Phytochemical Studies on Euphorbia hypericifolia

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Abstract:

Ten compounds were 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), 16*α*-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 their 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 shows 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 studies showed that several photochemicals have been isolated from this plant viz, taraxerol, *β*-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. Ultraviolet absorption spectra were recorded on a Perkin–Elmer Lambda Bio 20 UV spectrometer. IR spectra were 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 Fatehpur District in September. The plant was authenticated by comparison with data of the herbarium specimen deposited in the Herbarium of the Faculty of Botany, Abhinav Pragya Mahavidyalaya (Kanpur University) Fatehpur (Herbarium No. APM 587)

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, acetone and ethyl acetate fractions, four, five and two compounds were isolated in pure form respectively. From chloroform extract by eluting the column with petrol-ether/ $CHCl_3$ (5:4) *β* -sitosterol (EH-1, 14 mg),*n*-hexane/ethyl acetate (9:1) *β*-sitosterol-3-*O*- *β* -*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], β-sitosterol-3-*O*- β -*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: *β*-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)

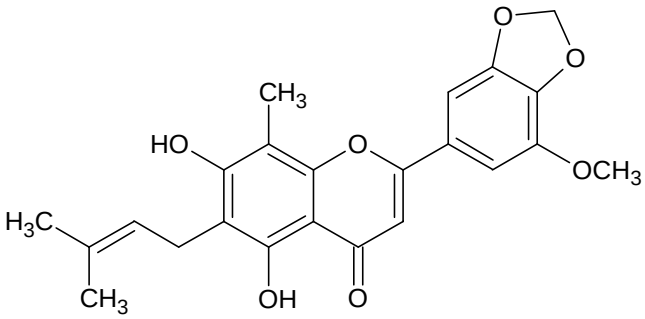
EH-7: *Ent*-kaur-15-en-19-oic acid [$C_{20}H_{30}O_2$] White crystals; mp: 189-191 ^{0}C; IR ν_{max} (KBr) cm^{-1}: 3000, 2940, 2850, 1700, 1270; EI-MS *m/z* (%): 302 ($[M]^+$, 5), 287 (12), 259 (45), 241 (22), 193 (51), 187 (24), 123 (48), 121 (50), 110 (30), 91 (75). ^{1}H NMR ($CDCl_3$) δ: 1.18 (3H, 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 correlations 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 were previously unknown from *Euphorbia hypericifolia* and further examination of the constituents of this plant is currently performed in progress.

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