

RESEARCH ARTICLE

Isolation and characterization of major phytoconstituents from the leaves of *Rhizophora mucronata* Lamk and *Acanthus ilicifolius* Linn.

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ABSTRACT

Rhizophora mucronata Lamk and *Acanthus ilicifolius* Linn, which are classified as true and associate mangrove respectively by botanical survey of India and as mangrove by others, are a tree and small flood tolerant shrub respectively. The present investigation deals with the characterization of major compounds purified from the leaves of *Rhizophora mucronata* and *Acanthus ilicifolius*. Results obtained by LCMS, FTIR and ¹HNMR studies indicated that purified compound from *Acanthus ilicifolius* contained 2S,3R,6S)-2-((4-(3-ethyl-4,5-dihydroxyphenyl)-5,7-dihydroxy-2-(hydroxymethyl)-6-methyl-1, 2, 3, 4-tetrahydronaphthalen-1-yl)methoxy)-4-hydroxy-6-(hydroxymethyl)-3,6-dihydro-2H-pyran-3-yl 3,4-dihydroxy-2-methoxybenzoate) and *Rhizophora mucronata* contained (R)-hexane-3-ol.

Keywords: *Rhizophora mucronata*; *Acanthus ilicifolius*; LCMS, UPLC, FTIR, ¹H NMR.

INTRODUCTION

Mangroves are part of a marginal ecosystem, able to tolerate extreme environmental factors such as high salinity and constant inundation (Vannucci, 2001).

There are about 59 species of mangroves and few mangrove associates are found in India. In west coast of India 34 species belonging 21 families are represented *Acanthus ilicifolius* and *Rhizophora mucronata* are being classified as an associate mangrove and true mangrove by Botanical survey of India (Banerjee *et al.*, 1989) and as a mangrove by others (Agoramoorthy *et al.*, 2007). Mangroves and mangrove associates possess novel agrochemical products, compounds of medicinal value, and biologically active compounds. (Bandaranayake, 2002). Extracts from different mangrove plants and mangrove associates are active against human and plant pathogens (Chandrasekara *et al.*, 2009). Mangroves plants contain number of phytochemicals that have disease preventive properties; they are a rich source of saponins, alkaloids, and flavonoides (Bandaranayake, 1995). A number of mangroves and associates contain substances, which show biological activities such as antiviral, antibacterial and antifungal properties.

Leaves of *Acanthus ilicifolius* contained Protein, Alkaloids, Resin, Steroids, Tannins, Glycosides, Reducing sugar, Carbohydrates, Saponins, Steroids, Sterols, Terpenoids, Phenol, Cardioglycosides and Catachol (Kokpol,

1984; Madhu and Madhu, 1997; Islam *et al.*, 2012). *Rhizophora mucronata* plant contained alkaloids, condensed and hydrolysable tannins, flavonoids, proteins, saponins, steroid, triterpens and flavonoids (Basak *et al.*, 1996; Madhu *et al.*, 1997, Rohini *et al.*, 2009; Nurdiani *et al.*, 2012).

Acanthus ilicifolius has traditionally been used for treatment of skin diseases, small pox, ulcers, snake bite and rheumatism. Its antiviral, antioxidant and anticarcinogenic activity has been demonstrated recently. *A. ilicifolius* shows significant analgesic activity.

Rhizophora mucronata has been used to treat heamaturia, diarrhea, diabetes, angina, hemorrhage, inflammation. Its anti-HIV activity has been demonstrated (Kirtikar *et al.*, 1987; Bandarnayake, 2002; Das *et al.*, 2009; Rohini *et al.*, 2009; Premanathan, 1999).

MATERIALS AND METHODS

Rhizophora mucronata (Lamk.) and *Acanthus ilicifolius* Linn plants were identified in the Botany laboratory of the Institute of science, Mumbai. Leaves of above test plants were collected in October / November from their wild growing stands from the selected sites. The fresh leaves of *Rhizophora mucronata* Lamk. and *Acanthus ilicifolius* Linn were washed with tap water and shade dried at room temperature ($28 \pm 2^\circ\text{C}$). The dried leaves were powdered in an electrical blender, and stored in clean dry and sterile jars until further use. The major phytochemical of *Acanthus ilicifolius* and *Rhizophora mucronata* were qualitatively analyzed and purified by using preparative HPLC. The reagents and solvents used were of reagent grade quality and were obtained from S. D. fine. Three fractions were eluted from the *Acanthus ilicifolius* out of which one major compound was selected for characterization. In *Rhizophora mucronata* out of two fractions eluted, one was selected for the study.

