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Research Article

CHEMICAL CONSTITUENTS OF *AVICENNIA ALBA* OF KRISHNA ESTUARY

Ramanjaneyulu MVV* **and Venkateswara Rao Battula**
Department of Engineering Chemistry, AU College of Engineering (Autonomous), Andhra University, Visakhapatnam 530 003, India

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Abstract: Phytochemical investigation on the aerial roots of *Avicennia alba* of Krishna estuary, resulted in the isolation of four triterpenoids, Lupeol (**1**), taraxerol (**2**), betulinic acid (**3**) and botulin (**4**). These are characterized by physical and spectral data and all are known compounds but we are reporting first time from this plant. Compound (**4**) was also possess antibacterial activity.

Keywords: *Avicennia alba*, Mangrove, Krishna estuary, Betulin, Bacteria.

INTRODUCTION

Avicennia alba, belonging to the family Avicenniaceae, is known to be a type of mangrove tree and grows in the tidal forests at the mouth of rivers. This species is found in India, Bangladesh, Burma, Malacca, Srilanka and often grown in southern Asia to southeast Asia and Australia (J.D. Hooker, 1982). This plant is used for fuel wood and timber in certain areas in the world (J.C.Th.uphof, 1968). The heartwood is used to make tonics. The bark and seeds of *A.alba* are used as a fish poison and resin used in birth control, ulcers treatment, skin diseases and also used to cure tumors (W.M. Bandaranayake, 1998).

Recently three new naphthoquinones and their analogues, named avicequinone – A, -B, -C and avicenol –A, -B, -C, respectively were reported (C Ito *et al.,* 2000). Deepanjan Banerjee et al. showed the presence of some antioxidant compound (Banerjee *et al.,* 2000). It provides a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins (S. Mandal, *et al.,* 2010). In 2011 Bangladesh workers confirmed the presence of alkaloids, steroids, flavonoids, saponins and tannins from leaf extract of *Avicennia alba* collected from Bangladesh and the extract possesses antinoceptive and antidiarrhoeal activities (Rahman *et al.,* 2011). Tannins, steroids, glycosides and anthroquinones were reported by Indian workers from leaves of *A. alba* on phytochemical screening which was collected from Godavary estuary, Andhra Pradesh, India (Nagababu *et al.,* 2012). In India mangrove forests are located in the alluvial deltas of the major rivers such as the Ganga, Mahanadi, Godavari, Krishna, Cauvery and also on the bay of Andaman and Nicobar Islands. As part of our investigations on mangroves for bioactive compounds we have exa, mined the aerial roots of *Avicennia alba* collected from Krishna estuary.

MATERIALS AND METHODS

Plant Material:

The roots of *Avicennia alba* were collected from the Thummalapalli, a village in Krishna estuary (Geographically located between 15°53'N and 80°34'E), Guntur district, Andhra Pradesh, India during January 2010. The plant was identified by Dr. T. Suvarna Raju, Department of Environmental Sciences, Andhra University and a voucher specimen has been deposited at the Applied Chemistry Labs, Andhra University.

Instrumentation:

Column chromatography was carried out using silica gel (100-200 mesh, Merck) and TLC analysis was performed on precoated Si Gel plates (Kiesegel 60F254, Merck). The melting points were measured on VEB analytic Dreader HMK hot plate and are uncorrected. IR spectrum was measured with FT-IR Perkin Elmer 1650. NMR spectra were recorded on JEOL JNM EX 90, at 90 MHz (1H); 22.5 MHz (13C) and Bruker Av III, at 400 MHz (1H); 100 MHz (13C). MS spectra (EI-MS) were obtained on Finnigan LCQ-Deca 70 eV.

Procedure:

Extraction and Isolation: The air dried and powdered roots (1.7kg) were extracted with dichloromethane (10 x 8L). Removal of the solvent from the combined extracts under reduced pressure gave a residue (10.0g). This residue was subjected to column chromatography over silica gel using solvents of increasing polarity from n-hexane through ethyl acetate to get 4 fractions. These are F1 (from 2% hexane in EtOAc), F2 (from 5% hexane in EtOAc) and F3,F4 (from 10% hexane in EtOAc) and are further purified by passing over a small column of silica gel or by repeated crystallization to afford four pure compounds **1-4**. These were characterized by a comparative study of their physical and spectral data with those reported for the compounds in the

***Corresponding Author:**
Dr. M.V.V.Ramanjaneyulu,
Department Of Chemistry,
K.G.R.L Degree College,
Bhimavaram-534201, A.P, India.

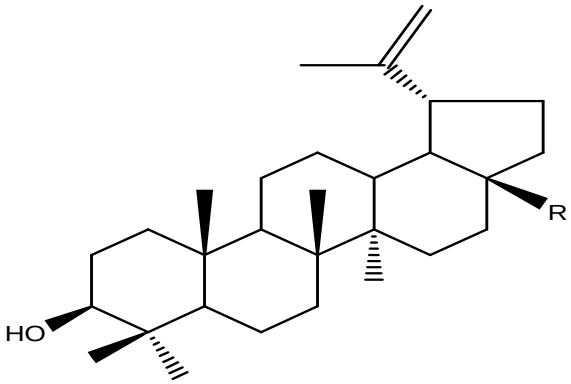
literature and by direct comparison with authentic samples wherever possible.

