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Research Article

Antioxidant and Anti-Inflamatory Activities of Phenolic Constituents from *Primula elatior L.* Aerial Part

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ABSTRACT

Eight phenolic compounds were isolated from the EtOAc fraction of the aerial parts of *Primula elatior* L. (Primulaceae) cultivated in Egypt. Their structures were established as kaempferol (1), quercetin (2), 5-hydroxy pyrogallol (3), gallic acid methyl ester (4), gallic acid (5), 4`-methoxy kaempferol-3-*O*-β-glucuronopyranoside (6), kaempferol-3-*O*-β-glucuronopyranoside (7), and quercetin-3-*O*-β-glucuronopyranoside (8). Three of these compounds (6-8) have been isolated for the first time from the genus *Primula*. Their structures were confirmed by comparison of their chromatographic properties, chemical and spectroscopic data (UV, ^{1}H, and ^{13}C NMR) with those reported in the literature. The isolated flavonoids 1, 2, and 6-8 were found to exhibit significant antioxidant and anti-inflammatory activities. This is the first report about the antioxidant and anti-inflammatory activities of compounds 6-8.

Keywords*: Primula elatior L.*, Primulaceae, phenolic, Antioxidant, Anti-inflammatory.

INTRODUCTION

Primula elatior L. belongs to family Primulaceae which comprises about more than 400 species[1]. *Primula* species are used for catharses of the upper respiratory tract[2]. They are found in cough teas, liquid- and dry extracts, and syrups[3]. Rhizomes and flowers have expectorant, anticonvulsant, antimitotic, antibacterial, and relaxant activities[2,4] , while seeds exhibited anti-hypercholesterolemic activity[5]. In European folk medicine, *Primula* species are used for bronchitis, cough, migraine, and sleeping disorders[3]. Previous phytochemical studies of *Primula* species led to isolation of flavonoids[6-9], saponins[2,10], carotinoids[11], phenolic compounds[5,12], and essential oil[13-15]. In the present study, we report the isolation and structure elucidation of eight phenolic compounds from the EtOAc fraction of the aerial parts *P. elatior*. Flavonoids are known to exhibit strong antioxidant and anti-inflammatory activities[16-18], which prompted us to evaluate the total MeOH extract (TME) as well as the isolated compounds for their antioxidant and anti-inflammatory activities.

MATERIALS AND METHODS

General: Melting points were uncorrected and carried out on an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT TSQ 7000 mass spectrometer. UV spectra were recorded in MeOH on a Shimadzu 1601 UV/VIS spectrophotometer. ^{1}H and ^{13}C NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on a Bruker Avance DRX 400 using DMSO-d_6 as solvent. NMR spectra were referenced to the solvent signals (2.49 ppm for ^{1}H and 39.9 ppm for ^{13}C). Column chromatographic separation were performed on silica gel 60 (0.04-0.063 mm), RP-18 (0.04-0.063 mm Merck), and Sephadex LH-20 (0.25-0.1 mm, Merck).The solvent systems used for TLC analyses were $CHCl_3$: MeOH (90:10, S1), $CHCl_3$: MeOH (85:15, S2), and $CHCl_3$: MeOH (80:20, S3). All solvents were distilled prior to use. Spectral grade solvents were utilized for chromatographic analysis. TLC was performed on precoated TLC plates with silica gel 60 F_{254} (0.2 mm, Merck).

Plant Material: Fresh aerial parts of *P. elatior* were collected in May 2009 from cultivated plants at the campus of Faculty of Pharmacy, Al-Azhar University, Assiut branch. The plant material was identified and authenticated by Prof. Dr. A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University. A voucher specimen (PE-2009) was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut branch.