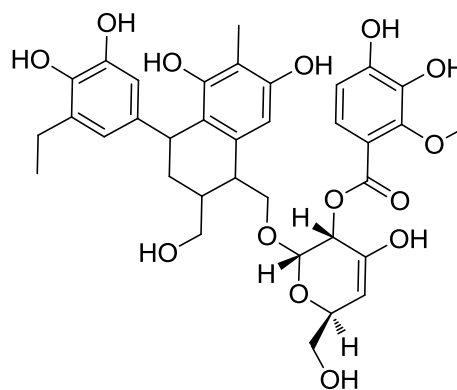
Structural elucidations were based on analyses of spectroscopic data by using LCMS or UPLC, FTIR and ^1H NMR. The reactions were monitored by HPTLC using on 0.25mm E-Merck silica gel 60 F₂₅₄ pre coated plates, which were visualized under U.V light. The LCMS were recorded on water Aquity SQD (UPLC) and MUX LCMS. The I.R spectra were recorded on a Perkin-Elmer Spectrum 100 FTIR Spectrometer using KBr discs. ^1H NMR spectra were recorded on NMR spectrometer 400 MHz instrument using Bruker Advance 400 MHz.

RESULTS AND DISCUSSIONS

The methanolic extracts from the leaves of *Rhizophora mucronata* and *Acanthus ilicifolius* were subjected to the preparative HPLC when three compounds from *Acanthus ilicifolius* and two compounds from *Rhizophora mucronata* were obtained.

One of the major compounds from *Acanthus ilicifolius* was selected and interpreted using LCMS, FTIR and ^1H NMR. The said compound's molecular weight was estimated to be 684.24 (Fig.1). Inspection of the IR spectrum indicated the presence of the hydroxyl stretching at 13369.41 cm^{-1} , Amide str. at 1747.39 cm^{-1} , Aromatic alkenes str. at $1604.66, 1595.02, 1579.59, 1575.73\text{ cm}^{-1}$, alkynes str. at 2923.88 cm^{-1} and ether str. at $1076.21, 1020.27\text{ cm}^{-1}$ (Fig. 2).

The ^1H NMR spectrum exhibited protons corresponding to an aromatic ring at δH 7.80 (2H), 7.49 (2H), 7.26 to 7.19 (4H), 6.74 (1H), 6.65–6.61 (2H). Three protons for methyl group at δH 1.13, in addition to 3.68 (3H) and 3.51 (3H) proton for methoxy group (Fig. 3).



Possible structure of the compound isolated from *Acanthus ilicifolius* leaves.

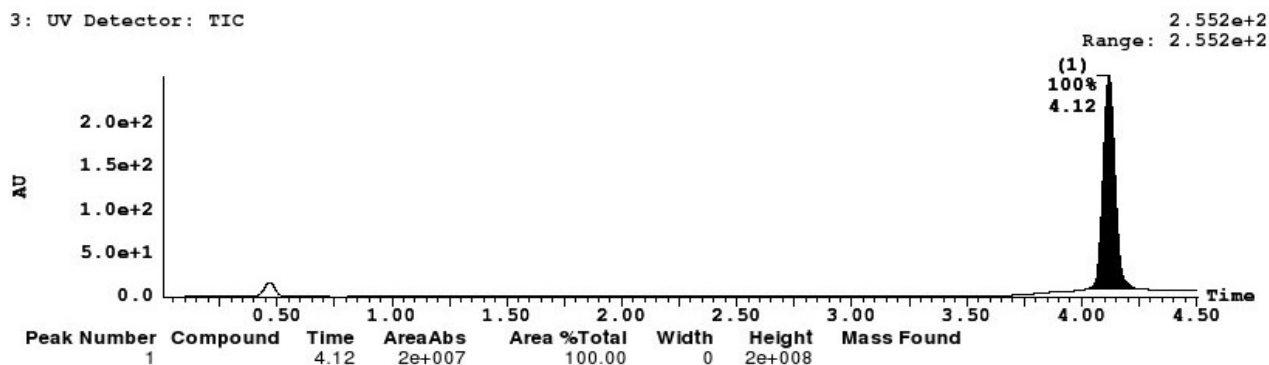
LCMS, FTIR and NMR were used for the characterization of Benzoxazinoids from *Acanthus ilicifolius* plants by Wahidulla and Bhattacharjee (2001) and by Huo Chang (2005) and lignin glycosides by Kanchanapoom *et al.* (2001)

The selected compound from *Rhizophora mucronata* was also interpreted by using UPLC, FTIR and ^1H NMR. The compound's molecular weight was estimated to be 102.7. (Fig. 4) Inspection of the FTIR spectrum indicated the presence of the hydroxyl stretching at 3300.7 cm^{-1} and 1380.2 cm^{-1} and carboxyl structure at 1460.5 cm^{-1} (Fig. 5).

