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Research Article

BIOACTIVE CHEMICAL CONSTITUENTS FROM THE PLANT *VERBENA OFFICINALIS* LINN.

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ABSTRACT

The ethyl acetate extracts of plant *Verbena officinalis* Linn. on chromatographic separation with various fractions afforded several compounds. In addition to lupeol and ursolic acid with one ursane type triterpenoic acid and two flavonoids were isolated. These compounds on the basis of spectral and mass studies were characterized as 3α, 24-dihydroxy-urs-12-en-28-oic acid (1), apigenin (2) and luteolin(3). These compounds were also reported to exhibit significant effect.

Keywords: Verbena officinalis, Verbenaceae, Triterpene, Flavonoids.

INTRODUCTION

Plant *Verbena officinalis* Linn. (Family: Verbenaceae) commonly known as Verveine. Verbena is found in moderate climatic region and is known for its anti-inflammatory, diuretic and expectorant properties in folk medicine. Pharmacological activities were found for several of its constituents. The plant has been used to treat acute dysentery, enteritis, amenorrhea and depression. The plant has also been used for its antidepressant and anticonvulsant effect as well as its use for the treatment of jaundice, cough, cold and digestive problems. It has also been used for healing liver and gallbladder diseases and nervous exhaustion. The aerial part of the plant has been effectively used to allevative conditions of anxiety, insomnia and nervous irritability. Various extracts of this plant have shown antifungal, antibacterial, antioxidant, analgesic, anti-rheumatic and nerve growth factor-potentiating activities[1-10].

In our previous paper[11] we reported the isolation of ursolic acid. With addition to lupeol, here we report three more compound isolated from the plant which were characterized as 3α, 24-dihydroxy-urs-12-en-28-oic acid (1), apigenin (2) and luteolin(3).

MATERIAL AND METHODS

The plant *Verbena officinalis* Linn. was grown from the seed in the beds ment for research purpose in the college campus in the month of Sept.- Oct.-2009. Fully grown plant (Feb.-March, 2010) were collected and dried in shade. A specimen was kept for record.

The air dried and coarsely powered plant material (3kg) and than sequential extracted with petroleum ether (60^0 -80^0C), ethyl acetate and methanol by the soxhlet apparatus (6 times x 1Lit. each).The fraction of each extract were mixed together and the excess of solvent was evaporated under reduced pressure. Out of these extracts only ethyl acetate(6 gm) extract was considered for further examination. A column of silica gel was prepared well stirred with petroleum ether. Than slurry of ethyl acetate extract was made and digested over this column. The column was eluted with different solvents like petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol and their mixtures of increasing polarity. From the eluent five compounds could be isolated out of which three were as major constituent. The compound 3α, 24-dihydroxy-urs-12-en-28-oic acid (1) was separated by chloroform – ethyl acetate (8:2) and was crystallized from methanol (m.p.-240^0C). The compounds apigenin (2) and luteolin(3) were isolated by chloroform – methanol mixtures (9:1) and (9:3) respectively(m.p.-176^0-178^0C). These compounds were characterized by comparing spectral data.

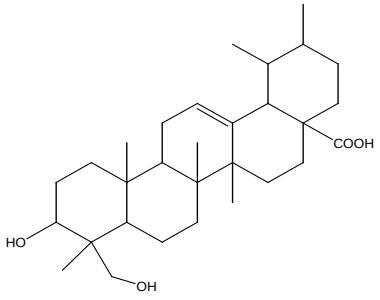
Compound **(1): –** ^{1}H NMR ($CDCl_3$), δ 2.20 (1H,d, J=11.0 Hz, H-18), δ 3.49 and 3.79 (2H,AB-q,J=11.0 Hz each d, H-24), δ 3.85 (1H,br s,H-3), δ 5.21 (1H,br s,H-12); ^{13}C NMR ($CDCl_3$) δ 32.9 (C-1), δ 23.4 (C-2), δ 72.0 (C-3), δ 41.8 (C-4), δ 51.2 (C-5), δ 19.1 (C-6), δ 32.7(C-7), δ 39.0 (C-8), δ 48.4 (C-9), δ 36.8 (C-10), δ 22.9(C-11), δ 126.8 (C-12), δ 139.2 (C-13), δ 41.9 (C-14), δ 29.4 (C-15), δ 23.1 (C-16), δ 47.7 (C-17), δ 52.7 (C-18), δ 38.8 (C-19), δ 39.2 (C-20), δ 31.3 (C-21), δ 37.7 (C-22), δ 21.9 (C-23), δ 66.1 (C-24), δ 16.6 (C-25), δ 18.9 (C-26), δ 24.1 (C-27), δ 181.2 (C-28), δ 16.9 (C-29), δ22.0 (C-30)

Compound **(2): –** I.R. cm^{-1} **:** 3410, 1700**;** MS, *m/*z = 270; RDAF at *m/z* = 152 [A] *m/*z = 118 [B]; ^{1}H NMR ($CDCl_3$), δ 7.12 (1H each, d, J=8.0 Hz, H-2′/H-6′), δ 6.67 (1H each, d, J=8.5 Hz, H-3′/H-5′), δ 6.71 (1H, s,H-3) δ 5.95 (1H,d, J=3.0 Hz, H-8), δ 5.95 (1H, d, J=3.0 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 161.0 (C-2), δ 95.7 (C-3), δ 186.65 (C-4), δ 160.0 (C-5), δ 96.6 (C-6), δ 162.0 (C-7), δ 95.8(C-8), δ 127.5 (C-1′), δ 122.8 (C-2′), δ 115.6 (C-3′), δ 152.2(C-4′), δ 116.2 (C-5′), δ 123.2 (C-6′), δ 160.7 (C-9), δ 105.2 (C-10)

