CHEMICAL CONSTITUENTS FROM *OROXYLUM INDICUM* (L.) KURZ OF NEPALESE ORIGIN

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Abstract: From the stem bark of *Oroxylum indicum*, three flavones namely baicalein (**1**), oroxylin (**2**) and pinostrobin (**3)** along with one sterol, Stigmast-7-en-3-ol (**4**) were isolated and their structures were established by the use of spectroscopic techniques. Baicalein (**1**) and oroxylin (**2)** were found to be active against brine shrimp with LC_{50} value 10.0 μg/ml and 36.0 μg/ml and also exhibited the antimicrobial activity on both Gram-positive and Gram-negative bacteria with MIC value 4.0 mg/ml and 8.0 mg/ml respectively.

Key words: *Oroxylum indicum;* Flavones; Baicalein; Brine-shrimp bioassay.

INTRODUCTION

Oroxylum indicum commonly called Indian tumplet flower (Nepali name: Tatelo, Karamkanda, saune tatal) belongs to the Bignoniaceae family and it is widely used in traditional medicine.[1, 2] Traditionally, the stem, bark, seeds and leaves used for the treatment of the diarrhea, typhoid and stomachache. It has also been used as antitussive, analgesic and anti-inflammatory agents for the treatment of cough, bronchitis and other lungs diseases. Considering its wide traditional uses we have carried out phytochemical studies on *Oroxylum indicum* to isolate its biologically active compounds. In the present study we have isolated four pure compounds (**1**-**4**) from the stem bark of *O. indicum*. The structure of isolated compounds was established on the basis of spectral analysis. Furthermore, the isolated compounds were showed the toxicity towards the brine-shrimp and they inhibited the growth of Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

General Experimental Procedure: Melting point was measured on electronic melting point apparatus (Ogawa Seiki Co. LTD, Tokyo, Japan) and was uncorrected. IR spectra were obtained on neat using Perkin Elmer 1310 Infrared spectrophotometer. Mass spectra were carried out on JEOL MSRoute. ^{1}H-NMR, ^{13}C- NMR spectra were obtained on Brukner WM400 NMR spectrometer. TLC was carried out on silica gel G precoated plates. Separation and purification were performed by column chromatography on silica gel (60-120 mesh) and precoated TLC plate.

Plant material

Stem bark of *Oroxylum indicum* was collected from temperate forest in July 2004. The plant was identified by Prof. Dr. R. P. Chaudhary, Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Extraction and Isolation

Air-dried powdered plant material (1 Kg) was taken in the Soxhlet extractor and extracted successively with hexane (3 L), dichloromethane (3 L), ethyl acetate (3 L) and methanol (3 L). The solvent was evaporated under reduced pressure in rotatory evaporator to get hexane, dichloromethane, ethyl acetate and methanol extracts. 5.0 gram of CH_2Cl_2 extract was subjected to column chromatography on silica-gel column (150 g, 60-120 mesh, 40 cm x 3 cm). The column was initially eluted with pure hexane and then by gradient of hexane-chloroform of increasing polarity and again with mixture of chloroform:ethyl acetate and finally ethyl acetate:methanol of increasing polarity. Repeated column chromatography of different fractions obtained from ethyl acetate-chloroform elution followed by preparative TLC afforded three pure compounds (**1-3)**. Ten gram of hexane extract was subjected to column chromatography on silica-gel column (150g, 60-120

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mesh, 40 cm x 3 cm). The column was initially eluted with pure hexane and then gradient of hexane- EtOAC of increasing polarity and finally with EtOAc. Repeated column chromatography followed by recrystallization of the 20% EtOAc in hexane fraction afforded compound **4**.

Brine-shrimp bioassay and antimicrobial activity

Brine-shrimp bioassay was carried out by the procedure developed by Meyer et al to determine the toxicity of fraction and compounds towards newly hatched brine shrimps nauplii.[3] In this method the compound/extracts having LC_{50} value less than 1000 *ì* g/ml are considered to be biologically active. The antimicrobial activity of extract/compounds was determined by agar well diffusion method developed by Dingle et al.[4] For this process *Bacillus subtillis* and *Staphylococcus aurous* were taken as Gram-positive bacteria while *E. coli* and *Salmonella typhi* were taken as Gram-negative bacteria.

RESULT AND DISCUSSION

Repeated column chromatography of the dichloromethane extract of *Oroxylum indicum* followed by preparative TLC afforded three pure compounds (**1-3)**. Similarly repeated column chromatography followed by recrystallization of the hexane extract yielded compound **4**.

