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Isolation of an antimicrobial compound from the tender shoots of Zanthoxylum oxyphyllum

Jitu Buragohain^{1*}, B. K. Konwar² and M. J. Bordoloi³

¹Department of Botany, Namrup College, P.O. Parbatpur, Dist. Dibrugarh, Assam (India) ²Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Dist. Sonitpur, Assam (India) ³Natural Product Chemistry Division, Regional Research Laboratory, Jorhat, Assam (India)

ABSTRACT

An antimicrobial compound has been isolated from the tender shoots of Zanthoxylum oxyphyllum, which has been assigned the structure 2-methylheptylisonicotinate based on IR, ${}^{1}H$ NMR and ${}^{13}C$ NMR spectra. The compound has been reported for the first time from Zanthoxylum oxyphyllum.

Key words: Zanthoxylum oxyphyllum, 2-methylheptylisonicotinate, NMR spectra, North East India.

INTRODUCTION

Natural extracts derived from plants are proven source of bioactive compounds with therapeutic indications against a wide spectrum of diseases and infections [1]. *Zanthoxylum oxyphyllum* Edgew is a scrambling shrub with aromatic smell and hooked prickles. The plant belongs to the family Rutaceae and is distributed in the temperate and subtropical Himalayas [2]. In North East India the plant has been used as traditional medicine. Tender shoots of this plant are taken as vegetable, which are useful against stomach trouble. If tender shoots are regularly eaten as vegetables, it is said to act as blood purifier and reduce the incidence of leucoderma. Fruits are used as spice and help in digestion. Kumar and Muller [3] reported that the MeOH extracts of the bark and roots of this plant with IC₅₀ values of 53 and 57 μ g/ml showed potent antiproliferative activity against the growth of human keratinocytes. Deshpande and Shastri [4] reported the presence of sesamin, eudesmin, epieudesmin, syringaresinol, y-fagarine, b-sitosterol and lupeol from the dried branches together with stem bark of the plant. Tiwari *et al.* [5] isolated 3,4-Bis (3', 4'-dimethoxyphenylmethyl) furan-2-one from the roots. In our effort for phytochemical and

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molecular studies on potential medicinal plants from North East India, we have investigated this plant species to isolate a few compounds from the tender shoots.

MATERIALS AND METHODS

Tender shoots of the plant were collected from Namrup $(27.18^{\circ} \text{ N} \text{ and } 95.33^{\circ} \text{ E})$, Assam. The plant species was identified by the Botanical Survey of India, Shillong. Voucher specimens were deposited in the herbarium of the department of Botany, Nmrup College, Assam. Collected tender shoots of *Zanthoxylum oxyphyllum* were shade dried and ground into fine powder in a grinder. 250 gm of this powder was extracted with 400 ml of MeOH in a Soxhlet apparatus at room temperature for 6 h and the solvent was evaporated to dryness in a rotary evaporator. The procedure yielded 3.04 g dark green methanol extract. The crude methanol extract was partitioned between ethyl acetate (EtOAc) and water in 1:1 proportion following the procedure described by Tori *et al.* [6]. The EtOAc soluble part was concentrated and the extract (1.02 g) was taken for further analysis. The crude EtOAc extract was chromatographed over silica gel (60-120 mesh size) with column size 45 cm x 20 mm and flow rate 1ml/min, and partitioned successfully in order of increasing polarity with n-Hexane and EtOAc. The fraction eluted with n-Hexane and EtOAc (90:10, v/v) afforded the compound **1a**. TLC was visualized by iodine spray followed by heating.

The IR spectrum of **1a** was recorded using potassium bromide (KBr) pellet in Nicolet Impact 410 FT-IR Spectrometer. NMR spectrum of **1a** was scanned on Varian Mercury 400 Spectrometer operating at 400 MHz for ¹H and 100 MHz for ¹³C nuclei respectively. The duterated chloroform (CDCl₃) was used as solvent and tetramethyl silane (TMS) was used as internal standard. MALDI TOF MS was run in a Micromass TofSpec 2E instrument using a nitrogen 337 nm laser with 4-nanosecond pulse. At least 40-50 shots were summed up. The matrix used was α -cyano-4 hydroxycinnamic acid dissolved in acetonitrile/methanol. Sample and matrix solutions were mixed together and 1 µl was spotted on MALDI target.

RESULTS

The compound was obtained as yellowish oil. MALDI TOF MS gave a molecular ion at m/z 379 (M⁺+144). The IR spectrum of the compound **1a** showed absorption band at 2958, 2926, 2854, 1729, 1600, 1580 cm⁻¹ indicated the presence of an aromatic system C=N and ester group. Absorption band at 1462, 1380, 1274, 123, 1072, 1039, 960 and 742 confirmed the long linear chain of the compound. The ¹H NMR spectrum (Fig. 1) showed two doublets of doublets each integrating to two protons at δ 7.49 and δ 7.68 indicated the presence of the pyridyl system in the molecule. The multiplet signal at δ 4.21 integrating to one proton confirmed the presence of the ester group. The doublet at δ 0.93 and triplet at δ 0.88 each integrating to three protons indicated the presence of four aromatic carbons, two methyl groups. The ¹³C NMR spectrum indicated the presence of four aromatic carbon and carbonyl carbon. The spectral data suggested that the compound **1a** was an alkaloid. Based on these evidences, the structure of the compound **1a** was assigned as 2-methylhptylisonicotinate (Fig 3). The NMR spectral data was in good agreement with the earlier report [7].

Analytical data of 1a

IR (KBr) cm⁻¹ 2958, 2926 (CH₃), 2854 (CH₂), 1729 (C=O), 1600, 1580, 1462, 1380, 1274, 1123, 1072, 1039, 960, 742

¹H NMR (CDCl₃) δ 0.88 (3H, H-7), 0.93 (3H, H-8), 1.25-1.41 (6H, overlapping signals of H-4, H-5 and H-6), 1.55 (1H, H-2), 4.21 (1H, H-1), 7.49 (doublet of doublets, 2H) and 7.68 ((doublet of doublets, 2H)

¹³C NMR (CDCl₃) δ 12.0 (C-7), 14.23 (C-8), 23.92 (C-6), 24.75 (C-5), 29.90 (C-4), 30.53 (C-3), 39.69 (C-2), 68.21 (C-1), 129.52 (C-3' and C-5'), 131.55 (C-2' and C-6'), 132.37 (C-1') and 167.49 (COO)



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Fig 2. Chemical structure of 1a

DISCUSSION

This is the first report of the extraction of the compound 2-methylhptylisonicotinate from *Zanthoxylum oxyphyllum*. The compound was first isolated from the culture filtrate of *Streptomyces* sp. 201 [7]. The compound was found to have high antibacterial and antifungal activities and is a natural analogue of the established antituberculotic drug, isoniazid [7]. The isolation of this compound from the plant indicated that the higher plant also synthesize same potential compounds like bacteria, which are antibacterial and antifungal. Though soil microorganisms or fungi produce most of the antibiotics, higher plants have also been a source of antibiotics [8]. Examples of these are the bacteriostatic antifungicidal properties of *Lichens*, the antibiotic action of allinine in *Allium sativum* (garlic) or the antimicrobial action of berberines of in goldenseal (*Hydrastis cndensis*) [8]. Although, the plant has been used to cure a number of ailments, so far, no report has been found about the use of the plant against tuberculosis. The plant can be of useful against tuberculosis. The isolation of **1a** from *Zanthoxylum oxyphyllum* thus provides a significant reference point for the therapeutic use of this plant and needs attention from the botanists, ethnopharmacologists and phytochemists.

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