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ISOLATION AND CHARACTERIZATION OF PROCATECHUIC ACID, GALLIC ACID AND 10-OCTADECENOIC ACID, METHYL ESTER FROM METHANOLIC EXTRACT OF BORASSUS FLABELLIFER LEAVES

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ABSTRACT

The aim of this study was to identify and characterize the bioactive constituents from methanolic leaf extract of *Borassus flabellifer* Linn. In the previous study, several steroidal saponins, poly saccharides and triterpenes were isolated from the fruit pulp and seeds, young shoots of *Borassus flabellifer* contains gum, albuminoids, fats and the fresh pulp contains vitamin A,B and C, the inflorescence contains borassoside, dioscin and also contains sucrose ,bitter compound flabelliferins. The present study was undertaken to explore the potential bioactive constituents from *Borassus flabellifer* leaves which have been evaluated using IR, MASS, GC-MS, NMR spectral studies for proper characterization and elucidation of structure. Three compounds were isolated and characterized. The compounds were concluded that procatechuic acid, gallic acid and 10-Octadecenoic acid,methyl ester.

KEYWORDS: Procatechuic acid, Gallic acid, 10-Octadecenoic acid,methyl ester, *Borassus flabellifer*.

1. INTRODUCTION

Borassus flabellifer L. belongs to the family Arecaceae, commonly known as Palmyra palm is a native of tropical Africa but cultivated and naturalized throughout India. The different parts of *Borassus flabellifer* are being used for medicinal properties. Trees, upto 30 m tall, Trunk straight, thick, dark or black colored with crown of leaves. Leaves large, fan shaped, up to 3m, cleft into many lobes, petioles long with hard, spinescent, serratures at margins flowers unisexual, Fruits a drupe, trigonous when young,(sub) globous when mature ;seeds are composed of white fleshy pulp, gradually harden on maturity.[1-2] In the previous study, several steroidal saponins, poly saccharides and triterpenes were isolated from the fruit pulp and seeds, young shoots of *Borassus flabellifer* contains gum, albuminoids, fats and the fresh pulp contains vitamin A,B and C, the inflorescence contains borassoside, dioscin and also contains sucrose ,bitter compound flabelliferins.[3] Plant yields a black gum, a good source of Vitamin B complex, extract in stroke, head ache, earache, epilepsy, scabies, syphilis, ulcers, vomiting. Bark used as dentifrices, flowers in uterus tumors. Fruits as tonic for asthmatic patients, gonorrhea and given in gas troubles. Roots used as cooling medicine and restorative, diuretic and anthelmintic, in gastritis.[4]

The purpose of the study is to identify and characterize the bioactive compounds from the leaves of *Borassus flabellifer*. In this paper we report that isolation and characterization of compounds known as procatechuic acid, gallic acid and 10-Octadecenoic acid, methyl ester.

2. MATERIALS AND METHODS

2.1Authentication

Borassus flabellifer leaves were collected from Perambalur district in Tamilnadu and authentication of the above species was carried out by Thirumala college of Pharmacy, Nizamabad, AP.

2.2 Extraction and isolation

The coarsely powdered drug (500g) was taken for extraction. The methanolic extract of Borassus flabellifer was prepared by using soxhlet apparatus. The crude extract was evaporated to dryness in a water bath to give dark brown mass. The methanolic extract around 80 gm was subjected to column chromatography on silica gel (60-120 mesh) using varying polarities, starting from Chloroform and Methanol to yield several fractions. The column was eluted firstly with pure chloroform and the methanolic extract was subjected to the column for isolation. The column eluted with chloroform and methanol (95:5, 90:10, 80:20 and 50:50). The elute from different solvents were concentrated to give dry residues. The obtained residues were further purified by

rechromatogram by using pure solvent chloroform. The samples were confirmed by using TLC, by their melting point, IR NMR and MASS spectroscopy.

2.3 Spectroscopic characterization

Different spectroscopic methods were used to elucidate the structure elucidation of isolated compounds. Among the spectroscopic techniques IR, ^{1}H NMR ,^{13}CNMR, MASS and GC-MS were carried out.

The infrared spectra were recorded on PPERKIN ELMER SYSTEM ONE FTIR/ATR (Model: Spectrun one: FT-IR Spectrometer) Scan range 1-o cm^{-1} with globar and mercury vapour lamp.

The mass spectra were recorded by using the GEOL GCMATE II GC-MS double focusing instrument, the maximum resolution 6000 and maximum calibrated mass: 1500 Daltons and source options are electron impact and chemical ionization.

