AkiNik

American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2013; 1 (2): 11-18

Received 20-10-2013
Accepted: 15-11-2013

Seham S. El-hawary
Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

Rabie H. El-sofany
Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

Azza R. Abdel-Monem
Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

Rehab S. Ashour
Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt

Amany A. Sleem
Department of Pharmacology, National Research Centre, Giza 12622, Egypt

Correspondence:
Azza R. Abdel-Monem
Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt
Email: azzaramy@yahoo.com

Seasonal variation in the composition of *Plectranthus amboinicus* (Lour.) Spreng essential oil and its biological activities

Seham S. El-hawary, Rabie H. El-sofany, Azza R. Abdel-Monem*, Rehab S. Ashour, Amany A. Sleem†

Abstract

The effect of seasonal variation on the yield and composition of the essential oils of the leaves and stems of *Plectranthus amboinicus* (Lour.) Spreng, growing in Egypt, collected along the four seasons at three months intervals viz., January, April, July and October was studied. The highest yield of essential oils of both the leaves and stems was obtained during spring (0.12% and 0.13% v/w fresh weight, respectively).The GC/MS analysis of the essential oil samples revealed a qualitative and quantitative variation in chemical composition. Concerning the leaves, δ-cadinene was the major component in the oils of spring (18.66%) and autumn (12.52%), while, β-caryophyllene (12.65%) and thymol (8.75%) were the major components in the oils of winter and summer, respectively. Concerning stems, α-humulene was the major component in the oil samples of winter (11.14%) and summer (12.70), while, β-copaene-4- α-ol (9.37%) and thymol (13.02%) were the major constituents in the oil samples of spring and autumn respectively. Further analysis of GC/MS data revealed that, the total sesquiterpenes were of higher percentage than the total monoterpenes in both the oil samples along the four seasons. The total oxygenated compounds were found highest in the volatile oil of the leaves collected in summer (53.57%) and volatile oil of the stems collected in spring (55.99%).The volatile oils of the leaves and stems exhibited variable antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic and antimicrobial activities. The LD_{50} of the volatile oils of both the leaves and stems were up to 0.05 ml/kg.

Keywords: *Plectranthus amboinicus* (Lour.) Spreng, essential oil, seasonal variations, antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic, antimicrobial.

1. Introduction

Genus *Plectranthus* belongs to family Lamiaceae. It comprises about 350 species worldwide, distributed in Tropical Africa, Asia and Australia. Several *Plectranthus* species are cultivated as ornamentals or as sources of essential oils, whereas others are used as edible tubers, or as food flavorings[1]. *Plectranthus amboinicus* (Lour.) Spreng (synonyms include *Plectranthus aromaticus* Roxb., *Coleus aromaticus* Benth. and *Coleus amboinicus* Lour.) is a perennial herb with a 3-10 years life span, and it is native to Indonesia[1]. The chemical composition of the volatile oil of *P. amboinicus* growing in different areas had been subjected to various investigations[2-9], qualitative and quantitative variability among the oil samples was greatly influenced by the techniques applied for extracting the oils[3] as well as, the locality of the plant [4]. The volatile oil of *P. amboinicus* was reported to exhibit antimicrobial[2, 8-11], insecticidal[4] and antileptospiral[12] activities. The present study was carried out to study the effect of seasonal variation on the yield and composition of essential oil samples hydrodistilled from the fresh leaves and stems of *P. amboinicus* growing in Egypt, collected all over the year at different time intervals. The LD_{50} of the oil samples was determined and their biological activities including antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic and antimicrobial activities were also studied.

2. Materials and methods

2.1. Plant material

Plant material of *P. amboinicus* were collected all over the years (2008-2010) along the four

seasons at three months intervals viz., January, April, July and October from El- Orman garden. The plant was kindly identified by Dr. Mohamed el Gebaly (Taxonomist) and Madam Treze (Taxonomist). A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

2.2. Preparation of the essential oils

Samples of fresh leaves and stems of *P. amboinicus* were subjected to hydrodistillation[13]. The essential oils obtained were separately dried over anhydrous sodium sulphate and kept in a refrigerator for analysis. The percentage of the oils was calculated on fresh weight bases.

2.3. Analysis of the essential oils

The oil samples were subjected to analysis by GC/MS technique using Agilent 6890 gas chromatograph equipped with Agilent mass spectrophotometric detector, with a direct capillary interface and fused silica capillary column HP-5 MS (30 m x 320 µm x 0.25 µm film thickness). The GC temperature program was started at 70 °C (3 min), then elevated to 260 °C at rate of 8 °C/min. the injector and detector temperature were set at 250 °C and 280 °C, respectively. Helium was used as a carrier gas at a flow rate 1 ml/min, pulsed splitless mode. The solvent delay was 3 minutes and the injection size was 1 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from m/z 50 to 500. The ion source temperature was 230 °C and the quadrupole temperature was 150 °C. The electron multiplier voltage (EM voltage) was maintained at 1050 V above auto tune. The instrument was manually tuned using perflourotributylamine (PFTBA). The volatile oils components were identified by comparing their mass fragmentation patterns with those of the available reference[14]. Compound identification was also confirmed by electronic Wiley and NIST mass spectral data base. The retention indices (RI) of the volatile oils components were determined relative to the retention times of series of hydrocarbons. Results are recorded in tables (2-5).

