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Original Article

EVALUATION OF PHYSICO-CHEMICAL PROPERTIES AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OBTAINED FROM THE FRUITS OF ZANTHOXYLLUM ACANTHOPODIUM DC. COLLECTED FROM MEGHALAYA, INDIA

M. MAJUMDER[1], H. K. SHARMA*[2], K. ZAMAN[2], W. LYNGDOH[3]

[1]Centre for Studies in Biotechnology, Dibrugarh University, Dibrugarh-786004, Assam, [2] Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam; [3] Lady Keane College, Shillong, Meghalaya, 793001.
Email: hemantasharma123@yahoo.co.in

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ABSTRACT

Objective: The fruits of the plant *Zanthoxylum acanthopodium* DC. have been used mostly in traditional purpose as spice, fish poison, treatment of abdominal colic and toothache etc. A study was carried out to extract the oil from the fruits and to study the physico-chemical, antibacterial activity of the oil.

Methods: The essential oil of the fruits of the plant was extracted by hydrodistillation method using Clevenger type apparatus. TLC and GC-MS study were carried out to characterize the oil. The antimicrobial activity of the extract was tested against *E. coli, Staphylococcus aureus, Bacillus subtalis* and *Streptococcus sp.*

Results: TLC characterisation of extracted oil sample with solvent system Hexane: Ethylacetate (4:1) as mobile phase reveals four (4) well separated spots. Analysis of the oil of *Z. acanthopodium* fruits by GC-MS resulted in the identification of 21 components. Among all the components, the predominant one is Eucalyptol (36.563%) followed by Limonine (16.903%), δ-3-carene (13.525%) and Methyl-cinnamate (9.366%).

Conclusion: The results demonstrated promising antibacterial activity and the extract was found to be more active against *Staphylococcus aureus* giving a wider zone of inhibition.

Keywords: Zanthoxylum acanthopodium, Fruits, Essential oil, Antibacterial activity, GC-MS, Meghalaya.

INTRODUCTION

Essential oils (EO) are natural in origin and have various medicinal and therapeutic properties for which it is used widely in pharmaceuticals. The plant *Zanthoxylum acanthopodium* DC. is an aromatic plant and the fruits have been used mostly in traditional purpose as spice, fish poison, treatment of stomachache and toothache etc and many more. The antibacterial properties of essential oils and their components are exploited in commercial products as dental root canal sealers [1], antiseptics [2] and feed supplements for lactating cows and weaned piglets [3]. Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition, insecticides [4]. Essential oils are used as flavoring agents. Flavors are added to food to enhance their taste and aroma. Essential oils and their terpene constituents may be accepted natural alternative to synthetic skin penetration enhancers. They are characterized by their relatively low price and promising penetration enhancing activities. Their toxicities are found to be relatively low compared with most synthetic penetration enhancers [5]. The rapid development of pathogen resistance to most of the known antibiotics is becoming a serious health problem. In view of this fact, the development of effective and potent antimicrobials from a natural source is an area of immense significance for Pharmaceutical scientist.

The state Meghalaya is rich in flora and report on the traditional herbal practices of Meghalaya highlighted the richness of the flora [6]. Traditional practices based on herbs exists among various tribes in Northeast India. Such practices based on herbs also exist among the tribes in the nearby areas of Meghalaya [7].

Literature survey revealed that very little work has work has been carried out on this plant growing in India. Previous study reported the chemical analysis of leaf oil [8]. We have already reported the isolation, chemical characterization of fruit oil and phytochemical screening of the aqueous extract of Zanthoxylum acanthopodium [9]. In the present study we have reported the detailed physico-chemical analysis of its fruit oil.

MATERIALS AND METHODS

Plant material and extraction [10, 11]

The ripe fruits of the plant *Zanthoxylum acanthopodium* DC., Family-Rutaceae was collected from Shillong (Meghalaya) during the month of January 2013 and cleaned properly before use. The fruits were crushed with a morter-pastle and EO was extracted by using Clevenger type apparatus by hydro-distillation method. The oil was dried with anhydrous sodium sulphate and stores in an airtight container at 0-4°C before doing analysis.

Physico-chemical properties of EO

The colour, odour and solubility of the EO in organic solvents were checked manually and the refractive index of the oil sample was determined using Abbes Refractometer.

Thin layer chromatography (TLC) characterization of EO

Glass plates of 5 × 20 cm size were coated with silica gel G to a layer thickness of 0.25 mm, dried and activated at 110°C for 30 min. 2-5 µl of the oil samples were spotted using capillary tube and the spot area was kept 2 cm above the base of the plate. The solvent system used for the EO sample was Hexane: Ethyl acetate in the ratio of 4:1 at room temperature (25°C) and inclining the plates at an angle of 750 in the chromatographic chamber. After completion of run the plates were removed from the chamber and allowed to dry in air. These plates were sprayed with freshly prepared Vanillin-Sulphuric Acid [0.5% Vanillin in Sulphuric acid- Ethanol (4:1)] and heated at 100°C for 5 min for the presence of the spots and the Rf values of the spots were calculated from the ratio of distance traveled by solute and distance traveled by solvent.