Extraction and Isolation: The air-dried aerial parts (1.75 kg) were crushed and macerated with MeOH (3 × 6 L, 72 h each) at room temperature. The combined extract was concentrated under reduced pressure to afford a dark green residue (52.5 g). The latter was suspended in distilled water (300 mL), and then partitioned between $CHCl_3$ (4 × 500 mL) and EtOAc (4 × 500 mL). Each fraction was concentrated under reduced pressure to give $CHCl_3$ (21.3 g), EtOAc (9.6 g), and aqueous (17.9 g) fractions. The EtOAc fraction (9.6 g) was subjected to VLC using $CHCl_3$:

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Table 1: The DPPH radical scavenging activity results.

Sample	Conc.	DPPH (% inhibition)
TME*	50	48.21
	100	68.14
	200	87.64
1**	50	72.89
2**	50	98.84
6**	50	59.88
7**	50	68.3
8**	50	84.95

Values are mean of 3 experimental

* *Conc. (µg/mL)*

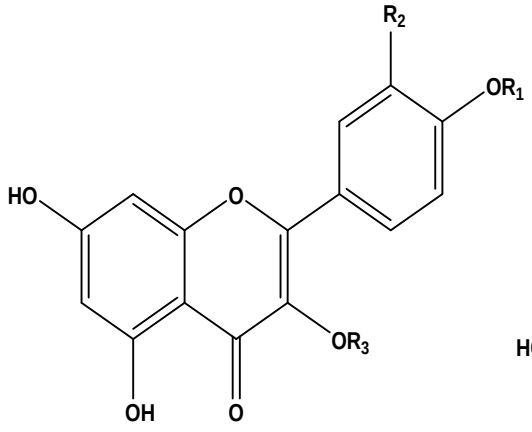
** *Conc. (µM)*

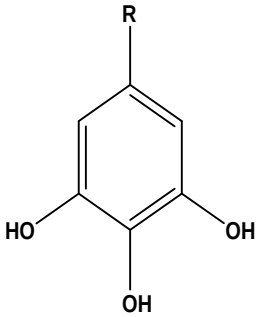
Table 2: The anti-inflammatory activity results.

Groups n = 6	Dose mg/kg	paw edema thickness (mm)				
		0 hr	1 hr	2 hr	4 hr	6 hr
Inflamed control		3.26±0.11	5.26±0.15	6.40±0.04	6.90±0.18	4.80±0.17
Inflamed + ASA	10	3.29±0.29	5.11±0.13 *	3.13±0.12 *	3.01±0.09 *	2.97±0.08 *
Inflamed + TME	50	4.11±0.13	4.01±0.21 *	3.86±0.13 *	3.30±0.15 *	3.12±0.19 *
	100	3.91±0.20	3.78±0.22 *	3.43±0.19 *	3.10±0.12 *	2.99±0.10 *
Inflamed + 1	10	4.16±0.18	3.97±0.11 *	3.42±0.09 *	3.13±0.08 *	2.96±0.10 *
Inflamed + 2	10	3.76±0.09	3.89±0.13 *	3.34±0.15 *	3.01±0.11 *	2.76±0.07 *
Inflamed + 6	10	3.68±0.09	3.76±0.09 *	3.21±0.11 *	3.10±0.04 *	2.99±0.07 *
Inflamed + 7	10	3.81±0.18	3.98±0.12 *	3.39±0.14 *	3.23±0.06 *	3.02±0.04 *
Inflamed + 8	10	3.38±0.15	4.18±0.12 *	3.62±0.18 *	3.09±0.09 *	2.85±0.09 *

Each value represents the mean ±S.E.M., n = 6

** Significant different from inflamed control group at P < 0.01.*





	R_1	R_2	R_3
1	H	H	H
2	H	OH	H
6	CH_3	H	Glucouronic acid
7	H	H	Glucouronic acid
8	H	OH	Glucouronic acid

