Polyphenolics content and biological activity of *Plectranthus amboinicus* (Lour.) spreng growing in Egypt (Lamiaceae)

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ABSTRACT

Background: Volatile oil, terpenoids, mainly diterpene and polyphenolic compounds including flavonoids and phenolic acids were previously isolated from different *Plectranthus* species. *Plectranthus amboinicus* (Lour.) Spreng growing abroad was subjected to phytochemical study resulted in isolation of several flavonoids, also the plant exhibited antioxidant, diuretic, anti-inflammatory, cytotoxic and antimicrobial activities. **Materials and Methods:** In this study ethyl acetate fraction of *Plectranthus amboinicus* (Lour.) Spreng leaves growing in Egypt was fractionated and chromatographed on silica gel and sephadex to isolate its phenolic constituents. The isolated compounds were identified using UV, ^{1}HNMR and ^{13}CNMR. Total phenolics and tannins content of the leaves, stems and roots of *Plectranthus amboinicus* (Lour.) Spreng were determined using Folin-Ciocalteau and Folin-Denis reagents, respectively. Phenolic compounds of the stems and roots were identified using UPLC-MS analysis. Leaves, stems and roots of this plant were tested for antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic and antimicrobial activities. **Results:** The isolated compounds were identified as 3-methoxy genkwanin, crisimaritin, *p*-coumaric acid, caffeic acid, taxifolin, rosmarinic acid, apigenin and 5-*O*-methyl-luteolin. The stems showed the highest concentration of the total polyphenolics followed by the leaves then the roots (9.6, 8.4 and 5.4 mg/g of gallic acid equivalents, respectively), while the roots recorded the highest tannins content followed by the leaves then the stems (126, 90 and 81 μg/g of tannic acid equivalents, respectively). UPLC-MS analysis revealed the presence of caffeic acid, rosmarinic acid, coumaric acid and chrysoeriol in the stems and roots, while luteolin, quercitin and eriodyctiol were detected only in the stems. The different extracts of the three organs exhibited antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic and antimicrobial activities with variable potency.

Keywords: *Plectranthus amboinicus*, Lamiaceae, phenolic compounds, antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic, antimicrobial

INTRODUCTION

Plectranthus is one of the oil-rich genera belonging to family Lamiaceae.[1] Diterpenoids, usually highly modified abietanoids, are the major group of secondary metabolites in this species.[2] Flavonoids, phenolic acids and phenolic acid esters had been isolated from different *Plectranthus* species.[2–5] Several flavonoids had been isolated from *Plectranthus amboinicus* (Lour.) Spreng growing in South America[6] (synonyms: *Plectranthus aromaticus* Roxb., *Coleus aromaticus* Benth. and *Coleus amboinicus* Lour.).[1] This plant was reported to possess variable biological activities, mainly, antioxidant,[7,8] diuretic,[8,9] anti-inflammatory,[10] cytotoxic[10] and antimicrobial[11] activities. No reports were found on the plant growing in Egypt, so this study was performed to investigate the phenolic content and biological activities of the Egyptian plant. The study includes isolation and identification of the major compounds of the ethyl acetate fraction of the leaves, quantitative determination of the total polyphenolics and tannins content of the leaves, stems and roots and identification of phenolic constituents in the stems and roots using high resolution UPLC-MS analysis.

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The antioxidant, anti-inflammatory, analgesic, diuretic, cytotoxic and antimicrobial activities of the different extracts of the leaves, stems and roots were studied.

MATERIAL AND METHODS

General Experimental

Electro thermal 9100 was used for determination of melting point, UV spectra was determined on Beckman Du-7 and Schimadzu 265 spectrophotometers, ^{1}H-(300 MHz) and ^{13}C-(75 MHz) NMR spectra were recorded on Varian Mercury apparatus at 25°C using TMS as an internal standard and chemical shifts were given in δ values. TLC was performed on precoated silica gel plates 60 F 254 (E-Merck), using solvent systems S_1 [$CHCl_3$: MeOH (98:2)], S_2 [$CHCl_3$: MeOH (95:5)], S_3 [$CHCl_3$: MeOH: Formic acid (90:10:2 drops)], S_4 [$CHCl_3$: MeOH: Formic acid (85:15: 2 drops)], S_5 [$CHCl_3$:MeOH:Formic acid (80:20:2 drops)] and S_6 [$CHCl_3$: MeOH (90:10)]. The chromatograms were visualized under UV light (at λ_{max} 254 and 366 nm) before and after exposure to ammonia vapor, as well as spraying with *p*-anisaldehyde/sulphuric acid spray reagent.

Plant Material

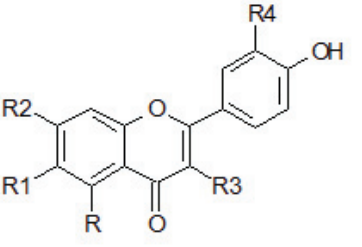
Plant material of *Plectranthus amboinicus* (Lour.) Spreng were collected all over the years (2008–2010) from El-Orman garden. The plant was kindly identified by Dr. Mohamed el Gebaly and Madam Treze (Taxonomist). A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Extraction and Isolation

Two kilograms of the air-dried and powdered leaves of *P. amboinicus* were percolated with 70% ethyl alcohol till exhaustion. The hydroalcoholic extract was evaporated under reduced pressure at a temperature not exceeding 60ºC to give 190 g (9.5%) dark green residue. The residue obtained was suspended in water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The solvent in each case was evaporated under reduced pressure to give *n*-hexane (29 g, 1.45%), chloroform (11.2 g, 0.56%), ethyl acetate (8.5 g, 0.43%) and *n*-butanol (7 g, 0.35%) fractions.