Table 1: The percentage yield and physical characters of the essential oils of *P. amboinicus*

Organ	Characters	Winter	Spring	Summer	Autumn
Leaves	% Yield	0.03	0.12	0.08	0.08
	Color	pale yellow	yellow	yellow	pale yellow
	Odour	characteristic, strongly aromatic	characteristic, strongly aromatic	characteristic, strongly aromatic	characteristic, strongly aromatic
	R.I. (20°C)	1.4982	1.4996	1.5081	1.5080
	Sp. gr. (20°C)	0.918	0.919	0.917	0.917
Stems	% Yield	0.01	0.13	0.09	0.01
	Color	colorless	pale yellow	colorless	pale yellow
	Odour	aromatic	aromatic	aromatic	aromatic
	R.I. (20°C)	1.4791	1.4779	1.4825	1.4696
	Sp. gr. (20°C)	0.909	0.910	0.874	0.846

Table 2: Identified components in the essential oils of the leaves of *P. amboinicus* at different seasons

Peak no.	Identified compounds	KI	Area %			
			Winter	Spring	Summer	Autumn
1	Octen-3-ol	1009	0.34	0.81	1.27	1.84
2	*α*-Terpinene	1017	0.22	0.04	0.79	0.08
3	*p*-Cymene	1023	1.82	1.73	2.55	0.84
4	*o*-Cymene	1025	--	--	--	1.01
5	γ-Terpinene	1062	1.63	0.96	3.24	--
6	Fenchone	1091	--	--	1.30	0.44
7	Linalool	1098	0.02	0.11	--	0.16
8	Terpinene-4-ol	1178	--	--	--	2.4
9	Thymol	1290	2.99	6.01	8.75	2.93
10	Carvacrol	1298	2.65	0.14	0.52	5.96
11	*α*-Terpinyl acetate	1345	--	--	--	0.1
12	*α*-Cubebene	1348	--	--	--	0.12
13	Thymol acetate	1350	1.69	1.91	0.21	0.24
14	*α*-Copaene	1374	6.85	2.64	0.23	5.04
15	*α*-Ylangene	1376	0.80	--	0.93	--
16	*β*-Bourbonene	1380	1.75	--	--	0.37
17	*β*-Elemene	1390	--	--	--	0.72
18	*α*-Gurjunene	1414	--	1.05	2.68	0.63
19	Isocaryophyllene	1411	--	--	0.97	--
20	*β*-Caryophyllene	1419	12.65	9.79	2.75	5.36
21	*β*-Copaene	1431	--	--	--	0.2
22	*trans*-*α*-Bergamotene	1434	1.65	0.75	--	--
23	γ-Elemene	1436	--	--	0.51	--
24	Aromadendrene	1445	0.29	--	--	--
25	*α*-Humulene	1454	8.01	8.21	--	6.25
26	Germacrene D	1469	--	--	--	3.90
27	γ-Muurolene	1478	--	--	--	0.12
28	*α*-Amorphene	1480	3.00	4.78	4.15	--
29	*β*-Selinene	1492	--	0.90	--	0.39
30	*epi*-Cubebol	1497	--	--	--	0.44

31	Valencene	1499	--	--	0.48	--
32	*α*-Muurolene	1501	--	2.87	--	2.24
33	*α*-Cuprenene	1505	1.32	--	--	0.29
34	γ-Cadinene	1510	--	--	--	0.55
35	*trans*-Cycloisolongifol-5-ol	1515	--	5.31	8.03	--
36	*δ*-Cadinene	1524	9.77	18.66	1.13	12.52
37	α-Cadinene	1542	--	--	--	0.23
38	*trans*-Cadinene ether	1557	--	--	3.93	--
39	*α*-Calacorene	1544	0.40	0.24	--	0.16
40	γ- Gurjunene epoxide	1545	--	0.28	5.20	1.24
41	*β*-Calacorene	1559	--	0.08	--	0.24
42	1(5),3-Aromadenedradiene	1560	3.18	1.61	7.1	--
43	Ledol	1561	--	0.14	--	0.15
44	Germacrene D-4-ol	1576	--	--	5.68	--
45	Himachalene epoxide	1577	8.59	4.85	7.9	1.58
46	Isospathulenol	1578	3.03	1.98	--	2.03
47	Spathulenol	1580	--	5.71	--	4.94
48	Caryophyllene oxide	1581	0.79	0.48	0.19	--
49	Cedrenol acetate	1583	--	0.31	--	0.32
50	Viridiflorol	1586	0.18	0.30	0.05	0.21
51	Salvia-4-(14)-en-1-one	1587	0.32	--	--	0.43
52	Humulene epoxide II	1589	1.51	0.09	--	2.13
53	*β*-Copaene-4-*α*-ol	1590	0.85	0.24	--	0.13
54	1,10-di-*epi* Cubenol	1621	0.30	0.27	1.05	1.27
55	*epi*-*α*-Cadinol	1645	0.79	0.80	--	--
56	epoxy-*allo*-Alloaromadendrene	1647	--	--	--	0.75
57	*epi*-*α*-Muurolol	1648	0.87	4.44	1.21	7.29
58	*α*-Cadinol	1652	--	5.67	1.96	12.48
59	*cis*-*α*-Copaene-8-ol	1659	0.67	--	0.98	--
60	9-*epi*-*E*-Caryophyllene-14-hydroxy	1668	0.41	--	1.39	--
61	Eudesm-4(15),7-diene-1- *β*-1-ol	1687	9.04	0.56	--	0.27
62	Shyobunol	1694	1.46	--	0.17	--
63	10-nor-Calamenene-10-one	1700	0.56	0.25	2.32	0.74
64	Oplopanone	1742	--	--	0.44	0.13
65	*α*-Muurolene-14-hydroxy	1785	1.17	1.14	2.29	0.34
66	Khusinol acetate	1820	0.42	0.17	--	0.09
67	18,20-Epoxypregn-5-en-3-*β*-yl acetate	1854	0.19	0.18	--	--
68	Cubitene	1876	0.51	--	--	--
69	Cembrene	1936	--	1.70	--	0.18
70	Phytol	1940	0.95	0.17	1.85	--
71	3*E*-Cembrene A	1972	0.42	0.21	1.7	0.08
72	Eicosane	2000	0.15	--	--	--
73	Kaurene	2044	--	--	0.47	--
74	Pentacosane	2500	0.30	--	0.31	--