	R
3	OH
4	$COOCH_3$
5	COOH

Fig. 1*. Chemical structures of the isolated compounds from Primula elatior*

MeOH gradient, to afford four fractions; PE-1 (2.6 g, $CHCl_3$: MeOH 75:25), PE-2 (1.3 g, $CHCl_3$: MeOH 50:50), PE-3 (1.7 g, $CHCl_3$: MeOH 25:75), and PE-4 (2.5 g, MeOH 100%). Fraction PE-1 (2.6 g) was subjected to silica gel column chromatography (0.04-0.063 mm; 150 g × 100 × 5 cm) using $CHCl_3$: MeOH gradient elution. Fractions of 100 mL were collected and monitored by TLC to obtain four subfractions PE-1-A to PE-1-D. Subfraction PE-1-D (395 mg) was subjected to different silica gel column (0.04-0.063 mm; 100 g × 50 × 3 cm) using $CHCl_3$: MeOH gradients to give 1 (34 mg, yellow crystals, $CHCl_3$: MeOH 92:8, subfraction PE-1-D). Fraction PE-2 (1.3 g) was chromatographed over Sephadex LH-20 column (0.25-0.1 mm; 100 g × 50 × 3 cm) using MeOH:$CHCl_3$ (90:10) as an eluent to obtain three subfractions PE-2-A to PE -2-C. Subfractions PE-2-A (270 mg), PE-2-B (511 mg),

and PE-2-C (410 mg) were subjected separately to different silica gel columns (0.04-0.063 mm; 100 g × 50 × 3 cm) using $CHCl_3$:MeOH gradients to get compounds; 2 (62 mg, yellow powder, $CHCl_3$:MeOH 90:10, subfraction PE-2-A), 3 (14 mg, brown residue, $CHCl_3$:MeOH 89:11, subfraction PE-2-B), 4 (9 mg, brown residue, $CHCl_3$:MeOH 85:15, subfraction PE-2-C), and 5 (11 mg, brown residue, $CHCl_3$:MeOH 85:14, subfraction PE-2-C). Silica gel column chromatography of fraction PE-3 (1.7 g) (0.04-0.063 mm; 200 g × 50 × 5 cm) using $CHCl_3$:MeOH gradients, 50 mL fractions were collected and monitored by TLC, to give two major subfractions PE-3-A (755 mg) and PE-3-B (422 mg). Subfraction PE-3-B was subjected to RP-18 column (0.04-0.063 mm; 100 g × 50 × 2 cm) using a MeOH: H_2O gradient to give 6 (69 mg, yellow residue, MeOH: H_2O 75:25). Fraction PE-4 (2.5 g) was subjected to silica gel column chromatography (0.04-0.063 mm; 25 g × 50 × 5 cm) using $CHCl_3$: MeOH gradients, 100 mL fractions were collected and monitored by TLC, to give impure 7 and 8, each one was purified on RP-18 column (0.04-0.063 mm; 100 g × 50 × 2 cm) using a MeOH: H_2O gradient to give 7 (41 mg, yellow residue) and 8 (32 mg, yellow residue).

Antioxidant activity: The antioxidant activity was determined as previously outlined[19,20] by the decrease in the absorption of each of the isolated compounds (50 μM) or TME (50, 100, and 200 μg/mL) in DPPH solution (4 mg was dissolved in HPLC MeOH 50 mL to obtain a concentration of 80 μg/mL) monitored at 517 nm using a spectrophotometer. The absorbance of DPPH in MeOH (with or without compounds) was measured after 2 min. The antioxidant activity of each compound was measured in relation to propyl gallate (as a reference antioxidant) set as 100 % antioxidant activity. Determinations were performed in triplicate. The antioxidant activity was calculated using the following equation:

Antioxidant activity:

$$100 \times \left(1 - \frac{\text{absorbance with compound}}{\text{absorbance of the blank}}\right)$$

Formalin-induced paw oedema in rats*:* Hind paw edema (skin edema) was induced by injection of 5% formalin (20 μL) solution into the subplanter region of the left hind paw[21]. Adult male albino rats 100-120 g were purchased from Animal House, Pharmacology Department, Faculty of Medicine, Assiut University. The inflamed animals were divided randomly into ten groups (6 for each);

inflamed control group, inflamed treated with acetyl salicylic acid (at a dose of 10 mg/kg subcutaneously), six groups of inflamed animals were treated with the tested compounds individually (at a dose of 10 mg/kg subcutaneously), and two groups were treated with TME at doses of 50 and 100 mg/kg subcutaneously (the plant extract was dissolved in sterile distilled water). The change in paw thickness in all tested animals was measured with Plethysmometer 7150 (UGO, Basil, Italy) at 0, 1, 2, 4, and 6 hours after formalin solution injection. The data were expressed as mean ± S.D. using the Student *t* test.

