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Research Article

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HPLC Profile of Phenolic Constituents, Essential Oil Analysis and Antioxidant Activity of Six *Plectranthus* Species Growing in Saudi Arabia

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ABSTRACT

Some plants used in Saudi folk medicine have little data about their phytochemical constituents. The HPLC-PDA profile of phenolic constituents, GC-MS composition of essential oil and DPPH antioxidant capacity were performed for six Plectranthus species (P. arabicus, P. asirensis, P. pseudomarrubioides, P. barbatus, P. hijazensis and P. aegyptiacus) growing in Saudi Arabia. The essential oil content of the aerial parts of these species was varied from 0.1% to 0.3% v/w. Their volatile oils compose mainly of monoterpene hydrocarbons (18.79%, 8.52%, 39.14%, 71.27%, 0.97%, 26.77%), oxygenated monoterpenes (33.72%, 8.16%, 25,51%, 0.75%, 4.59%, 58.49%), sesquiterpene hydrocarbons (9.27%, 26.06%, 2.55%, 0%, 30.48%, 9.11%), and oxygenated sesquiterpenes (30.23%, 33.38%, 24.36%, 0%, 44.2%, 0%) in (P. arabicus, P. asirensis, P. pseudomarrubioides, P. barbatus, P. hijazensis and P. aegyptiacus) respectively. Thymol was the only phenolic component detected in P. aegyptiacus with high concentration 58.49%. The HPLC profiles of these plants were recorded and it imply for close similarity with minor differences between the six species of Plectranthus. Rosmarinic acid and gallic acid were detected in the studied species. All samples showed significant anti-free radical activity in comparison with trolox and ascorbic acid.

Keywords: *Plectranthus*; Phytochemical analysis; HPLC-PDA; Essential oil; Antioxidant activity

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INTRODUCTION

The genus *Plectranthus* is one of the largest genera of family Lamiaceae, which belongs to the subfamily *Nepetoideae*, tribe *Ocimeae*, subtribe *Plectranthinae*. It comprising about 300 species distributed in both tropical and warm regions of the world [1,2]. Plants belong to the genus of *Plectranthus* are herbs or sub-shrubs. Many of them were classified formerly in the genus *Coleus Lour*., afterward they transferred to the genus *Plectranthus* [3]. At the same time, many plants were classified formerly as *Plectranthus* and later on reclassified in different closely related genera like *Solenostemon*, *Englerastrum* and *Coleus* [4]. Study of phytochemical composition of some species belong to this genus may help in its chemotaxonomic classification. In the Flora of Saudi Arabia, Collonette [5] described *Plectranthus* by seven species: *P. arabicus* Bruce, *P. montanus* Benth, *P. aegyptiacus* (Forssk.) C. Chr, *P. comosus* Sims, *P. barbatus* Andrews, *P. pseudomarrubioides* Willemse and *P. asirensis* Wood. However, Chaudhary [6] accepted only 6 species viz. *P. arabicus, P. montanus, P. aegyptiacus*, *P. lanuginosus* (Hochst. ex Benth.) Agnew, *P. barbatus*, and *P. asirensis*.

In Saudi Arabia, *Plectranthus* species are used economically as traditional medicine for their biological benefits in the primary health care system. Certain species of *Plectranthus* are famous for their biological activities as antiseptic, for wounds, remedy for stomach, intestine, liver disorders, heart problems and respiratory diseases [7-9]. It is well known that, certain plants belong to the genus *Plectranthus* have a great biosynthetic capacity to form diverse phytochemical classes from cell secondary metabolism, mainly diterpenes, phenolics and triterpenoids [10,11]. There are many selective and sensitive analytical methods were developed to illustrate the phenolic compounds profile in medicinal plants and accurate characterization of them [12]. Until now there are no phytochemical studies on most of *Plectranthus* species growing wildly in Saudi Arabia. Only a few studies have reported on the anatomy and phytochemistry in *Plectranthus* species such as *P. aegyptiacus and P. asirensis* [10,13]. Therefore, this study aims to investigate phytochemical analysis of its volatile distillates to determine their main constituents using GC-MS and determining the HPLC-PDA profile of each individual plant extract using comparative analysis with some phenolic and flavonoid standards identified from other *plectranthus* plants worldwide. Moreover, in order to help taxonomists in chemosystematics evaluation of these plants and because of ethnomedical importance of different *Plectranthus* species in Saudi Arabia.

MATERIALS AND METHODS

Plant Material

Plants were collected during the flowering stages from different places in Saudi Arabia (Appendix 1, Figure 1). The identification was simplified according to Collonette, 1999 and Chaudhary, 2001 [5,6]. Voucher specimens were deposited at the Herbaria of King Saud University (Appendix 1), Kew and Edinburgh herbarium (KSU, K and E).

