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Isolation of triterpenoids from the leaf of *Avicennia marina*

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ABSTRACT

A simple and sensitive method for isolation of triterpenoids from the leaf of Avicennia marina. Soxhlet extraction method was used to get petroleum ether extract. The separations of triterpenoids were carried out by column chromatography and preparative thin layer chromatography. The structure of these compounds was determined by spectroscopic analysis such as FT-IR, UV, Mass spectra, ^{13}C NMR.

KEYWORDS

Avicennia marina;
Triterpenoids;
Lupeol.

INTRODUCTION

Avicennia marina is commonly known as gray mangrove and Vernacular name is Tella mada belongs to the family Aviceniaceae. It is grown as a shrub or tree to a height of three to ten meters or up to 14 meters in tropical regions. It is more wildly grown in the saline intertidal zone of sheltered coast lines. It has been reported to tolerate extreme weather condition, high winds. The bark and leaf of this plant have antimicrobial activity. *Avicennia marina is* a medium-sized tree growing in tropical region. The 15 species in the single genus of Avicenniaceae family are found on tropical coasts as constituents of mangrove vegetation[1]. The previous phytochemical investigations on the different species of *Avicennia* resulted in the isolation of essential oil and sugars like arabinose, glucose and ribose. Among other compounds alkaloids, flavonoids, steroids, terpenoids and iridoids are most considerable components[2]. In India, *Avicennia marina* is widely distributed in saline intentidal zone of sheltered coast lines. These plants are used in traditional medicine to treat skin diseases[3]. The earlier studies on this plants resulted in the isolation of Iridoid glucosides, fatty acids, sterols and hydrocarbons[4,5], also in vitro antimalarial activity and cytotoxicity of *A. marina* were reported[6]. The detail phytochemical studies have been carried out *Avicennia marina* and nothing has been reported on the triterpenoids of this species. Since this plant has good medicinal properties, the present work has been undertaken to isolate and identify secondary metabolites. In this paper the isolation and structural elucidation of the lupeol by using spectroscopic techniques like UV, IR, ^{1}H NMR, ^{13}CNMR and EIMS are being reported.

EXPERIMENTAL

Apparatus

Melting points were determined on a kolfer hot-stage apparatus. UV spectrum was taken using a Shimadzu UV-pharmspec 1700 Spectrometer. IR spectra were recorded on Shimadzu FTIR-8400 S Spectrometer. ^{1}H NMR and ^{13}C NMR spectra were ob-

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tained on Bruker WP 200 SY and AM 200 SY instruments (^{1}H, 200. 132 MHz; ^{13}C, 50.32 MHz) using TMS as internal standard and $CDCl_3$ as solvent. Electron impact mass spectra (EIMS) were recorded using Shimadzu QP 2010 MS Spectrometer and optical rotations were measured on an optical activity Petroleum ether specifically refers to the bp 40-60° fractions.

Plant materials

The Leaf and bark of *Avicennia marina* was collected from Thane district of Maharastra. A voucher specimen was deposited at the Herbarium of the Department of Botany, Nashik.

Extraction and isolation

The plant material was dried in shade. The powder of leaf (1Kg) of *A. marina* was extracted in a Soxhlet apparatus for five days in contact with petroleum ether. This extract was concentrated *in vacuo* and subjected to flash column chromatography over silica gel (Merck Kieselgel GF_{254}). Elution of the column first with chloroform, increasing amounts of EtOAc in chloroform and finally with methanol yielded a number of fractions. The proportion of solvent systems used to obtain fraction were chloroform -EtoAc (95:5),. Fraction gave lupeol (100mg) upon multiple preparative TLC using chloroform -EtOAc (95:5) and (90:10) respectively.