Similarly, the air-dried and powdered stems (1 kg) and roots (200 g) of *P. amboinicus* were extracted with 70% ethyl alcohol to yield 75 g (7.5%) and 25 g (12.5%), respectively, then fractionated to produce *n*-hexane (9 g, 0.9% and 6 g, 3%), chloroform (3 g, 0.3% and 2 g, 1%), ethyl acetate (2 g, 0.2% and 3 g, 1.5%) and *n*-butanol (5 g, 0.5% and 4 g, 2%) fractions of stems and roots, respectively.

Ethyl acetate fraction (7.5 g) of the leaves was fractionated on sephadex LH-20 using 100% methanol as eluent. Fractions of 3 ml each were collected. The obtained fractions were subjected to TLC, similar fractions were pooled and rechromatographed on sephadex LH-20 and/or silica gel 60, which afforded eight compounds (1–8).



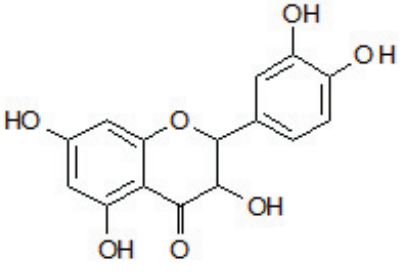
Compound	R	R1	R2	R3	R4
1	OH	H	OCH_3	OCH3	H
2	OH	OCH_3	OCH_3	H	H
7	OH	H	OH	H	H
8	OCH_3	H	OH	H	OH

Compound 1: 240 mg, yellow powder, soluble in chloroform, R_f = 0.56 in S_1, UV λ_{max} nm: MeOH (269–343), $NaOCH_3$ (270–396), $AlCl_3$ (276–387), $AlCl_3$/HCl (277–385), NaOAc (269–352), NaOAc/Boric acid (269–352), ^{1}HNMR (DMSO), δ ppm: 7.59 (2H, d, J = 7.2, H-2' & H-6'), 6.95 (2H, d, J = 8.7 Hz, H-3' & H-5'), 6.80 (1H, br.s, H-8), 6.37 (1H, br.s, H-6), 3.90 (3H, s, 7-OMe), 3.87 (3H, s, 3-OMe), ^{13}C NMR (DMSO), δ ppm: 181.89 (C-4), 165.07 (C-2), 161.10 (C-5), 148.01 (C-9), 120.76 (C-2'), 120.45 (C-6'), 103.31 (C-10), 92.66 (C-8), 55.98 (3 &7- OCH_3).

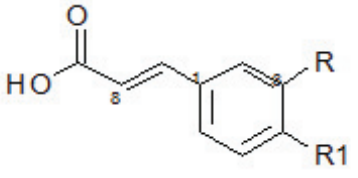
Compound 2: 50 mg, yellow powder, soluble in chloroform, R_f = 0.56 in S_2, UV λ_{max} nm: MeOH (277–335), $NaOCH_3$ (274–376), $AlCl_3$ (301–361), $AlCl_3$ / HCl (300–359), NaOAc (274–336), NaOAc / Boric acid (275–336), ^{1}HNMR (DMSO), δ ppm: 7.98 (2H, d, J= 8.7 Hz, H-2' & H-6'), 6.95 (2H, d, J=8.7 Hz, H-3'& H-5'), 6.93 (1H, s, H-8), 6.84 (1H, s, H-3), 3.93 (3H, s, 7-OCH_3), 3.73 (3H, s, 6-OCH_3), ^{13}C NMR (DMSO), δ ppm: 182.13 (C-4), 164.00 (C-2), 158.53 (C-5), 128.445 (C-2' & C-6'), 115.91 (C-3' & C-5'), 102.61 (C-3), 91.51 (C-8), 59.90 (6-OCH_3), 56.39 (7-OCH_3).

Compound 3: 24 mg, white crystals, soluble in methanol, m.p. 209–213 °C, R_f = 0.64 in S_4, ^{1}HNMR (CD_3OD), δ ppm: 7.57 (1H, d, J = 16.2 Hz, H-7), 7.41 (2H, d, J = 8.4 Hz, H-2, H-6), 6.79 (2H, d, J = 8.4 Hz, H-3, H-5), 6.24 (1H, d, J = 15.9 Hz, H-8), ^{13}C NMR (CD_3OD), δ ppm: 171.05 (C-9), 161.07 (C-4), 146.70 (C-7), 131.08 (C-2, C-6), 127.26 (C-1), 116.82 (C-3, C-5), 115.58 (C-8).

Compound 4: 90 mg, buff powder, soluble in methanol, R_f = 0.72 in S_3, ^{1}HNMR (DMSO), δ ppm: 7.37 (1H, d, J = 15.6Hz, H-8), 7.01 (1H, br. s, H-2), 6.93 (1H, d, J = 8.4 Hz, H-6), 6.74 (1H, d, J = 8.1 Hz, H-5), 6.13 (1H, d, J=16.2 Hz, H-7).^{13}C NMR (DMSO), δ ppm: 167.80 (C-9), 148.05 (C-4), 145.50 (C-3), 144.42 (C-7), 125.64 (C-1), 121.03 (C-6), 115.69 (C-5), 115.12 (C-8), 114.56 (C-2).