KI: Kovats index -: not detected

Table 3: Identified components in the essential oils of the stems of *P. amboinicus* at different seasons

Peak no.	Identified compounds	KI	Area %			
			Winter	Spring	Summer	Autumn
1	*α*-Terpinene	1017	--	--	--	0.08
2	*p*-Cymene	1023	--	--	--	0.20
3	γ-Terpinene	1062	--	--	--	0.16
4	Fenchone	1091	--	--	0.44	--
5	Linalool	1098	--	--	0.11	--
6	Terpinene-4-ol	1178	--	--	0.03	0.86
7	Thymol	1290	3.99	7.10	7.58	13.02
8	Carvacrol	1298	3.19	3.96	1.51	5.39
9	Thymol acetate	1350	3.49	2.66	1.68	1.03
10	*α*-Copaene	1374	3.29	2.26	3.38	2.56
11	*β*-Elemene	1390	--	--	--	0.44
12	*α*-Gurjunene	1414	--	--	--	0.14
13	*β*-Caryophyllene	1419	3.01	6.48	12.57	12.67
14	*β*-Gurjunene	1433	0.62	1.42	1.25	0.14
15	*α*-Muurolene	1434	1.94	2.97	1.25	6.27
16	*trans*- *α*-Bergamotene	1434	--	--	--	1.06
17	γ-Elemene	1436	--	--	0.15	--
18	Aromadendrene	1445	0.61	--	1.44	--
19	*α*-Humulene	1454	11.14	8.27	12.70	5.26
20	Germacrene D	1469	--	1.21	--	0.15
21	*α*-Amorphene	1480	--	2.89	4.88	0.95
22	*β*-Selinine	1492	8.89	0.70	0.37	2.55
23	*trans*-Murrola-4(14),5-diene	1494	--	0.19	--	6.46
24	Cadina-1,4-diene	1497	--	--	--	0.54
25	Ledene	1498	0.2	0.19	0.06	1.03
26	*α*-Muurolene	1501	1.94	2.97	--	--
27	*α*-Cuprenene	1505	--	--	1.57	0.30

28	*δ*-Cadinene	1524	5.58	8.21	7.35	9.51
29	*α*-Calacorene	1544	0.14	0.15	0.19	--
30	*trans*-Cadinene ether	1557	0.22	1.03	--	--
31	Ledol	1560	0.45	0.33	--	0.19
32	1(5),3-Aromadendradiene	1560	2.79	--	--	0.89
33	Germacrene D-4-ol	1576	0.97	2.98	0.87	--
34	Himachalene epoxide	1577	6.55	6.94	4.42	0.81
35	Isospathulenol	1578	0.27	--	1.50	--
36	Spathulenol	1580	0.66	0.5	0.42	0.62
37	Caryophyllene oxide	1581	--	0.70	--	1.09
38	Cedrenol acetate	1583	0.16	0.22	0.45	--
39	Viridiflorol	1586	--	--	0.49	0.21
40	Humulene epoxide II	1589	0.50	--	2.41	--
41	*β*-Copaene-4- *α*-ol	1590	6.79	9.37	8.72	4.96
42	1,10-di-*epi*-Cubenol	1621	1.53	1.40	--	1,95
43	1-*epi*-Cubenol	1633	0.41	--	--	0.42
44	*epi*-*α*-Cadinol	1645	6.32	5.53	1.89	5.82
45	*α*-Cadinol	1652	6.23	7.43	5.41	9.01
46	14-hydroxy-9-*epi* E-Caryophyllene	1657	3.01	2.07	--	--
47	*cis*-*α*-Copaene-8-ol	1659	0.23	0.79	--	0.50
48	Eudesma-4(15),7-dien-1- *β*-ol	1687	0.26	0.45	1.37	0.21
49	10-nor-Calamenene-10-one	1700	0.41	0.57	0.69	0.24
50	Oplopanone	1742	--	--	0.32	--
51	14-hydroxy-*α*-Muurolene	1785	0.87	--	--	0.31
52	Khusinol acetate	1820	0.36	1.96	1.12	--
53	Cembrene	1936	0.24	0.53	0.52	0.17
54	Phytol	1940	0.88	0.98	0.68	--
55	3*E*-Cembrene A	1972	--	0.46	0.07	--
56	Kaurene	2044	0.22	0.15	0.16	--
57	Eicosane	2000	0.19	0.54	--	--
58	Docosane	2200	0.24	--	--	--
59	Tricosane	2300	1.10	0.11	--	--
60	Tetracosane	2400	0.55	--	--	--
61	Pentacosane	2500	0.96	--	0.12	--

