INTRODUCTION

Plant *Verbena officinalis* Linn. (Family: Verbenaceae) commonly known as Verveine. Verbenas are 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 alleviate conditions of anxiety, insomnia and nervous irritability. Various extracts of this plant have shown antioxidant, antibacterial, antioxidant, analgesic, anti-rheumatic and nerve growth factor-potentiating activities.1-3.

In our previous paper1 we reported the isolation of ursolic acid. With addition to lupon, here we report three more compounds 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 meant 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 (3 kg) and than sequential extracted with petroleum ether (60-80°C), ethyl acetate and methanol by the soxhlet apparatus (5 times x 1 Lit: 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 study.

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 'H NMR spectrum a broad signal appeared at δ 5.21 clearly designated to vinylic proton H-1. Another downfield signal for one proton at δ 3.85 (each doublet) was assignable to hydroxymethylene protons H-3α, 24. The pentacyclic triterpenes is of ursane type. The compound was characterized as 3α, 24-dihydroxy-urs-12-en-28-oic acid.

**Keywords:** Verbenas officinalis, Verbenaceae, Triterpenes, Flavonoids.

**ABSTRACT**

The ethyl acetate extracts of plant *Verbena officinalis* Linn. on chromatographic separation with various fractions afforded several compounds. In addition to lupon and ursolic acid with one ursane type triterpenoid 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.
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 C15 H16 O5. 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 one is ring B. By comparison of above data it is confirmed as Apigenin.

The ¹H NMR spectrum of the compound showed the peaks at δ 143.7 and δ 182.65 assignable to C-14 and C-13 of ring B. The compounds were having two hydroxyl groups to which hydroxyl groups are attached.

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

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 C15 H16 O5. 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 ¹³C NMR spectrum which exhibited a downfield fraction (1700 cm⁻¹).

On the basis of above observations and comparison of data it was characterized as Luteolin.

REFERENCES