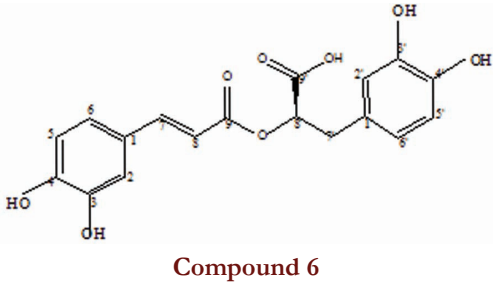


Compund 5



Compound	R	R1
3	H	OH
4	OH	OH

Compound 5: 52 mg, yellow powder, soluble in methanol, R_f = 0.51 in S_4, UV λ_{max} nm: MeOH (292–329), $NaOCH_3$ (247 sh., 328), $AlCl_3$ (280 sh., 312, 375), $AlCl_3$/HCl (312, 375), NaOAc (290 sh., 329), NaOAc/Boric acid (293, 336 sh), ^{1}HNMR (CD_3OD), δ ppm: 6.97 (1H, d, J=2.1 Hz, H-6'), 6.86 (1H, dd, J = 8.5 & 2.1 Hz, H-2'), 6.81 (1H, d, J = 8.4 Hz, H-3'), 5.92 (1H, d, J = 2.1 Hz, H-8), 5.88 (1H, d, J = 2.4 Hz, H-6), 4.93 (d, J = 11.4, H-3), 4.51 (d, J = 11.7 Hz, H-2), ^{13}C NMR (CD_3OD), δ ppm: 198.30 (C-4), 168.79 (C-5), 165.27 (C-7), 164.46 (C-9), 147.10 (C-4'), 146.27 (C-3'), 129.86 (C-1'), 120.89 (C-6'), 116.09 (C-2'), 115.88 (C-5'), 101.79 (C-10), 97.34 (C-6), 96.31 (C-8), 85.08 (C-2), 73.65 (C-3).



Compound 6

Compound 6: 50 mg, colorless amorphous solid, soluble in methanol, R_f = 0.41 in S_5, ^{1}HNMR (DMSO), δ ppm: 7.39 (1H, d, J = 15.9 Hz, H-7), 7.03 (1H, d, J=1.8 Hz, H-2), 6.96 (1H, dd, J=8.1 & 1.8 Hz, H-6), 6.74 (1H, d, J=8.1 Hz, H-5), 6.66 (1H, d, J=1.8 Hz, H-2'), 6.07 (1H, d, J= 8.1 Hz, H-5'), 6.51 (1H, dd, J=8.1 & 1.8 Hz, H-6'), 6.18 (1H, d, J=15.9 Hz, H-8), 4.94 (1H, dd, J=8.7 & 3.9 Hz, H-8'), 3.02 (1H, dd, J=15 & 3.9 Hz, H-7a'), 2.88 (1H, dd, J= 13.5 & 9.3 Hz, H-7b').

Compound 7: 170 mg, yellow powder, soluble in methanol, R_f = 0.57 in S_6, UV λ_{max} nm: MeOH (266, 338), $NaOCH_3$ (277, 323, 390), $AlCl_3$ (277, 386), $AlCl_3$/HCl (277, 385), NaOAc (272, 376), NaOAc / Boric acid (269, 338), ^{1}HNMR (DMSO), δ ppm: 7.87 (2H, d, J = 7.5 Hz, H-2' & H-6'), 6.89 (2H, d, J = 7.8 Hz, H-3' & H-5'), 6.70 (1H, br.s., H-8), 6.42 (1H, s, H-3), 6.13 (1H, br. s, H-6).

Compound 8: 80 mg, yellow powder, soluble in methanol, R_f = 0.45 in S_6, UV λ_{max} nm: MeOH (256, 268 sh, 349), $NaOCH_3$ (274, 405), $AlCl_3$ (274, 310 sh, 421), $AlCl_3$/HCl (270, 381), NaOAc (273, 376), NaOAc/Boric acid (264, 301, 373), ^{1}HNMR (CD_3OD), δ ppm: 7.37 (2H, m, H-2' & H-6'), 6.89 (1H, d, J=9.3 Hz, H-5'), 6.54 (1H, s, H-3), 6.43 (1H, d, J=2.1 Hz, H-8), 6.20 (1H, d, J=1.8 Hz, H- 6).

Quantitative Determination of the Total Polyphenolics Content

Total phenolics content of the leaves, stems and roots of *Plectranthus amboinicus* (Lour.) Spreng were determined by Folin-Ciocalteau reagent, using gallic acid as standard.[12]

Quantitative Determination of the Tannins Content

Tannins content of the leaves stems and roots of *Plectranthus amboinicus* (Lour.) Spreng was determined by Folin-Denis reagent using tannic acid as standard.[13]