Chemicals: DPPH, (Aldrich Co., USA), propyl gallate, (Sigma Co., USA), 5% formalin (Sigma Co., USA), acetyl salicylic acid (Sigma Co., USA).

Animals: Adult male albino rats (120-150 g body weight) were used. All animal procedures were conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines and Institutional Ethical Committee Approval was obtained. The study protocol was approved by the Animal Ethical Committee of Assiut University. The animals were housed under standardized environmental conditions in the pre-clinical Animal House, Pharmacology Department, Faculty of Medicine, Assuit University. The animals were fed with standard diet and free access to water. They were kept at 24-28°C, 60-70% relative humidity, 12 hr day and night cycle for one week to acclimatize to the environmental conditions.

STATISTICAL ANALYSIS

All data were expressed as S.D. using the Student *t* test and the statistical significance was evaluated by one-way analysis of variance ANOVA. The values were considered to be significantly different when *P* values were less than 0.01.

RESULTS AND DISCUSSION

General: Two flavonoid aglycones (1 and 2), three phenolic compounds (3-5) and three flavonoid glucouronosides (6-8) were isolated from the EtOAc fraction of the aerial parts of *P. elatior* L. cultivated in Egypt (Fig. 1). Compounds 6-8 have been isolated for the first time from the genus *Primula*. Their structures were established by physical, chemical, and spectral data (UV, ^{1}H, and ^{13}C NMR), as well as comparison with authentic samples. The isolated flavonoids 1, 2, and 5-8 were found to exhibit significant antioxidant and anti-inflammatory activities. This is the first report about the antioxidant and anti-inflammatory activities of compounds 6-8.

Compound 1: It was obtained as yellow needles (EtOH), R_f = 0.64 (S1), m. p. 276-278 °C; UV λ_{max} (MeOH): 263 and 365 nm. ^{1}H NMR (DMSO-d_6) δ: 8.08 (2H, *J* = 8.4 Hz, H-2`, 6`), 6.93 (2H, d, *J* = 8.4 Hz, H-3`, 5`), 6.40 (1H, d, *J* = 1.6 Hz, H-8), 6.19 (1H, d, *J* = 1.6 Hz, H-6)[22].

Compound 2: It was obtained as yellow needles (EtOH), R_f = 0.59 (S1), m. p. 313-314 °C; UV λ_{max} (MeOH): 257 and 373 nm. ^{1}H NMR (DMSO-d_6) δ: 7.67 (1H, d, *J* = 2.2 Hz, H-2`), 7.54 (1H, dd, *J* = 8.4, 2.2 Hz H-6`), 6.90 (1H, d, *J* = 8.4 Hz, H-5`), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6).$^{13}$C NMR (DMSO-$d_6$) δ: 176.7 (C-4), 163.9 (C-7), 160.6 (C-5), 156.2 (C-9), 156.0 (C-2), 147.5 (C-4`), 144.9 (C-3`), 135.6 (C-3), 121.9 (C-1`), 119.9 (C-6`), 116.5 (C-5`), 116.0 (C-2`), 102.9 (C-10), 98.1 (C-6), 93.2 (C-8)[22].

Compound 3: It was obtained as brown residue, R_f = 0.86 (S2).^{1}H NMR (DMSO-d_6) δ: 7.29 (2H, s, H-2, 6). ^{13}C NMR (DMSO-d_6) δ: 154.3 (C-1), 143.3 (C-3, 5), 130.3 (C-4), 102.0 (C-2, 6)[23].