Peak no.: Peak number KI: calculated Kovate index M+: Molecular weight --: not detected

Table 4: Calculated percentage of different classes of components of leaves essential oils at different seasons

Oil samples	Winter	Spring	Summer	Autumn
% of identified components	94.51	98.54	86.68	92.55
% of unidentified components	5.49	1.46	13.32	7.45
Monoterpene hydrocarbons	3.67	2.73	6.58	1.93
Sesquiterpene hydrocarbons	49.67	51.58	20.93	39.33
Total hydrocarbons	53.34	54.31	27.51	41.26
Oxygenated monoterpenes	7.35	8.17	10.78	12.23
Oxygenated sesquiterpenes	30.96	32.99	42.79	37.36
Total oxygenated compounds	38.31	41.16	53.57	49.59
Total monoterpenes	11.02	10.9	17.36	14.16
Total sesquiterpenes	80.63	84.57	63.72	76.69
Major constituent	*β*-caryophyllene (12.65%)	*δ*-cadinene (18.66%)	Thymol (8.75%)	*δ*-cadinene (12.52%)

Table 5: Calculated percentage of different classes of components of stems essential oils at different seasons

Oil constituents	Winter	Spring	Summer	Autumn
% of identified components	91.4	96.67	90.14	98.17
% of unidentified components	8.60	3.33	9.86	1.83
Monoterpene hydrocarbons	--	--	--	0.44
Sesquiterpene hydrocarbons	40.15	37.91	47.16	50.03
Total hydrocarbons	40.15	37.91	47.16	50.47
Oxygenated monoterpenes	10.67	13.72	11.35	20.30
Oxygenated sesquiterpenes	36.20	42.27	30.08	27.23
Total oxygenated compounds	46.87	55.99	41.43	47.53
Total monoterpenes	10.67	13.72	11.35	20.74
Total sesquiterpenes	76.35	80.18	77.24	77.26
Major constituent	*α*-Humulene (11.14%)	*β*-Copaene-4-*α*-ol (9.37%)	*α*-Humulene (12.70%)	Thymol (13.02%)

2.4. Biological study

The volatile oils of the leaves (collected in summer) and stems (collected in spring), containing the highest percent of oxygenated compounds (53.57% and 55.99%, respectively) were chosen to test the pharmacological and antimicrobial activities.

Experimental animals

Albino mice (25-30 gm body weight) were used for the toxicity

study and analgesic effect. Adult male albino rats of Sprague Dawely Strain weighing 130–150 gm were used for the determination of antioxidant, anti-inflammatory and diuretic activities. The rats were kept on standard laboratory diet under hygienic conditions. Water was supplied *ad libitum*.

Reference drugs and kits

Vitamin E: Pharco Pharmaceutical Co., Egypt.
Indomethacin: Epico, Egyptian Int. Pharmaceutical industries Co., Egypt.
Carrageenan: Sigma Co., USA.
Glutathione kits: Biodiagnostic, Egypt.
Moduretic drug (hydrochlorothiazide): Kahira Pharma and Chemical Ind. Co., Egypt.
Doxorubicin®: Sigma-Aldrich Co., US.
Discs of ceftriaxon and clotrimazole: 5 µg/disc, Oxoid Chemical Co., UK

Determination of median lethal dose (LD_{50})

The LD_{50} of the chosen volatile oils of the leaves and stems was estimated following Karber procedure[15].

Antioxidant activity

The antioxidant activity was estimated by determination of glutathione level in blood of alloxan- induced diabetic rats[16]. Rats were divided into five groups (six animals each), one group was kept as a negative control while for the other groups, diabetes mellitus was induced by intra-peritoneal injection of a single dose of alloxan (150 mg/kg b. wt.) followed by an overnight fasting[17]. A group of diabetic rats was kept non-treated, another group received the reference drug (Vitamin E, 7.5 mg/kg b. wt.) and the other two groups received the volatile oils of the leaves and stems of *P. amboinicus* (0.01 ml). The rats were kept one week before the determination of glutathione in blood. The results obtained are recorded in table (6).