High Resolution UPLC-MS Analysis

The ethyl acetate fraction of the stems and roots of *Plectranthus amboinicus* (Lour.) Spreng were subjected to LC/ESI-MS, to investigate the major fingerprint ions. Chromatographic separations were performed on an Acquity UPLC system (Waters) equipped with a HSS T3 column (100 × 1.0 mm, particle size 1.8 μm; Waters) applying the following binary gradient, at a flow rate of 150 μL min^{-1}: 0 to 1 min, isocratic 95% A (water/formic acid, 99.9/0.1 [v/v]), 5% B (acetonitrile/formic acid, 99.9/0.1 [v/v]); 1 to 16 min, linear from 5 to 95% B; 16 to 18 min, isocratic 95% B; 18 to 20 min, isocratic 5% B. The injection volume was 3.1 μL (full loop injection). Eluted compounds were detected from m/z 100 to 1000 using a MicrOTOF-Q hybrid quadrupole time-of-flight mass spectrometer (Bruker Daltonics) equipped with an Apollo II electrospray ion source in positive and negative

ion modes using the following instrument settings: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 litres min^{-1}, 190 °C; capillary, –5500 V (+4000 V); end plate offset, –500 V; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp; in-source CID energy, 0 V; hexapole RF, 100 Vpp; quadrupole ion energy, 5 eV; collision gas, argon; collision energy, 10 eV; collision RF 200/400 Vpp (timing 50/50); transfer time, 70 µs; prepulse storage, 5 µs; pulser frequency, 10 kHz; spectra rate, 3 Hz. Internal mass calibration of each analysis was performed by infusion of 20 µL 10 mM lithium formiate in isopropanol/water, 1/1 (v/v), at a gradient time of 18 min using a diverter valve. Identification of phenolic compounds was carried out by comparing retention times and mass spectra with those of authentic standards and/or based on accurate mass of pseudomolecular [M – H] or [M + H] ions. The major peaks observed have been tabulated in Table 1.

Plant Extracts for Biological Study

Alcoholic extracts of the leaves, stems and roots were prepared by macerating 100 g of each organ, separately, in 70% ethyl alcohol till exhaustion. The hydroalcoholic extract in each case was evaporated under reduced pressure to obtain a semisolid residue.

Aqueous extracts were prepared by boiling 100 g of the powdered leaves, stems and roots, separately, with bidistilled water. The aqueous extracts were dried by lyophilization.

Lyophilized juice was prepared by mixing about 500 g of fresh leaves with distilled water (least amount), with the help of mixer. The fresh juice was filtered and concentrated to a dry residue using lyophilizer.

The residues of different extracts were dissolved in tween 80 in selected doses based on their respective LD_{50} or dissolved in dimethyl sulphoxide at a concentration of 200 mg/ ml then 50 µl (containing 10 mg of each extract) were screened for the antimicrobial activity.

Experimental Animals

Albino mice 25–30 g body weight was used for the toxicity study and for analgesic effect.

Adult male albino rats of Sprague Dawely Strain weighing 130–150 g were used for the determination of antioxidant, anti-inflammatory and diuretic activities (according to the ethics of national research center). The rats were kept on standard laboratory diet under hygienic conditions. Water was supplied *ad lib.*

Cancer cell lines: Hepatocellular carcinoma cell line (HEPG2) and breast carcinoma cell line (MCF7), obtained from National Cancer Institute, Kasr El Ainy, Cairo, Egypt.

Micro-organisms: Gram positive bacteria [*Streptococcus mutans* (clinical isolates), *Lactobacillus acidophilus* (clinical isolates), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 6538) and methicillin resistance *staphylococcus aureus* (MRSA) (ATCC 12692)], Gram negative bacteria [*Klebsiella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 9027) and *E. coli* (ATCC 8739)], filamentous fungi [*Aspergillus flavus* (ATCC 15517) and *Aspergillus niger* (ATCC 16404)], and yeast [*Candida albicans* (ATCC 10231) and *Candida parapsilosis* (ATCC 22019)], available in stock culture of the Microbiology Department, Faculty of Pharmacy, Al-Azhar university, were used for antibiotic sensitivity testing.

Reference Drugs and Kits

Vitamin E: Pharco Pharmaceutical Co.

Indomethacin: Epico, Egyptian Int. Pharmaceutical industries Co., A.R.E. Carrageenan: Sigma Co.

Biodiagnostic glutathione kit.

Table 1. Identified Peaks in the Ethyl Acetate Fractions of Stems and Roots of *P. Amboinicus* by UPLC-MS.

	Polyphenols	Rt Min	M/Z	Ion	Formula	Error ppm	RDB	Organ
1	Caffeic acid	5.85	179.0342	[M–1]	$C_9H_7O_4$	4.4	6.5	S, R
2	Eriodyctiol	6.15	287.1491	[M–1]	$C_{14}H_{23}O_6$	3.5	3.5	S
3	Rosmarinic acid	6.22	359.0781	[M–1]	$C_{18}H_{15}O_8$	–2.5	11.5	S, R
4	Coumaric acid	6.27	163.0398	[M+1]	$C_9H_7O_3$	–5.2	6.5	S, R
5	Luteolin	6.52	285.0407	[M–1]	$C_{15}H_9O_6$	-8.5	11.5	S
6	Chrysoeriol	9.61	301.1406	[M+1]	$C_{18}H_{21}O_4$	9.3	8.5	S, R
7	Quercetin	9.48	303.1555	[M+1]	$C_{15}H_9O_7$	–	–	S

S: Stems, R: Root, -: not determined

Moduretic drug (hydrochlorothiazide): Kahira Pharma and Chemical Ind. Co.

Doxorubicin®: Sigma-Aldrich Co., US.

Discs of ceftriaxon and clotrimazole: 5 µg/disc, Oxoid Chemical Co., UK

Toxicity Study

The LD_{50} of the different extracts was estimated following Karber's procedure.[14]

Antioxidant Activity

The antioxidant activity was calculated by the determination of glutathione in blood of alloxan- induced diabetic rats adopting the method of Beutler *et. al.*,[15] using vitamin E as a positive control.

