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PHYTOCHEMICAL STUDIES ON EUPHORBIA HYPERICIFOLIA

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ABSTRACT

Ten compounds have been isolated from the roots of *Euphorbia hypericifolia* viz., β -sitosterol (EH-1), β-sitosterol-3-*O*-β-*D*-glucopyranoside (EH-2), ursolic acid(EH-3), oleanolic acid (EH-4), 16α-hydroxy-*ent*-kauran-19-oic acid (EH-5) , 16α, 17-dihydroxy-*ent*-kauran-19-oic acid (EH-6) , *ent*-kaur-15-en-19-oic acid (EH-7), *ent*-kaur-15-en-17-ol-19-oic acid(EH-8),rutin(EH-9)5'-methoxy-8-methyl-6-prenyl-5,7-dihydroxy- 3',4'-methylenedioxy-flavone (EH-10). Compounds EH-3 to EH-10 was isolated for the first time from this plant and the structures were elucidated by spectroscopic analysis.

KEYWORDS: *Euphorbia hypericifolia, Euphorbiaceae,* flavonoid

Introduction

As part of our investigations on the chemical constituents of medicinal plants this paper deals with the isolation and characterization of several compounds isolated from the roots of *Euphorbia hypericifolia*, some of them have been isolated for first time from this plant. *Euphorbia hypericifolia* (*Euphorbia indica* Lam., Syn., *Chamaesyce glomerifera*, *Euphorbia glomerifera*, Graceful Sandmat , is an annual herb with milky sap. Stems are hairless, erect, and often red. Oppositely arranged leaves are oblong-elliptic, 1 - 2.5 cm long, 4 - 8 mm wide, margin slightly toothed. The species name *hypericifolia* means, having leaves like Hypericum, that is, St. John's Wort. Flowers are minute, clustered into cup-like cyathia (A cythium is a flower-like object which is not the actual flower). Cyathial appendages are petal-like, 4, white to pink, each with a minute gland at the base. Capsules are smooth, generally widest below the middle. *Euphorbia hypericifolia* is an important medicinal plant used in our traditional system of medicine to treat various diseases. The whole plant is used in colic and colic, diarrhea and dysentery. The leaves are used as astringent, antidysentric, antileucorrhoeic in menorrhagia. Previous study showed that several photochemical have been isolated from this plant viz., taraxerol, ß-sitosterol, stigmasterol, campesterol and kaemferol, quercetin, quercetrin (quercetin-3-rhamnoside), rhamnetin-3-galactoside, rhamnetin-3-rhamnoside and ellagic acid [1-5].

Material and methods

General Methods

Melting points were determined on a Perfit apparatus and are uncorrected. Ultraviolet absorption spectrum was recorded on a Perkin–Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed using the KBr disc method on a Perkin–Elmer 1710 infrared Fourier transform spectrometer. NMR spectra were recorded on Bruker AVANCE DRX-300 (300, 100 MHZ). Chemical shifts are shown as values (ppm) with tetramethylsilane (TMS) as internal reference. FAB-MS was recorded on a JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried out using silica gel (60–120 mesh). Chemicals of analytical-reagent grade were purchased from E-Merck (India)

Plant Material

The roots of *Euphorbia hypericifolia* were collected from the rural areas of Lucknow District in September. The plant was authenticated by comparison with data of the herbarium specimen deposited in the Herbarium of the Faculty of Botany, Isabella Thoburn College, Lucknow (Herbarium No. APM 287)