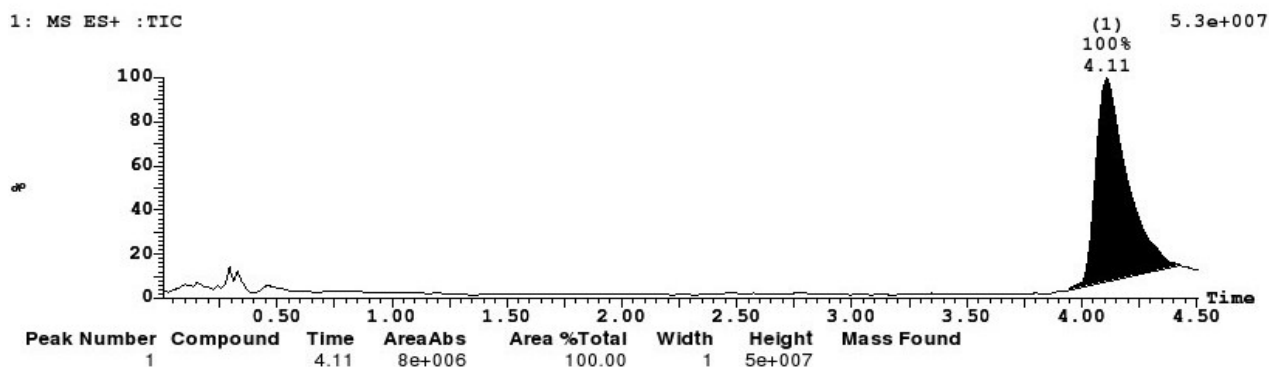
Sample Report:

Sample 10 Vial 1:G,7 ID *Acanthus ilicifolius* Peak 1 File MS1_18 MAY 2012_A10 Date 18-May-2012 Time 22:36:53 Description Sanj

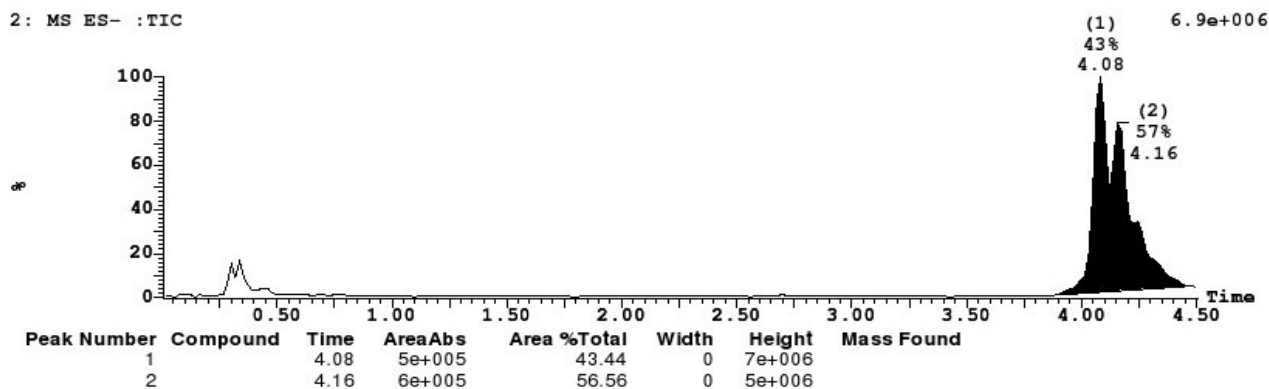
3: UV Detector: TIC



1: MS ES+ :TIC



2: MS ES- :TIC



Peak ID	Compound	Time	Mass Found
1		4.11	

1: (Time: 4.11)