The 1HNMR and 13CNMR spectra were recorded by using Bruker AVANCE III 500 MHz (AV500 MHz) multinuclei solution NMR Spectrometer with 11.7 Tesla super conducting long hold magnet, activity shielded with standard bore (narrow bore: 5-5.4 cm) built in cryoshims, 34 channel room temperature shims, amplifier power 300W, HP Work station with LCD TFT monitor and Windows XP based TOPSPIN-2 software with an additional software for processing. The IR, MASS and NMR spectral studies were carried out in Sophisticated Analytical Instrumental Facility (SAIF), Indian Institute of Technology Madras**.,** Chennai. Melting points were measured by SRS Optimelt MPA 100 instrument.

3. RESULTS AND DISCUSSION

Two compounds were shown from polar fraction of methanolic extract of *Borassus flabellifer*. The two compounds were separated from Chloroform: Methanol (8:2). Further separated compounds were confirmed by using TLC method. The solvent system used for the separation is Ethyl acetate: Benzene (9:11), the R_f values were found to be 0. 40 and 0.44 for gallic acid and 3, 4 dihydroxy benzoic acid (Procatechuic acid) respectively shown pink colour spots when sprayed Vanillin sulphuric acid reagent. The spectral details are as follows. The isolated compound gave blue colour with Ferric chloride reagent.

Compound1

A nearly white coloured fine powder with melting point 220 to 230. It is soluble in water.

IR Spectroscopy

The absorption band Fig 1 shows the following frequencies are 3401-(OH) broad band, 2934 along with broad band, 1634 (C=O) corboxylate.1416, 1249(C=O), 597(O=C-H).

MASS

The M+ ion peak is 154.12; other fragmentation peaks are 141, 137, 125, 119, 113, 109, 101, 97, 91 and 81. The results are shown in Fig 2.

^{1}H-NMR

The proton NMR peaks are 7.419, 7.415, 7.387, 7.384, 7.372, 7.368, 6.935 and 6.919.

7.419 chemical shift of 1H. , 7.415, 1H 2-6 coupling and 6.935, 1H 5-6 coupling. The results are shown in Fig 3.

^{13}C-NMR

The ^{13}C- NMR spectrum Fig 4 shows the following peaks.

188.36, 165.46 (C-7), 156.29 (C-4), 141.30 (C-3),139.08 (C-5), 135.41(C-1) 116.19 (C-2), 106.98, 105.88, 104.80, 55.58, 40.46, 40.39, 40.30, 40.13, 39.96, 39.80, 39.63, 39.46.

Compound 2

IR Spectroscopy

The absorption band Fig5 shows the following frequencies:

3383-(OH) broad band, 2923 along with broad band, 1652 (C=O) corboxylate.1403&1260(C=O), (O=C-H).Other peaks are 1543, 1058, 679 and 568.

MASS

The M+ ion peak is 170.12 and other m/z peaks are 162, 154, 144, 137, 125, 119, 113, 109, 97 and 91. The results are shown in Fig 6.

^{1}H-NMR

The proton NMR peaks are 9.44(OH, 2H), 8.885(OH, H), 5.007, 8.003, 7.981, 7.945, 7.941, 7.439, 7.199, 7.196, 6.894, 6.890, (aromaticity, 2H), 5.764, 3.73 and3.693. The results are shown in Fig 7.

^{13}C-NMR

The ^{13}C- NMR spectrum Fig 8 and the 13CNMR peaks are 188.60 (C-6) ,165.47 (C-7), 156.13 (C-4), 148.16 (C-3), 135.31 (C-1) 129.54, 116.63 (C-2), 104.98.

Compound 3

Two to three drops of oil obtained from *Borassus flabellifer* [Chloroform: Methanol (80:20)] fraction. The compound was tested by GC-MS. The Fig 9 shows the GC-MS of oil. There are 4 peaks of retention times are 11.27, 12.23, 13.38 and 14.87, The 11.27 peak indicates the major constituent of the oil. The Fig 10 shows the GC MS spectra of compound 3 10-Octadecenoic acid, methyl ester.

All spectral data were compared with earlier reported studies.[5-8]

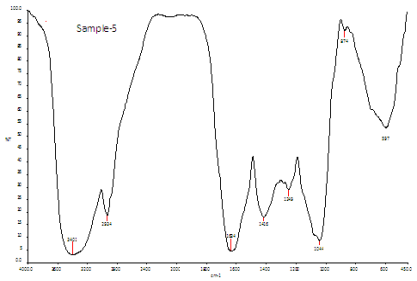


Fig 1: IR Spectrum of compound 1.