Gas chromatography-mass spectrometry (GC-MS) analysis of EO

The GC-MS analysis of the extracted oil was performed on a Parkin Elmer Clarus 680/600 chromatogram with build in auto sampler

using a fused DB-5 capillary column [length 30 m × 0.25 mm internal diameter (ID), film thickness 0.25 µm], equipped with a Elite-5 MS capillary column and FID detector. The oven temperature was programmed from 50° -260° C at a rate of 5°C/min. The injector temperature was set at 250°C and the injection volume was 1.0 µL. The run time was 46 min. the gas used was helium with a flow rate of 1.1 mL/min [12].

Antibacterial susceptibility testing of EO

Antimicrobial susceptibility testing was performed by the determination of zone of inhibition of bacterial growth in Mueller-Hinton agar after application of a specified amount of the EO sample. Gram-negative bacteria *Escherichia coli* and gram-positive bacteria *Staphylococcus aureus, Bacillus subtalis* and *Streptococcus sp.* were obtained from Centre for Studies in Biotechnology, Dibrugarh University for the test and were performed by "disc diffusion method" devised by Kirby and Bauer. Among all the media available, Mueller-Hinton agar is considered to be the best for routine susceptibility testing. Immediately after autoclaving, Mueller-Hinton agar was allowed to cool to 45-50°C. 25 ml of this sterilized media was poured into previously sterilized petri dishes (90 mm Diameter) to get a uniform depth of approximately 4 mm.

Working broth culture was prepared by inoculating 5 ml nutrient broth with pure stock cultures and incubated in a shaker incubator at 37°C until it was achieved the turbidity of the 0.5 McFarland standards. Within 15 minutes after adjusting the turbidity of the inoculums suspension, inoculums were added to the solidified media in the Petri plates and were spread gently with the help of a sterilized glass spreader to disperse the microorganisms homogenously. The previously impregnated discs were placed onto the surface of the inoculated agar plates ensuring complete contact with the agar surface. Each test plate comprised of four discs containing one standard (ciprofloxacin), one vehicle control (Pet ether) and two different concentrations of the EO sample. The plates were than incubated in an inverted position at 37°C for 18 hours. Then zone of inhibition was measured to the nearest whole millimeter [13-15].

RESULTS

Physicochemical properties of EO

Physicochemical properties such as colour, odour, taste, solubility and refractive index of the extracted oil were determined. After proper observation, the EO of the fruit was found colorless and have a characteristic odour and pungent taste with anesthetic and burning sensation. The oil was found soluble in acetone, chloroform, DMSO, ethyl acetate, hexane, methanol, petroleum ether and toluene. Refractive index was found to be 1.437 at 21°C (Table 1).

TLC of the EO

TLC was produced with the aim of identifying the individual substances in the oil and also for testing purity of the separated mixtures. The Rf values indicates the position at which a substance is located in a chromatogram. It is appropriate to regard R_f value as a guide for identification of compounds. The results are shown in Table 2.

GC-MS analysis of EO

Analysis of EO of *Z. acanthopodium* fruit by GC-MS resulted in the identification of 21 components of the major peaks determined by Gas Chromatogram. The results of the GC-MS analysis is depicted in Fig1 and the identified compounds are tabulated in Table 3.

Antimicrobial Susceptibility Testing

The result in Table 4 showed that the EO has varying antimicrobial activity against all the bacterial strains tested. The EO was found to be more active against *Staphylococcus aureus* (Gram +ve) showing wider zone of inhibition. There was no inhibition of growth in vehicle control (i.e. Petroleum ether). The demonstration of activity against both gram-positive and gram-negative bacteria is an indication that the plant can be a source of bioactive substances that could have a broad spectrum of activity.