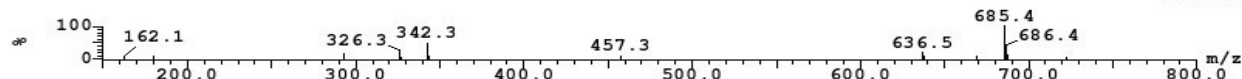
1: MS ES+
6.0e+006



Peak ID	Compound	Time	Mass Found
1		4.11	

1: (Time: 4.12)

2: MS ES-
1.0e+006



Peak ID	Compound	Time	Mass Found
2		4.16	

2: (Time: 4.16)

2: MS ES-
8.1e+005

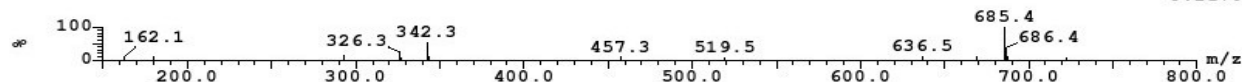
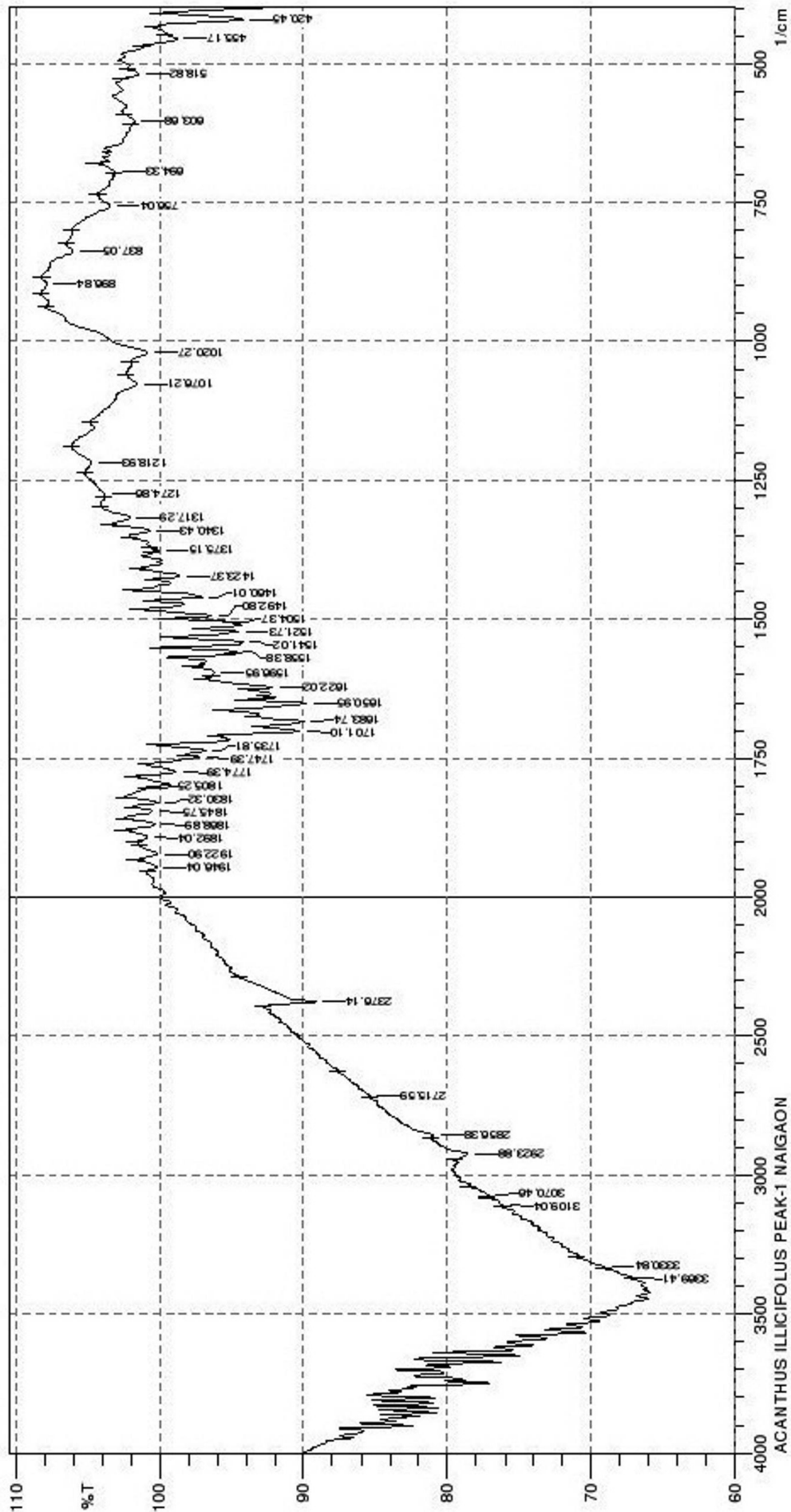