Extraction and isolation

The air-dried plant material (roots and stem bark) of *Euphorbia hypericifolia* (2.0 Kg) was extracted with methanol. Different extracts were combined and concentrated under reduced pressure. The residue (120 g) was suspended in methanol/water (1 L, 1:9, v/v) solution and extracted with petroleum ether, chloroform, acetone and ethyl acetate to give their extracts and aqueous phases. Out of these extracts hexane, chloroform, acetone and ethyl acetate extracts were considered for further investigation. These extracts were separately column chromatographed using silica gel and were eluted with different solvent system of increasing polarity. Several fractions were obtained in each of chromatography. These fractions were monitored with TLC and the fractions of similar TLC results were combined together. These combined fractions on rechromatography afforded several compounds. From chloroform four, from acetone five and from ethyl acetate only two compounds could be isolated in pure form. From chloroform extract by eluting the column with petrol-ether/ $CHCl_3$ (5:4) ß -sitosterol (EH-1, 14 mg),*n*-hexane/ethyl acetate (9:1) ß-sitosterol-3-*O*-ß-*D*-glucopyranoside (EH-2, 7 mg), $CHCl_3$/MeOH (9:4), Oleanolic acid (EH-3, 13 mg) and from the eluent $CHCl_3$/MeOH (9:7) Ursolic acid (EH-4, 15 mg) was isolated. From acetone extract by eluting the column with petrol-ether/ ethyl acetate (6:4) 16α-Hydroxy-*ent*-kauran-19-oic acid (EH-5, 15.4 mg), chloroform/ ethyl acetate (8:2) 16α, 17-Dihydroxy-*ent*-kauran-19-oic acid (EH-6, 1.2 mg), petrol-ether/ ethyl acetate (3:2) *Ent*-kaur-15-en-19-oic acid (EH-7, 10 mg), petrol-ether/ ethyl acetate (8:2) *Ent*-kaur-15-en-17-ol-19-oic acid (EH-8, 8.6 mg). Similarly ethyl acetate extract on elution with petrol-ether/ ethyl acetate (8:2) Rutin (EH-9, 2.0 mg) and acetone/ ethyl acetate (7:5) yielded 5'-methoxy-8-methyl-6-prenyl-5, 7-dihydroxy- 3',4'-methylenedioxy-flavone (EH-10, 5.4 mg). Compounds could be readily identified by direct comparison of their UV, IR, MS and NMR data with those published data for the *β*-sitosterol [6], ß-sitosterol-3-*O*-ß-*D*-glucopyranoside [7] , ursolic acid [8], Oleanolic acid [9], four diterpenoid viz., 16α-hydroxy-*ent*-

kauran-19-oic acid, 16α, 17-Dihydroxy-*ent*-kauran-19-oic acid, *ent*-kaur-15-en-19-oic acid, *ent*-kaur-15-en-17-ol-19-oic acid [10-15], rutin [16] respectively.

EH-1: **ß-sitosterol [**$C_{29}H_{50}O$**] :** UV (MeOH) λ_{max}: 205 nm; EIMS *m/z* 414 $[M]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.52 (1H, *m*, H-3), 5.35 (1H, *m*, H-6), 0.68 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-19), 0.91 (3H, *d*, *J* = 6.4 Hz, Me-21), 0.83 (3H, *d*, *J* = 6.8 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.9 Hz, Me-27), 0.85 (3H, *t*, *J* = 7.8 Hz, Me-29); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 29.9 (C-8), 50.3 (C-9), 36.7 (C-10), 21.3 (C-11), 40.0 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.6 (C-19), 36.3 (C-20), 19.0 (C-21), 34.1 (C-22), 26.3 (C-23), 46.0 (C-24), 29.3 (C-25), 20.0 (C-26), 19.2 (C-27), 23.2 (C-28), 12.2 (C-29).

EH-2: **β-sitosterol-3-*O*-β-*D*-glucopyranoside** $C_{35}H_{60}O_6$**:** white crystal; mp 280-282 °C; IR ν_{max} (KBr) cm^{-1}: 3460, (OH), 3035, 1654 (C=C); EIMS *m/z* (%): 576 [M]+ (5), 414 [M –Glc]+ (17), 399 [M –Glc–Me]+ (15), 396 [M –Glc–H2O]+ (24), 381 (14), 329 (14), 303, 275, 273, 255; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.34 (1H,*d*, *J* = 2.1 Hz, H-1'), 5.14 (1H,*d*, *J* = 5.6 Hz, H-1'), 4.53(1H, s, H-6'), 4.27(1H, s, H-3'), 4.52(1H, s, H-4'), 4.03(1H, s, H-2'), 3.96(1H, s, H-5'), 3.85(1H, s, H-3), 1.02 (3H,s, H-19), 0.92 (3H,*d*, *J* = 6.4 Hz, H-21), 0.86 (3H,*d*, *J* = 7.3 Hz, H-29), 0.83 (3H,*d*, *J* = 6.8 Hz, H-26), 0.81 (3H,*d*, *J* = 6.7 Hz, H-27), 0.68 (3H,s,H-18);

