



## PHYTOCHEMICAL CONSTITUENTS FROM THE BARK OF *VITEX NEGUNDO* LINN

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### ABSTRACT

The acetone extracts of plant *Vitex negundo* Linn on chromatographic separation with various fractions afforded four compounds. These compounds on the basis of spectral and mass studies were characterized as 5-hydroxy- 3, 6, 7, 3',4'- pentamethoxy flavone, 5,3'-dihydroxy-7,8,4'-trimethoxy flavanone, 3 $\beta$ -acetoxy-olean-12-en-27- oic acid and 3 $\beta$ -hydroxy-olean-5,12-dien-28-oic acid. These compounds were also reported to exhibit significant effect.

**Keywords:** *Vitex negundo*, verbenaceae, acetone extract, triterpenic acid and flavonoids.

### INTRODUCTION

Plant *Vitex negundo* Linn. (Family: Verbenaceae) is a large aromatic shrub commonly known as "Nirgundi" in Hindi. It is an erect 2-5m in height, found throughout the tropical, semi-tropical and warm temperature regions of India. It is also abundant along the bank of rivers in moist places and near the deciduous forests and the rural areas of the country. It is widely planted as a hedge plant along the road, between the fields and usually not browsed by cattle. All the parts of the plant are used as medicine but the leaves and roots are more important and sold as drugs. Its leaves are reported to possess medicinal, pesticidal and antibacterial properties. The roots are used in rheumatism, dyspepsia, dysentery, piles and considered as tonic, febrifuge, expectorant, antihelmintic and diuretic. The flowers are astringent and are employed in fever, diarrhea and liver complaints. The dried fruits are vermifuge and the bark is used in toothache.<sup>1-10</sup> The chemical constituents of the essential oil of *V.negundo* leaves have been reported which indicated viridifloral to be its chief constituents. Other compounds reported from the leaves include alkaloids, aromatic acids, flavonoids, iridoids and terpenoids<sup>11-14</sup>. Thus it was thought worthwhile to carryout phytochemical examination of its bark for the identification of bioactive molecules.

In our previous paper<sup>15</sup> we reported the isolation of 5-hydroxy- 3, 6, 7, 3', 4'- pentamethoxy flavone and 3 $\beta$ -acetoxy-olean-12-en-27- oic acid. Here we report two more compound isolated from the plant which were characterized as 5,3'-dihydroxy-7,8,4'-trimethoxy flavanone(**1**) and 3 $\beta$ -hydroxy-olean-5,12-dien-28- oic acid(**2**).

### MATERIALS AND METHODS

The fresh bark of *Vitex negundo* Linn.(Family: Verbenaceae) was collected from the Village-Nekapur, a local area of the Distt., Shahjahanpur in the month of January 2009. The plant was identified by Head, Deptt. of Botany and a specimen was kept for record.

The air dried, coarsely powdered (2kg) of *V. negundo* was sequentially extracted with petroleum ether (60<sup>0</sup>-80<sup>0</sup>C), acetone and methanol by the soxhlet apparatus (5 times x 1 lit each). The fractions of each extract were mixed together and the excess of solvent was evaporated under reduced pressure to get their mass. Out of these extracts only acetone extract was considered for further examination.

The semisolid brownish mass (6 gm) obtained from acetone extract was dissolved in small amount of acetone and a slurry was made with (6 gm) of silica gel. The slurry was loaded on a column of silica gel (120 gm) and eluted with petroleum ether, benzene, chloroform, ethyl acetate, methanol and their mixtures of different proportions of increasing polarity. Several fractions were obtained which were monitored by TLC and the fractions showing single spot on TLC were combined together. Four compounds were isolated from different pools of identical fractions.