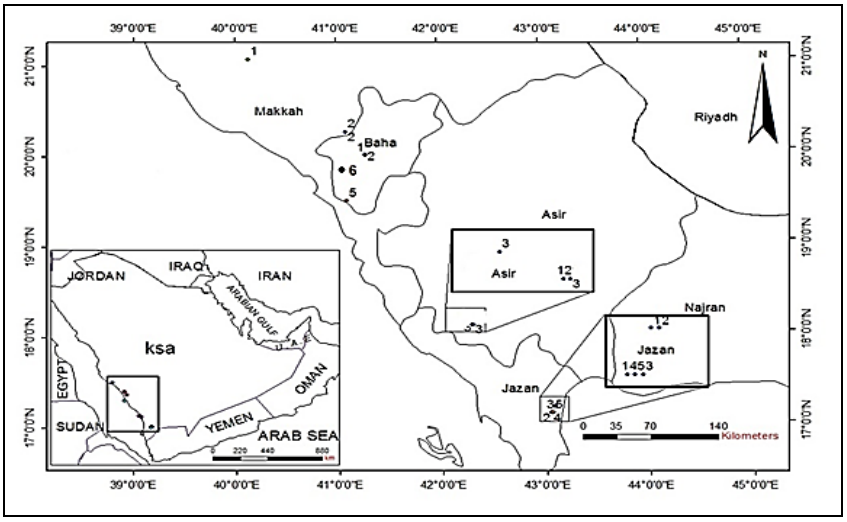

Figure 1: Map of distribution of *Plectranthus* in Saudi Arabia; 1. *P. barbatus*; 2. *P. asirensis; 3. P. aegyptiacus;* 4. *P. arabicus,* 5; *P. pseudomarrubioides;* 6. *P. hijazensis*

Preparation of Extracts

The air-dried leaves of each plant (200 g/each) were grinded to fine powder using electrical grinder and extracted separately with 75% methanol (500 ml × 3). The collected methanol extracts were dried under *vacuum* to give 15 g, 10 g, 13 g, 12 g, 16 g and 13 g of *P. arabicus, P. asirensis, P. pseudomarrubioides, P. barbatus, P. hijazensis* and *P. aegyptiacus* respectively. All extracts were kept frozen until usage.

Preparation of Essential Oil

Two hundred grams of each fresh plant were shopped into small pieces of average size about 4-6 mm diameter then placed in a round flask with flat bottom 1000 ml. Deionized water was added and subjected to hydro-distillation for 3 hours, using a Clevenger-type apparatus. The percentages of essential oils were calculated as volume (ml) of essential oil per 100 g of fresh plant material. The obtained essential oils were dried over anhydrous sodium sulphate. They stored in an amber color, air tight bottle and kept in a refrigerator at 3-5^{o}C until usage for further analysis.

GC–MS Analysis of Essential Oil

GC-MS analysis was carried out on a Shimadzu GCMS-QP2010 ultra interfaced with Quadrupole mass spectroscopy fitted with Rtx-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm, RESTEK, USA) the column temperature was programmed 45^{o}C, initial hold time of 3 min, then raised to 250^{o}C by 4^{o}C/min, the injector temperature was 250 $^{\circ}$C, transfer line and source temperatures were 250°C. Injection in split mode with

1:10 ratio; carrier gas He was used at 10 psi constant pressure; ionization energy 75°C; mass scan range 35-500 amu. Before GC–MS analysis the oils (water free) were dissolved in *n*-hexane with a ratio of 1:200. Identification was done by comparison of mass spectra with a built-in electronic library database (NIST08, Wiley9) and confirmation preformed using the arithmetic index (IUPAC, 1997) [14], compared to published values as calculated relative to series of C8- C30 n-alkanes [15] while % calculated from FID data. Identification of some major compounds as thymol was confirmed by built in library and co-injection of standard samples. GC-FID analyses were done on Shimadzu chromatograph (model QP2010 series) by using a flame ionization detector (FID).

HPLC-PDA Profile of Phenolic Constituents

The analytical method was developed by some modifications of previous study described by of Chanjuan *et al*., [16]. Qualitative and quantitative determination of some phenolic acids and flavonoids in the studied species of *Plectranthus* were performed using the HPLC fingerprints of them using Waters Alliance HPLC (Waters Corp, Milford, MA, USA) equipped waters e2995 separation module and waters 2998 PDA and Empower 2 software. Elution was done using gradient mobile phase system, which composed of methanol (A) and 0.7% (v/v) acetic acid (B), the gradient program was as follow: 15% A up to 15 min, then changed to obtain 45, 65, and 15% B in 55, 61, and 65 min, respectively. Monitoring of UV absorbance was at 254, 280, 320, 520, and 630 nm for standards and extracts with photodiod array detector (PDA). All injection volumes of extracts and standard were 20µl. Column: Stainless steel, reverse phase, RP-C_{18} column, 5µm, 4.6 mm × 150 mm X Bridge. Detector: PDA (210-800 nm). Flow Rate: 0.6 ml/min. Sample volume (loop): 10-100 µl. The extracts were subjected to solid phase extraction using RP-C_{18} silica gel finger column eluted with water followed by washing with methanol: water 75:25. The aliquot samples were filtered through a 0.45 ml pore membrane filter prior to injection. The standards used were; galic acid (1), caffeic acid (2), *trans*-ferulic acid (3), rutin (4), rosmarinic acid (5), scutellarein (6), acacetin (7), 7-O-methyltaxifolin (8), cinnamic acid (9), 7, 3`-di-O-methyltaxifolin (10). The standards 1-7 and 9 were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany), while standards 8 and 10 were kindly obtained from our colleague Ehab Ragab which were isolated previously from *Pulicaria jaubertii* [17]. For data validation of the fingerprinting all experiments were repeated three times. Identification of peaks was achieved by congruent retention times and UV-PDA profile against standards. Calibration curves of external standards (within the range of 12.5-200 µg /ml) were performed for quantification of the identified phenolic components (flavonoids and phenolic acids).