EH-3: **Oleanolic acid [**$C_{30}H_{48}O_3$**]:** UV (MeOH) λ_{max}: 215 nm; EIMS *m/z* 456 [M]+; 1H NMR (400 MHz, $CDCl_3$) *δ*: 5.24 (1H, *t*, *J* = 3.6 Hz, H-12), 3.21 (1H, *dd*, *J* = 10.2/4.4 Hz, H-3), 2.82 (1H, *dd*, *J* = 12.7/4.3 Hz, H-18), 0.96 (3H, *s*, Me-23), 0.78 (3H, *s*, Me-24), 0.84 (3H, *s*, Me-25), 0.76 (3H, *s*, Me-26), 1.25 (3H, *s*, Me-27), 0.87 (3H, *s*, Me-29), 0.93 (3H, *s*, Me-30); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 38.6 (C-1), 26.7 (C-2), 78.5 (C-3), 39.2 (C-4), 55.5 (C-5), 18.3 (C-6), 32.6 (C-7), 39.6 (C-8), 48.1 (C-9), 37.0 (C-10), 22.7 (C-11), 122.4 (C-12), 144.1 (C-13), 42.0 (C-14), 27.7 (C-15), 22.8 (C-16), 46.7 (C-17), 41.5 (C-18), 46.1 (C-19), 30.4 (C-20), 33.7 (C-21), 32.3 (C-22), 28.8 (C-23), 14.7 (C-24), 15.1 (C-25), 16.5 (C-26), 25.2 (C-27), 180.4 (C-28), 32.8 (C-29), 23.3 (C-30).

EH-4: **Ursolic acid [**$C_{30}H_{48}O_3$**]:** UV (MeOH) λ_{max}: 215 nm; EIMS *m/z* 456 $[M]^+$; ^{1}H NMR (300 MHz. $CDCl_3$) *δ*: 5.28 (1H, *t*, *J* = 3.6 Hz, H-12), 3.21 (1H, *dd*, *J* = 10.2/4.4 Hz, H-3), 2.18 (1H, *d*, *J* = 11.7 Hz, H-18), 1.19 (1H, *m*, H*a*-22), 2.00 (1H, *dd*, *J* = 13.0/4.0 Hz, H*b*-22), 1.25 (3H, *s*, Me-23), 0.98 (3H, *s*, Me-24), 0.77 (3H, *s*, Me-25), 1.08 (3H, *s*, Me-26), 1.14 (3H, *s*, Me-27), 0.93 (3H, *d*, *J* = 6.5 Hz, Me-29), 0.91 (3H, *d*, *J* = 5.9 Hz, Me-30); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 39.2 (C-1), 27.5 (C-2), 78.5 (C-3), 38.7 (C-4), 55.5 (C-5), 18.3 (C-6), 33.1 (C-7), 39.6 (C-8), 47.8 (C-9), 36.9 (C-10), 16.6 (C-11), 125.7 (C-12), 138.4 (C-13), 41.7 (C-14), 29.5 (C-15), 24.1 (C-16), 47.7 (C-17), 53.1 (C-18), 39.2 (C-19), 39.2 (C-20), 30.5 (C-21), 36.9 (C-22), 28.0 (C-23), 15.2 (C-24), 14.8 (C-25), 16.4 (C-26), 23.1 (C-27), 180.4 (C-28), 22.9 (C-29), 22.8 (C-30).

EH-5: **16α-Hydroxy-*ent*-kauran-19-oic acid [**$C_{20}H_{32}O_3$] White powder; mp: 277-279 ^{0}C; EI-MS *m/z* (%): 320 ([M]+, 2), 302 (20), 123 (100), 121 (45), 109 (65); IR ν_{max} (KBr) cm^{-1}: 3500, 2930, 1700; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.84 (3H, s, H-20), 1.08 (3H, s, H-17), 1.25 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.3 (C-1), 18.3 (C-2), 37.4 (C-3), 42.5 (C-4), 55.7 (C-5), 21.5 (C-6), 39.6 (C-7), 44.2 (C-8), 55.0 (C-9), 38.6 (C-10), 18.0 (C-11), 25.9 (C-12), 47.2 (C-13), 37.2 (C-14), 56.8 (C-15), 76.7 (C-16), 23.6 (C-17), 28.9 (C-18), 179.3 (C-19), 15.5 (C-20);