Fig. 1 Lcms Spectrum of the compound isolated from the leaves of *Acanthus ilicifolius*

Fig. 2. IR Spectrum of the compound isolated from the leaves of *Acanthus illicifolius*



Sample Name : ACANTHUS ILLICIFOLUS

Date/Time; 5/22/2012 9:19:19 PM

No of Scans : 32 Resolution : 4.0

Fig. 3: ¹H NMR Spectrum of the compound isolated from the leaves of *Acanthus ilicifolius*

Acanthus ilicifolius Naigoan_Peak-I in DMSO

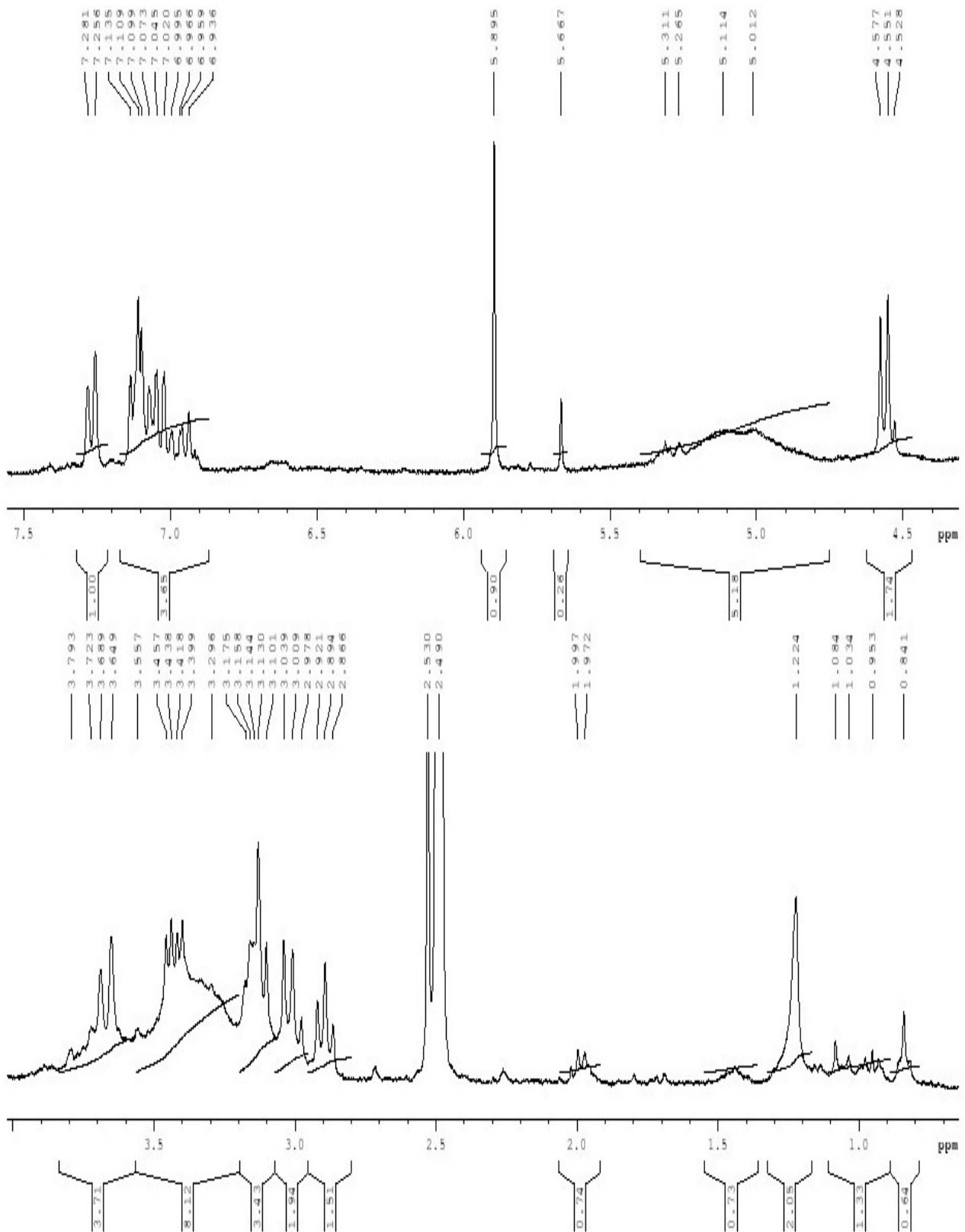


Fig. 4 UPLC spectrum of the compound isolated from the of leaves of *Rhizophora mucronata*