DPPH Radical Scavenging Assay

The antioxidant activities of six *Plectranthus* species growing in Saudi *Arabia, P. arabicus, P. asirensis, P. pseudomarrubioides, P. barbatus, P. hijazensis* and *P. aegyptiacus* were estimated based on the inhibition (%) of the stable DPPH free radical [18]. Methanolic solution of DPPH was prepared in a concentration of 0.002%. Equal volumes (1 ml) of 0.002% DPPH and the plants extracts solution (in concentrations ranged between 10-200 µg/ml) were mixed together then incubated at room temperature in dark for 30 minutes. The absorbance of the mixture was measured by UV/Vis-Spectrophotometer at 515 nm (6800 UV/Vis. Spectrophotometer, JENWAY, UK.). 0.002% DPPH solution was used as control and methanol as blank. Trolox and Ascorbic acid were used as references; they were used in concentrations of (10-200 µg/ml). The DPPH radical scavenging activity of samples was calculated from the following equation:

$$\text{Inhibition (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100$$

Where: $\text{Abs}_{\text{control}}$ is the absorbance of DPPH solution (1 ml) + methanol (1 /ml); $\text{Abs}_{\text{sample}}$ is the absorbance of the DPPH solution with sample extract

RESULTS AND DISCUSSION

The wide diversity of ethnomedical uses of *Plectranthus* taxa [4] in Arab peninsula and apparent shortage of phytochemical data about most plants of this important taxa growing in Saudi Arabia. Also the earlier conflicts in its taxonomy are the main reasons to focus some light on certain species of *Plectranthus*. Only *P. barbatus* was extensively studied for its phytochemicals which focus on diterpenes and essential oil [19,20].

Chemical Identification of Essential Oils

Essential oils of *Plectranthus* plants were obtained by hydrodistilation from the aerial parts with variable yields ranged from 0.1% to 0.3% v/w. The yield of essential oil obtained from *P. arabicus* was 0.2%; *P. asirensis*, 0.1%; *P. pseudomarrubioides*, 0.3%; *P. barbatus*, 0.25%; *P. hijazensis*, 0.15% and *P. aegyptiacus*, 0.2% v/w. The highest yield was recorded for *P. pseudomarrubioides* while the lowest was obtained from *P. asirensis.* It was observed that the oils obtained from *P. arabicus*, *P. pseudomarrubioides* and *P. barbatus* were characterized by colorless with nicotine like odor. The oil obtained from *P. hijazensis* has rose like odor, while

P. aegyptiacus has a strong thymol odor which agreed with its content (58.49% thymol of its essential oil) (Table 1).

Table 1: Chemical composition of essential oils of six *Plectranthus* species growing in Saudi Arabia

