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ISOLATION AND CHARACTERIZATION OF PALMITIC ACID, 2-(TETRADECYLOXY) ETHYL ESTER AND BETA SITOSTEROL FROM METHANOLIC EXTRACT OF BAUHINIA PURPUREA LEAVES

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ABSTRACT
The aim of this study is to identify and characterize the bioactive compounds from methanolic leaf extract of *Bauhinia purpurea*. The literature survey of *Buhinia purpurea* revealed that it contains major classes of secondary metabolites are glycosides, flavanoides, saponins, triterpenoids, phenolic compounds, oxepins, phytol, leutin, sterols, monoterpenes quercetin, quercetrin, rutin, apigenin, astralagin, isoquecetin, fat, protein, carbohydrates and 15% oil, with linoleic, palmitic , stearic acid and oleic acids. The plant has wide folk medicinal use in traditional medicine. The air dried leaf powder was pulverized to powder and subjected to soxhlet extraction using methanol as solvent and compounds were isolated by using column chromatography. The isolated compounds were subjected to spectral studies such, IR, MASS, NMR (H-NMR& C-13 NMR) and GCMS for proper characterization and elucidation of the structure. The compounds were concluded that Palmitic acid, 2-(tetradecyloxy) ethyl ester and beta sitosterol.
KEYWORDS: Betasitosterol, Palmitic acid 2-(tetradecyloxy) ethyl ester, Sterols, Bauhinia purpurea.

1. INTRODUCTION

Bauhinia purpurea Linn belongs to the family Fabaceae. It is known as Mandharai in Tamil. It is used in the indigenous system of medicine. Bauhinia species are small to medium size tree growing 17 meter tall.[1] *Bauhinia purpurea* contains major class of secondary metabolites are glycosides, flavanoides, saponins, triterpenoids, phenolic compounds, oxepins, phytol, leutin, betasitosterol, monoterpenes, etc.[2] *B.Purpurea* has been traditionally used by the Malaysian, Indian, Sri Lankan and Pakistani people to treat ailment like ulcer, wound, glandular swelling and stomach ulcer. The decoction of the root is used for expelling gases, flatulence and gripping pain from the stomach and bowel, the bark of the plant is used as an astringent in the treatment of diarrhoea. Its decoction of flower is used as laxative.[3] Plant used in dropsy, convulsion, intoxication and tongue blackness.[4]

The purpose of the study is to identify and characterize the bioactive compounds from the leaves of *Bauhinia purpurea*. In this paper we report that isolation and characterization of compounds known as beta sitosterol and palmitic acid 2-(tetradecyloxy) ethyl ester.

2. MATERIALS AND METHODS

2.1Authentication

Bauhinia purpurea leaves were collected from Mathura district in UP and authentication of the above species was carried out byTirumala College of Pharmacy, Nizamabad, AP.

2.2 Extraction and isolation

The coarsely powdered drug (500g) was taken for extraction. The methanolic extract of *Bauhinia purpurea* 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}C-NMR, MASS and GC-MS were carried out.

2.4 Methods
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
Compound1
A slightly yellow colored oily like semisolid compound, melting point 26 was isolated from methanolic extract of *Bauhinia purpurea* leaves with solvent system Chlorofom: Methanol (9:1) by column chromatography.

FT-IR Spectroscopy
IR absorption spectrum showed absorption peaks at 3373cm^{-1} ester,(S,)2925cm^{-1}, Alkene (S), 2854cm^{-1} (M), 1609 & 1511cm^{-1}(S), carboxylic acids, other peaks 1446, 1374, 1274. 1214, 1155, 1114, 1075, 890, 837, 805, 775, 716 and 636 cm^{-1}

The results are shown in Fig 1.

GC-MS
The GC-MS spectra showed the following peaks. The m/z ion peak is 310, the fragmentations are: 286, 276, 238,227, 212,200,185,160, 148, 124, 82 and 57.

The peaks are matching with the data from the GC-MS NIST library. The following peaks shown as 300, 283,257, 239,225,194, 182, 155, 141, 125, 97, 82 and 57. The results are shown in Fig 2.

^{1}H-NMR
The chemical shift values obtained from proton NMR were 5.1, 3.78, 3.74, 3.73, 3.69, 3.65, 3.51, 3.4 and 3.2 ppm. The results are shown in Fig 3.

^{13}C-NMR
The13C-NMR signals obtained from NMR Spectrum were 188.73, 168.89, 168.5, 165.48, 164.91, 156.05, 155.91, 135.25, 135.22, 129.45, 116.81, 105.09, 103.68, 55.73, 52.36, 40.41, 40.24, 40.08, 39.91, 39.74, 39.58 and 39.41ppm. The results are shown in Fig 4. The compound is confirmed from the above spectral data as palmitic acid, 2-(tetradecyloxy) ethyl ester.