The animals were divided into 11 groups (6 animals each). One group was kept as a negative control while for the other groups, diabetes mellitus was induced according to the method described by Eliasson and Samet[16] in which a single dose of 150 mg alloxan / kg b.wt. was injected intraperitoneal in each animal followed by an overnight fasting.

A group of diabetic rats was kept non- treated, another group received daily the reference drug (Vitamin E) and the other groups received the tested samples daily in the given doses (see Table 2). Blood samples were taken after a week for the determination of glutathione. The results obtained were recorded in Table 2.

Anti-inflammatory Activity

It was carried out according to the rat paw oedema method.[17] Ten groups of male albino rats were used (6 animals each). The first group received 1 ml saline orally (negative control). The second group was given indomethacin orally (positive control). The other groups received the tested samples in the dose given in Table 3. One hour later, oedema was induced in the right hind paw by a sub planter injection of 0.1 ml of 1% carrageenan solution in saline while 0.1 ml saline was injected 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 (increase in the paw oedema) after acute inflammation and the percentage of inhibition were calculated. The results obtained were recorded in Table 3.

Analgesic Activity

Swiss male albino mice were divided into ten groups of six animals each. First group of the animals received 1 ml saline and served as control. Second group served as positive control received indomethacin, while the rest groups received the tested samples. All the samples were administered orally 30 minutes prior to the administration of acetic

Table 2. Antioxidant Activity of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Groups		Blood glutathione (mg %)	% change from control	% of relative Potency**
Diabetic	Control (1 ml saline)	36.2±1.4	–	–
treated with	Diabetic non treated	21.2± 0.4*	41.44	–
Leaves	Alcoholic 100 mg/kg b. wt	34.6 ±1.3	4.42	96.64
	Aqueous 100 mg/kg b. wt	34.1 ±1.1	5.80	95.25
Stems	Alcoholic 100 mg/kg b. wt	31.8 ±1.2	12.15	88.82
	Aqueous 100 mg/kg b. wt	30.9 ±0.8	14.64	86.31
Roots	Alcoholic 100 mg/kg b. wt	29.7± 0.8*	17.96	82.96
	Aqueous 100 mg/kg b. wt	29.2 ±0.7*	19.34	81.56
Lyophilized juice of leaves 50 mg/kg b. wt		34.1± 0.6	5.80	95.25
Ethyl acetate fraction of leaves 100 mg/kg b. wt		35.4 ±1.5	2.20	98.88
Diabetic treated with 7.5 mg/ kg b. wt vitamin E		35.8 ±0.9	1.10	100.00

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

Table 3. Acute Anti-inflammatory Effect of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Groups		Dose	% oedema		% of relative potency**
			Mean ± S.E.	% of change	
Control		1 ml saline	61.4± 1.7	–	–
Leaves	Alcoholic	100 mg/kg b.wt	28.3 ±0.4*	53.91	83.37
	Aqueous	100 mg/kg b.wt	32.1 ±1.1*	47.72	73.80
Stems	Alcoholic	100 mg/kg b.wt	36.9 ±1.4*	39.90	61.71
	Aqueous	100 mg/kg b.wt	39.4 ±1.8*	35.83	55.41
Roots	Alcoholic	100 mg/kg b.wt	43.8 ±2.1*	28.66	44.32
	Aqueous	100 mg/kg b.wt	47.8 ±1.6*	22.15	34.26
Lyophilized juice of leaves		50 mg/kg b.wt	37.2 ±1.3*	39.41	60.95
Ethyl acetate fraction of leaves		100 mg/kg b.wt	26.2 ±0.3*	57.33	88.66
Indomethacin		20 mg/kg b.wt	21.7 ±0.3*	64.66	100.00

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

acid injection (0.2 ml of 0.6% v/v, interperitoneal).[18] Each mice 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 for analgesic activity. The results are given in Table 4.

Diuretic effect

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 negative control. The last group received moduretic drug as a positive control. The other groups received the tested samples, in the doses given in Table 5.

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.[19] The results are shown in Table 6.

Table 4. Analgesic Activity of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Groups		Dose	Number of abdominal constrictions	% of inhibition	% of relative potency**
Control		1 ml saline	48.1 ± 1.3	–	–
Leaves	Alcoholic	100 mg/kg b.wt.	19.2 ± 0.4*	60.08	90.33
	Aqueous	100 mg/kg b.wt.	25.7 ± 0.6*	46.57	70.01
Stems	Alcoholic	100 mg/kg b.wt.	38.6 ± 0.9*	19.75	29.69
	Aqueous	100 mg/kg b.wt.	35.8 ±1.2*	25.57	38.44
Roots	Alcoholic	100 mg/kg b.wt.	31.4 ± 1.1*	34.72	52.20
	Aqueous	100 mg/kg b.wt.	34.1 ± 0.8*	29.11	43.77
Lyophilized juice		50 mg/kg b.wt.	28.7 ± 0.6*	40.33	60.64
Ethyl acetate fraction of leaves		100 mg/kg b.wt.	22.3 ± 0.3*	53.64	80.65
Indomethacin		20 mg/kg b.wt.	16.1 ± 0.2*	66.51	100.00

