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

Seham S El-hawary, Rabie H El-sofany, Azza R Abdel-Monem*, Rehab S Ashour and Amany A Sleem[1]

Department of Pharmacognosy, Faculty of pharmacy, Cairo University, Cairo 11562, Egypt
[1]Department of Pharmacology, National Research Centre, Giza 12622, Egypt

Submission Date: 26-4-2012; Review Completed: 30-5-2012; Accepted Date: 24-7-2012

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.

***Corresponding author.**

E-mail: azzaramy@yahoo.com

DOI: 10.5530/pj.2012.32.9

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

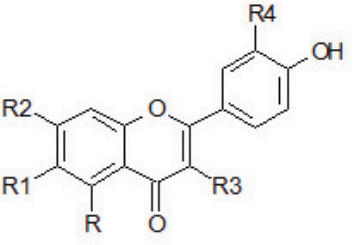
Plant material of *Plectranthus amboinicus* (Lour.) Spreng were collected all over the years (2008–2010) from El-Orman garden. The plant was kindly identified by Dr. Mohamed el Gebaly and Madam Treze (Taxonomist). A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of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