

Phytochemical and Antimicrobial Analysis on the Extracts of *Oroxylum indicum* Linn. Stem-Bark

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ABSTRACT

The stem bark of *Oroxylum indicum* (Fam. *Bignoniaceae*) was extracted with methanol. The methanol extract was fractionated with pet ether, chloroform, hexane and carbon tetrachloride using modified Kupchan partitioning method. Extensive chromatographic separation and purification with the organic solvent was done. One compound was extracted from the n-hexane fraction of stem bark of *O. indicum* which was then confirmed as flavonoid type of compound by using ^1H NMR spectrometric analysis. Three fractions (hexane, carbon tetrachloride, chloroform), obtained from methanolic crude extract, were tested for antimicrobial activity by standard disc diffusion method. The extracts were found to show antimicrobial activities towards the gram-positive and gram-negative bacteria, and some fungi. The results were compared with same microbial strains against a standard antibiotic disc of ampicillin (10 μg /disc).

Keywords: *Oroxylum indicum*, Kupchan partitioning method, Flavonoid, antimicrobial activity, Disc diffusion method

The family *Bignoniaceae* includes 120 genera and almost 850 species. The members of *Bignoniaceae* are distributed in tropical and subtropical zones of Asia and America. The major types of phytochemicals so far detected are naphthaquinones and iridoids, which are used in the treatment of tumors and are used as antiseptic [1]. The antitumor activity of *Bignoniaceae* is due mainly to its naphthaquinones, among them lapachol have been considered as candidates for clinical use [1]. *Oroxylum indicum* (also known as Shivnak, Sonapatha, Shyonaka or Midnight horror) is a small tree of *Bignoniaceae* family having few branches. Seeds in several rows, very thin, compressed, rounded, surrounded by a transparent broad wing [2]. Previous chemical studies of *Oroxylum indicum* led to the isolation of ellagic acid [3], 5,7-Dihydroxy flavone (chrysin) [4], 5-hydroxy-8-methoxy-7-O- β -D-glucopyranuronosyl flavone [5], Stigmast-5-en-3-ol [6], 5,6,7-trihydroxy flavone (baicalein) [7,8], 4',5-Dihydroxy-7-methoxy isoflavone (pratensol) [9], 3-(4-hydroxy phenyl)-2-propenoic acid [10]. In this work, we report the isolation of an additional compound, a flavonoid, 3, 4', 5, 7-tetrahydroxy-flavonol, as well as demonstrate the antimicrobial effects of the extract.

MATERIALS AND METHODS

General experimental procedure

Usually the intact plant/plant part(s) is collected as a whole and sun-dried. In fresh condition, it is then oven-dried at reduced temperature (not more than 50°C) to make suitable for grinding purpose. The coarse powder is then stored in airtight container with marking for identification and kept in cool, dark and dry place for use. Extraction can be done in two ways – cold extraction and hot extraction. Chromatographic techniques are the most useful in the isolation and purification of compounds from plant extracts. Nuclear magnetic resonance (NMR) spectroscopy is used to determine the structure of the compound.

Plant materials

The stems of *Oroxylum indicum* were collected from Chittagong in September 2004 and were taxonomically identified by Mrs. Mahbuba Begum, Chief Scientific Officer, Bangladesh National Herbarium and one voucher specimen has been deposited there.

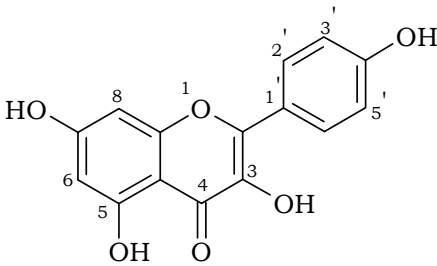


Fig 1. Proposed structure of 3,4',5,7 tetrahydroxy flavonol (kaempferol)

Extraction and isolation

Extraction of dried and powdered stem bark of *O. indicum* was done by cold extraction process by using methanol as a solvent [11]. The air dried and pulverized plant material (100.0gm) was cold extracted with methanol and was successively partitioned with *n*-hexane, carbon tetrachloride and chloroform using modified Kupchan partitioning method [12]. After that the fractions were evaporated by roto-dryer at low temperature (40–50°C) to dryness, they were used to isolate compound and to find out the antimicrobial activity. Deposited portion of the *n*-hexane fraction was then subjected to PTLC (Stationary phase – Silica gel PF₂₅₄, Mobile phase – Toluene:Ethyl acetate = 95:5 with few drops of acetic acid, multiple developments, Thickness of plates – 0.5mm) [13].

Table 1. ¹H NMR (400MHz, CDCl₃) spectral data for the compound

Protons	Compound δ_H in ppm (CDCl ₃)
H-6	6.46
H-8	6.27
OH-5	12.76
H-2', H-6'	7.88
H-3', H-5'	7.53

The plate was developed twice for better resolution. Three sets of band were scrapped and then eluted initially using a 50:50 mixture of ethyl acetate (EtOAc) and chloroform followed by 100% EtOAc. The 6th band from the top of the developed plate after scrapping and elution yielded the compound (2.10 mg).

