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Research Article

Bioactive Compounds from the Bark of *Vitex negundo* Linn.

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ABSTRACT

Plant *Vitex negundo* (Family : Verbenaceae) commonly known as "Nirgundi" is an important medicinal plant used in rheumatism, dyspepsia, dysentery and piles. It also possesses antibacterial property. Various extracts of this plant have shown anti inflammatory, antifungal and antibacterial activities. From the acetone extract of the bark of the plant two bioactive compounds were isolated which on basis of ^{1}H and $^{13}CNMR$ studies were characterized as 5-hydroxy-3, 6, 7,3' 4'- pentamethoxy flavone and 3β-acetoxyolean-12-en-27 oic acid.

Keywords: *vitex negundo, acetone extract,* 5-hydroxy-3,6,7,3'4'-pentamethoxy flavone and 3β-acetoxyolean-12-en-27 oic acid.

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

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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, diarrhoea and liver complaints. The dried fruits are vermifuge and the bark is used in toothache.[1-9] 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[10-13]. Thus it was thought worthwhile to carryout phytochemical examination of its bark for the identification of bioactive molecules.

MATERIAL AND METHODS

Plant Material

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.

Extraction and Isolation

The air dried and coarsely powdered bark (2kg) was sequentially extracted with petroleum ether (60^0-80^0C), 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. Out of these extracts only acetone extract was considered for further examination. A column of silica gel was prepared well stirred with petroleum ether. Then a slarry of acetone extract (6gm) was made and digested over this column. The column was eluted with different solvents like petroleum ether, benzene, chloroform, ethyl acetate, methanol and their mixtures of increasing polarity. Form the eluent two compounds could be separated. One compound (1) was obtained by eluting the column with benzene-methanol (1:1) while other compound (2) was obtained by eluent chloroform-methanol (8:2). These compounds were further purified by crystallization. The compounds were then characterized by m.p., solubility and different spectral studies like IR, NMR(^{1}H and ^{13}C).

The compound (1) crystallized from methanol-chloroform as white needles, m.p.-161^0C and was characterized as 5-hydroxy-3,6,7,3′,4′-pentamoethoxy flavone by comparing spectral data ^{1}HNMR (DMSO) δ 12.68 (1H, s,O-H), δ7.81 (2H,m,2′-H & 6′-H), δ 6.98 (1H,d,J=9.0 H_z,5′-H), δ 6.48 (1H,s ,8-H), δ 3.95 (9H,s,3 x OCH_3), δ 4.01 (3H,s,OCH_3), δ 3.92 (3H,s,OCH_3); ^{13}CNMR (DMSO), δ 159.7 (C-2), δ 133.1 (C-3), δ

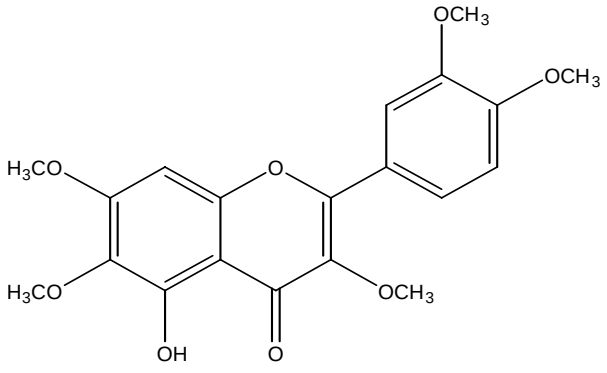
181.2 (C-4), δ 156.7 (C-5), δ 139.3 (C-6), δ 151.3 (C-7), δ 92.3 (C-8), δ 150.1 (C-9), δ 104.3 (C-10), δ 124.3 (C-1'), δ 111.7 (C-2'), δ 146.9 (C-3'), δ 153.2 (C-4'), δ 112.3 (C-5'), δ 122.3 (C-6'), δ 61.7 (6-0CH_3), δ 58.3 (7-OCH_3), δ 62.1(3-OCH_3), δ 57.1 (3'-OCH_3), δ 55-9 (4'-OCH_3).