Ant-inflammatory activity

It was carried out according to the rat paw oedema method[18, 19]. Four groups of rats, each group consists of six animals, were used. The first group received 1 ml saline orally (negative control). The second group was given indomethacin; orally in a dose of 20 mg/kg b. wt. (positive control). The other two groups received the essential oils of the leaves and stems of the plant at a dose of 0.01 ml. One hour later, oedema was induced by a sub planter injection of 0.1 ml carrageenan (1% solution in saline) in the right hind paw and 0.1 ml saline in the left hind paw. Three hours after the induction of inflammation, the rats were sacrificed; both paws were excised and weighed separately using an electric balance. The mean response and the percentage of inhibition were determined. Results obtained are recorded in table (7).

Analgesic activity

Mice were divided into four groups each of six animals. Animals were acclimatized to the laboratory conditions for at least one hour before testing and were used only during one experiment. First group of the animals received 1 ml saline and served as negative control. Second group served as positive control received indomethacin (20 mg/kg b. wt.), while the rest two groups received the volatile oils of the leaves and stems of the plant at a dose of 0.01 ml. The reference drug and the tested samples were administered orally 30 minutes prior to the administration of acetic acid injection (0.2 ml of 0.6% v/v, interperitoneal)[20].

Table 6: Antioxidant activity of the essential oils of *P. amboinicus*

Groups	Blood glutathione (mg %)	% of change	% of relative Potency**
Control (1 ml saline)	36.2±1.4	-	-
Diabetic non treated	21.2± 0.4*	41.44	-
Diabetic treated with volatile oil of leaves (0.01 ml)	35.1 ±1.1	3.04	98.04
Diabetic treated with volatile oil of stems (0.01 ml)	33.5 ±0.7	7.46	93.57
Diabetic treated with vitamin E (7.5 mg/kg b. wt.)	35.8 ±0.9	1.10	100.00

* Statistically significant from control at $P < 0.01$
** % of potency as compared to vitamin E.

Table 7: Acute anti-inflammatory effect of the essential oils of *P. amboinicus*

Groups	% oedema		% of relative potency**
	Mean ± S.E.	% of change	
Control (1 ml saline)	61.4± 1.7	---	---
Volatile oil of leaves (0.01ml)	25.3 ±0.6*	58.88	91.06
Volatile oil of stems (0.01 ml)	33.7±0.9*	45.11	69.76
Indomethacin (20 mg/kg b. wt.)	21.7 ±0.3*	64.66	100.00

* Statistically significant from control at $P < 0.01$
** % of potency as compared to indomethacin.

Table 8: Analgesic activity of the essential oils of *P. amboinicus*

Groups	Number of abdominal constrictions	% of inhibition	% of relative potency**
Control (1ml saline)	48.1 ± 1.3	-	-
Volatile oil of leaves (0.01 ml)	26.8 ± 0.4*	44.28	66.58
Volatile oil of stems (0.01 ml)	33.9 ± 0.7*	29.52	44.38
Indomethacin (20 mg/kg b.wt.)	16.1 ± 0.2*	66.51	100.00

* Statistically significant from control at $P < 0.01$
** % of potency as compared to indomethacin

Each mouse was then placed in an individual clear plastic observation chamber and the total number of writhes/30 minutes was counted for each mice and the percentage protection was calculated. The results are shown in table (8).

Diuretic activity

Twenty four rats were divided into four groups each of six animals. The animals were held into metabolic cages, fasted for 18 hours prior to experiment allowing only water during the fasting period. After completion of the fasting period, the first group received 1ml saline on the day of the experiment and kept as normal control. The last group received moduretic drug (5 mg/kg b. wt.) as positive control. The rest two groups received 0.01 ml of the volatile oils of leaves and stems. After treatment, the urine was collected in measuring cylinder and measured at 2, 4 and 24 hours after the dose was administered. The collected urine volume of the respective test groups was compared with the standard group. The sodium and potassium concentrations were measured[21]. Results are recorded in tables (9, 10).

Table 9: Diuretic activity of the essential oils of *P. amboinicus*

Groups	Volume of urine in ml		
	2 hrs	4 hrs	24 hrs
Control (1ml saline)	0.8 ± 0.01	1.8 ±0.1	7.2 ±0.3
Volatile oil of leaves (0.01) ml)	2.3 ± 0.1	3.5±0.3*	10.2±0.6*
Volatile oil of stems (0.01 ml)	1.7 ± 0.03	2.1±0.4	8.4±0.2*
Moduretic drug (5 mg/kg b. wt.)	3.9 ±0.6	6.4±1.2*	16.4±1.8*

* Statistically significant from control at P < 0.01

Table 10: The effect of the essential oils of *P. amboinicus* on serum electrolyte concentrations

Groups	Serum electrolytes concentration	
	K^+ mmol/L	Na^+ mmol/L
Control (1ml saline)	4.3 ±0.2	169.2 ±3.1
Volatile oil of leaves (0.01 ml)	4.1 ±0.3	134.2 ±3.4*
Volatile oil of stems (0.01 ml)	3.9 ±0.4	148.3 ±3.6*
Moduretic drug (5 mg/kg b.	5.4 ±0.5	151.1±2.6

* Statistically significant from control group at P < 0.01

Table 11: Cytotoxic activity of the essential oils of *P. amboinicus*

Sample	HEPG2 IC_{50} µg/ml	MCF7 IC_{50} µg/ml
Doxorubicin ®	0.9	0.7
Volatile oil of leaves	7.85	4.65
Volatile oil of stems	4.70	8.76

Statistical analysis

The data obtained were statistically analyzed using Student's t- test [22].

