Steriod and Triterpenoid from *Anogeissus latifolia*

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ABSTRACT: 3-β-hydroxy-28-acetyltaraxaren (**1**) and β-sitosterol (**2**) were isolated from an ethyl acetate extract of the stem bark of *Anogeissus latifolia*. The ethyl acetate and methanol extracts when subjected to antimicrobial screening showed significant inhibitory activity to microbial growth, while the ethyl acetate showed demonstrated significant cytotoxicity to brine shrimp with LC_{50} of 0.50 μg/ml.

Key words: *Anogeissus latifolia*, Combretaceae, 3-β-hydroxy-28-acetyltaraxaren, β-sitosterol, antimicrobial, cytotoxiciy, disc diffusion

INTRODUCTION

Anogeissus latifolia (Local name- Dhai, Family- Combretaceae) is a small to medium-sized tree up to 36 meters tall, which grows all over Chittagong division in Bangladesh. The bark has been reported to be useful in the treatment of skin diseases, snake and scorpion bite, stomach diseases, colic, cough and diarrhoea.[1] The wound healing and free radical scavenging activities of the plant have also been documented.[2] Previous phytochemical investigations with *A. latifolia* led to the isolation of (+) leucocyanidin, ellagic acid and glycosides of ellagic and flavellagic acids.[3,4]

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MATERIALS AND METHODS

General experimental procedure. The ^{1}H NMR spectra were recorded using a Varian VXR-500S (500 MHz) instrument. For NMR studies deuterated chloroform was used and the δ values for ^{1}H spectra were referenced to the residual nondeuterated solvent signal.

Plant material. Stem bark of *A. latifolia* was collected from Dhaka in the month of September 2005. A voucher specimen for this collection has been deposited in the herbarium of the Department of Botany, University of Dhaka.

Extraction and isolation. The air-dried and powdered plant material (490.0 g) was successively extracted with 0.7 litre of ethyl acetate followed by methanol (0.7 litre). The extracts were filtered separately through a fresh cotton plug and finally with a Whatman No.1 filter paper. The filtrates were then evaporated individually under reduced pressure at 40 ºC using a Rotary Evaporator to have concentrates of ethyl acetate (2.7 g) and methanol

(4.5 gm) extracts. The ethyl acetate extract was fractioned by vacuum liquid chromatography (VLC) over silica gel (Kieselgel 60H) and the column was eluted with *n*-hexane, ethyl acetate and methanol mixtures of increasing polarities to give a total of 18 fractions, each 100 ml. Compound **1** (20 mg) and compound **2** (15 mg) were obtained as colorless needles upon evaporation of solvents from fraction-8 and 11, respectively.

3-β-hydroxy-28-acetyltaraxaren (1). Colorless crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26 (1H, m, *J*=14.0 Hz, H-15), 4.13 & 4.09 (each 1H, d, *J*= 7.2 Hz, H_2-28), 3.21 (1H, dd, *J*=10.4, 6.0 Hz, H-3), 2.03 (3H, s, OAc-28), 1.25 (3H, s, H_3-29), 1.07 (3H, s, H_3-30), 1.02 (3H, s, H_3-27), 0.98 (3H, s, H_3-25), 0.92 (3H, s, H_3-26), 0.77 (3H, s, H_3-23), 0.75 (3H, s, H_3-24).

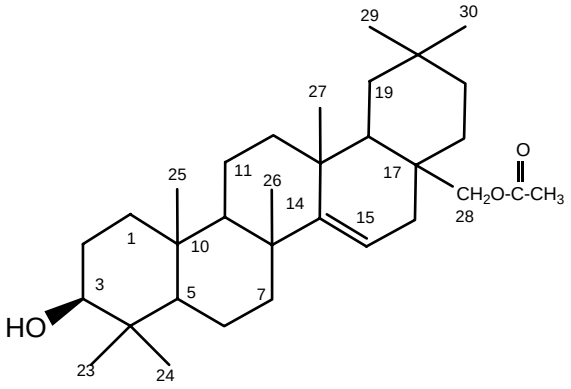
β-Sitosterol (2). Colorless crystals; ^{1}H NMR spectral data was identical to previously reported values.[9]

Bioassays. The preliminary antimicrobial activity of the extractives was determined at 400 µg/disc by the disc diffusion method[5] against a number of Gram positive and Gram negative bacteria and fungi (Table 1). The bacterial and fungal strains used in this experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Here, standard Kanamycin (30 µg/disc) was used as the reference. On the other hand, DMSO solutions of the plant extracts were assayed for cytotoxicity against *Artemia salina* in a one-day *in vivo* assay, the experimental details of which could be found elsewhere.[6] For the experiment 4 mg of each of the extract was dissolved in DMSO and serially diluted to get solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml. In the cytotoxicity screening vincristine sulfate was used as the standard.