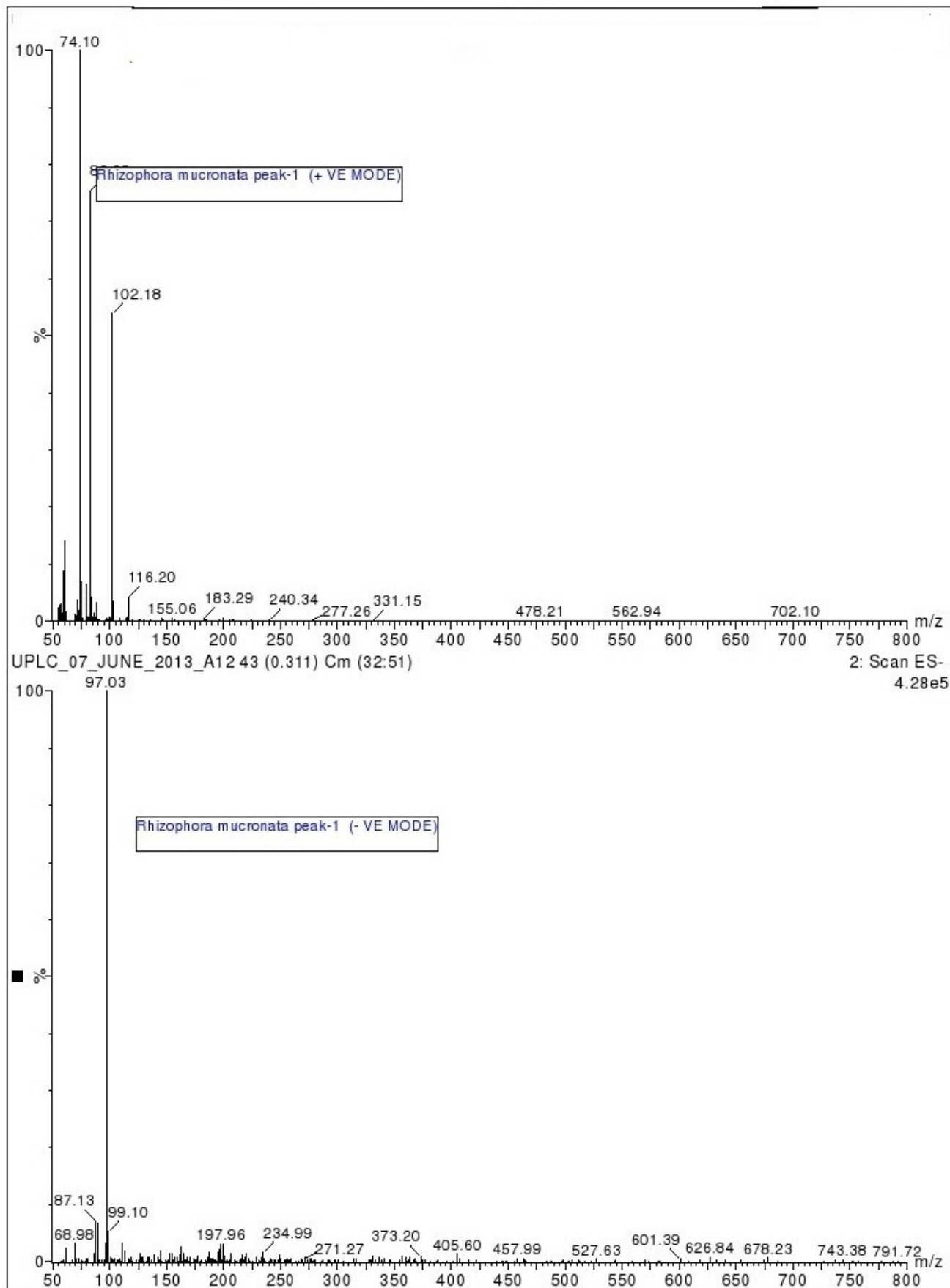