S No	Compound	RRI	*P. arabicus*	*P. asirensis*	*P. pseudomarrubioides*	*P. barbatus*	*P. hijazensis*	*P. aegyptiacus*
1	Octane	800		1.15		0.4	0.36	
2	2-Hexenal (E)	846		0.76				
3	3-Hexen-1-ol (Z)	852		1.32		6.06		
4	2-Hexen-1-ol (Z)	860				0.97		
5	1-Hexanol	864				1.15		
6	Nonane	900				0.75		
7	*α*-Thujene	926		0.38		0.13		
8	*Tetrahydrocitronellene*	936			11.85	0.92		
9	*α*-Pinene	939	7.9	2.38	10.76	0.21		
10	Camphene	953			2.09	0.14		
11	Verbenene	962		0.32				
12	Sabinene	977			1.34	0.31		
13	*β*-Pinene	980	2.36	0.4		0.21		
14	L-Octen-3-ol	982	3.39	9.34	1.34	11.88		1.25
15	3-Octanone	987		0.58		0.85		
16	*β*-Myrcene	989	0.7		0.43	14.9	0.97	0.97
17	3-Octanol	994				2.41		
18	3-Hexen-1-ol, acetate, (Z)	998		0.56				
19	*δ*-3-Carene	1001	6.77		0.69	0.73		
20	(+)4-Carene	1008				23.21		
21	*α*-Terpenene	1017			2.89	9.55		7.46
22	*β*-Cymene	1020			2.85			
23	L-Limonene	1032	1.06	0.44	3.67	3.04		
24	1,8-Cineole	1036	10.41			0.75		
25	Benzeneacetaldehyde	1038		0.42		0.2		
26	*Cis*-Ocimene	1042						1.47
27	*Trans-β*-Ocimene	1050		4.6	1.27	1.85		
28	*γ*-Terpinene	1063			1.3	16.07		16.87
29	L-Fenchone	1083	1.1		10.56			
30	L-Linalool	1101		2.73	1.84		1.05	
31	Nonanal	1103		1.07			0.16	
32	*δ*-Fenchyl Alcohol	1118	1.04		1.89			
33	*Trans-p*-Mentha-2-en-1-ol	1134	0.43					
34	p-Menth-1,5-dien-8-ol	1136		0.37				
35	Camphor	1141			1.14			
36	Verbenol	1143		1.63			0.27	
37	*Iso*-Borneol	1158			1.18			
38	Pinocarvone	1160			1.12			
39	Borneol	1162					0.22	
40	*α*-Phellandren-8-ol	1164		0.64				
41	Neo-Thujan-3-Ol	1166			1.89			
42	Terpinen-4-ol	1168			4.59		0.71	
42	P-Cymen-8-ol	1182			0.81		0.13	
44	*α*-Terpeneol	1189		0.81	0.25		0.22	
45	(-)-Myrtenal	1195					0.93	
46	*Cis*-Piperitol	1197		0.5				
47	Decanal	1205					0.2	
48	Neral	1235		0.4				
49	Geraniol	1248					0.2	
50	Myrtenol	1250	2.82					
51	Linalyl acetate	1254	0.6					
52	Geranial	1268		0.51			0.25	
53	Nerylformte	1285			0.24			
54	Thymol	1292						58.49
55	Myrtinyl acetate	1316					0.41	
56	*α*-Cubebene	1345					0.29	
57	*α*-Terpenyl acetate	1355	13.2					
58	6-Epi-α-Cubebene	1359					0.27	
59	*α*-Copaene	1377		0.46	0.2		0.2	
60	*Trans*-Caryphyllene	1406		0.33	0.27		3.29	9.11
61	*α*-Gurjunene	1410					1.23	

62	10-(Acetylmethyl)-(+)Carene	1412		0.57				
63	*β*-cedrene	1416		1.81				
64	*α*-Pinene, 10-(2-Oxopropyl)	1418	4.12					
65	*γ*-Maaliene	1420					1.4	
66	*Cis*-caryophyllene	1423	3.17	5.65			16.98	
67	*β*-Gurjunene	1429		2.82	0.26		0.71	
68	*Trans*-*α*, *α*- Bergamotene	1432		1.47	0.7			
69	Aromadendrene	1442					2	
70	*α*-Humulene	1452	5.49	3.14	0.32			
71	Allo-aromadendrene	1460		0.26			0.89	
72	*β*-Acoradiene	1468	0.61					
73	*β*-Himachalene	1460		1.8				
74	Germacrene-D	1482					0.51	
75	*β*-Ionone	1487		6.95	0.25		1.83	1.08
76	*α*-Muurolene	1500		3.46	0.23		1.31	
77	*γ*-Cadinene	1509		2.85				
78	α-Bulnesene	1512			0.8			
79	Seesquicineole	1515		0.35			0.37	
80	*δ*-Cadinene	1524		5.47			2.71	
81	Nerolidol (Z)	1532	0.76					
82	*Cis*-Sesquisabinene hydrate	1538					0.27	
83	7-Epi-*Cis*-Sesquisabinene hydrate	1542		13.03	0.77		0.65	
84	Germacrene *D*-4-ol	1574	0.94		0.47			
85	Spathulanol	1577	2.1					
86	*Trans*-Sesquisabinene	1579		2.52	0.41			
87	Caryophyllene oxide	1582		1.77	1.02		28.4	
88	*Cis*-Z-*α*-Bisabolene epoxide	1590	1.08					
89	Globulol	1596			2.38		0.31	
90	Veridiflorol	1599	1.23				3.88	
91	Humulene oxide	1614					1.87	
92	*α*-Acorenol	1620		2.11				
93	T-Cadinol	1622					0.54	
94	Aromandrene oxide (2)	1639	1.7	0.83	0.91		0.74	
95	*δ*-Cadinol	1642		1.84			0.17	
96	Tau-muurolol	1646					0.69	
97	Bisabolol oxide B	1656	1.11	1.19			3.68	
98	7-Epi-B-Eudesmol	1662			1.15			
99	*β*-Bisabolol	1674		6.28				
100	*α*-Bisabolol	1680			17.02			
101	6-*Epi*-shyobunol	1682	3.66					
102	1-Tetradecanol	1684					0.4	
103	*β*-Bisabolene	1686	16.69					
104	Farnesol	1688					0.6	
105	Geranyltiglate	1710					0.72	
106	Dehydronerolidol	1790	0.96					
107	Sclareol oxide	1876					0.45	
108	Manoyl oxide	1986					1.96	
109	Palmitic acid methyl ester	1920					1.96	
110	Benzoic acid pentdecyl ester	1946					0.48	
	Total Identified (% v/v)		96.2	88.93	93.15	96.69	87.84	96.7
	Number of identified components		28	45	39	23	47	8
	Monoterpene hydrocarbons		18.79	8.52	39.14	71.27	0.97	26.77
	Oxygenated monoterpenes		33.72	8.16	25.51	0.75	4.59	58.49
	Sesquiterpene hydrocarbons		9.27	26.06	2.55		30.48	9.11
	Oxygenated sesquiterpenes		30.23	33.38	24.36		44.2	
	Others		4.19	12.81	1.59	24.67	7.6	2.33