Compound 4: It was obtained as brown residue, R_f = 0.78 (S2), UV λ_{max} (MeOH): 263 and 365 nm.^{1}H NMR (DMSO-d_6) δ: 6.96 (2H, d, *J* = 1.8 Hz, H-2, 6), 3.25 (3H, s, OCH_3).

^{13}C NMR (DMSO-d_6) δ: 167.4 (C-7), 145.4 (C-3, 5), 137.9 (C-4), 120.5 (C-1), 108.8 (C-2, 6), 49.0 (OCH_3)[24].

Compound 5: It was obtained as brown residue, R_f = 0.71 (S2).^{1}H NMR (DMSO-d_6) δ: 6.94 (2H, d, *J* = 1.5 Hz, H-2, H-6). ^{13}C NMR (DMSO-d_6) δ: 167.3 (C-7), 145.4 (C-3, 5), 137.8 (C-4), 130.7 (C-1), 108.6 (C-2, 6)[25,26].

Compound 6: It was obtained as brown residue, R_f = 0.77 (S3), UV λ_{max} (MeOH): 255 and 358 nm.^{1}H NMR (DMSO-d_6) δ: 8.04 (2H, *J* = 8.8 Hz, H-2`, 6`), 6.87 (2H, d, *J* = 8.8 Hz, H-3`, 5`), 6.43 (1H, d, *J* =2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.41(1H, d, *J* = 7.0 Hz, H-1``), 3.58 (3H, s, $OCH_3$). $^{13}$C NMR (DMSO-$d_6$) δ: 177.1 (C-4), 170.1 (C-6``), 164.1 (C-7), 161.0 (C-5), 160.9 (C-4`), 156.9 (C-9), 156.2 (C-2), 133.0 (C-3), 130.7 (C-2`, 6`) 120.9 (C-1`), 114.9 (C-3`, 5`), 103.7 (C-10), 101.1 (C-1``), 98.6 (C-6), 93.5 (C-8), 75.7 (C-3``), 75.4 (C-5``), 73.8 (C-2``), 71.3 (C-4``), 55.7 (OCH_3)[22].

Compound 7: It was obtained as yellow residue, R_f = 0.71 (S3), UV λ_{max} (MeOH): 261 and 364 nm.^{1}H NMR (DMSO-d_6) δ: 8.04 (2H, *J* = 8.8 Hz, H-2`, 6`), 6.89 (2H, d, *J* = 8.8 Hz, H-3`, 5`), 6.43 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.0 Hz, H-6), 5.46 (1H, d, *J* = 7.7 Hz, H-1``). $^{13}$C NMR (DMSO-$d_6$) δ: 177.2 (C-4), 170.3 (C-6``), 164.3 (C-7), 161.1 (C-5), 160.0 (C-4`), 156.4 (C-9), 156.2 (C-2), 133.1 (C-3), 130.8 (C-2`, 6`) 120.6 (C-1`), 115.2 (C-3`, 5`), 103.7 (C-10), 101.3 (C-1``), 98.7 (C-6), 93.6 (C-8), 76.0 (C-3``), 75.5(C-5``), 73.7 (C-2``), 71.4 (C-4``)[22].

Compound 8: It was obtained as yellow residue, R_f = 0.66 (S3), UV λ_{max} (MeOH): 258 and 362 nm. ^{1}H NMR (DMSO-d_6) δ: 7.67 (1H, d, *J* = 2.2 Hz, H-2`), 7.43(1H, dd, *J* = 8.8, 2.2 Hz, H-6`), 6.80 (1H, d, *J* = 8.8 Hz, H-5`), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.27 (1H, d, *J* = 6.94 Hz, H-1``). $^{13}$C NMR (DMSO-$d_6$) δ: 177.5 (C-4), 171.0 (C-6``), 164.5 (C-7), 160.9 (C-5), 156.3 (C-9), 156.2 (C-2), 148.4 (C-4`), 145.5 (C-3`), 133.8 (C-3), 120.6 (C-1`), 117.9 (C-6`), 115.3 (C-5`), 115.0 (C-2`), 103.6 (C-10), 102.5 (C-1``), 98.6 (C-6), 93.6 (C-8), 76.4 (C-3``), 74.5 (C-5``), 74.0 (C-2``), 71.6 (C-4``)[22] .