EH-6: **16α, 17-Dihydroxy-*ent*-kauran-19-oic acid [**$C_{20}H_{32}O_4$] White crystals; mp: 261-263 ^{0}C; EI-MS *m/z* (%): 336 ([M]+, 5), 305 (100), 287 (30), 259 (43), 123 (40), 109 (62), 107 (50); IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1700, 1240, 1040; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (3H, s, H-20), 1.36 (3H, s, H-18), 4.07 and 4.17 (2H, d, *J* = 11.0 Hz, H-17); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.1 (C-1), 19.8 (C-2), 37.7 (C-3), 42.9 (C-4), 57.0 (C-5), 23.1 (C-6), 42.8 (C-7), 44.9 (C-8), 56.2 (C-9), 40.3 (C-10), 19.0 (C-11), 26.7 (C-12), 46.0 (C-13), 38.2 (C-14), 52.8 (C-15), 82.7 (C-16), 66.5 (C-17), 29.5 (C-18), 180.2 (C-19), 17.0 (C-20)

EH-7: ***Ent*-kaur-15-en-19-oic acid [**$C_{20}H_{30}O_2$] White crystals; mp: 189-191 ^{0}C; IR ν_{max} (KBr) cm^{-1}: 3000, 2940, 2850, 1700, 1270; EI-MS *m/z* (%): 302 ($[M]^+$, 5), 287 (12), 259 (45), 241 (22), 193 (51), 187 (24), 123 (48), 121 (50), 110 (30), 91 (75). ^{1}H NMR ($CDCl_3$) δ: 1.18 (3H, s, H-20), 1.35 (3H, s, H-18), 1.70 (3H, s, H-17), 2.62 (1H, br s, H-13), 5.12 (1H, s, H-15); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 41.2 (C-1), 19.7 (C-2), 38.7 (C-3), 44.3 (C-4), 56.8 (C-5), 21.5 (C-6), 44.5 (C-7), 49.9 (C-8), 48.1 (C-9), 40.1 (C-10), 19.2 (C-11), 25.3 (C-12), 44.3 (C-13), 39.9 (C-14), 135.5 (C-15), 142.3 (C-16), 15.5 (C-17), 29.5 (C-18), 179.9 (C-19), 15.9 (C-20)

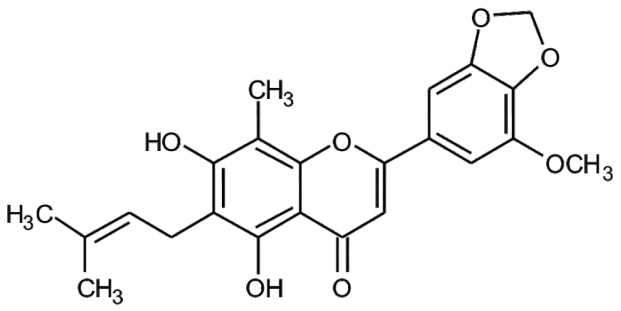
EH-8: ***Ent*-kaur-15-en-17-ol-19-oic acid [**$C_{20}H_{30}O_3$] White powder; mp: 278-280 C; IR ν_{max} (KBr) cm^{-1}: 2930, 1720, 1325; EI-MS *m/z* (%): 318 ([M]+, 12), 258 (45), 123 (45), 121 (51), 110 (34), 91 (67).^{1}H NMR ($CDCl_3$) δ: 1.20 (3H, s, H-20), 1.35 (3H, s, H-18), 2.67 (1H, br s, H-13), 4.50 (2H, d, *J* = 1.5 Hz, H-17), 5.62 (1H, s, H-15); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.9 (C-1), 19.5 (C-2), 38.5 (C-3), 43.7 (C-4), 56.6 (C-5), 20.5 (C-6, t), 43.9 (C-7), 48.9 (C-8), 47.9 (C-9), 39.9 (C-10), 18.8 (C-11), 25.5 (C-12), 41.3 (C-13), 39.6 (C-14), 135.1 (C-15), 147.9 (C-16), 60.4 (C-17), 28.0 (C-18), 180.3 (C-19), 15.6 (C-20);