Note: The order of compounds is listed according the elution time; compounds with relative abundance less than 0.13% are not listed

The identified compounds were 110 representing 96.2%, 88.9%, 93.15%, 96.69%, 87.84% and 96.70% of the total composition of the oils in *P. arabicus*, *P. asirensis*, *P. pseudomarrubioides*, *P. barbatus*, *P. hijazensis* and *P. aegyptiacus* respectively (Table 1). The number of identified components in essential oils were ranged from 8 compounds in *P. aegyptiacus* to 47 compounds in *P. hijazensis*.

The concentration of the major components were *α*-pinene 10.76%; 1,8-cineole 10.41%; *γ*-terpinene 16.87%; L-fenchone 10.56%; thymol 58.59%; *α*-terpenyl acetate 13.20%; *trans*-caryphyllene 9.11%; *cis*-caryophyllene 16.98%; 7-epi-*cis*-sesquisabinene hydrate 13.03%; caryophyllene oxide 28.40%, *α*-bisabolol 17.02% and *β*-

bisabolene16.69%. The analysis of these data confirmed the presence of monoterpene hydrocarbons in all of the investigated species (18.79%, 8.52%, 39.14%, 71.27%, 0.97%, 26.77%), oxygenated monoterpenes (33.72%, 8.16%, 25.51%, 0.75%, 4.59%, 58.49%), sesquiterpene hydrocarbons (9.27%, 26.06%, 2.55%, 0%, 30.48%, 9.11%), oxygenated sesquiterpenes (30.23%, 33.38%, 24.36%, 0%, 44.2%, 0%) in *P. arabicus*, *P. asirensis*, *P. pseudomarrubioides*, *P. barbatus*, *P. hijazensis* and *P. aegyptiacus* respectively. l-octen-3-ol is common in all studied species except *P. hijazensis*, while *β*-myrcene is common in all except *P. asirensis*. *α*-pinene, l-limonene, *β*-ionon, *trans*-caryphyllene and aromadendrene oxide (2) were detected in *P. arabicus*, *P. asirensis*, *P. pseudomarrubioides* and *P. barbatus*. There are 17 compounds (octane, *β*-pinene, 3-octanone, *δ*-3-carene, *α*-terpenene, *trans*-*β*-ocimene, *γ*-terpinene, L-linalool, *α*-terpeneol, copaene, *cis*-caryphyllene, *β*-gurjunene, *α*-humulene, *α*-muurolene, 7-epi-*cis*-sesquisabinene hydrate, caryophyllene oxide, bisabolol oxide B) recorded at least in 3 studied species (Table 1). It is clear that sesquiterpene hydrocarbons detected in all samples except *P. barbatus*. It was observed that oxygenated sesquiterpenes was not detected in *P. aegyptiacus* and *P. barbatus*. The previous results are not in accordance with Costa [21] who identified two oxygenated sesquiterpenes and 10 sesquiterpenes hydrocarbons from *P. barbatus* which may be of other chemotype. Twenty eight components were representing 96.2% of the total oil content were identified in *P. arabicus*; The main constituents were *β*-bisabolene (16.69%), 2-pinen-10-ol acetate (13.20%), 1,8-cineole (10.41%), *α*-pinene (7.90%), *δ*-3-carene (6.77%) and *α*-humulene (5.49%). While previous study determined 1,8-cineole as most abundant compound [22]. The main constituents of essential oil of *P. asirensis* were sesquisabinene (26.06%), *β*-Ionone (6.95%), *β*-bisabolol (6.28%), 7-epi-*cis*-Sesquisabinene hydrate (13.03%), *δ*-cadinene (5.47%), *trans*-*β*-ocimene (4.60%), *β*-bisabolene (6.28%), *α*-humulene (3.14%), and *cis*-caryophyllene 5.6%. In our study, thymol was not detected in *P. asirensis*, this result is against the previous literature [13]. Thirty nine components were identified in *P. pseudomarrubioides* essential oil which was representing 93.15% of the total oil content; the main constituents were *α*-bisabolol (17.02%), tetrahydrocitronellene (11.85%), *α*-pinene (10.76%), L-fenchone (10.56%), L-limonene (3.67%) and terpinen-4-ol (4.59%). Twenty-three compounds were the main detected constituents in the oil of *P. barbatus*. These compounds were representing 96.69% of its essential oil content; (+)4-carene (23.21%), *γ*-terpinene (16.07%), L-limonene (3.04%), *β*-myrcene (14.90%), L-octen-3-ol (11.88%) 3-hexen-1-ol (6.06%) and 3-octanol (2.41%). In contrast the major oil content of Italian sample of *P. barbatus* leaves studied by Gelmini *et al*., (2015) were anethol (18.76%), *β*-caryophyllene (12.24%), D-germacrene (9.35%) and 1-octen-3-ol (8.13%) [23]. Main constituents of *P. hijazensis* were caryophyllene oxide (28.40%), *cis*-caryophyllene (16.98%), veridiflorol (3.88%), *trans*-caryphyllene (3.29%) and bisabolol oxide B (3.68%) among other 47 compounds comprising (87.84%) of the total oil components. Tow diterpenes were also identified as sclareol oxide (0.45%), and manoyl oxide (1.96%). The identified components of *P. aegyptiacus* were 96.7% of its oil content. Thymol (58.49%) represented the main identified component in this oil, which is responsible for the odor of its oil. The other major components were *α*-terpenene (7.46%), *γ*-terpenene (16.87%) and *trans*-caryphyllene (9.11%). Our study may supports the previous thought about the qualitative and quantitative variations of essential oil constituents of the investigated *Plectranthus* species which may be varied with the source of plants, time of the plants collection, proportions of distilled parts, variations in metabolism and biosynthetic pathways [24, 25].