Cytotoxic activity

The cytotoxic activity of the samples under investigation was tested against hepatocellular (HEPG2) and breast (MCF7) carcinoma cell lines, obtained from National Cancer Institute, Kasr El Ainy, Cairo, Egypt, using Sulphorhodamine B assay (SRB)[23]. The oil samples were dissolved separately in saline solution in a concentration of 100 µg/100 µl, tween 80 was used to help dissolution of insoluble materials. The obtained IC_{50} were compared with that of doxorubicin as reference drug (table 11).

Antimicrobial Screening

A series of bacterial and fungal strains available in stock culture of Microbiology Department, Faculty of Pharmacy, Al-Azhar university, were used for antibiotic sensitivity testing comprising: Gram positive bacteria [*Streptococcus mutans* (clinical isolates), *Lactobacillus acidophilus* (clinical isolates), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 6538) and methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 12692)], Gram negative bacteria [*Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 8739)], filamentous fungi [*Aspergillus flavus* (ATCC 15517) and *Aspergillus niger* (ATCC 16404)], and yeast [*Candida albicans* (ATCC 10231) and *Candida parapsilosi*s (ATCC 22019)].

The previously prepared essential oils were diluted 1/3 v/v in dimethyl sulphoxide (E-Merck), 30 µl of each (containing 10 µl of pure oil) were used in the test. Dimethyl sulphoxide (50 µl) was used as a negative control.

The agar diffusion method[24] was applied using trypticase soy agar (Difco) medium inoculated with the bacterial or fungal suspension of the test organisms. Discs 5 mm in diameter were impregnated with the oils or the control. Then the discs were placed onto the surface of the culture medium.

Table 12: Antibacterial activity of the essential oils of *P. amboinicus*

Microorganisms	Diameter of zone of inhibition (mm) Relative potency (%)		
	Leaves	Stems	Ceftriaxon
Streptococcus mutans	15 (214%)	21 (300%)	7 (100%)
Lactobacillus acidophilus	13 (162%)	12 (150%)	8 (100%)
Bacillus subtilis	22 (244%)	15 (166%)	9 (100%)
Staphylococcus aureus	10 (143%)	11 (157%)	7 (100%)
MRSA	11 (157%)	12 (171%)	7 (100%)
E. coli	8 (133%)	R	6 (100%)
Klebsiellapneumoniae	R	7 (100%)	7 (100%)
Pseudomonas aeruginosa	R	R	6 (100%)

R= resistant, *The results are the mean of 3 readings.

Table 13: Antifungal activity of the essential oils of *P. amboinicus*

Micro-organisms	Diameter of zone of inhibition (mm) Relative potency (%)		
	Leaves	Stems	Clotrimazole
Candida albicans	43 (215%)	18 (90%)	20 (100%)
Candida parapsilosis	38 (200%)	13 (68%)	19 (100%)
Aspergillus flavus	42 (221%)	13 (68%)	19 (100%)
Aspergillus niger	44 (259%)	10 (59%)	17 (100%)

R= resistant

*The results are the mean of 3 readings.

Discs of ceftriaxon and clotrimazole were used as standard antibacterial and antifungal agent, respectively.

The plates were incubated at 35-37 ºC for 24-48 hours in case of bacteria, 25 ºC for 48 hours in case of filamentous fungi, while yeasts were incubated at 30 ºC for 24-48 hours.

After incubation, the diameters of inhibition zones were recorded in millimeters and the results were compiled in tables (12 and 13).

The minimum inhibitory concentrations (MIC) of the tested samples against *Lactobacillus acidophilus* and *Streptococcus mutans* were also determined by microdilution method[25].

3. Results and discussion

Physical characters of the essential oils

The color, odor, yield, refractive index (20 °C) and the specific gravity (20 °C) of each of the hydrodistilled oil were separately examined. The essential oils prepared from the leaves of *P. amboinicus* were obtained as pale yellow to yellow oils with characteristic, strongly aromatic odor while that of the stems were obtained as colourless to pale yellow oils with aromatic odor. The percentage yield of the oil at different seasons vary greatly (table 1). Samples of leaves collected at January, April, July and October yielded 0.03%, 0.12%, 0.08% and 0.08% v/w (calculated on fresh weight basis), respectively. The minimal yield was obtained in winter and the maximal yield in spring. While, that of the stems gathered along the four seasons, (January, April, July and October) yielded 0.01%, 0.13%, 0.09%, 0.01% v/w (calculated on fresh weight basis), respectively. The minimal yield was obtained in autumn and winter, while the maximal yield in spring. During spring, the leaves and stems produced the highest oil yield (0.12% and 0.13% v/w fresh weight, respectively).There was no significant difference in the refractive index, specific gravity and solubility in ethanol (70%) of the different oils collected in different seasons.