The compound (**1**) was obtained as light yellow amorphous powder by elution with benzene-methanol(3:2) m.p.-150<sup>0</sup>C and was characterized as 5,3'-dihydroxy-7,8,4'-trimethoxy flavanone by comparing spectral data <sup>1</sup>HNMR (DMSO)  $\delta$  10.88 (1H, br, -OH, C-5),  $\delta$  6.83-  $\delta$  6.94 (3H,m, H-2',5', 6'),  $\delta$  5.99 (1H,s,-OH, C-3'),  $\delta$  5.77 (1H,s ,H-6),  $\delta$  5.47 (1H, dd, methine),  $\delta$  3.73-  $\delta$  3.77 (9H,s, 3x-OCH<sub>3</sub>,7,8,4'),  $\delta$  3.24 -  $\delta$  3.02 (2H,dd,methylene); <sup>13</sup>CNMR (DMSO),  $\delta$  73.5 (C-2),  $\delta$  49.1 (C-3),  $\delta$  197.6 (C-4),  $\delta$  151.3 (C-5),  $\delta$  93.8 (C-6),  $\delta$  154.0 (C-7),  $\delta$  143.0 (C-8),  $\delta$  148.2 (C-9),  $\delta$  103.6 (C-10),  $\delta$  134.6 (C-1'),  $\delta$  117.6 (C-2'),  $\delta$  143.1 (C-3'),  $\delta$  148.1 (C-4'),  $\delta$  116.9 (C-5'),  $\delta$  121.2 (C-6'),  $\delta$  56.3 (7-OCH<sub>3</sub>),  $\delta$  58.1 (8-OCH<sub>3</sub>),  $\delta$  60.0 (4'-OCH<sub>3</sub>).

The compound (**2**) from methanol was crystallized as white crystalline product by elution with chloroform-methanol(7:3) mp.-198<sup>0</sup>C and was characterized as 3 $\beta$ -hydroxy-olean-5,12-dien-28- oic acid by comparing spectral data <sup>1</sup>HNMR (DMSO),  $\delta$  5.37 (1H, H-6),  $\delta$  5.29 (1H, H-12),  $\delta$  3.23 (1H, H-3)  $\delta$  1.14 (3H, -CH<sub>3</sub>),  $\delta$  1.11 (6H,2x-CH<sub>3</sub>),  $\delta$  0.98 (6H,2x-CH<sub>3</sub>),  $\delta$  0.93 (3H,-CH<sub>3</sub>),  $\delta$  0.81 (3H,-

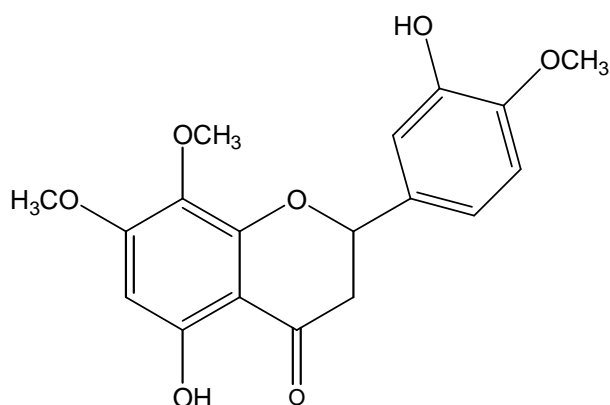


CH<sub>3</sub>); <sup>13</sup>CNMR (CDCl<sub>3</sub>) δ 33.2 (C-1), δ 30.0 (C-2), δ 84.7 (C-3), δ 43.0 (C-4), δ 147.6 (C-5), δ 121.1 (C-6), δ 34.3 (C-7), δ 37.2 (C-8), δ 53.1 (C-9), δ 32.6 (C-10), δ 26.3 (C-11), δ 122.8 (C-12), δ 149.0 (C-13), δ 40.0 (C-14), δ 26.2 (C-15), δ 28.6 (C-16), δ 52.2 (C-17), δ 31.9 (C-18), δ 40.3 (C-19), δ 22.6 (C-20), δ 33.2 (C-21), δ 21.2 (C-22), δ 19.1 (C-23), δ 21.3 (C-24), δ 26.2 (C-25), δ 25.3 (C-26), δ 19.1 (C-27), δ 180.3 (C-28), δ 23.2 (C-29), δ 25.3 (C-30).