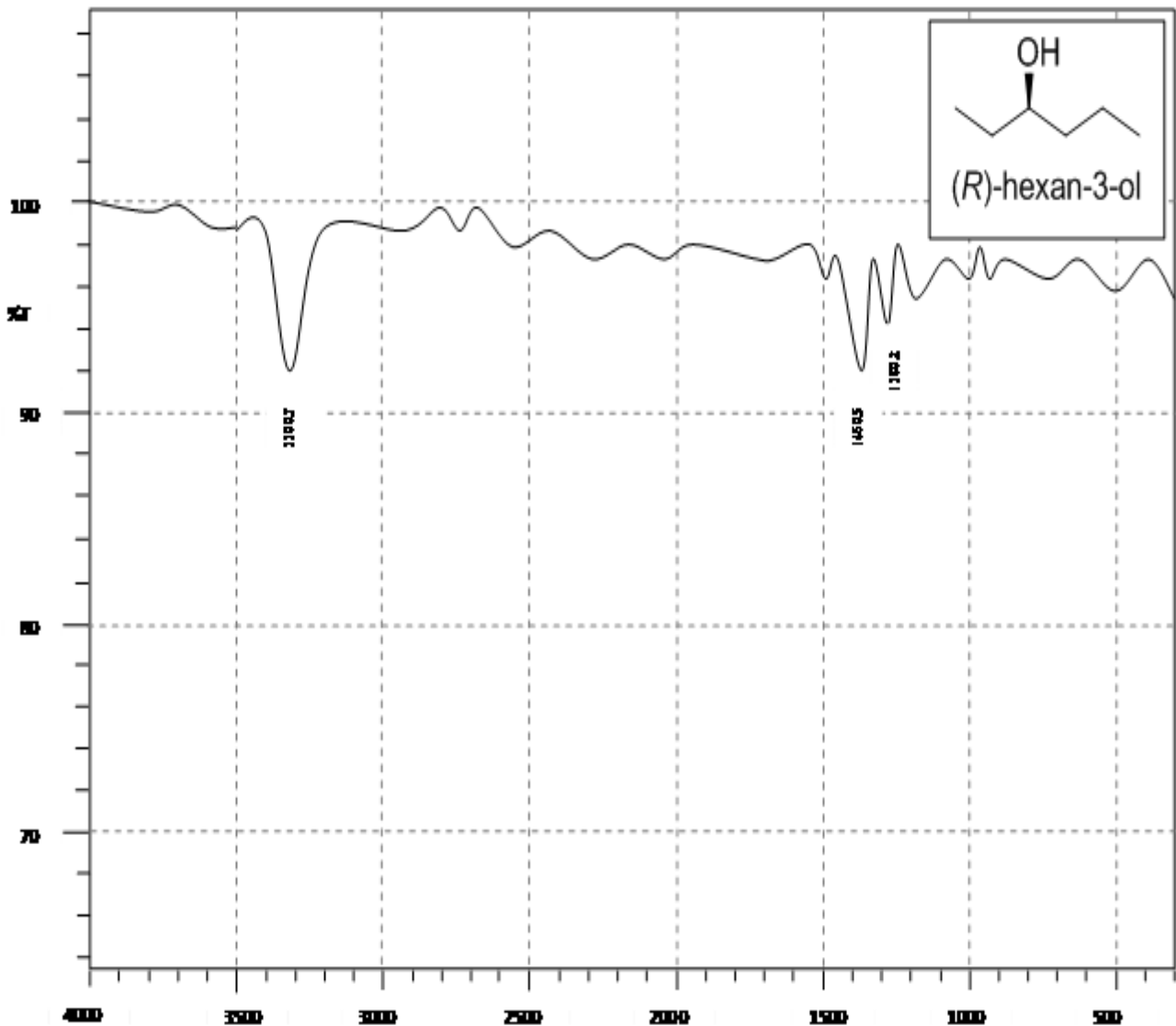