GC/MS analysis of the essential oil

From table (2), the GC/MS analysis of the essential oils of the leaves, collected along the four seasons, showed a qualitative and quantitative variation in chemical composition. The total number of the identified constituents in the volatile oil samples of winter, spring, summer and autumn comprise 43, 43, 38 and 51 components, constituting 94.51%, 98.54%, 86.68% and 92.55%, respectively, of the total oil composition. δ-Cadinene was the major constituent in the oils of spring and autumn, representing 18.66% and 12.52%, respectively. While, β-caryophyllene (12.65%) was the major constituent in the oil of winter and thymol (8.75%) was the major constituent in the oil of summer.

Concerning the oils of the stems collected along winter, spring, summer and autumn, they comprises 42, 38, 38 and 39 identified components, constituting 91.40%, 96.67%, 90.14% and 98.17%, respectively, of the total oil composition. α-Humulene was the major component in winter and summer, constituting 11.14% and 12.70%, respectively, while β-copaene-4-α-ol (9.37%) and thymol (13.02%) were the major constituents in spring and autumn, respectively.

The GC/MS analysis of the essential oil samples of the leaves, in different seasons, revealed the presence of 16 common components with different percentage while those of the stems have 19 common components, with different percentages. Also, the essential oils of the leaves and stems, showed 10 common components with different percentages. The percentages of total hydrocarbons, monoterpene and sesquiterpene hydrocarbons as well as the percentages of total oxygenated compounds, oxygenated monoterpenes and sesquiterpenes were calculated and are compiled in tables 4 and 5. The total sesquiterpenes were of higher percentage than the total monoterpenes and the total oxygenated sesquiterpenes were of higher percentage than the total oxygenated monoterpenes in both the leaves and stems along the four seasons, where the leaves in summer showed the highest total oxygenated compounds (53.57%), the stems in spring showed the highest total oxygenated compounds (55.99%).

The present study is the first assessment of seasonal variation in the oils from leaves and stems of *P. amboinicus*, the observed chemo variation may be attributed to environmental and seasonal influence.

Determination of median lethal dose (LD_{50})

The LD_{50} of the volatile oils of both the leaves and stems were up to 0.05 ml/kg. From this result it could be concluded that the oils are non-toxic and safe according to Buck[26].

Antioxidant activity

The reduced level of blood glutathione in diabetic rats was greatly restored by the volatile oils relative to vitamin E, so they could be considered as powerful antioxidant. The volatile oil of the leaves was found to possess higher antioxidant power (98.04%) than that of the stem (93.57%) as compared to vitamin E (100%).

Anti-inflammatory activity

The volatile oil of the leaves posses powerful anti-inflammatory activity with potency 91.06%, while that of the stem posses moderate activity with potency 69.76% compared to indomethacin (100%).

Analgesic activity

The oils of both the leaves and stems exhibited analgesia at the tested dose. The oil of the leaves exhibited higher analgesic activity (66.58%) than that of the stem (44.38%) compared to indomethacin (100%).

Diuretic activity

The volatile oils of both the leaves and stems showed significant increase in urine volume, as compared to the moduretic drug, after 24 hours.

Concerning the serum electrolyte level, the two oils showed significant decrease in serum sodium level as compared to the moduretic drug.

Cytotoxic activity

The essential oils of both the leaves and stems showed weak cytotoxic activity comparing to doxorubicin on liver and breast

carcinoma cell lines.

Antimicrobial activity

The two oil samples, exhibited powerful antibacterial activity against all the tested Gram positive bacteria comparing to ceftriaxon. The minimum inhibitory concentration of the essential oils of the leaves and stems against *Lactobacillus acidophilus* (50 µl/ml) and *Streptococcus mutans* (6.25 and 12.25 µl/ml, respectively) indicates a pronounced activity of the two oil samples against these oral pathogens, this support their incorporation in toothpaste or mouth wash preparations[10]. Concerning Gram negative bacteria, the oil of the leaves showed antibacterial activity against *E. coli*, while the oil of the stems showed activity against *Klebsiella pneumoniae*.

The oil of the leaves showed high antifungal activity against all the tested fungi and yeast compared to clotrimazole as standard.

4. Conclusion

The observed pharmacological activities could be attributed to the individual or combined action of bioactive constituents present in the oil. Sesquiterpenes which represent the major class of components in all the analyzed oil samples were reported to possess certain biological activities viz., *α*-humulene exhibited anti-inflammatory[27] and cytotoxic activities[28] and *β*-caryophyllene showed a pronounced synergistic effect for cytotoxic drugs[29]. The antioxidant activity is mainly due to the phenolic constituents of the oil samples as thymol[30].

Acknowledgements

The authors thank Dr. Amany Abdallah El-sharif, Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt, for performing the antimicrobial study.

