinicus* (Lour.) Spreng. J. Essent. Oil Res. 2010; 22(2):183-185.
21. Tewari G, Pande C, Kharkwal G, Singh S, Singh C. Phytochemical study of essential oil from the aerial parts of *Coleus aromaticus* Benth. Nat. Prod. Res. 2012; 26(2):182-185.
22. Singh G, Singh OP, Prasad YR, Lamposona MP, Catalan C. Studies on essential oils. Part 33. Chemical and insecticidal investigations on leaf oil of *Coleus amboinicus* (Lour). Flavour Frag. J. 2002; 17(6):440-442.
23. Joshi RK, Badakar V, Kholkute SD. Carvacrol Rich Essential Oils of *Coleus aromaticus* (Benth.) from Western Ghats Region of North West Karnataka, India. Adv. Environ. Biol. 2011; 5(6):1307-1310.
24. Gonçalves TB, Braga MA, Oliveira FFM, Santiago GMP, Carvalho CBM, Cabral PB *et al*. Effect of subinihibitory and inhibitory concentrations of *Plectranthus amboinicus* (Lour.) Spreng essential oil on *Klebsiella pneumonia*. Phytomed. 2012; 19(11):962-968.
25. Senthilkumar A, Venkatesalu V. Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against *Anopheles stephensi*: a malarial vector mosquito. Parasitol Res. 2010; 107(5):1275-1278.
26. Kweka EJ, Senthilkumar A, Venkatesalu V. Toxicity of essential oil from Indian borage on the larvae of the African malaria vector mosquito, *Anopheles gambiae***.** Parasites & Vectors. 2012; 5:277. DOI: 10.1186/1756-3305-5-277.
27. Hassani MS, Zainati I, Zrira S, Mahdi S, Oukessou M. Chemical Composition and Antimicrobial Activity of *Plectranthus amboinicus* (Lour) Spring. Essential Oil from Archipelago of Comoros. J. Essent. Oil Bear. Pl. 2012; 15(4):637-644.
28. Gurib-Fakim A, Sewraj MD, Narod F, Menut C. Aromatic Plants of Mauritius: Volatile Constituents of the Essential Oils of *Coleus aromaticus* Benth., *Triphasia trifolia* (Burm. f.) and *Eucalyptus kirtoniana* (F. Muell.). J. Essent. Oil Res. 1995; 7(2):215-218.
29. Lawrence BM. Chemical components of Labiatae oils and their exploitation, In: Advances in Labiate Science. Ed., Royal Botanic Gardens Kew, Whitstable, UK. 1992, 399-436.
30. Grayer RJ, Kite GC, Goldstone FJ, Bryan SE, Paton A, Putievsky E. Intraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochem. 1996; 43(5):1033-1039.
31. Haque IU. Analysis of volatile constituents of *Coleus aromaticus*. J. Chem. Soc. Pak. 1988; 10(3):369-371.
32. Manjamalai A, Alexander T, Grace VMB. Bioactive Evaluation of the Essential Oil of *Plectranthus amboinicus* by GC-MS Analysis and Its Role as A Drug for Microbial Infections and Inflammation. Int J Pharm Pharm Sci. 2012; 4(3):205-211.
33. Afolayan AJ, Ashafa AOT. Chemical composition and antimicrobial activity of the essential oil from *Chrysocoma ciliata* L. leaves. J. Med. Plant Res. 2009; 3(5):390-394.
34. Manjamalai A, Grace VMB. Volatile constituents and antioxidant property of essential oil from *Plectranthus amboinicus* (Lour). Int J Pharm Bio Sci. 2012; 3(4):B445-B458.