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

Table 5. Diuretic Effect of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Groups		Dose	Volume of urine in ml		
			2 hrs	4hrs	24hrs
Control		1 ml saline	0.8 ± 0.01	1.8 ± 0.1	7.2 ± 0.3
Leaves	Alcoholic	250 mg/kg b.wt.	2.6 ± 0.1	4.3 ± 0.1*	12.3 ± 0.9*
	Aqueous	250 mg/kg b.wt.	2.2 ± 0.02	2.6 ± 0.3	10.6 ± 0.8*
Stems	Alcoholic	250 mg/kg b.wt.	1.8 ± 0.01	3.1 ± 0.1	8.1 ± 0.4*
	Aqueous	250 mg/kg b.wt.	1.5 ± 0.01	2.3 ± 0.7	7.7 ± 0.2*
Roots	Alcoholic	250 mg/kg b.wt.	1.4 ± 0.01	1.9 ± 0.4	8.3 ± 0.3*
	Aqueous	250 mg/kg b.wt.	1.1 ± 0.03	1.9 ± 0.3	7.9 ± 0.3*
Lyophilized juice of leaves		250 mg/kg b.wt.	1.3 ± 0.02	2.9 ± 0.6*	9.7 ± 0.5*
Ethyl acetate fraction of leaves		250 mg/kg b.wt.	2.1 ± 0.1	3.8 ± 0.2*	11.7 ± 0.9*
Moduretic drug		5 mg/kg b.wt	3.9 ± 0.6	6.4 ± 1.2*	16.4 ± 1.8*

* Statistically significant from control group at $P < 0.01$

Table 6. The Effect of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots on Serum Concentrations of Sodium and Potassium.

Groups		Serum electrolytes concentration	
		K^+ mmol/L	Na^+ mmol/L
Control		4.3 ± 0.2	169.2 ± 3.1
Leaves	Alcoholic	4.1 ± 0.1	121.9 ± 2.8*
	Aqueous	4.5 ± 0.2	139.9 ± 3.7*
Stems	Alcoholic	3.9 ± 0.1	138.2 ± 3.6*
	Aqueous	3.7 ± 0.1	141.1 ± 4.1
Roots	Alcoholic	4.2 ± 0.1	143.7 ± 2.8
	Aqueous	3.8 ± 0.1	132.3 ± 4.7*
Lyophilized juice		3.8 ± 0.3	133.5 ± 2.8
Ethyl acetate extract		4.4 ± 0.2	156.7 ± 5.2
Moduretic drug		5.4 ± 0.5	151.1 ± 2.6

* Statistically significant from control group at $p < 0.01$

Statistical Analysis

The data obtained were statistically analyzed using the Student's t- test.[20] The data was presented as mean ± standard error.

In vitro Cytotoxic Activity against Human Cell Lines

The potential cytotoxicity agaist hepatocellular and breast carcinoma human cell lines was tested by Sulphorhodamine B assay (SRB)[21] using doxorubicin as standard and IC_{50} were determined (see Table 7).

Antimicrobial Activity

The agar diffusion method[22] was applied using trypticase Soy agar (Difco) medium inoculated with the bacterial or fungal suspension of the test organisms.

Table 7. Results of Cytotoxic Activity of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Sample		HEPG2 IC_{50} µg/ml	MCF7 IC_{50} µg/ml
Doxorubicin®		0.9	0.7
Leaves	Alcoholic	24.5	22.5
	Aqueous	25.8	18.2
Stems	Alcoholic	21.9	20.8
	Aqueous	29.1	17.5
Roots	Alcoholic	24.2	11.5
	Aqueous	23.6	15.9
Lyophilized juice of leaves		10.1	6.8

Discs (5 mm) were impregnated with the tested samples or dimethyl sulphoxide (50 µl) as a negative control and discs of ceftriaxon and clotrimazole were used as reference standards for the antibacterial and antifungal activities, respectively. Then, the discs were placed onto the surface of the culture medium. The plates were incubated at 35–37°C for 24–48 hours in case of bacteria and at 25°C for 48 hours in case of filamentous fungi, while yeasts were incubated at 30°C for 24–48 hours. After incubation, the diameters of inhibition zones were recorded in mm and the results were compiled in Tables 8 and 10. The minimum inhibitory concentrations (µg/ml) of the different samples against the oral pathogens *Lactobacillus acidophilus* and *Streptococcus mutans* were also determined by microdilution method,[23] Table 9.

RESULTS AND DISCUSSION

The isolated compounds (1–8) were identified, based on their physical and spectral data and by

Table 8. Antibacterial Activity of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Microorganisms	Inhibition zone (mm) (potency %)							
	Alcoholic extracts of			Aqueous extracts of			Lyophilized juice	Reference standard Ceftriaxon
	Leaves	Stems	Roots	Leaves	Stems	Roots		
Streptococcus mutans	9 (128%)	9 (128%)	20 (286%)	20 (286%)	23 (329%)	27 (386%)	25 (357%)	7 (100%)
Lactobacillus acidophilus	10 (125%)	8 (100%)	13 (163%)	11 (138%)	8 (100%)	7 (88%)	8 (100%)	8 (100%)
Bacillus subtilis	11 (122%)	7 (77%)	7 (77%)	R	R	R	12 (133%)	9 (100%)
Staphylococcus aureus	10 (142%)	9 (128%)	11 (157%)	13 (186%)	8 (114%)	9 (129%)	7 (100%)	7 (100%)
MRSA	13 (186%)	10 (143%)	12 (171%)	14 (200%)	8 (114%)	7 (100%)	7 (100%)	7 (100%)
E. coli	R	R	R	11 (183%)	8 (133%)	7 (116%)	R	6 (100%)
Klebsiella pneumonia	R	R	R	R	R	R	R	7 (100%)
Pseudomonas aeruginosa	R	R	R	R	R	R	R	6 (100%)

R = resistant

*The results are the mean of 3 readings.