Compound 2
Three compounds were separated from methanolic extract from *Bauhinia purpurea*. The TLC shown two spots in the mixture were separated by using Chloroform: Methanol (97:3) solvent system. Further these compounds were confirmed by TLC method. The solvent system is Hexane: Acetone (3:1) by using 10% H_2SO_4 as detecting agent. The R_f value matches with the compounds beta-sitosterol 6.2, stigmasterol 6.0 and lupeol 6.3. Melting point of these compounds were found that 139,165 and 217 viz .beta sitosterol, stigmasterol and lupeol. The compound betasitosterol was confirmed by further spectral characterization.

FT-IR Spectroscopy
The resulted spectral graphs are presented in Fig 5. The IR spectral analysis revealed that a broad peak at 3395cm^{-1} (S) indicating the presence of OH group. The peaks at 2924& 2853cm^{-1} indicating the presence of C=O and C-O stretching respectively. The peak at 1075cm^{-1} indicating cycloalkane. Other peaks are 1736, 1645, 1461, 1408, 1167, 887, 721, 604 cm^{-1}. These data are compared with the literatures which are reported earlier.

MASS
The MS of this compound suggested that the molecular mass is 414 (Molecular formula $C_{29}H_{58}O$) having characteristic fragments observed at m/z414, 390, 368,346, 334, 310, 297, 279,254,212, 197, 169, 153, 136, 118, 108 and 93.The results are shown in Fig 6.

^{1}H-NMR
The proton NMR spectrum 7 which are exhibited a broad triplet at 6.560, 6.551 and 6.541 and multiplet at 3.79, 3.73 and 3.70 corresponding to H-6 olefinic proton and H 30 proton respectively. Rest of the protons appeared in the high field region in between 2 to3 ppm. The figure 7 shows the NMR spectra of the compound 2.

^{13}C-NMR
The ^{13}C- NMR spectrum Fig.21 shows the following peaks.

188.77, 165.44, 164.03, 158.48, 156.02, 135.25, 129.38, 116, 110.61, 105.15, 55.74, 40.41, 40.33, 40.24, 40.16, 40.07, 39.91, 39.74, 39.57 and 39.41. The spectra shown in figure 8.

All spectral data were compared with earlier reported studies.[5-10]

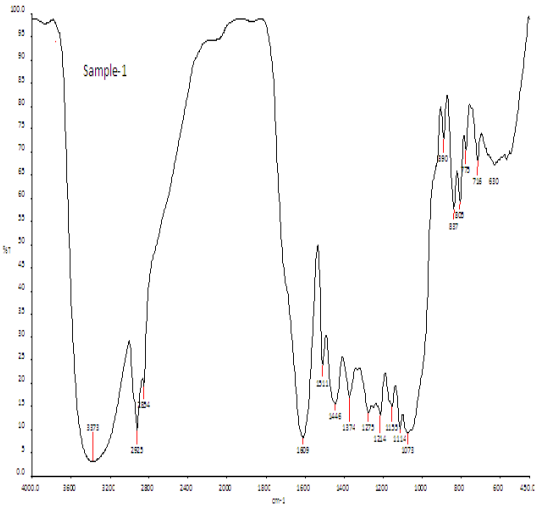


Fig 1: IR Spectrum of compound 1.

