SUPPLEMENTARY MATERIAL

Chemical composition and Antimicrobial activity of fatty acid methyl ester of *Quercus leucotrichophora* fruits

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Abstract

Natural fats and dietary oils are chief source of fatty acids and are well known to have antimicrobial activities against various microbes. The chemical composition and antimicrobial activities of fatty acids from fruits of white Oak (*Quercus leucotrichophora*) are yet unexplored and therefore the present study for the first time determines the fatty acid composition and the antibacterial and antifungal activities of fatty acid methyl esters (FAME) of the white Oak plant found along the Himalayan region of Uttarakhand, India. The GCMS analysis revealed the presence of higher amount of saturated fatty acids than unsaturated fatty acids. FAME extract of fruits of *Q. leucotrichophora* demonstrated better antibacterial activity against Gram-positive bacteria than the Gram-negative bacteria. The present studies clearly establish the potential of the fruits of *Q. leucotrichophora* for use in soap, cosmetics and pharmaceutical industries.

Keywords: White Oak, Fatty Acid Methyl Esters, *Quercus leucotrichophora*, antibacterial activity, antifungal activity

1. EXPERIMENTAL

1.1 Collection and identification of plants material

The fruits of *Q. leucotrichophora* were collected from Nagnath Pokhari, District Chamoli Garhwal, Uttarakhand, India. The plant was identified from Taxonomy Laboratory, Department of Botany, H. N. B. Garhwal University, Srinagar Garhwal, Uttarakhand and the voucher specimen (GUH8835) was kept in the Departmental Herbarium.

1.2 Extraction

The air dried fruits of the plant were exhaustively extracted with 85% aqueous ethanol repeatedly until the extractive became colorless. All the ethanolic extracts were mixed together and concentrated under reduced pressure in a rotary evaporator. The ethanolic extract was fractionated with hexane and ethyl acetate using soxhlet's apparatus. The hexane extract was concentrated and studied for fatty acid analysis.

1.3 Preparation of methyl ester

The extraction and methylation procedure was based on that describe by Jenkins and coworkers (1977) with some modifications (Jenkins, Kuhn & Daly, 1977). Hexane extract (10g) was treated with 10ml of alkaline methanol [5g NaOH, 50ml H₂O, 50ml MeOH] for 12-14 hours at 104-110°C, the residue was removed by centrifugation and the unsaponified material was removed with three 15 ml portions of n-hexane after acidification to pH 1.5 with sulfuric acid. The fatty acids were extracted with four 15 ml portions of n-hexane, dried over anhydrous sodium sulfate and concentrated by reducing the volume to 2-3ml. The fatty acids were refluxed with 10ml 2 N methanolic H₂SO₄ at 60°C for 3 hours. After addition of 10ml distilled water on reaction mixture the fatty acid methyl esters (FAME) were extracted with 4, 10ml portions of n-hexane dried over anhydrous sodium sulfate and concentrated by reducing the volume up to 4-5ml. The fatty acid methyl esters were stored at 3-4°C prior to analysis.

1.4 GC-MS analysis of FAMES

Qualitative and quantitative analysis of fatty acid methyl esters (FAME) was performed on Perkin-Elmer make Clarus-500 GC-MS equipped with data handling system. Analytical condition were Perkin-Elmer[®] Phase Elite-I (Crossbond 100% dimethyl polysiloxane) capillary column (60m×0.25mm, film thickness 0.25 µm), injector and detector temperatures were 210°C and 280°C, respectively while the helium was used as carrier gas. Oven temperature was held for 5 min at 50°C with 5 min solvent delay, then programmed at 3°C /min up to 220°C /min, and then maintained isothermally at 220°C for 20 min. GC-MS was operated in EI mode at 70 eV. Constituents were identified on the basis of comparison of

linear retention indices of extracted samples with those of authentic samples and by comparison of their mass spectral fragmentation patterns matching against the commercial library mass spectra (NIST, Pfleger, Wiley, etc.) and home-made library mass spectra generated from pure substances and components of known volatile components.

1.5 Antimicrobial activity

Two strains of Gram-positive bacteria (*Bacillus subtilis*, and *Staphylococcus aureus*) and two strains of Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were used for evaluation of antimicrobial activity.

1.5.1. Antimicrobial susceptibility assay

In vitro antifungal activity was determined by using antifungal assay agar and Sabouraud's dextrose agar (obtained from Himedia Ltd., Mumbai, India). The selected bacteria/yeasts (24h culture) were mixed with physiological saline and the turbidity was adjusted to a Mac Farland turbidity standard of 0.5. The agar diffusion method was used for antibacterial susceptibility tests (Baur, Kirby, Sherris & Turck, 1966). Plates were prepared by pouring freshly prepared Mueller Hinton agar for bacteria into petri plates and allowed to solidify, to which 0.1ml of standardized inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 5 min, then the discs were placed on the inoculated agar. The content was thoroughly mixed and then allowed to solidify. The test solution was prepared with known weight of crude extracts, dissolved in 5% dimethyl sulphoxide. Ciprofloxacin (5µg/disc) was used as standard for antibacterial activity. The experiments were carried out in triplicate. The plates were incubated at 37°C/24h (Oyedemi & Afolayan, 2005). At the end of incubation, zone of inhibition was measured in all the plates.

1.5.2. Minimum inhibitory concentration (MIC) Assay

The MIC method was performed to quantify the antimicrobial efficacy against tested microorganisms. The highest dilution of a plant extract that still retained an inhibitory effect against the growth of a microorganism is known as MIC (Misra & Dixit, 1978). Minimum inhibitory concentration of the FAME extract was tested in Mueller Hinton broth for bacteria by two fold serial dilution method. The test extract was dissolved in 5% DMSO to obtain 4mg stock solutions. 0.5mg of stock solution was incorporated into 0.5ml of Mueller Hinton broth for bacteria to get a concentration of 2, 1, 0.5, 0.25, 0.125 and 0.06mg/ml, 50µl of standardized suspension of the test organism were transferred to each tube. A control tube, containing the microorganisms but not the FAME extract, was also prepared. The culture tubes were incubated at 37°C for 24h.

Hexadecanoic acid

9,12-Octadecadienoic acid methyl ester

6-Octadecenoic acid methyl ester

14-Methylpentadecanoic acid methyl ester

Fig S1: Chemical structure of fatty acids of *Quercus leucotrichophora* fruits

Fig S2: Chromatogram of fatty acid methyl ester of *Quercus leucotrichophora* fruits

References

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