Bioassay: Antibacterial activity test was carried out by measuring growth inhibition zone of *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Rhodococcus rhodochrous* and *Proteus vulgaris*, at various concentrations, using agar well diffusion method. The minimum inhibition concentration (MIC), was determined by dilution method (Murray P.R *et al.,* 1995; Olurinola P.F., 1996)

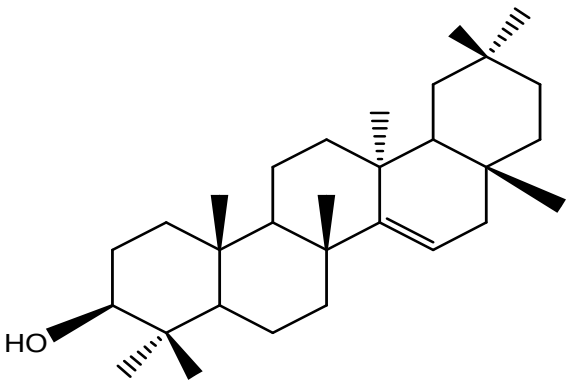
RESULTS AND DISCUSSION

Compound.1:

It was obtained as colourless needles from methanol, m.p.200-202°C, $[\alpha]_D$ + 25° (c 1.2, $CHCl_3$). This compound was analysed for C_{30} $H_{50}O$ from its molecular on at m/z 426 in its EI mass spectrum. It gave positive Liebermann-Burchard test for triterpenoids. Its IR absorptions showed hydroxyl (3500 cm^{-1}) and methylene (880 cm^{-1}) functional groups. The ^1H NMR spectral data indicated the presence of seven tertiary methyls (δ 0.76, 0.79, 0.83, 0.94, 0.97, 1.03 and 1.68), two exocyclic methylene protons at δ4.56 and 4.68 (1H each as singlet) and hydroxyl methine proton (3α-H) at δ 3.19 (1H, dd, J=11.2 and 5.2 Hz). Its ^{13}C NMR spectral data revealed the presence of seven methyl, eleven methylene and six methine carbons. The ^{13}C NMR (Table 1) and DEPT experiments confirmed olefinic carbons at 110.0(t), 151.0(s) and an oxygenated carbon at δ 79.0(d). A search in literature showed that the above data is identical to the data reported for triterpene lup-20(29)-en-3β-ol (lupeol) (S.B. Mahato *et al.,* 1994). Thus the compound **1** as identified as Lupeol (**1**). This triterpene is the first report from the species.



Lupeol (**1**): R = CH_3
Betulinic acid (**3**): R= COOH
Betulin (**4**): R = CH_2OH



Taraxerol (**2**)

Compound.2:

It was obtained as colourless needles from methanol, m.p. 280°C, $[\alpha]_D$ + 3.0° (c,1.4, $CHCl_3$). This compound was analysed for $C_{30}H_{50}O$ from its molecular in at m/z 426 in its EI mass spectrum. It gave positive Liebermann-Burchard test for triterpenoids. Its IR spectrum showed absorptions at 3450 cm^{-1} for hydroxyl and 1640, 845cm^{-1} for unsaturation. The ^1H NMR Spectral data showed a trisubstituted olefinic proton (15-H) at δ 5.55 (dd, J=8.0, J=4.0 Hz) and a hydroxymethine proton (3α-H) at 3.25(m), eight tertiary methyls at δ 1.15, 1.0, 0.98, 0.96(9H), 0.87 and at δ 0.85. The ^{13}C NMR spectral data (Table 1) revealed the presence of two olefinic carbons at δ 158.0(s) and δ 116.7 (d) characteristic of 14-15 double bond, a carbon with secondary hydroxyl at δ 79.4(d).

All the spectral (IR, ^1H NMR and ^{13}C NMR and physical characteristics (m.p., $[\alpha]_D$) of compound 2 agreed well with those of β-Taraxerol. Therefore the compound 2 was identified as β-taraxerol (**2**) (S.B.Mahato *et al.,* 1994; K. Ogihara *et al.,* 1987; Corbett R.E. *et al.,* 1972). This is first report from the plant.

Compound.3:

Compound 3 obtained as colourless needles from methanol-chloroform, m.p. 307-309°C, $[\alpha]_D$ + 15.2° (c1.0, pyridine) and analysed for C_{30} $H_{48}O_3$ from its molecular ion at m/z 456 in its mass spectrum. It gave a positive Liebermann-Burchard test for triterpenoids. Its IR spectrum showed bands at 3445 (hydroxyl), 1685 (carbonyl) and 885 cm^{-1} (methylene). The ^1H NMR spectrum showed five tertiary methyls at δ 99.0, 98.0 95.0, 83.0, 76.0 and one methyl group on double bond at δ 1.70. The spectrum also showed peaks for a pair of olefinic protons at δ 4.75 and 4.62 (each one H, br-s; exomethylene group), a carbinolic proton at δ 3.19 (dd, J=12.0, 6.4 Hz) referring to its α–orientation and a peak at δ 3.0 (1H, m).