BIOLOGICAL DISCUSSION

The total MeOH extract and isolated compounds showed a concentration dependent scavenging activity by quenching DPPH radicals (Table **1**), maximum inhibition (87.64 % for TME at 200 µg/mL and 98.84 for isolated compounds at 50 µM) of DPPH. This high antioxidant capacity may be due to the high concentration of phenolics which are common in *P. elatior*. Furthermore, the TME of *P. elatior* and compounds **1**, **2**, and **6-8** were tested for their anti-inflammatory effects using the induced paw edema test. All the tested compounds as well as TME exhibited potent anti-inflammatory effects. The TME exhibited anti-inflammatory activity more than aspirin at dose 100 mg/kg. Compound **2** showed higher activity more than aspirin in the same dose followed by **8** (Table **2**) after 6 hr. Thus, the free OH group at C-3`, 4` may be essential for the anti-inflammatory activity. As well as, **1** showed significant activity followed by **6** and **7**. These results are in accordance with previous studies that attributed the anti-inflammatory activity of flavonoids to the C-2, 3 double bond and the presence of a methoxy group at C-4`, and the pyran ring[27]. The activity of TME may be due to the presence of different classes of terpenes and flavonoids, especially, flavonoids are known to inhibit the enzyme prostaglandin synthesis, more specifically the endoperoxide and reported to produce anti-inflammatory activity (Table **2**)[28].

REFERENCES

1. Budzianowskia J, Morozowska M, Wesołowska M. Lipophilic flavones of *Primula veris* L. from field cultivation and in vitro cultures. *Phytochemistry* 2005; 66: 1033-1039.
2. Adamsa M, Berset C, Kessler M, Hamburger M. Medicinal herbs for the treatment of rheumatic disorders-A survey of European herbals from the 16th and 17th century. *J. Ethnopharmacol.* 2009; 121: 343-359.
3. Teuscher E, Melzig MF, Lindequist U. Biogene Arzneimittel. Ein Lehrbuch der Pharmazeutischen Biologie. Edn 6, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 2004.
4. BaşBülBül G, Özmen A, Biyik HH, Sęn Ö. Antimitotic and antibacterial effects of the *Primula veris* L. flower extracts. *Caryologia* 2008; 61: 88-91.
5. Fouad MA, Abdel-Hafeez AS. Chemical and biological studies of the methanolic extract of *Primula elatior* seeds. *Bull. Pharm. Sci. Assuit University* 2009; 32: 111-123.
6. Ahmad VU, Shah MG, Noorwala M, Mohammad FV. Isolation of 3,3'-Dihydroxychalcone from Primula macrophylla. *J. Nat. Prod.* 1992; 55: 956-958.
7. Wollenweber E, Mann K, Iinuma M, Tanaka T, Mizuno M. 5,2′,5′- trihydroxyflavone and 2′,β-dihydroxychalcone from *Primula pulverulenta. Phytochemistry* 1989; 28: 295-296.
8. Wollenweber E, Mann K, Iinuma M, Tanaka T, and Mizunod M8,2′- Dihydroxyflavone from *Primula pulverulenta*. *Phytochemistry* 1988; 27: 1483-1486.
9. Vitalini S, Flamini G, Valaguzza A, Rodondi G, Iriti M, and Fico G. *Primula spectabilis* Tratt. aerial parts: Morphology, volatile compounds and flavonoids. *Phytochemistry* 2011; 72: 1371-137.
10. Coran SA, Mulas S. Validated determination of primula saponins in primula root by a high-performance-thin-layer-chromatography densitometric approach. *Journal of Pharmaceutical and Biomedical Analysis* 2012; 70: 647-651.
11. Yamamizo C, Hirashima M, Kishimoto S, Ohmiya A. Carotenoid composition in the yellow and pale green petals of *Primula* species. *Bull. Natl. Inst. Flor. Sci.* 2011; 11: 67-72.
12. Müller A, Ganzera M, Stuppner H. Analysis of phenolic glycosides and saponins in *Primula elatior* and *Primula veris* (*primula* root) by liquid chromatography, evaporative light scattering detection and mass spectrometry. *Journal of Chromatography A* 2006; 1112: 218-223.
13. Lodhia MH, Bhatt KR, Thaker VS. Antibacterial activity of essential oils from palmarosa, evening