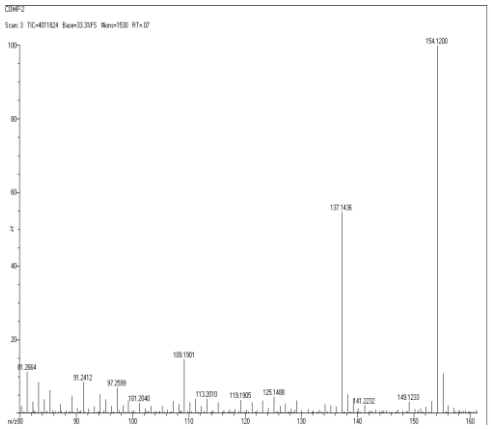


Fig 2: Mass spectrum of compound1

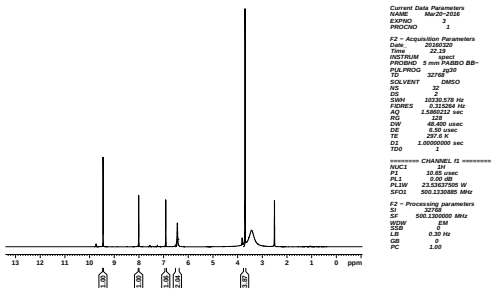


Fig 3: H-NMR spectrum of compound 1

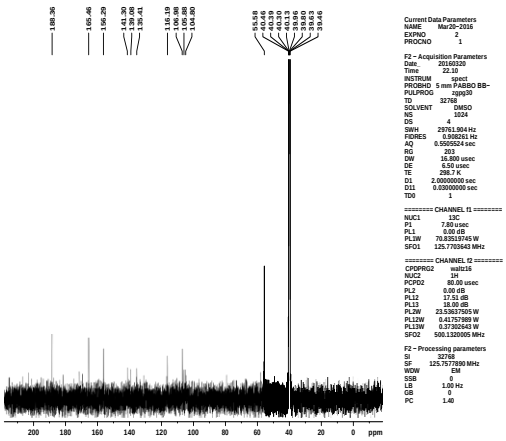


Fig 4: C-13 NMR Spectrum of Compound 1

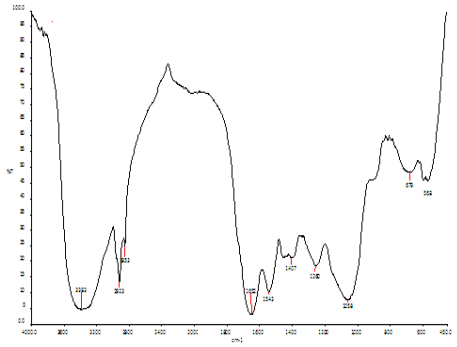


Fig5: IR spectrum of Compound 2

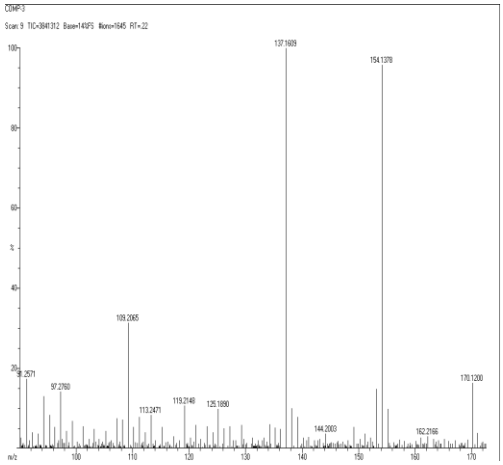


Fig 6: Mass spectrum of Compound 2

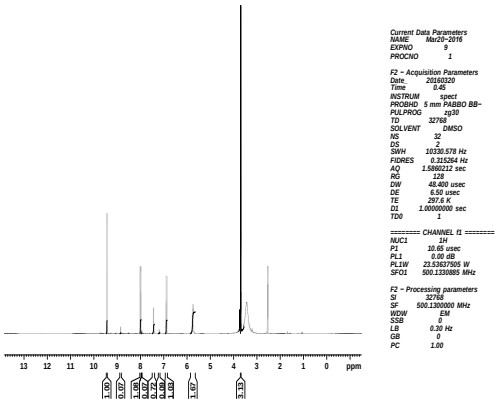


Fig 7:H NMR spectrum of Compound 2

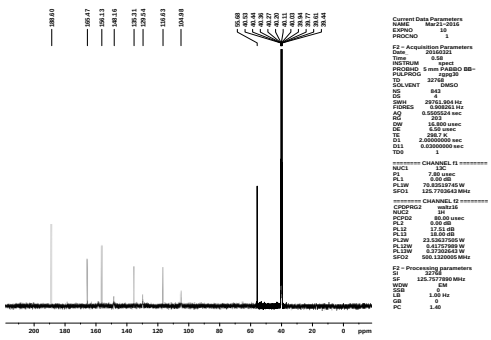


Fig 8: C 13 NMR spectrum Compound 2

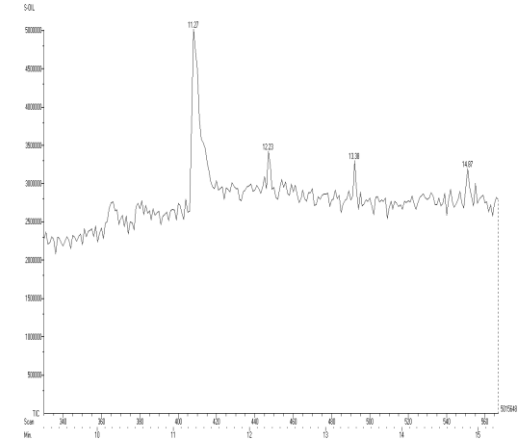


Fig 9: GC-MS of Oil

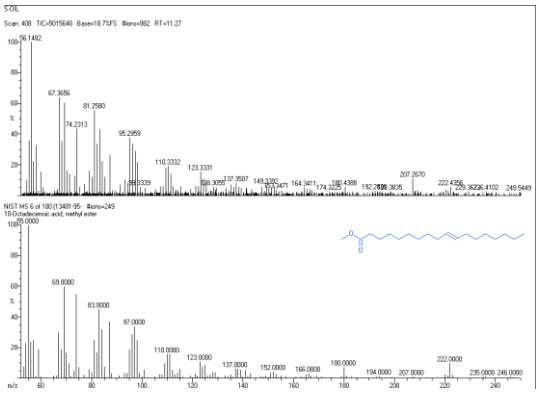
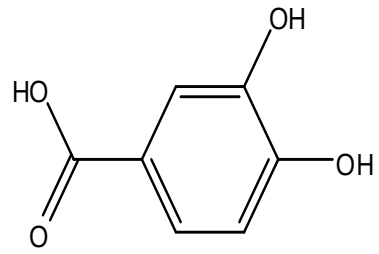
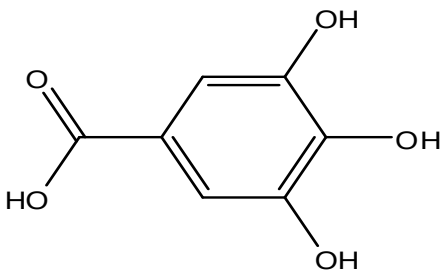


Fig 10: GC-MS of Compound 3

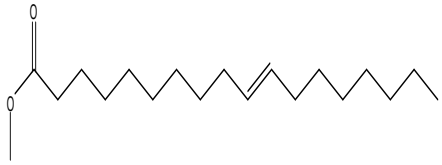
Structure of Isolated compounds



3,4-dihydroxy benzoic acid



3,4,5-trihydroxy benzoic acid



10-octadecenoic acid, methyl ester

4. CONCLUSION

3,4 Di hydroxy benzoic acid (Procatechuic acid), 3,4,5 Tri hydroxy benzoic acid (Gallic acid) and 10-Octadecenoic acid, methyl ester were isolated and characterized from methanolic extract of *Borassus flabellifer* leaves. From the above spectral details concluded that the two compounds are tannins, compound 1 is 3, 4 Dihydroxy benzoic acid (Procatechuic acid) and compound 2 is 3, 4, 5 Trihydroxy benzoic acid. Two to three drops of oil obtained from *Borassus flabellfer* [Chloroform: Methanol (80:20)] fraction. The compound was tested by GC-MS. The Fig 9 shows the GC-MS of oil. There are 4 peaks of retention times are 11.27, 12.23, 13.38 and 14.87, The 11.27 peak indicates the major constituent of the oil. The Compound3 (10=octadecenoic acid,methyl ester) is isolated from oil, the fig 10 shows the GC MS of compound 3.

5. ACKNOWLEDGEMENT

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