HPLC-PDA Profile of Phenolic Constituents

The secondary metabolites produced by plants such as phenolic compounds, were reported to have a vast array of medicinal activities like anti-inflammatory, antimicrobial, antiviral and high antioxidant properties [12]. HPLC is known to be accepted internationally as a reliable method for identification of herbs and herbal products. It has the ability to characterize both marker constituents and unknown constituents in a complex herbal system [26, 27]. HPLC is considered to be the principle analytical technique, for quantitative and qualitative determination of the targeted analytes within complex matrices. In this study, HPLC-DAD was used to determine fingerprint chromatograms for the investigated molecules; the method was practical and reliable. The high-tech system which was utilized during this research was equipped with a powerful and precise photodiode array detector (PDA). The peak purity was examined and the selected peaks were identified against standards comparing their UV spectra and retention times. In the present work HPLC-DAD chromatographic study was used as a rapid method for exploring and characterization of some untapped plants of *Plectranthus* taxa growing in Saudi Arabia. In addition, exploring the presence of some phenolic acids and flavonoids against standard samples was performed. The extracts of the studied *Plectranthus* species were subjected to solid phase elution using RP-C_{18} silica gel finger column eluted with water at first to exclude sugars and high polar constituents. The method of HPLC-DAD developed for this study showed appropriate separation of standards. The data obtained for the ten-individual standards phenolic compounds at 280-800 nm is shown in Table 2.

Table 2: Elution of individual flavonoids and phenolic acids standards using HPLC-DAD system

Key no.	Compound name	Retention times	Maximum absorbance (210–800 nm)
1	Gallic acid	4.897	230, 270
2	Caffeic acid	7.014	295, 330
3	Ferulic acid	24.548	220, 228, 238, 324
4	Rutin	25.167	256, 356
5	Rosmarinic acid	26.712	222, 292, 331
6	Scutellarein	32.46	216, 275, 331
7	Acacetin	34.407	268, 302, 330
8	7-*O*-methyltaxifolin	36.624	290, 332
9	Cinnamic acid	40.26	220, 266
10	7, 3`-di-*O*-methyltaxifolin	50.518	290, 330

Table 3: Quantification of individual flavonoids and phenolic acids at 280 nm using HPLC-UDAD system in *Plectranthus* taxa

Plant name	Total area mAUs	Identified compounds	Time (min)	Area (mAUs)	Area(%) in the extract	Quantity (µg/g extract)
P. arabicus	4188086	Gallic acid	4.664	859814	20.53	160.21
		Caffeic acid	6.455	11314	0.27	7.34
		Ferulic acid	24.521	80978	1.93	12.58
		Rosmarinic acid	26.515	323179	7.72	32.76
P. asirensis	10242138	Gallic acid	4.675	1317139	12.86	240.71
		Ferulic acid	24.483	241096	2.35	35.58
		Rutin	25.095	152450	1.49	140.02
		Rosmarinic acid	26.147	4222250	41.22	7000.22
P. pseudomarrubioides	9306597	Gallic acid	4.665	906698	9.74	170.51
		Rosmarinic acid	26.36	2040006	21.92	350.84
		7-O-methyltaxifolin	37.362	188194	2.02	2.36
P. barbatus	4023545	Gallic acid	4.664	1027211	25.53	190.04
		Rosmarinic acid	26.087	1947079	48.39	3350.05
P. hijazensis	3502512	Gallic acid	4.675	1563521	44.64	290.87
		Caffeic acid	6.406	14417	0.41	0.93
		Ferulic acid	24.428	380342	10.86	55.07
		Rutin	25.18	210128	6	1954.02
		Rosmarinic acid	26.732	880625	25.14	1510.1
P. aegyptiacus	4140382	Gallic	4.666	1331547	32.16	240.43
		Rosmarinic acid	25.98	219747	5.31	202.55