References

1. Lukhoba CW, Simmonds MSJ, Paton AJ. Plectranthus: A review of ethnobotanical uses. J. of Ethnopharmacology 2006; 103:1-24.
2. Hafez SS. Essential oil of *Coleus aromaticus* Benth. Zag. J Pharm Sci 1994; 3(3):93-96.
3. Pino J, Gracia J, Martinez M. Comparative chemical composition of the volatiles of *Coleus amboinicus* produced by steam distillation, solvent extraction and supercritical carbon dioxide extraction. J Essent Oil Res 1996; 8:373-375.
4. Valera D, Rivas R, Avila JL, Aubert L, Amelot MA, Usubillaga A. The essential oil of *Coleus amboinicus* Lour., chemical composition and evaluation of insect antifeedant effects. Ciencia 2003; 11(2):113-118.
5. Mallavarapu GR, Laxmi R, Srinivasaiyer R. Essential oil of *Coleus aromaticus* Benth. from India. J Essent Oil Res 1999; 11(6):742-744.
6. Vera R, Mondon J, Pieribattesti J. Chemical composition of essential oil and aqueous extract of *Plectranthus amboinicus*. Planta Med 1993; 59(2):182-183.
7. Baslas R, Kumar P. Chemical examination of essential oil of *Coleus aromaticus* Benth. Journal of Indian Chemical Society 1981; 58:103-104.
8. Koba K, Grade D, Raynaud C, Caumont J. Chemical composition and antimicrobial properties of the leaf essential oil of *Coleus aromaticus* Benth. from Cambodia. International Journal of Essential Oil Therapies 2007; 1:16-20.
9. Prudent D, Perineau F, Bessiere J, Michel G, Baccou J. Analysis of the essential oil of wild oregano from Martinique(*Coleus aromaticus* Benth.), evaluation of its bacteriostatic and fungistatic properties. J Essent Oil Res 1995; 7:165-173.
10. Koba K, Nenonene AY, Sanda K, Grade D, Millet J, Caumont JP *et al*. Antibacterial Activities of *Coleus aromaticus* Benth. (Lamiaceae) essential oil against oral pathogens. J Essent Oil Res 2011; 23:13-17.
11. Murthy PS, Ramalakshmi K, Srinivas P. Fungitoxic activity of Indian borage (*Plectranthus amboinicus*) volatiles. Food Chemistry 2009; 114:1014-1018.
12. Nirmala DK, Periyanayagam K, Ismail M. *In vitro* antileptospiral activity of *Plectranthus amboinicus* (Lour.) Spreng. Pharmacology online 2008; 2:95-98.
13. Egyptian Pharmacopoeia. English Text, Edn 3, University Press, Cairo, 1984.
14. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publ Carol Stream, Illinois, 2001.
15. Karber G. Beitrag zur kollektiven Behandlung pharmakologischer Reihen versuche. Arch Exp Path Pharmacol 1931; 162:480-483.
16. Beutler E, Duron O, Kelly B. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61:882-888.
17. Eliasson SG, Samet GM. Alloxan induced neuropathies: lipid changes in nerve and root fragment. Life Sciences 1969; 81(1):493-498.
18. Winter GA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti inflammatory drugs. Proc Soc Exp Biol Med 1962; 111:544-547.
19. Vogel HG. Drug Discovery and Evaluation. Springer- Verlag, Berlin, Heidelberg, Germany, 2002.
20. Koster R, Anderson M, De-Beer E. Acetic acid for analgesic screening. Fed Proc 1959; 18:412.
21. Goldstein S, Brown MS. Methods for Urine Analysis. Edn 5, M. C. Graw-Hill, New York, 1964.
22. Snedecor WG, Cochhran GW. Statistical Methods. Iowa State, University Press, Ames Iowa, 1971.
23. Skehan P, Storeng R, Scudiero D, Monks A, Mc Mahom JM, Vistica D. New colorimetric cytotoxicity assay for anticancer-drug Screening. J Nat Cancer Inst 1990; 82(13):1107-1112.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1966; 45:493-496.
25. Hammer KA, Carson CF, Riley TV. *In vitro* activity of *Melaleuca alternifolia* (tea tree) oil against dermatophytes and other filamentous fungi. J Antimicrobial Chemother 2002; 50:195-199.
26. Buck WB, Osweiter GD, Gelder VAG. Clinical and Diagnostic Veterinary Toxicology. Edn 2, Kendall/ Hund Publishing Company, Iowa, 1976.
27. Fernandes ES, Passos GF, Medeiros R, Cunha DFM, Ferreira J, Campos MM. Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. Eur J Pharmacol 2007; 569:228-236.
28. Kim JY, Oh TH, Kim BJ, Kim SS, Lee NH, Hyun CG. Chemical composition and anti-inflammatory effects of essential oil from *Farfugium japonicum* flower. J Oleo Sci 2008; 57:623-628.
29. Legault J, Pichette A. Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol 2007; 59(12):1643-1647.
30. Chizzola R, Michitsch H, Franz C. Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes. J Agric Food Chem 2008; 56:6897–6904.