EH-9: **Rutin [**$C_{27}H_{30}O_{16}$ **] :** UV (MeOH) λ_{max}: 365, 255 nm, MS *m/z :* 611[M+H]+ ; ^{1}H-NMR (400 MHz, DMSO-d6): 7.55 (1H, d, H-6'), 7.54 (1H, dd, H-2'), 6.82 (1H, d, H-5'), 6.36 (1H, d, H-8), 6.17 (1H, d, H-6), 5.32 (1H, d, H-1''), 5.02 (1H, d, H-1'''), 1.01 (3H, d, H-6'''); ^{13}C-NMR (100 MHz, DMSO-d6): δ 156.4 (C-2), 133.3 (C-3), 177.4 (C-4), 161.2 (C-5), 98.7 (C-6), 164.1 (C-7), 93.6 (C-8), 156.6 (C-9), 104.0 (C-10), 121.2 (C-1'), 115.2 (C-2'), 144.8 (C-3'), 148.4 (C-4'), 116.3 (C-5'), 121.6 (C-6'), 101.2 (C-1''), 74.1 (C-2''), 75.9 (C-3''), 70.1 (C-4''), 76.5 (C-5''), 67.0 (C-6''), 100.7 (C-1'''), 70.4 (C-2'''), 70.5 (C-3'''), 71.9 (C-4'''), 68.2 (C-5'''), 17.7 (C-6''')

Results and discussion

Compound EH-10 was isolated as yellow needles mp ~170 ^{0}C. In mass spectra molecular ion peak obtained at m/z =410 corresponds to the molecular formula $C_{23}H_{22}O_7$. The compound gave a positive Shinoda test, and an alcoholic solution of the compound gave green color with ferric chloride, indicative that the compound was a flavonoid with a free hydroxyl function at C-5 [17-22]. The IR spectrum exhibited strong absorption bands at 1635 cm (chelated C=O) and 3440 cm (strong H-bonding OH). The UV spectrum of the compound exhibited absorption maxima at 272 and 333 nm, characteristic of flavonoids [23]. The MS gave the prominent fragments at m/z: 355 [M-55], 367 [M-43] and 354 [M-56], suggested the presence of a prenyl unit. The ^{1}H NMR spectra of the compound displayed a signal at δ 12.98 assignable to a strongly bonded phenolic hydroxyl group. The ^{1}H-NMR spectrum of the compound sharp singlet at δ 1.67 (6H, 2 x CH_3) revealed the presence of gem-dimethyl group whereas the presence of -CH_2- and -CH= protons attached to the aromatic ring was indicated by a doublet at δ 3.51 (J = 7Hz) and a triplet at δ 5.35 (J =7Hz) respectively indicated the presence of C-prenyl unit. In addition a signal appeared at δ 6.08 assignable to a methylenedioxy group. The structure was further supported by its 13C NMR spectrum [23], which demonstrated a downfield signal at δ182.03 clearly assigned to carbonyl carbon C-4. The fragment peaks at *m/z* 176 from a retro-Diels-Alder reaction in the EIMS and *m/z* 180 from a retro-Diels-Alder reaction, followed by a rearrangement, were consistent with the ring B substituted with methylenedioxy and methoxy groups at 3', 4' and 5' positions, respectively. Moreover in the low field region signals appeared at 7. 24 (d, 1H, J=2.1 Hz) and 7.30(d 1H, J=2.1 Hz) were assigned to the 2', 6' protons respectively. The ^{1}H NMR spectrum further showed signals at (3.94, s, 3H) and (2.36, s, 3H) attributes to the presence of a methoxy and an aromatic methyl group. HMBC spectrum showed that methoxyl protons (δ3.94) existed long-range heteronuclear correlations with δ 147.6 (C-5), confirmed the position of methoxyl group at C-5. In the HMBC experiment the corre-

lations of OCH_2O protons δ 6.07 (2H, s) and carbons at C-3' (δ 142.8) and C-4' (δ 146.3) confirmed the existence of methylenedioxy group at C-3' and C-4'. Long-range correlations were deduced between H-1"(δ 3.26, d, 2H, J=7.2 Hz) and C-5 (δ 156.8) and C-7(δ 155.3), and also between H-2"(δ 5.16, t, 1 H, J=7.2 Hz) and C-6 (δ 110.2) corroborate the presence of prenyl unit at C-6 (δ 110.2). Furthermore the HMBC experiments indicated the long-range correlations between proton (δ 2.36) of aromatic methyl and C-7(δ 155.3), C-8(δ 105.7) and C-9(δ 151.3) and between OH (δ 10.32) and C-7 (δ 155.3), and also between OH (δ 12.88) and C-5 (δ 156.8). From these spectral data compound was identified as 5'-methoxy-8-methyl-6-prenyl-5, 7-dihydroxy- 3',4'-methylenedioxy-flavone.



