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Phytochemical Investigation of Conical Prickles On The Stem Bark (Petroleum Ether Extract) Of *Zanthoxylum Rhetsa*

Dr Mity Thambi

Department of chemistry, Catholicate College, Pathanamthitta, Kerala

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Abstract: *Zanthoxylum rhetsa* commonly called Mullilam is a lofty, deciduous tree with pale corky bark covered with conical prickles on stems and branches. It is commonly found in the evergreen monsoon forests of the foothills of Assam and Meghalaya and in the Eastern and Western Ghats in peninsular India. The petroleum ether extract from *Zanthoxylum rhetsa* was analyzed by GC-MS. Out of the compounds identified long chain aliphatic esters were dominated. The compounds present in this fraction found to possess diverse biological properties from different plant origin.

Keywords: *Zanthoxylum rhetsa, Spathulenol, skimmianine, evodiamine*

INTRODUCTION

Zanthoxylum, commonly called “prickly ash” or Satin wood and also known as *Mangangtein*, is the largest genus in the family *Rutaceae* and comprises about 200 species of trees and shrubs with a worldwide, but predominantly tropical distribution. Traditionally different species of *Zanthoxylum* were used for the treatment of tooth ache, urinary and venereal diseases, and rheumatism. *Zanthoxylum* species are reported to have many medicinal properties and phytochemical studies on the genus have shown it to be a rich source of alkaloids, aliphatic and aromatic amides, lignans, coumarins, sterols, carbohydrate residues etc. Some of these are anticancer, molluscidal, anticonvulsant, anti-sickling, anesthetic, antibacterial, antihypertensive and anti inflammatory properties[1]. *Zanthoxylum rhetsa* is a lofty, deciduous tree, up to 35 m. tall, with pale corky

bark covered with conical prickles on stems and branches, commonly known as Mullilam found in shaded moist localities of tropical regions of India at an altitude upto 1,800 m. Commonly found in the evergreen monsoon forests of the foothills of Assam and Meghalaya and in the Eastern and Western Ghats in peninsular India. The fruits and stem bark of this plant are used traditionally as a stimulant, astringent, stomachic and digestive and prescribed for urinary infection, dyspepsia, heart troubles, tooth ache, asthma and bronchitis[2]. Paknikar and Pai[3, 4] reported that the plant also bears significant antinociceptive and antidiarrhoeal activities. Shafi and coworkers identified 118 compounds from the leaf essential oil of *Z.rhetsa*, and they described the aroma of the essential oil[5, 6]. Compounds isolated from *Z.rhetsa* include dihydroavicine, rhetsinine[8], N-methylflindersine, zanthobungeanine, dictamine, rutaecarpine, γ-fagarine, skimmianine, evodiamine , canthin-6-one[7], rhetsine, rhetine, chelerythrine[8,] and hydroxyevodiamine[9] from bark, arborine and dictamine from fruits[10] and rutaecarpine from seeds[11].

From the literatures on the genus *Zanthoxylum* it is very much evident that all the species belonging to this genus are rich sources of a variety of chemical compounds with exceptional attributes. Not many are thoroughly studied and there is much more to be explored regarding their chemistry and biological activity. This makes the plant more interesting and this study reasonable. The already reported compounds from *Z.rhetsa* mainly belong to the flavanoids, alkaloids and terpenes with interesting structural backbone and are found to possess remarkable properties. The plant show marked regional variations with respect to the compounds present in them, a novelty in the chemical profile or a change in the amount of useful molecules can always be expected. The conical prickles on the stem bark of the tree were selected for investigation. Such modifications on the exterior of the plants are expected to function in protection of plants against adverse situations including pathogen attack. Hence the chemical compounds present in them are likely to be biologically active even at lower concentrations. No work had been reported on the petroleum extract of conical prickles of *Zanthoxylum rhetsa*. The present work reports the components present in the petroleum ether extract of the conical prickles on the stem bark of *Zanthoxylum rhetsa*.

MATERIALS AND METHODS

All melting points were determined on Toshniwal melting point apparatus and are uncorrected. UV spectra were obtained in a JASCO UV spectrometer. IR spectra (KBr) were taken on a JASCO FT-IR spectrometer. GC-MS analysis was done by Varian 4000 GC-MS. The columns were prepared as slurry with suitable solvents and a gradient elution was carried out by mixing of solvents with different polarity. Silica gel G Merck for preparative thin layer chromatography using Stahl apparatus**.**

Plant Material: The prickles on the bark of the stem of *Zanthoxylum rhetsa* were collected from Calicut university campus, Malappuram district, Kerala. It was identified by Dr. A.K Pradeep, Department of Botany, University of Calicut. A voucher specimen was deposited in the Herbarium, Department of Botany, University of Calicut.