## RESULTS, DISCUSSION AND CONCLUSION

**Compound (1):-** The compound was obtained as yellow colored amorphous powder by eluting the column with benzene-methanol (3:2). The <sup>1</sup>H NMR spectrum of the compound showed three sets of one proton double doublets at δ 5.47, δ 3.24 and δ 3.02 which are in conformity of flavanone skeleton. The spectrum also exhibited the signals in aromatic region. A singlet at δ 5.77 was ascribed for H-6 proton indicating that the ring A is trisubstituted. Other signals in aromatic region were appeared as three proton multiplets at δ 6.83- δ 6.94 could be due to H-2', H-5' and H-6' that ascertaining the disubstitutions in ring B. The spectrum also exhibited three proton singlets at δ 3.73- δ 3.77 for three methoxy groups. The signal at δ 5.99 was ascribed for hydroxy group at C- 3' of ring B while the other signal at downfield region at δ 10.88 was significantly due to cheated hydroxyl group at C-5.

The compound was further supported by <sup>13</sup>C NMR spectrum which exhibited a downfield signal at δ 197.6 for carbonyl carbon C-4. The signals for five substituted carbon atoms for hydroxyl and methoxy groups were appeared in the range δ 154 – δ 143. The three signals for methoxy carbon were appeared at δ 56.3 – δ 60.0. The signals for C-2 and C-3 were appeared at δ 73.5 and δ 49.1 respectively further in confirmatiy of flavanone skeleton. On the basis of above observation and comparison of data the compound-1 was characterized as **5, 3'-dihydroxy-7, 8, 4'-trimethoxy flavanone**<sup>12,16</sup>.



**Compound - 1**

**Compound (2):-** The compound was obtained as white crystalline solid by eluting the column with chloroform-methanol (7:3). The I.R. spectrum of the compound

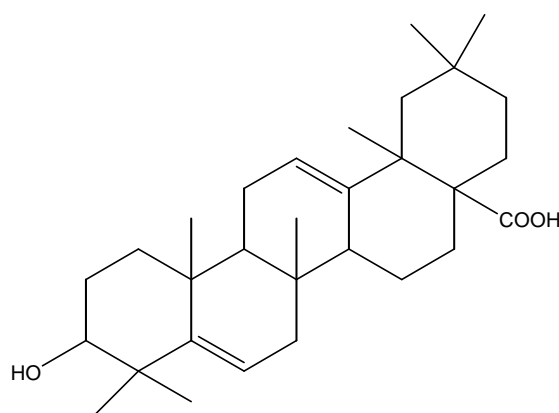
showed bands at 3200 cm<sup>-1</sup>(OH), 1700 cm<sup>-1</sup> (C=O, COOH) and 1390 cm<sup>-1</sup>, 1375 cm<sup>-1</sup>(gem- dimethyl).

The <sup>1</sup>H NMR spectrum showed two one proton signals in downfield region at δ 5.37 and δ 5.29 for two vinylic proton H-6 and H-12 respectively. A multiplet at δ 3.23 was reasonably assigned to H-3 proton.

The structure of the compound was further supported by <sup>13</sup>C NMR spectrum. Signals at δ 122.8 and δ 149.0 in the <sup>13</sup>C NMR spectrum assignable to C-12 and C-13 supported the presence of carbon-carbon double bond at C-12. The structure also displayed the two additional olefinic carbon signals at δ 147.6 and δ 121.1 for C-5 and C-6. Another downfield signal at δ 180.3 was clearly assigned to carboxyl group. The signal at δ 84.7 was ascribed for hydroxy carbon C-3.

The molecular ion peak at m/z =454 appeared in mass spectrum corresponds to the Molecular formula C<sub>30</sub> H<sub>46</sub> O<sub>3</sub>. The retro Diel's Alder fragmentation was observed in the mass spectrum of the compound with ions at m/z =248 and m/z= 206 indicating a triterpenoid oleanane skeleton with a C=C at C-12 and carboxylic group at C-28. Another significant fragments obtained at m/z =203 and m/z =189 were reasonably due to loss of -COOH and H<sub>2</sub>O from two fragments respectively.

On the basis of these finding the structure of the compound was illustrated as **3β-hydroxy-olean-5, 12-dien-28- oic acid**.



**Compound - 2**

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