5'-methoxy-8-methyl-6-prenyl-5, 7-dihydroxy- 3',4'-methylenedioxy-flavone

Conclusion

From the survey of the literature to the best of our knowledge EH-2 to EH-10 was previously unknown from *Euphorbia hypericifolia* and further examination of the constituents of this plant is currently in progress.

References

1. Khare C.P., (Ed.) 2007 Indian Medicinal Plants, an Illustrated Dictionary, Springer Science, Springer Verlag: Berlin/Heidelberg, Germany
2. Khare C.P., (Ed.) 2007 Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany Springer Science, Springer Verlag: Berlin/Heidelberg, Germany
3. Schmelzer G.H., (Ed.) 2008 Plant Resources of Tropical Mrica 11(1) *PROTA* Foundation, *Wageningen*, Netherlands / *Backhuys Publishers*.
4. Chopra R.N., Nayer S.L., Chopra I.C., 2006 Glossary of Indian medicinal plants. Academic Publishers Kolkata, India
5. Rizk A.M., Rimpler H., Ismail S.I., 1977 Flavonoids and Ellagic-Acid from Euphorbia-Hypericifolia Euphorbia-Indica. Fitoterapia 48, 99-100.
6. Greca, M. D., Monaco, P., Previtera, L., *J*. 1990 *Nat. Prod*., 53, *1430-1435*
7. Hui, B.W., M.L. Sung., 1968 Aust. J. Chem. **21**, 2137-2140.
8. Lin, C.–N., Chung, M.-I., Gan K.-H., Chiang, J.-R., 1987 Phytochemistry 26, 2381-2384.
9. Maillard, M., Adewunmi C.O., Hostettmann, K. 1992 **Phytochemistry 31**, 1321-1323.
10. Jiang, B., Lu, Z.-Q., Hou, A.-J., Zhao, Q.-S., Sun, H.-D. **1999** *J. Nat. Prod*., 62 (7), 941–945.
11. Chen, C.-Y. , Chang, F.-R. , Cho, C.-P. , Wu, Y.-C., 2000 *J. Nat. Prod*., 63 (7), 1000–1003.
12. Jiang, B., Yang, H., Li M.-L., Hou, A.-J., Han, Q.-B., Wang, S.-J., Li, S.-H., Sun, H.-D., 2002 *J. Nat. Prod*. 65 (8), 1111–1116.
13. Han, Q.-B., Zhao, A.-H., Zhang, J.-X., Lu Y., Zhang, L.-L., Zheng, Q.-T., and Sun, H.-D, 2003 *J. Nat. Prod. 66* (10), 1391–1394.
14. Jia Z.-J., Shi J-G., Yang L 1994 *J. Nat. Prod*. 57 (6), 811–816.
15. Silva, E.A., Takahashi, J.A., Boaventura, M.A.D., Oliveira, A.B., 1999 *Phytochemistry*, 52, 3, 397-400.
16. Harborne, J. B., Mabry, T. J. 1982 The Flavonoids: Advances in Research**,** Chapman and Hall Ltd., New York.
17. Intekhab, J., Aslam, M. 2010 *Monatsh. Chemie - Chem. Mon.*, 141, 1263; and references therein.
18. Intekhab, J., Aslam, M. *2011Arabian Journal of Chemistry* 4, 79–81; and references therein.
19. Mabry, T.J., Markham, K.R., 1975. In: Harborne, J.B., Mabry, T.J., Mabry, H. (Eds.), The Flavonoids, Chapman and Hall, London.
20. Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids, Springer-Verlag, New York.
21. Markham, K.R., 1982. Techniques of Flavonoid Identification, Academic Press, London.
22. Markham, K.R., Mabry, T.J., 1975. Ultraviolet–visible and proton magnetic resonance spectroscopy of flavonoids, In: Harborne, J.B., Mabry, T.J., Mabry, H. (Eds.), The Flavonoids. Chapman and Hall, London, 45–77.
23. Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids, Elsevier, Amsterdam.