Compound **(3): –** I.R. cm^{-1} **:** 3400, 1690**;** MS, *m/*z = 286; RDAF at *m/z* = 152 [A] *m/*z = 134 [B]; ^{1}H NMR ($CDCl_3$), δ 7.09 (1H,d, J=8.0 Hz, H-2′), δ 6.69 (1H, d, J=9.0 Hz, H-6′), δ 6.54 (1H,d,J=8.5 Hz,H-5′), δ 6.71 (1H, s,H-3) δ 5.99 (1H,d, J=3.0 Hz, H-8), δ 5.99 (1H, d, J=3.0 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 160.8 (C-2), δ 98.0 (C-3), δ 182.65 (C-4), δ 161.8 (C-5), δ 95.2 (C-6), δ 160.2 (C-7), δ 95.8(C-8), δ 128.0 (C-1′), δ 114.8 (C-2′), δ 144.4 (C-3′), δ 143.7(C-4′), δ 117.0 (C-5′), δ 120.2 (C-6′), δ 159.9 (C-9), δ 104.0 (C-10)

RESULTS, DISCUSSION AND CONCLUSION

Compound (1) obtained from the eluent chloroform – ethyl acetate (8:2) was found to be a triterpenoid since it gave a positive Liebermann Burchard test for pentacyclic triterpenoid. The I.R. spectrum of the compound showed the presence of hydroxyl, carboxylic and unsaturation. In its ^{1}H NMR spectrum a broad signal appeared at δ 5.21 clearly designated to vinylic proton H-12. Another downfield signal for one proton at δ 3.85 was assigned to H-3 proton. An AB type signals at δ 3.49 and δ 3.79 (each doublet) were assignable to hydroxymethylene protons H-24. The pentacyclic triterpene is of ursane type was supported by ^{13}C NMR spectrum which exhibited downfield signals at δ 126.8 and δ 139.2 for C-12 and C-13. The downfield signal at δ 181.2 was undoubtedly assigned to carboxylic function. On the basis of these spectral studies the compound was characterized[12] as 3α, 24-dihydroxy-urs-12-en-28-oic acid.

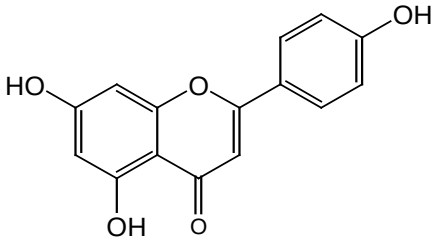


(Compd.1)

Compound (2) obtained from the eluent chloroform-methanol (9:1) was found to be a flavone since it gave a positive Shinoda test. The molecular ion peak obtained in mass spectra at m/z 270 corresponds to the molecular formula C_{15} H_{10} O_5. The I.R. spectrum showed the presence of hydroxyl group (3410 cm^{-1}) and carbonyl fraction (1700 cm^{-1}).

The ^1H NMR spectrum of the compound showed the peak in aromatic range between δ 5.95 to δ 7.12. The peak observed at δ 6.71 as singlet was clearly assigned to H – 3 proton. The^1H NMR signals at δ 5.95 corresponding to two protons were assignable to H-6 and H-8 protons of ring A. Rest of the aromatic proton signals were of ring B. The compound is tri-substituted in which the two hydroxyl groups are attached in ring A and only one is at ring B was further supported by mass spectrum which gave two fragment at m/z 152 [A] and m/z 118 [B] formed due to retro Diel's Alder fragmentation. The structure of the compound was further supported by ^{13}C NMR spectrum which exhibited a downfield signal at δ 186.65 assignable to C-4. The other downfield signals at δ 152.2 - δ 160.0 were due to C-5, C-8 and C-4′ to which hydroxyl groups are attached.

On the basis of these observations it is clear that the compound is tri hydroxy flavone in which two – OH groups are attached to ring A and one is ring B. By comparison[13-15] of above data it is confirmed as Apigenin.

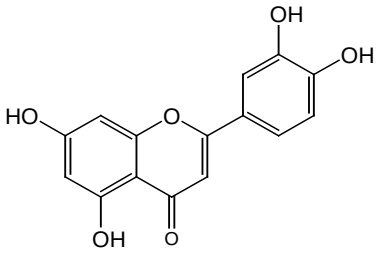


(Compd.-2)

Compound **(3)** obtained from the eluent chloroform-methanol (9:3) was found to be a flavone since it gave a positive Shinoda test. The molecular ion peak obtained in mass spectra at m/z 286 corresponds to the molecular formula C_{15} H_{10} O_6. The I.R. spectrum showed the peaks at 3400 cm^{-1} (-OH group) and 1690 cm^{-1}(-CO group).

The ^1H NMR spectrum of the compound showed the peak in aromatic range between δ 5.99 to δ 7.09. The peak observed at δ 6.71 as singlet was clearly assigned to H – 3 proton. The signals at δ 5.99 corresponding to two protons were assignable to protons H-6 and H-8 of ring A. Rest of the aromatic proton signals were of ring B. The compound is tetra substituted in which both their rings A and B are having two hydroxyl groups each. It was further supported by mass spectrum which gave two fragment at m/z 152 [A] and m/z 134 [B] formed due to Retro Diel's Alder fragmentation. The structure of the compound was further supported by ^{13}C NMR spectrum which exhibited a downfield signal at δ 182.65 assignable to C-4. The other downfield signals at δ 143.7 - δ 161.8 were due to C-5, C-8, C-3′ and C-4′ to which hydroxyl groups are attached.

On the basis of above observations and comparison[13-15] of data it was characterized as Luteolin.



(Compd.-3)

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