RESULTS AND DISCUSSION

Compound **1** and **2** was isolated from the ethyl acetate extract of the stem bark of *A. latifolia* by chromatographic separation. The structures of the isolated compounds were deduced by analysis of spectroscopic data as well as by comparison with previously reported values.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** displayed well resolved peaks between δ 2.75 to 5.40. The multiplets centered at δ 5.26 could be assigned to the olefinic proton, H-15. The spectrum showed a three proton singlet at δ 2.03 which could be assigned to an acetyl group. The doublets (*J* = 7.2 Hz) centered at δ 4.09 and 4.13 were assignable to the oxymethylene protons at C-28. The relatively downfield shifts of these protons were demonstrative of theirs attachment to an acetyl group. The typical double doublets (*J* = 10.4, 6.0 Hz) centered at δ 3.21 was ascribed to the oxymethine proton at C-3. In addition, the ^{1}H NMR spectrum exhibited seven methyl group resonances as singlets as at δ 0.75 & 0.77, 0.92, 0.98, 1.02 and 1.07 & 1.25 which could be assigned to the methyl groups at C-4 (2 × Me), C-8, C-10, C-13 and C-20 (2 × Me). However, the exact assignment could not be made due to lack of 2D NMR (^{1}H-^{1}H cosy, HSQC and HMBC) data. The ^{1}H NMR spectral data of compound **1** and myricadiol were also identical except the acetate ester as C-28.[7] On this basis, compound **1** was identified as as 3-β-hydroxy-28-acetyltaraxaren. Although it has been reported form many plants, [8] this is the first report of its occurrence form *A. latifolia.*



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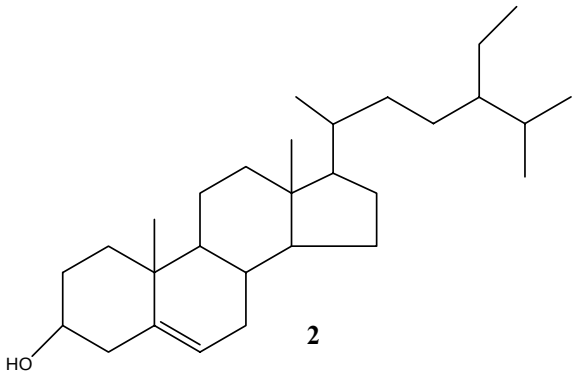

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Compound **2** was characterized as β-sitosterol by compairing of its ^{1}H NMR spectral data with reported values[9] as well as by Co-TLC with an authentic sample.

In the antimicrobial screening, the extractives of the *A. latifolia* exhibited antimicrobial activity. The zone of inhibition produced by the ethyl acetate and methanol extract ranged from 7-14 mm and 8-14 mm, respectively (Table 1). The methanol extract (ME) of

Table 1. Antimicrobial activity of *A. latifolia* extractives (400 µg/ disc) and kanamycin (30 µg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)		
	EA	ME	KAN
Gram positive bacteria			
Bacillus cereus	10	10	25
B. megaterium	10	10	30
B. subtilis	10	-	23
Staphylococcus aureus	10	10	26
Sarcina lutea	10	08	24
Gram negative bacteria			
Escherichia coli	-	08	22
Pseudomonas aeruginosa	10	10	20
Salmonella paratyphi	08	10	25
S. typhi	12	13	25
Shigella boydii	-	10	-
S. dysenteriae	10	-	25
Vibrio mimicus	14	14	28
V. parahemolyticus	10	-	25
Fungi			
Candida albicans	11	-	25
Aspergillus niger	13	-	25
Sacharomyces cerevacae	10	10	25

EA: Ethyl acetate extract; ME: methanolic extract; KAN: standard kanamycin disc; diameter of zone of inhibition less than 7 mm was considered inactive.

the bark of *A. latifolia* showed mild to moderate activity against most of the test organisms, whereas the growth of *V. mimicus* (14 mm), *S. typhi* (13) was moderately inhibited. In the same time, mild inhibitory activity was observed against *B. cereus* (10 mm), *S. boydii* (10 mm), *B. megaterium* (10 mm), *S. aureus* (10 mm), *S. paratyphi* (10 mm) and *P. aeruginosa* (10 mm). On the other hand, the ethyl acetate extract showed moderate inhibitory activity against *V. mimicus* (14 mm) and mild activity against all of the tested gram negative bacteria and most of the gram positive bacteria. In case of gram positive bacteria, the growth of *B. cereus* (10 mm), *B. megaterium* (10 mm) and *S. aureus* (10 mm) was inhibited. Among the gram negative bacteria, *V. mimicus* (14 mm) was found to be very sensitive to the extract and moderate sensitivity was observed against *S. typhii* (12 mm). In case of fungal strains, the ethyl acetate and methanol extract showed

moderate inhibition of growth of *C. alibicans* and *A. niger*.

Following the procedure of Meyer,[6] the lethality of the ethyl acetate and methanol extracts to brine shrimp was determined on *A. salina*. Table 2 shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC_{50} obtained from the best-fit line slope were found to be 0.32, 0.50 and 2.13 µg/ml for vincristine sulfate, ethyl acetate and methanol extract, respectively. In comparison with the positive control, the cytotoxicity exhibited by the ethyl acetate and methanol extract was significant. However, the purified compounds could not be tested due to lack of adequate amount of samples.

The results of antimicrobial and cytotoxicity screenings are consistent with the folk uses of *A. latifolia* by the local people.

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