Extraction: The conical prickles collected were already in the dried form. Coarsely powdered 5kg of the plant material and extracted with 3x7L of light petroleum ether. The extraction was carried out in a round bottom flask by boiling the material in the solvent with a water condenser, over a water bath. Reflux the material until the solvent started to boil and the hot content was left standing overnight. Then filtered and collected the extract and added fresh solvent to residue. The process is repeated four times to complete the extraction.

Fractionation: The extract was adsorbed on 250gm silica gel. This was then loaded on a preparative column. (Dimension usually being 7cm x100cm; d x1) The column was packed with silica gel as the stationary phase which was wetted using petroleum ether to achieve least polarity to the mobile phase during the beginning of elution. The mobile phase for elution was fixed based on the TLC analysis. The elution was carried out by gradient elution technique; the gradation of the mobile phase polarity was achieved by homogenous mixing of the solvents with different polarity. The different fractions collected were again analyzed using TLC.

The petroleum ether extract - GC-MS analysis: The concentrated petroleum ether extract after removal of the solvent, yielded 40g of light yellow residue. This when dissolved in petroleum ether and adsorbed on silica gel. It was loaded on a preparative column using silica gel wetted with petroleum ether as the stationary phase. Mixture of petroleum ether and ethyl acetate in the ratio 8:1 gave TLC with different colored spots. This fraction, a light yellow colored waxy material (ZW), on TLC analysis was found to be mixtures of a large number of compounds and was analyzed by GC-MS.

RESULTS AND DISCUSSION

Characterization of ZW: Large numbers of spots were observed on TLC examination of ZW, hence it was subjected to GC-MS analysis. UV spectra of ZW showed characteristic peaks at 210, 232, 280 and 372 nm. In IR spectra characteristic peaks were seen at 3444 cm^{-1}, 2918.73 cm^{-1}, 2853 cm^{-1}, 1735 cm^{-1}, 1706.69 cm^{-1}, 960 cm^{-1}, 720.28 cm^{-1}. On GC-MS analysis of this fraction eleven aliphatic esters and two alcohols were found to be present. The components were identified by library search spectrum. The results of GC-MS analysis of ZW is given in **Table 1.**

Table 1: Composition of ZW

Sl.No	%	Compound
1	1.427	Methyl dodecanoate
2	0.5	Spathulenol
3	0.690	Humulane-2,6-dien-3-ol
4	4.584	Methyl 12-methyltridecanoate
5	10.788	Methyl pentadecanoate
6	10.073	Methyl 9-octadecanoate
7	4.293	Methyl 16-methylheptadecanoate
8	0.728	Methyl 8,11,14-docosatrienoate
9	0.646	Methyl eicosanoate
10	2.993	Methyl docosanoate
11	20.603	Methyl tetracosanoate
12	1.029	Methyl pentacosanoate
13	1.534	Methyl hexacosanoate

Out of the thirteen compounds identified from the GC-MS analysis the esters which is present in high percentage are methyl tetracosanoate (20.603%), methyl pentadecanoate (10.788%), methyl 9-octadecanoate (10.073%), methyl 12-methyltridecanoate (4.584%), Methyl 16-methylheptadecanoate (4.293%).The compounds present in the fraction previously reported from different plant sources possess diverse biological properties. These compounds were reported as flavoring agent, perfumery, anticancer agent and antibacterial activity. Methyl dodecanoate is used as a food additive permitted for direct addition to food for human consumption as a synthetic flavoring substance and adjuvant. Spathulenol inhibit the human ABCB1 efflux pump [12] and also seen in flavored and perfumery products. Spathulenol shows immunomodulatory effects on peripheral blood lymphocytes[13.] Methyl pentadecanoate which is used in the treatment of neurological disorders [14]. Methyl pentacosanoate which shows antibacterial activity[15.]

CONCLUSION

It can be concluded that the petroleum ether extract from the bark of Zanthoxylum rhetsa growing in Malabar region of Kerala shows different long chain esters. The esters and alcohol present in the extract shows different biological properties such as anticancer, antibacterial, flavouring agent and perfumery.

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*** Corresponding author: Dr Mity Thambi**

Assistant Professor, Catholicate College, Pathanamthitta, Kerala