The ^{13}C NMR data (Table 1) showed olefinic carbons at 150.6 (s) and δ 109.3 (t), hydroxy methine carbon at δ 79.0(d) and carbonyl carbon at δ 179.5(s).

A search in literature showed that the above data, physical and spectral characteristics, of compound3 well agreed with the data reported for Betulinic acid (S.B. Mahato *et al.*, 1994). Thus the compound 3 was identified as Betulinic acid (**3**).

Compound.4:

This was obtained as colourless needles from methanol, m.p. 250-251°C, $[\alpha]_D$ + 20.0° (c1.2, $CHCl_3$) and analysed for $C_{30}H_{50}O_2$ from its molecular on at m/z 442 in its EI mass spectrum. It gave a positive Liebermann-Burchard test for triterpenoids and IR spectrum showed hydroxyl (3450 cm^{-1}) and methylene (880 cm^{-1}) absorptions. The ^{1}H NMR spectra showed five tertiary methyls and one methyl on double bond and two exocyclic methylene protons at δ 4.58 and δ 4.68 (1H each as singlet), a hydroxy methine proton (3α-H) at 3.19 (dd, J = 11.2,4.8 Hz). Further Two hydroxy methylene protons appeared at δ 3.4 (1H, d, J=10.4 Hz) and δ 3.8 (1H, d, J = 10.4 Hz). The ^{13}C NMR data (Table 1) showed olefinic carbons at δ 150.2(s) and δ 109.2 (t), hydroxy methine carbon at δ 79.0(d) and hydroxy methylene carbon at δ 60.2 (t). All the physical and spectral characteristics (IR, ^{1}H NMR and ^{13}C NMR) were in full agreement with the data of pentacyclic triterpene, Betulin (**4**) to show their identity (S.B. Mahato *et al.*, 1994). This is first report from the species.

Table.1: ^{13}C NMR data of compounds 1, 2, 3 and 4

Carbon no.	Compound 1 δ_C	Compound 2 δ_C	Compound 3 δ_C	Compound 4 δ_C
1	38.6	38.4	38.6	38.7
2	27.4	27.1	27.0	27.1
3	79.0	79.4	79.0	79.0
4	38.6	38.8	38.7	38.7
5	55.4	55.4	55.3	55.3
6	18.3	18.6	18.2	18.3
7	34.5	35.0	34.2	34.3
8	40.7	38.6	40.5	40.9
9	50.7	48.6	50.4	50.4
10	37.3	37.5	37.0	37.2
11	21.0	17.4	20.8	20.8
12	25.2	35.6	25.4	25.2
13	38.2	37.4	38.2	37.3
14	42.6	158.0	42.3	42.7
15	28.1	116.7	30.7	28.0
16	35.7	36.6	32.0	29.2
17	43.0	37.8	56.1	47.8
18	48.4	49.1	46.1	48.8
19	47.3	41.1	49.5	47.8
20	151.0	29.6	150.6	150.2
21	29.6	33.6	29.9	29.8
22	40.0	33.0	37.2	34.0
23	28.0	28.6	27.5	27.4
24	15.3	15.4	15.2	15.3
25	16.1	15.4	15.8	16.1
26	16.0	29.8	16.0	16.0
27	14.5	25.8	14.5	14.7
28	18.0	29.9	179.5	60.2
29	110.0	33.3	109.3	109.2
30	19.3	21.3	19.3	19.1

Compounds 1, 3 and 4 were measured at 100 MHz in $CDCl_3$; Compound 2 was measured at 22.5 MHz in $CDCl_3$,

Table.2: Inhibition zone (mm) and MIC of Betulin againt test bacteria

Bacteria	Concentration % (b/v) in ethyl acetate			MIC
	Control	0.25	0.50	1.00	(µg/mL)
Bacillus subtilis	0.00	10	10	13	220
Staphylococcus aureus	0.00	11	11	15	130
Micrococcus luteus	0.00	9	9	12	150
Rhodococcus rhodochrous	0.00	7	8	11	240
Proteus vulgaris	0.00	6	6	8	250

Based on the antibacterial activity test, it is shown that this compound is more sensitive against S. aureus among selected bacteria. The minimum inhibition concentrations (MIC) against *B. subtilis, S. aureus M. luteus, R. rhodochrous* and *P. vulgaris* are 220, 130, 150, 240 and 250µg/mL respectively. The minimum inhibition concentration (MIC) of betulin against *S. aureus* was 130µg/mL whereas with *P. vulgaris was* 250µg/mL. Therefore it is shown that the antibacterial activity of betulin is more against *S. aureus* and least against *P. vulgaris.*

CONCLUSION

Chemical examination *of Avicennia alba* of Krishna estuary, afforded four triterpernoids, Lupeol, taraxerol, betulinic acid and betulin. All are known compounds but we are reporting first time from this plant. Isolated compound betulin was showed moderate antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*

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