The HPLC chromatograms (Figure 2) showed that the close similarity with minor differences between the six species of *Plectranthus* and showed the presence of gallic and rosmarinic acids in all species under investigation. Also, it was observed that gallic acid is detected in all species under investigation. These results came in agreement with many other species of *Plectranthus* taxa from which these acids were previously identified [4]. The calculated concentrations of these acids were; *P. arabicus* (160 µg/g, 32 µg/g), *P. pseudomarrubioides* (170 µg/g, 350 µg/g); *P. aegyptiacus* (240.43 µg/g, 202.55 µg/g); *P. hijazensis* (290 µg/g, 1510 µg/g); *P. barbatus* (190 µg/g, 3335 µg/g); and *P. asirensis* (240 µg/g, 7000 µg/g) extracts of galic and rosmarinic acid respectively. Caffeic acid was detected only in *P. arabicus* (7.3 µg/g). Trans-ferulic acid was present in *P. arabicus*, *P. hijazensis* and *P. asirensis* with concentrations of (12 µg/g, 55 µg/g and 35 µg/g extract respectively). Rutin was present in *P. hijazensis* and *P. asirensis* (by concentrations of 1954 µg/g and 140 µg/g respectively). The 7-O-methyltaxifolin was detected only in *P. pseudomarrubioides* (2.36 µg/g). Cinnamic acid and flavonoids like scutellarein and acacetin were not detected in all studied plant samples, although scutellarein 4-O-methyl ether 7-O-glucuronide was reported previously to be isolated from *P. barbatus* [18]. From the previous data, we can conclude that rosmarinic acid and gallic acid may be used as a useful tool for chemotaxonomy of *Plectranthus* taxa growing in Saudi Arabia.

The HPLC-DAD profile and essential oil analysis in *Plectranthus* taxa offer a set of characters useful for the taxonomy of the *Plectranthus*. The present study showed high diversity in concentration, distribution and components of volatile oil. It provided some evidence for infra generic classification and partly corresponds with the phylogenetic and ethno-botanical uses [4, 28], morphology [29, 30] and phytochemistry [9]. Our results indicated some similarity among the endemic *P. asirensis* and *P.hijazensiss* should be homogeneous group. A remarkable result from this study that *P. hijazensis* is morphologically close to *P. asirensis*; therefore, some samples of *P. hijazensis* may be incorrectly determined as *P. asirensis* or *P. lanuginosus* in the past. Also, present investigations have shown that *P. hijazensis* differs from *P. asirensis* as it was not containing *α*-pinene, *β*-pinene, L-limonene, trans-*β*-ocimene, *α*-humulene, neral, *α*-acorenol, *β*-bisabolol and many other constituents (Table 1). *As well as P. hijazensis detected to contain β*-myrcene, L-linalool, aromadendrene, veridiflorol and tow diterpene compounds (i.e. manoyl oxide and sclareol oxide) which are not identified in *P. asirensis*.

Furthermore, this study was identifying *P. arabicus* with several unique compounds as a separate group and suggesting that should be treated as a separate subgenus.

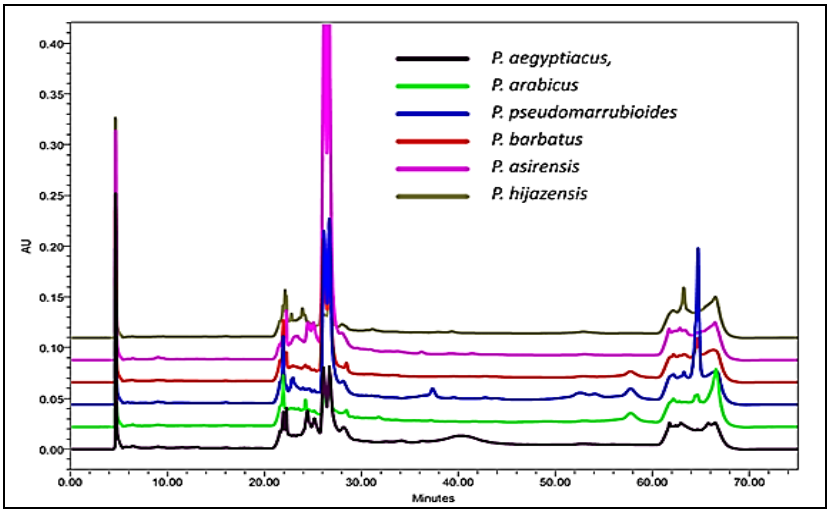

Figure 2: Typical HPLC-DAD fingerprint overlay chromatogram of six *Plectranthus* species growing in Saudi Arabia