The compound (2) crystallized from methanol as white crystalline product mp. 197^0C and was characterized as 3β-acetoxyolean-12-en-27-oic acid by comparing spectral data ^{1}HNMR ($CDCl_3$), δ 5.26 (t,1H, J=2.1 Hz, H-12), δ 4.38 (t,1H, J=7.5 Hz, H-3α), δ 2.01 (3H, s, OAc) C-Me signals at δ 1.04 (3H, s), δ 0.94 (6H,s), δ 0.84 (6H, s), δ 0.78 (3H,s), δ 0.62 (3H, s); ^{13}CNMR ($CDCl_3$) δ 36.6 (C-1), δ 29.0 (C-2), δ 82.9 (C-3), δ 37.9 (C-4), δ 52.1 (C-5), δ 17.9 (C-6), δ 33.7(C-7), δ 39.0 (C-8), δ 51.7 (C-9), δ 36.3 (C-10), δ 24.1(C-11), δ 124.9 (C-12), δ 147.2 (C-13), δ 46.5 (C-14), δ 22.7 (C-15), δ 24.1 (C-16), δ 32.6 (C-17), δ 49.5 (C-18), δ 46.1 (C-19), δ 31.6 (C-20), δ 32.9 (C-21), δ 39.1 (C-22), δ 28.1 (C-23), δ 22.3 (C-24), δ 15.5 (C-25), δ 17.9 (C-26), δ 181.3 (C-27), δ 25.9 (C-28), δ 33.9 (C-29), δ23.7 (C-30), δ 171.2 (oAc), δ 22.0 (Me).

RESULT AND DISCUSSION

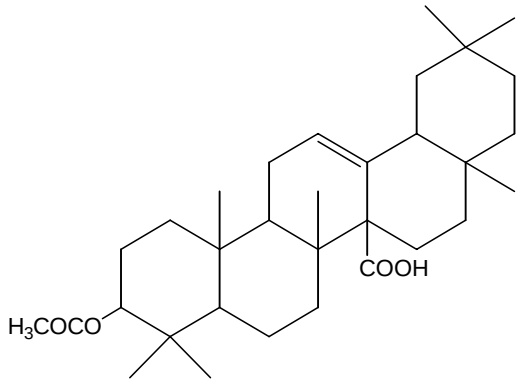
In the ^{1}HNMR spectrum of compound (**1**) a one proton singlet appearing at δ 6.48 was assigned to H-8 proton of ring B. The appearance of singlet also indicated that the ring B is penta substituted. Other benzenoid ring protons appeared at δ 7.81 and δ 6.98 were designated for H-2′, 6′ and H-5′ of ring C respectively. In addition the spectrum also exhibited signals of methoxy protons at δ 4.01-3.92. In ^{13}CNMR spectrum a downfield signal appeared at 178 was designated for carbonyl carbon C-4. Another downfield signal appeared at 152 was assigned to phenolic carbon C-5. The methoxy carbon atom resonates between 140-150.

On the basis of these spectral studies the compound was characterized as **5-hydrorxy-3, 6, 7. 3′, 4′-pentamethoxy flavone.**[14,15]



(Com. 1)

The Compound (**2**) exhibited positive triterpenoid test. In its ^{1}H NMR spectrum a triplets appeared at δ 5.26clearly designated to vinylic proton H-12. Another downfield signal for one proton at δ 4.38 assigned to H-3 α-proton. The proton of acetoxy group appeared in the range of δ 2.01. In the $^{13}CNMR$ spectrum of the compound a down field signal at 181.3 was indicative of carboxylic carbon C-27. Another down field signal at 171.2 was designated for acetoxy carbon. The methylene carbon C_{12} and C_{13} appeared at 124.9 at 147.2 respectively. The signal appeared at δ 82.9 was designated to C-3 to which acetoxy group is attached. On the basis of these spectral studies the compound was characterized as **3β-acetoxyolean-12-en-27- oic acid.**[16]



(Com.2)

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