primrose, lavender and tuberose. *Indian Journal of Pharmaceutical Sciences* 2009; 71: 134-136.
14. Nan P, Peng S, Shi S, Rena H, Yang J, Zhong Y. Interpopulation congruence in Chinese *Primula ovalifolia* revealed by chemical and molecular markers using essential oils and ISSRs. *Z. Naturforsch*. 2003; 58 c: 57-61.
15. Na P, Peng S, Zhang Y, Zhong Y. Composition of volatile oil of *Primula obconica* in Central China. *Nat. Prod. Lett.* 2002; 16: 249-53.
16. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 1996; 20: 933-956.
17. Heim KE, Tagliaferro AR. Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*. 2002; 13: 572-584.
18. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radic. Biol. Med.* 1997; 22: 749-760.
19. Al-Musayeib NM. Mohamed GA, Ibrahim SRM, Ross SA. Lupeol-3-O-decanoate, a new triterpene ester from *Cadaba farinosa* Forssk. growing in Saudi Arabia. *Med. Chem. Res.* 2013; 22: 5297-5302.
20. El-Kumarasamy Y, Byres M, Cox PJ, Delazar A, Jaspars M, Nahar L, Shoeb M, Sarker SD. Isolation, structure elucidation, and biological activity of flavone 6-c-glycosides from *Alliaria petiolata*. *Chemistry of Natural Compounds* 2004; 40: 122-128.
21. Githinji CG, Paul MM, Titus IK, David KK. Analgesic and anti-inflammatory activities of 9-Hexacosene and Stigmasterol isolated from *Mondia whytei*. *Phytopharmacology* 2012; 2: 212-223.
22. Campeol E, Cioni PL, Flamini G, Rossi B, Cremonini R. Flavonoids analysis of four *Vicia* species of Narbonensis complex in two different vegetative phases. *Caryologia* 2003; 56: 365-371.
23. Gerothanassis IP, Exarchou V, Lagouri V, Troganis A, Tsimidou M, Boskou D. Methodology for identification of phenolic acids in complex phenolic mixtures by high-resolution two-dimensional nuclear magnetic resonance. Application to methanolic extracts of two *Oregano* Species. *J. Agric. Food Chem.* 1998; 46: 4185-4192.
24. Mehta BK, Sharma KMS,Dubey A. 4-Ethylgallic acid from two *Mimosa* species. *Phytochemistry* 1988; 27: 3004-3005.
25. Azizuddin, Makhmoor T, Choudhary MI. Radical scavenging potential of compounds isolated from *Vitex agnus-castus*. *Turk. J. Chem*. 2010; 34: 119-126.
26. Eldahshan OA. Isolation and structure elucidation of phenolic compounds of Carob Leaves grown in Egypt. *Current Research J. Biol. Sci*. 2011; 3: 52-55.
27. Ibrahim SRM, Mohamed GA, Al-Musayeib NM. New Constituents from the Rhizomes of Egyptian *Iris germanica* L. *Molecules* 2012; 17: 2587-2598.
28. Das S, Haldar PK, Pramanik G, Suresh RB. Evaluation of Anti-Inflammatory Activity of *Clerodendron infortunatum* Linn. Extract in Rats. *Global Journal of Pharmacology* 2010; 4: 48-50