Table 9. The Minimum Inhibitory Concentrations (MIC) of the Different Extracts of *P. Amboinicus* Leaves, Stems and Roots against *Lactobacillus Acidophilus* and *Streptococcus Mutans.*

Sample		MIC (µg/ml)	
		Lactobacillus acidophilus	Streptococcus mutans
Alcoholic	Leaves	12.5	3.85
	Stems	100	3.85
	Roots	100	3.85
Aqueous	Leaves	100	50
	Stems	50	6.25
	Roots	100	50
Lyophilized juice of the leaves		50	50

comparing these data with the published one, as 5,4'-dihydroxy-3,7-dimethoxy flavone (3-methoxy genkwanin),[24,25] 5,4'dihydroxy-6,7-dimethoxy flavone (crisimaritin),[24,25,26] *p*- coumaric acid (hydroxy cinnamic acid),[27] caffeic acid,[1] 3,5,7,3',4' pentahydroxy flavanone (taxifolin),[26] rosmarinic acid,[1] apigenin[26] and 5-*O*-methyl-luteolin.[24,25] These compounds were isolated for the first time from the leaves of *Plectranthus amboinicus* (Lour.) Spreng cultivated in Egypt, while, 3-methoxy genkwanin, *p*-coumaric acid and 5-*O*-methyl-luteolin were isolated for the first time from this plant.

Quantitative Determination of Total Polyphenolics Content

The stems showed the highest concentration of total polyphenolic compounds followed by the leaves then the roots (9.6, 8.4 and 5.4 mg/g, expressed in gallic acid equivalents, respectively).

Quantitative Determination of the Tannins Content

The concentration of tannins was found highest in the roots, followed by the leaves then the stems (126, 90 and 81 μg/g of tannic acid equivalents, respectively).

Identification of Phenolic Constituents in *Plectranthus Amboinicus* (Lour.) Spreng Stems and Roots using High Resolution UPLC-MS Analysis

The LC-MS analysis of the ethyl acetate fractions of the stems and roots of *Plectranthus amboinicus* (Lour.) Spreng revealed the presence of caffeic acid, rosmarinic acid and coumaric acid in the stems and roots which were isolated from the leaves in the present study. Chrysoeriol was also detected in the two organs under investigation, while luteolin, quercitin and eriodyctiol were detected only in the stems.

Determination of Median Lethal Dose (LD_{50})

The LD_{50} of the tested extracts was up to 5 g/kg b. wt. so, they could be considered safe according to Buck, *e.t al.*.[28] This may explain its extensive utilization in traditional medicine.

Antioxidant Activity

The reduced level of blood glutathione in diabetic rats was greatly restored by the different tested samples relative to vitamin E. The ethyl acetate fraction of the leaves showed the highest antioxidant power with potency 98.88% as compared to vitamin E. The alcoholic and aqueous extracts and the lyophilized juice of the leaves, also possess high antioxidant activity, with potency 96.64%, 95.25% and 95.25%, respectively.

Anti-inflammatory Activity

The ethyl acetate fraction and the alcoholic extract of the leaves showed the most potent anti-inflammatory activity with relative potency 88.66% and 83.37%, respectively, as compared to indomethacin. The other tested extracts showed anti-inflammatory activity with moderate potency.

Analgesic Activity

All the tested extracts exhibited analgesia at the tested doses. The alcoholic extract and the ethyl acetate fraction of the leaves

Table 10. Antifungal Activity of the different Extracts of *P. Amboinicus* Leaves, Stems and Roots.

Microorganisms	Inhibition zone (mm) (potency %)							
	Alcoholic extracts of			Aqueous extracts of			Lyophilized juice	Reference standard Clotrimazole
	Leaves	Stems	Roots	Leaves	Stems	Roots		
Candida albicans	R	R	R	15 (75%)	R	R	R	20 (100%)
Candida parapsilosis	R	R	R	16 (84%)	R	R	R	19 (100%)
Aspergillus flavus	R	R	R	R	R	R	R	19 (100%)
Aspergillus niger	R	R	R	R	R	R	R	17 (100%)

R = resistant

*The results are the mean of 3 readings.

showed the highest analgesic activity with potency 90.33% and 80.65%, respectively, as compared to indomethacin.

Diuretic Activity

The alcoholic extract, ethyl acetate fraction and aqueous extract of the leaves showed the highest increase in urine volume as compared to the moduretic drug after 24 hours. Concerning the serum electrolyte level most of the tested extracts showed significant decrease in serum sodium level as compared to the moduretic drug. Meanwhile, no significant effect was observed on K^+ level.

According to the results obtained, the ethyl acetate fraction of the leaves is the best choice as a diuretic with good electrolyte balance.

Cytotoxic Activity

All the tested samples showed high IC_{50} on hepatocellular and breast carcinoma cell lines compared to doxorubicin, so they could be considered as inactive as cytotoxic drugs.

Antibacterial Activity

The results of antibacterial screening revealed that all the tested extracts, exhibited powerful antibacterial activity against Gram positive bacteria, especially the oral pathogens *Streptococcus mutans and Lactobacillus acidophilus*. So, the minimum inhibitory concentration (MIC) of the different extracts against these two micro-organisms was determined. The alcoholic extract of the leaves recorded the lowest MIC against *Streptococcus mutans* and *Lactobacillus acidophilus* (12.5 and 3.85 μg/ml, respectively), this may suggests the incorporation of this extract in toothpaste or mouth wash preparations.