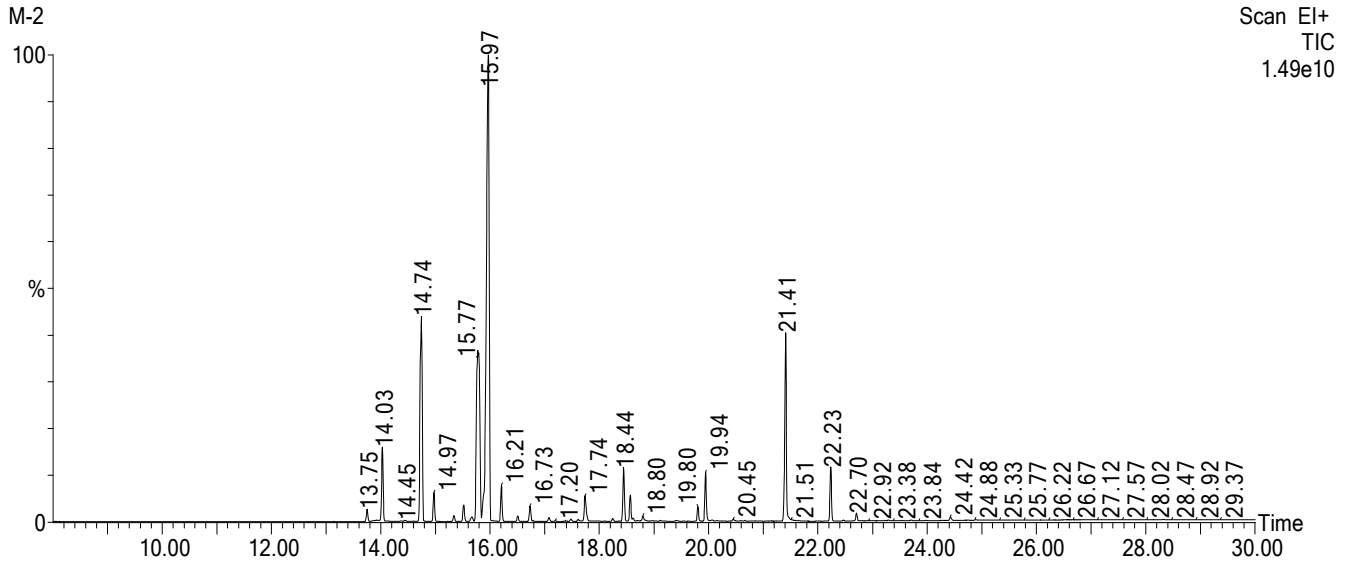

Fig. 1: GC-MS Chromatogram of EO of *Z. acanthopodium* fruit

Table 1: Physicochemical properties of EO

Sl No	Parameters	Result
1	Colour	Colourless
2	Odour	Characteristic un-pleasant odour
3	Taste	Characteristic pungent taste with anesthetic and burning sensation
4	Solubility	Soluble in Acetone, Chloroform, DMSO, Ethyl acetate, Hexane, Methanol, Petroleum ether and Toluene.
5	Refractive index	1.437 at 21^{0}C

Table 2: TLC profile of Essential Oil (EO)

Mobile phase	No. of Spots	R_f value
Hexane: Ethyl acetate (4:1)	4	0.93,0.76,0.63, 0.50

Table 3: Identified components (%) of Essential oil (EO)

Peak No.	Components	RT (min)	%Area
1	α-thujene	13.751	0.548
2	α-pinene	14.031	3.466
3	comphene	14.446	0.048
4	δ-3-carene	14.741	13.525
5	β-Terpinene	14.971	1.327
6	α-phellandrene	15.336	0.291
7	α-terpinene	15.516	0.803
8	Limonine	15.777	16.903
9	Eucalyptol	15.967	36.563
10	γ-terpinene	16.207	1.563
11	β-terpineol	16.507	0.260
12	Ocimine	16.732	0.790
13	α-thujol	17.737	1.503
14	(-) Terpineol	18.443	2.484
15	Estragole	18.568	1.141
16	Myrtenyl acetate	19.803	0.646
17	Anethole	19.943	2.209
18	Citronellyl proponoate	20.448	0.103
19	Methyl-cinnamate	21.409	9.366
20	β-caryophyllene	22.234	2.460
21	α-caryophyllene	22.704	0.373

Table 4: Determination of zone of inhibition of Essential oil (EO)

S. No.	Microorganism used	Zone of Inhibition (mm)		
		Direct EO	50% diluted EO	Standard Ciprofloxacin
1	*Escherichia coli*	13mm	11mm	27mm
2	*Staphylococcus aureus*	16mm	12mm	28mm
3	*Bacillus subtalis*	11mm	10mm	27mm
4	*Streptococcus sp.*	11mm	10mm	19mm

DISCUSSION

The percentage yield of volatile oil in the fruits of Zanthoxylum acanthopodium obtained by hydrodistillation was 0.35% which is quite high as compared to leaf oil (0.2%) as reported by Rana et al. The nauseating aromatic odour and burning anaesthetic taste of the fruit oil is very characteristic to this species which is uncommon properties than other essential oil of plant origin. The GC-MS analysis revealed 21 identified compounds out of 32 traceable peaks in the gas chromatogram. The components were identified based on the elution pattern on DB-5 column and comparing with library data. Among all the components, the predominant one is 1,8- cineol (36.563%) followed by Limonine (16.903%), δ-3-carene (13.525%) and Methyl-cinnamate (9.366%).

The oil was found to contain predominantly monoterpenes (90.48%) and trace of sesquiterpines (9.5%). There was more amount of monoterpene hydrocarbons (63.16) than oxygenetad monoterpene (36.84%). The fruit oil from Indonesian plant reported to contain geranyl acetate as main constituents [16], while linalool was reported as major compound in the leaf oil from Indian plant [8]. The fruits oil exhibited varying degree of antibacterial activity against both Gram positive and Gram negative bacteria however it showed maximum activity against *Staphylococcus aureus.*

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