Lupeol

White crystals (MeOH), mp 210-212°; $[\alpha]_D$+ 30.4° (C, 0.58 in $CHCl_3$); IR ν_{max}: 3610, 3070, 3015, 1640, 1520, 1380, 1217, 1020, 887 cm^{-1}; EIMS m/z (rel. int.): 426 [M^+] (2), 411 [M^+ - CH_3] (3), 408 [M^+ - H_2O] (3), 218 (5), 207 (6), 189 (58), 163 (80), 135 (57), 107 (68), 105 (55), 79 (54), 41 (100); ^{1}H NMR: δ_H: 0.75, 0.78, 0.81, 0.92, 0.94, 1.02 (Me-28, Me-23, Me-24, Me-25, Me-26, Me-27), 1.67 (3H, br d, J = 0.5 Hz, Me-30), 3.18 (1H, dd, J = 9.6, 6.2 Hz, Hα-3), 4.56 (1H, d, J = 0.4 Hz, Ha-29), 4.67 (1H, dq, J = 0.4, 0.5 Hz, Hb-29); ^{13}C NMR: δ_C: 38.0 (C-1), 27.4 (C-2), 79.0 (C-3), 38.7 (C-4), 55.3 (C-5), 55.3 (C-5), 18.3 (C-5), 18.3 (C-6), 34.2 (C-7), 40.1 (C-8), 50.4 (C-9), 37.7 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 42.8 (C-17), 48.2 (C-17), 48.2 (C-18), 48.0 (C-19), 150.9 (C-20), 28.5 (C-21), 40.0 (C-22), 28.1 (C-23), 15.4 (C-24), 16.1 (C-25), 15.9 (C-26), 14.6 (C-27), 18.0 (C-28), 109.5 (C-29), 19.4 (C-30).

RESULTS AND DISCUSSION

The petroleum ether extract of the leaf and bark of *A. marina* afforded triterpenoids The isolated compounds were identified by spectroscopic analysis as well as by comparisons of their spectral data with previously reported values. *Triterpenoids from the leaf* Lupeol was isolated as white crystals from methanol and gave mp 210-212° $[\alpha]_D$ + 30.4° (C, 0.58 in $CHCl_3$). Its IR spectrum exhibited hydroxyl [ν_{max}: 3610, 1020cm^{-1}] and exomethylene [ν_{max}: 3070, 1640, 887 cm^{-1}] absorption. The mass spectrum displayed a molecular ion [M^+] peak at m/z 426 corresponding to $C_{30}H_{50}O$ together with fragments at m/z 411 [M^+–15] and 408 [M^+ –18] which were due to the loss of methyl group and a molecule of water from the molecular ion peak. The mass spectrum also showed a base peak at m/z 41 [$C_3H_5^+$] arising from the loss of the side chain of lupeol. The ^{1}H NMR spectrum exhibited six tertiary methyl singlets at [δ_H: 0.75, 0.77, 0.80, 0.92, 0.94 and 1.02], a methine group at [δ_H: 1.66 (br d, J = 0.5 (Hz)], a secondary carbinol group at [δ_H: 3.20 (dd, J = 9.6 and 6.2 Hz)] and an exomethylene group at [δ_H: 4.58 (1H, d, J = 0.4 Hz) and [δ_H: 4.65 (1H, dq, J = 0.4 and 0.5 Hz)] typical of pentacyclic triterpenoid[8,9] of the lupeol (**1**). The structural assignment of was further substantiated by its ^{13}C NMR spectrum which showed seven methyl groups at [δ_C: 28.0 (C-23), 19.3 (C-30), 18.0 (C-28),16.1(C-25),15.9(C-26),15.4 56 Haque et al. (C-24), 14.5 (C-27)], an exomethylene group at [δ_C: 150.8 (C-20), 109.3 (C-29)] and a secondary hydroxyl bearing carbon at [δ_C: 78.9 (C-3)], in addition to ten methylene, five methine and five quaternary carbons. The shielding of C-23 methyl of could be due to the influence of the adjacent C-3 hydroxyl group. These data were in close agreement with those reported for lupeol[7-10] and further confirmed the identity of as lupeol.

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CONCLUSIONS

The plant Avicennia Marina has good medicinal properties, isolation of triterpenoids from the leaf deserve the analytical merits. Method is simple, rapid, selective and obtained reproducible results. Present work has been undertaken to isolate and identify secondary metabolites.

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