Concerning Gram negative bacteria most of the extracts showed no or moderate activity compared to ceftriaxon except the aqueous extracts of the leaves, stems and roots which showed significant activity against *E. coli*.

Antifungal Activity

Only the aqueous extract of the leaves showed moderate activity against *Candida albicans* (15 mm, 75%) and *Candida parapsilosis* (16 mm, 84%), compared to clotrimazole, while no activity was observed against the tested filamentous fungi. The authors thank Dr. Mohamed Farag, Assistant Professor, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, for performing the UPLC-MS analysis. Also, we are sincerely gratitude for 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. Journal of Ethnopharmacology. 2006; 103:1–24.
2. Adel-Mogib M, Albar HA, Batterjee SM. Chemistry of genus *Plectranthus*. Molecules. 2002; 7:271–301.
3. Kumaran A, Karunakaran RJ. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. Food-Chemistry. 2007; 100:356–61.
4. Grayer RJ, Eckert MR, Veitch NC, Kite GC, Marin PD, Kokubun T, Simmonds MS, Paton AJ. The chemotaxonomic significance of two bioactive caffeic acid esters, nepetoidins A and B, in the Lamiaceae. Phytochemistry. 2003; 64:519–28.
5. Juch M, Rüedi P. Isolation, structure, and biological activities of long chain catechols of *Plectranthus sylvestris* (Labiatae). Helvetica Chimica Acta. 1997; 80:436–48.
6. Brieskorn CH, Riedel W. Flavonoids from *Coleus amboinicus*. Planta Medica. 1977; 31:308–10.
7. Patel RD, Mahobia NK, Singh MP, Singh A, Sheikh NW, Alam G, Singh SK. Antioxidant potential of leaves of *Plectranthus amboinicus* (Lour.) Spreng. Der Pharmacia Lettre. 2010; 2:240–5.
8. Palani S, Raja S, Naresh R, kumar BS. Evaluation of nephroprotective, diuretic, and antioxidant activities of *Plectranthus amboinicus* on acetaminophen induced nephrotoxic rats. Toxicology Mechanisms and Methods. 2010; 20:213–21.
9. Patel RD, Mahobia NK, Gendle R, Kaushik B, Singh SK. Diuretic activity of leaves of *Plectranthus amboinicus* (Lour.) Spreng in male albino rats. Pharmacognosy Research. 2010, 2:86–8.
10. Gurgel AP, da Silva JG, Grangeiro AR, Oliveira DC, Lima CM, da Silva AC, Oliveira RA, Souzal A. *In vivo* study of the anti-inflammatory and antitumor activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng Lamiaceae. Journal of Ethnopharmacology. 2009; 125:361–3.
11. Pritima RA, Pandian RS. Antimicrobial activity of *Coleus aromaticus* (Benth.) against microbes of reproductive tract infections among women. African. J. Infect. Dis. 2008; 1:18–24.
12. Malick CP, Singh MB. Plant Enzymology and Histoenzymology (eds). Kalyani Publishers, New Delhi, 1980. Through: Rasineni GK, Siddavattam D, Reddy AR. Free radical quenching activity and polyphenols in three species of *Coleus*, J of Medicinal plants research. 2008; 2 (10):285–91.
13. Shanderl SH. Method in food analysis. Academic Press, New York, 1970. Through: Rasineni GK, Siddavattam D, Reddy AR. Free radical quenching activity and polyphenols in three species of *Coleus*. J of Medicinal plants research. 2008; 2 (10):285–91.
14. Karber G. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Naunyn- Schmiedeberg's Arch. Exp. Path. Pharmacol. 1931; 162:480.
15. Beutler E, Duron O, Kelly B. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963; 61:882–8.
16. Eliasson SG, Samet GM. Alloxan induced neuropathies: lipid changes in nerve and root fragments. Life Sciences. 1969; 81:493–8.

17. Winter GA, Risley EA, Nuss GW. Carrageenen-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. BioI. Med. 1962; 111:544–7.
18. Koster R, Anderson M, de Beer E. Acetic acid for analgesic screening. Fed. Proc. 1959; 18:412.
19. Goldstein S, Brown MS. Methods for urine analysis 5th Ed. M.C. Grawihill Book Co., New York, 1964.
20. Snedecor WG, Cochhran GW. Statistical Methods, 6th Ed. Iowa State, University Press. Ames, Iowa, 1971.
21. Skehan P, Storeng R, Scudiero D, Monks A, Mc Mahom JM, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer - drug Screening. J. Nat. Cancer Inst. 1990; 82:1107–12.
22. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1966; 45:493–6.
23. 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; 50:195–9.
24. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. Springer Verlag, New York, 1970.
25. Agrawal PK. Carbon-13 NMR of flavonoids, Elsevier Science Publishers B.V, 1989.
26. Brieskorn CH, Riedel W. Flavonoids from *Coleus amboinicus*. Planta Medica. 1977; 31:308–10.
27. Bergman M, Varshavsky L, Gottlieb HE, Grossman S. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. Phytochemistry. 2001; 58:143–52.
28. Buck WB, Osweiter GD, van Gelder AG. Clinical and Diagnostic Veterinary Toxicology, 2nd ed., 52011, Kendall/ Hund Publishing Company, Iowa, 1976.