Structure elucidation

¹H-NMR spectra were obtained from Bangladesh Council of Scientific and Industrial Research (BCSIR) with Bruker NMR spectrophotometer (400MHz, with TMS as the internal reference). Silica gel (kieselgel G 60, mesh 70-230, particle size 0.043-0.063 mm) was used for column chromatography. PTLC was done on using coated glass plates (kieselgel 60 PF₂₅₄, Merck).

Antimicrobial Study (disc diffusion method)

Collected fractions, *n*-hexane, carbon tetrachloride,

Table 2. Antibacterial activity of the hexane, chloroform and carbon tetrachloride fractions

Test organism	Diameter of Zone of Inhibition (mm)			
	Hexane extract	CHCl ₃ extract	CCl ₄ extract	Ampicillin
	200 µg/disc	200 µg/disc	200 µg/disc	10 µg/disc
Gram positive bacteria				
Bacillus cereus (BTCC-19)	10	11	20	10
Bacillus megaterium (BTCC-18)	12	12	16	19
Bacillus subtilis	10	11	14	08
Staphylococcus aureus (BTCC-43)	10	10	17	10
Sarcina lutea (ATCC-9341)	11	09	15	17
Gram negative bacteria				
Escherichia coli (BTCC-172)	10	11	18	09
Pseudomonas aeruginosa (BTCC-1252)	11	09	18	15
Salmonella paratyphi	12	11	16	09
Salmonella typhi	09	12	17	15
Shigella boydii	09	11	11	11
Shigella dysenteriae	08	10	10	06
Vibrio mimicus	13	18	16	08
Vibrio parahemolyticus	11	12	16	12
Fungi				
Saccharomyces cevevaceae	09	09	11	07
Candida albicans	09	13	21	10
Aspergillus niger	12	14	13	09

chloroform and methanol extracts were tested in an antimicrobial susceptibility study using a standard disc diffusion method [14, 15]. In this investigation, 16 microorganisms (5 Gram-positive bacilli, 8 Gram-negative bacilli and 3 fungi) were obtained from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. Ampicillin (10 µg/disc) and blank sterile filter paper disc (diameter, 6 mm) were used as positive and negative controls, respectively. Nutrient agar medium (DIFCO) was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures. The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously-marked zones in the agar plates, pre-inoculated with test bacteria. The discs were then incubated on the plate aerobically at 37°C for 24 hours. The diameter of inhibition zone around each disc was measured and recorded at the end of the incubation period.

RESULTS AND DISCUSSION

Phytochemical analysis

The compound was obtained as yellow gum. It appeared as a dark quenching spot on TLC plate under UV light at 254nm. It also appeared as yellow spot under UV light at 366nm. Spraying the developed plate with vanillin-sulfuric acid followed by heating at 110°C for several minutes gave yellow color.

The ¹H NMR spectrum (400 MHz, CDCl₃) of the compound displayed signals characteristic of a 4'-monooxygenated flavonol. The spectrum also indicated the presence of an aromatic proton, three phenolic hydroxyl groups of which one is hydrogen-bonded. The ¹H NMR spectrum exhibited two two-proton doublets at δ 7.88 and δ 7.53. The doublet at δ 7.88 assignable to flavonol 2' and 6' protons while the other doublet at δ 7.53 assignable to flavonol 3' and 5' protons. A broad singlet at δ 12.76 could be attributable for a hydroxyl group at C-5 and the relatively deshielded nature of this hydroxyl group suggested that this hydroxyl group is hydrogen-bonded with the C-4 carbonyl group. Two singlets at δ 6.46 & δ 6.27 could be assigned to H-6 & H-8 respectively of ring-C (Table 1). So, on the basis of the spectral data and assignment the structure of the compound can be proposed as Fig 1.

Antimicrobial study

From the study, the zones of inhibition produced by the hexane extract, chloroform extract and carbon tetrachloride extract were between 08 – 13 mm, 09 – 18 mm and 10 – 20 mm respectively at a concentration of 200 µg/disc in case of bacterial strain but the methanol extract showed no sensitivity (Table 2). The hexane extract showed significant activity against *Bacillus megaterium* (12mm), *Salmonella paratyphi* (12mm), *Vibrio mimicus* (13mm), *Vibrio parahemolyticus* (11mm), *Pseudomonas aeruginosa* (11mm). The chloroform extract showed prominent activity against

Vibrio mimicus (18mm); significant activity against *Bacillus megaterium* (12mm), *Salmonella paratyphi* (11mm), *Vibrio parahemolyticus* (12mm), *Salmonella typhi* (12mm), *Shigella boydii* (11mm), *Bacillus cereus* (11mm), *Escherichia coli* (11mm), *Bacillus subtilis* (11mm). The carbon tetrachloride extract demonstrated prominent zones of inhibition >15mm, thus demonstrating significant activity against almost all bacterial strains except *Shigella dysenteriae* (10mm), *Shigella boydii* (11mm), which showed lower susceptibility to the crude extract.

The crude extracts of *Oroxylum indicum* were also tested for antifungal activity against three fungi. The extracts had inhibitory effects against all three fungi with the carbon tetrachloride fraction being most active against *Candidia albicans*, while the chloroform and hexane fraction were most active against *Aspergillus niger*.

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