The compound **1** was obtained as a brown yellow crystal having melting point 260-262°C. The EI mass spectrum showed M^+ peak at *m/z* 270 corresponding to molecular formula C_{15} H_{10} O_5. IR spectrum had a broad absorption at 3500 cm^{-1} due to presence of OH group and an absorption peak at 1710 cm^{-1} due to presence of carbonyl group. Its ^{1}H-NMR profile was consistent with that of a flavone structure. The singlet peak at *ä* 12.66 was assigned for highly deshielded phenolic proton. The double doublet at ä 8.06 was assigned for aromatic protons H2‘ and H6‘. A complex pattern at ä 7.55-7.62 were due to the aromatic protons H-3‘, H-4‘ and H-5‘ showing ortho coupling (H3‘, H4‘ and H-4‘, H-5‘, J = 7.17) and meta coupling (H-3‘, H-5‘ J = 1.8). Two singlets at ä 6.93 and 6.63 were assigned for the H-3 and H-8 proton respectively. The ^{13}C NMR peaks were assigned as 95.2 (C8), 105.4 (C10) 105.5 (C3), 126.6 (C2‘ and C6‘), 129.3 (C3‘ and C5‘), 131.4 (C6), 131.7 (C1’), 132.1 (C4’), 148.30 (C9), 151.18 (C5), 155.82 (C2) 163.63 (C7) and 183.09 (C4) respectively. Further comparison of these spectral data with those of published in the literature[5], the compound **1** was identified as baicalein (5,6,7-trihydroxy flavone).

The compound **2** was isolated as brownish yellow crystals. Analysis of ^{1}H- and ^{13}C-NMR spectrum of the compound **2** revealed that it is differed from compound **1** on position C-6 where a hydroxyl group replaced by methoxy group in compound **2**. Therefore, the compound **2** was identified as oroxylin (5,7-dihydroxy-6-methoxy flavone).

Compound **3** was obtained as a yellowish crystal having melting point 230-232°C. The mass spectrum showed M^+ peak at m/z 270 corresponding to molecular formula $C_{16}H_{14}O_4$. Its ^{1}H-NMR signals were assigned as *ä* 3.88 (OCH_3), 6.68 (1H, H- 6), 6.76 (1H, H-8), 7.45 (3H, H-3’, H4’, H5’) 7.89 (1H, H-5’), 7.91 (1H, H-2’) respectively. ^{13}C-NMR peaks were assigned as *ä* 58.6 (OCH_3), 94.9 (C9), 100.1 (C7), 105.1 (C3), 106.1 (C5), 126.7 (C1’, C4’), 129.3 (C2’, C6’), 131.8 (C5’), 131.9 (C3’), 158.5 (C10), 163.7 (C6), 166.1 (C8), and 182.6 (C4), respectively. From these spectral evidences the compound **3** was identified as pinostrobinÿ5-hydroxy-7-methoxy flavanone).

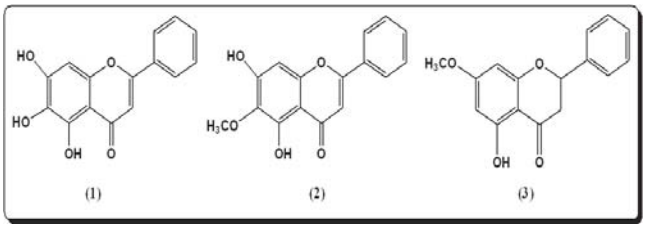

Fig. 1. Structure of isolated compounds

Compound (**4)** was obtained as a white crystalline solid with melting point 199°C. Its ^{1}H- and ^{13}C-NMR spectra were similar to that of stigmast-7-en-3-ol. This was further confirmed by co-TLC and co-melting point of authentic sample of Stigmast-7-en-3-ol previously isolated in our lab.

Brine-shrimp bioassay and antimicrobial activity

Different extracts and isolated compounds were tested to brine-shrimp bioassay and antimicrobial activity to determine the pharmacological potential. The brine-shrimp bioassay revealed that the dichloromethane, hexane and methanol fractions have potent activity against brine-shrimp with LC_{50} value 60, 100 and 106 µg/ml, respectively. Baicalein (**1**) and oroxylin (**2)** were found to be active against brine shrimp with LC_{50} 10.0 μg/ml and 36.0 μg/ml. Baicalein (**1**) and oroxylin (**2)** also exhibited antimicrobial activity on both Gram-positive and Gram-negative bacteria with MIC value 4.0 mg/ml and 8.0 mg/ml respectively.

CONCLUSION

The dichloromethane and hexane fractions of *O. indicum* were subjected to different chromatographic techniques to afford four compounds. The isolated compounds were identified as baicalein (**1**) oroxyline (**2**), pinostrobin (**3**) and stigmast-7-en-3-ol (**4**). Biological screening by Brine shrimp bioassay and antimicrobial activity test on different fractions and compounds obtained from stem bark of *O. indicum* were carried out. The brine-shrimp bioassay revealed that the dichloromethane, hexane and methanol fractions have potent activity against brine-shrimp with LC_{50} value 60, 100 and 106 µg/ml, respectively. Baicalein (**1**) and oroxylin (**2)** were found to be active against brine shrimp with LC_{50} 10.0 μg/ml and 36.0 μg/ml. Baicalein (**1**) and oroxylin (**2)** also exhibited antimicrobial activity on both Gram-positive and Gram-negative bacteria with MIC value 4.0 mg/ml and 8.0 mg/ml respectively. To the best of our knowledge, pinostrobin and stigmast-7-en-3-ol were isolated for the first time from this plant.

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