Fig. 5: IR spectrum of the compound isolated from the of leaves of *Rhizophora mucronata*

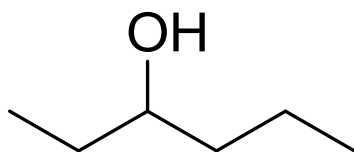


Sample Name: *Rhizophora mucronata*

Date/Time: 11/11/2012 4:43:28 PM

No of Scans: 32 Resolution: 4.0

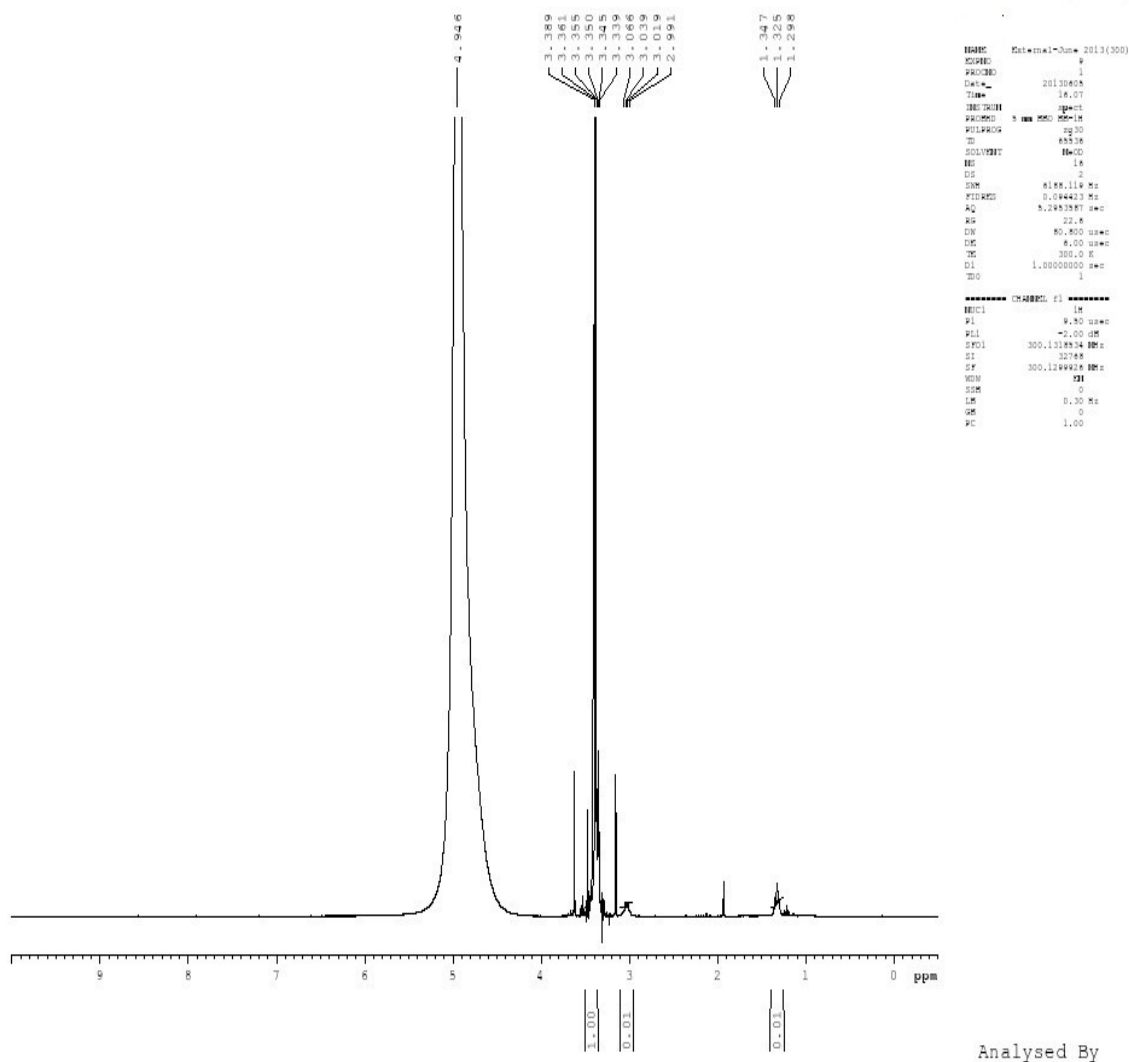
The ¹H NMR spectrum exhibited protons corresponding to methyl group at δH 1.32(6H), methylene group at 1.93 (6H), methane at 3.03 (1H) and hydroxyl group at 3.3(1H) (Fig. 6)



Possible structure of the compound isolated from *Rhizophora mucronata*

All the above data indicated that the said compound per IUPAC nomenclature could be (R)-hexane-3-ol. The structure of the same is being depicted as below.

HPLC method for purification and isolation and H and C NMR for characterization of compound in the bark of *Rhizophora apiculata* plant was also used by Rahim *et al.*, (2008).

Fig. 6: H NMR spectrum of the compound isolated from the leaves of *Rhizophora mucronata*

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