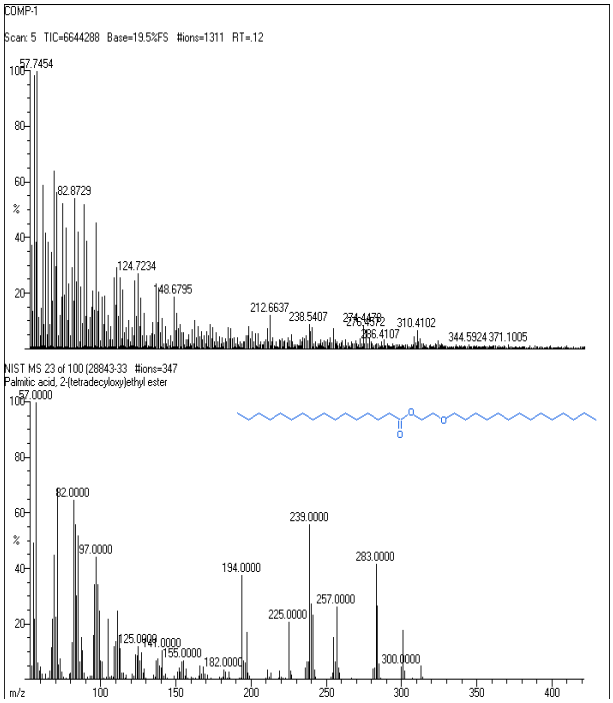


Fig 2: GC-MS of Compound1

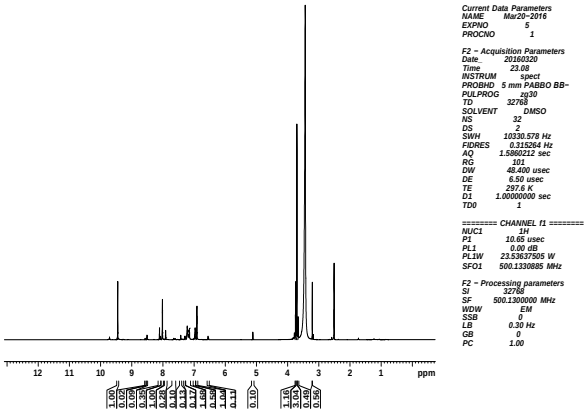


Fig 3: H-NMR xpectrum of compound1

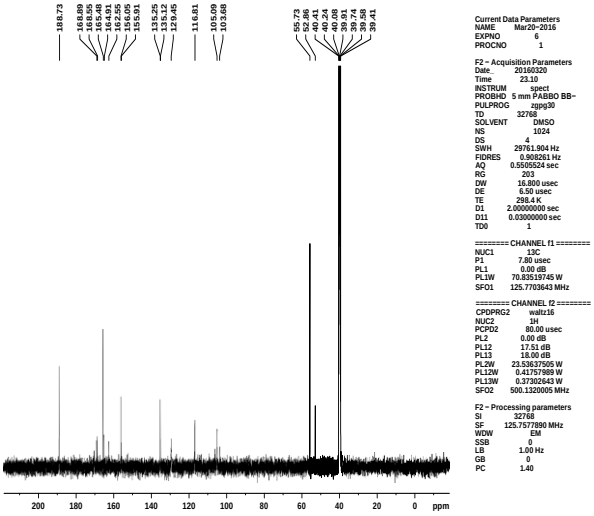


Fig 4: C-NMR of compound 1

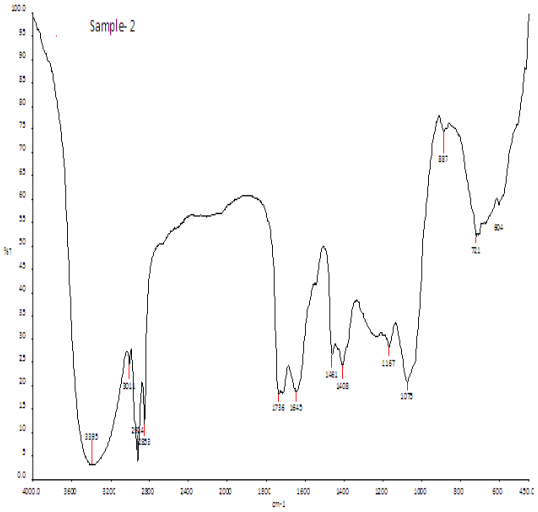


Fig 5: IR Spectrum of compound 2

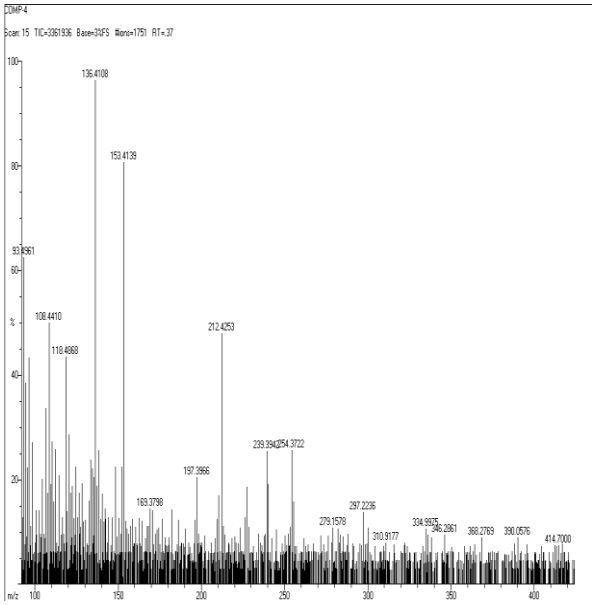


Fig 6: MS of the compound 2

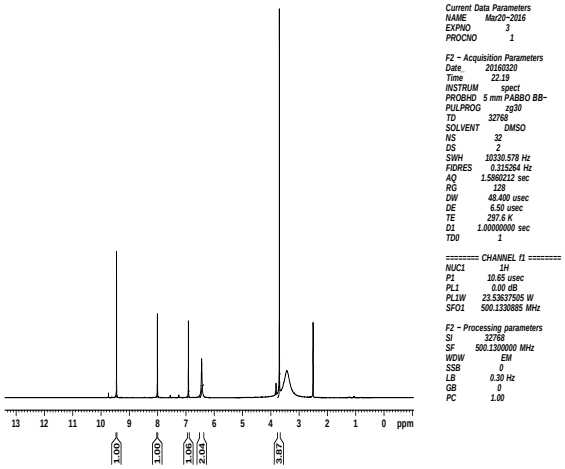


Fig 7: H-NMR Spectra of compound 2

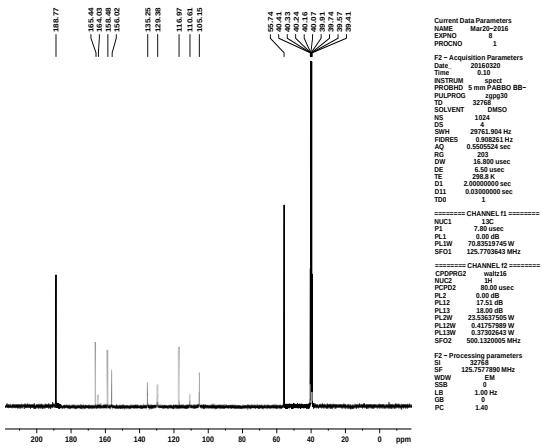
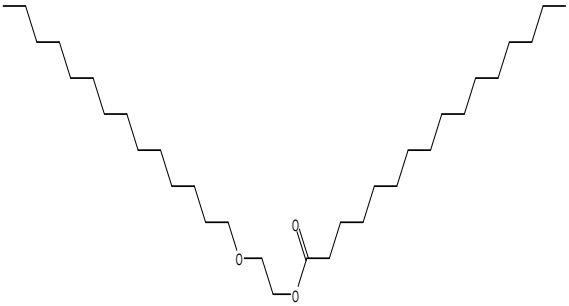


Fig 8: C-13 NMR Spectra of Compound 2

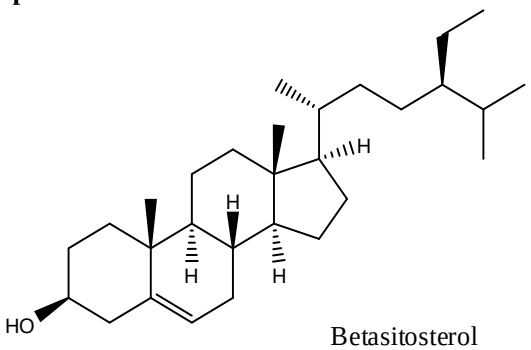
Structures of Isolated compounds

Compound 1



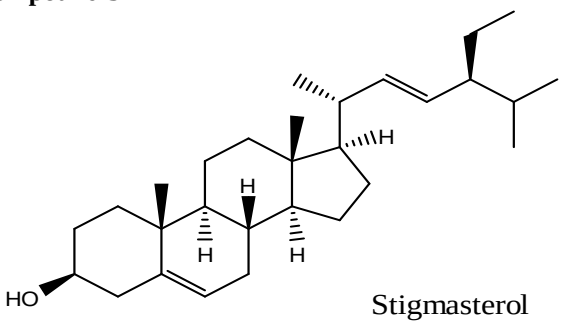
Palmitic acid, 2-(tetradecyloxy)ethyl ester

Compound2



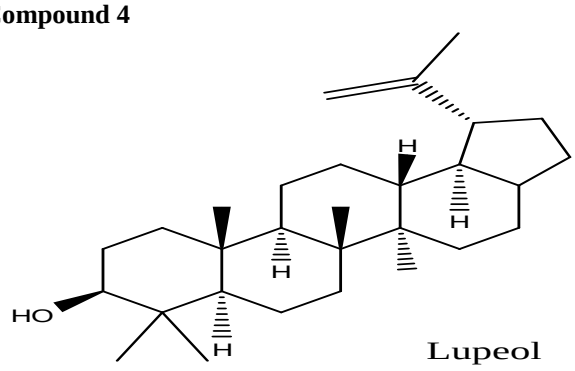
Betasitosterol

Compound 3



Stigmasterol

Compound 4



Lupeol

4. CONCLUSION

The GC-MS result of compound 1 is matching with NIST Library data. MS of this compound 2 suggested

that the molecular mass is 414. The proton NMR spectrum which exhibited a broad triplet at 6.560,6.551 and 6.541 and multiple at 3.79, 3.73 and 3.70 corresponding to H-6 olefinic proton and H 30 proton respectively. From the above information we concluded that the compound1 is palmitic acid, 2-(tetradecyloxy) ethyl ester and the compound 2 is beta sitosterol. Another two compounds 3 and 4 were confirmed by TLC using n-Hexane: Acetone (3:1) having R_f value of 6.0 was confirmed as Compound 3 as stigma sterol and the compound containing Rf value 6.3 was confirmed as compound 4 as lupeol.

5. ACKNOWLEDGEMENT

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