Free Radical Scavenging Activity

The drug formulations which containing natural antioxidant agents are used for treatment of many diseases like Alzheimer's disease, diabetes, stroke, atherosclerosis, and cancer [31]. The antioxidant activity of medicinal plants might be attributed to its phenolic contents especially flavonoids, lignans, catechins and anthocyanin [32]. Free radical scavenging activities of the studied plant extracts were analyzed by the DPPH assay in comparison with trolox and ascorbic acid as standards. The results of free radical scavenging activity (Figure 3) for all samples showed significant antiradical activity in comparison with the two standards trolox and ascorbic acid (Figure 3). *P. asirensis* (40 µg/ml) showed the highest antioxidant activity (93.62 %) in comparison with trolox (95.39%) and ascorbic acid (95.55%). There is no significant increase at higher concentrations of this plant extracts.

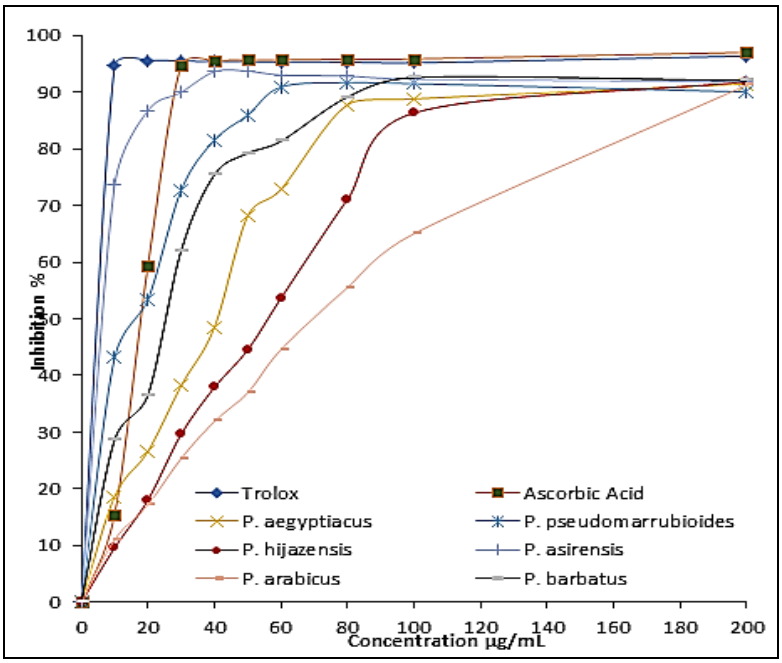

Figure 3: DPPH free radical scavenging activity of *P. arabicus, P. asirensis, P. pseudomarrubioides, P. barbatus, P. hijazensis*and *P. aegyptiacus* with comparison of trolox and ascorbic acid

The second effective one against DPPH was *P. pseudomarrubioides* at concentration of 90.91 µg/ml. The lowest activity was recorded for *P. arabicusas* as shown in Figure 3. It was observed that, the antioxidant capacity of all samples was concentration dependent up to concentrations of 100 µg/ml, while the activity above this limit was concentration independent. These results indicate that further exploration of biological activities of these plants is needed.

CONCLUSION

The HPLC-DAD profile and essential oil analysis showed the relation between the phytochemicals of *Plectranthus* species growing in Saudi Arabia which may offer a light to researchers for exploring of some untapped plants, also this study concludes that rosmarinic acid and gallic acid may be used as a useful tool for chemotaxonomy of *Plectranthus* taxa growing in Saudi Arabia. *P. aegyptiacus* contains high content of thymol (58.49 %), so it may be used as economic source of thymol. Most of *Plectranthus* plants have a significant free radical scavenging activity even in low concentration.

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APPENDIX 1

List of *Plectranthus* taxa and specimens studied [5,6].

1. *Plectranthus arabicus* E. A. Bruce: Saudi Arabia, Jazan, GabalFayfa, Abdel Khalik s. n. (UQU).
2. *Plectranthus asirensis* J. R. I. Wood: Saudi ArabiaJazan, GabalFayfa, Abdel Khalik& Al-Ozekii s. n. (UQU).
3. *Plectranthus barbatus* Andrews**:** Saudi Arabia, Jazan, GabalFayfa, Abdel Khalik s. n. (UQU).
4. *Plectranthus pseudomarrubioides* R. H. Willemse: Saudi Arabia, Jazan, GabalFayfa, Abdel Khalik& Al-Ozekiis.n. (UQU).
5. *Plectranthus aegyptiacus* (Forssk.) C. Chr: Saudi Arabia, Jazan, in area of GabalFayfa, Abdel Khaliks.n. (UQU).
6. *Plectranthus hijazensis* K. Abdel Khalik: Saudi Arabia, Al Baha, in area of Saad Medhas, Abdel Khalik& Howldars.n s. n. (UQU).

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