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Isolation and characterisation of *Pterocarpus santalinus* heartwood extract

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ABSTRACT

Recently there has been increasing interest in plants and plant derived compounds as medicinal agents. Bioactive compounds typically occur in small amounts and have more subtle effects. These bioactive compounds influence cellular activities that modify the risk of disease rather than prevent deficiency diseases. Many important bioactive phytocompounds have been extracted and identified from the heartwood of Pterocarpus santalinus. A wide array of biological activities and potential health benefits of Pterocarpus santalinus have been reported, including antioxidative, antidiabetic, antimicrobial, anticancer, antiinflammatory and protective effects on the liver, gastric mucosa, and nervous system. All these protective effects were attributed to bioactive compounds present in Pterocarpus santalinus. Pterocarpus santalinus Linn. heartwood was extracted with ethanol and blackish brown residue was obtained and percentage yield of was found to be 15% w/w. Pterocarpus santalinus Linn. heartwood extract was subjected to qualitative analysis for various phytochemical constituents and revealed the presence of glycosides, flavonoids, alkaloids, tannins, phenols, saponins and sterols. The extract was subjected to column chromatography for isolation of compounds and characterized by ^{1}H-NMR, ^{13}C-NMR and LC-MS Spectral analysis. Based on the spectral data's, the proposed structure of the compound was characterized as 6-Hydroxy-7-methoxycoumarin with $C_{10}H_8O_4$ and the melting point 204^{o}C was confirmed by comparison of its spectral data with reported values.

Keywords: *Pterocarpus santalinus*, Nuclear Magnetic Resonance, Mass spectrometer, Column chromatography.

INTRODUCTION

Herbs have been playing major role in curing various diseases [1]. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain [2]. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has often been reported [3]. *Pterocarpus santalinus* Linn. belongs to the family Fabaceae, commonly known as Red Sandal wood, Red Sanders [4] and widely distributed in India and china[5]. Various pharmacological activity of these plant has be showen by the researches such as; heartwood is rubbed with water, honey, ghee and oil applied as collyrium to alleviate defects of vision; Treat skin diseases; bone fracture, leprosy, spider poisoning, scorpionsting, hiccough, ulcers, general debility and metal aberrations; Wood paste is applied on boils and other skin eruptions, infections, inflammation, forehead to relieve headache; Decoction of fruits is used to cure chronic dysentery and to check dermatological conditions including psoriasis; Wood and bark brew taken orally relieves chronic dysentery, worms, blood vomiting, weak vision

and hallucination; Wood powder is used to control hemorrhage, bleeding piles and inflammation; The antibacterial, anticancer, hepatoprotective, diaberes and wound healing properties of this drug have been established recently [6]. The phytochemical analysis of *Pterocarpus santalinus* Linn. heartwood showed many active constituents such as Santalin, β-sitosterol, pterocarpol, pterocarptriol, ispterocarpolone, pterocarpo-diolones with β-eudeslol and cryptomeridol [7]. Though the species has more number of active principles and increasing demand. Hence the present study is to reviews about active principles present in ethanolic extract of *Pterocarpus santalinus* Linn. heartwood.

MATERIALS AND METHODS

Collection and authentication of plant material

The *Pterocarpus santalinus* Linn. heartwood was collected from Tirupathi, Andhra Pradesh, India and authenticated by Dr. Madhava Chetty, Asst Prof, Botany Dept, Sri Venkateshwara University, Tirupati.

Preparation of *Pterocarpus santalinus* Linn. heartwood extract

Pterocarpus santalinus Linn. heartwood powder (500 gm) was defatted with petroleum ether and successively extracted further with ethanol in a Soxhlet extractor.

Preliminary phytochemical investigations of the *Pterocarpus santalinus* Linn. heartwood extracts [8]

Pterocarpus santalinus Linn. heartwood extract were subjected to qualitative analysis for various phytochemical constituents.

Isolation and characterization of *Pterocarpus santalinus* Linn. heartwood extract.

The extract were subjected to column chromatography for isolation of compounds and characterized by ^{1}H-NMR, ^{13}C-NMR and LC-MS Spectral analysis [9].

RESULTS

Preparation of *Pterocarpus santalinus* Linn. heartwood extract

The percentage yield of ethanol extract was found to be 15% w/w.

Preliminary phytochemical analysis of *Pterocarpus santalinus* Linn. heartwood extract

The ethanol extract of *Pterocarpus santalinus* Linn. heartwood revealed the presence of glycosides, flavonoids, alkaloids, tannins, phenols, saponins and sterols (**Table 1**).

Table 1: Details of preliminary phytochemical screening of ethanol extract of *Pterocarpus santalinus* Linn. heartwood

Sl No.	Constituents	Test	Ethanolic Extract
1	Alkaloids	a) Mayer's reagent	Present
		b) Dragendorff's reagent	Present
		c) Hagner's reagent	Present
		d) Wagner's reagent	Present
2	Steroids	a) Libermann's burchard test	Present
		b) 5% KOH	Present
3	Phenols	a) Ferric chloride	Present
		b) 10% Sodium chloride	Present
4	Tannins	a) 10% Lead acetate solution	Present
		b) 10% NaCl	Present
		c) Aqueous bromine solution	Present
5	Flavonoids	a) Amyl alcohol + Sodium acetate+ Ferric chloride	Present
		b) Conc. H2SO4	
		c) Magnesium turning test	Present
		Glacial acetic acid + Ferric	Present
6	Glycosides	chloride + Conc.H2So4	Present
		Foam test	
7	Saponins		Present

Isolation and charactesrisation of compounds of *Pterocarpus santalinus* Linn. heartwood extract.

Pterocarpus santalinus Linn. heartwood extract was fractionated using pet ether (4x300 ml) benzene (3x300 ml) and chloroform (3x300 ml). The extract yielded a brownish solid, which was non homogenous in TLC and hence was further subjected to separation and purification by column chromatography and characterized by ^{1}H-NMR, ^{13}C-NMR and LC-MS Spectral analysis [21]. Compound was isolated from the *Pterocarpus santalinus* Linn. heartwood by repeated chromatographic separation and purification over silica gel.

Column chromatographic analysis

Pterocarpus santalinus Linn. heartwood extract (15g) residue was chromatographed in silica gel column (60-120 mesh, 300 gm, 100x5 cm) using gradient elution with the solvents of increasing polarity (Hexane: EtOAC]. Fractions of 100 ml were collected each time and the homogeneity was examined on TLC with suitable solvents. Fractions 1-5, 6-13, 14-31, 32-57, 58-67 and 68-82 each of which on concentration yielded residues with varying intensities of yellowish colour. This was tested individually by TLC and further purification was not carried out because of paucity of the samples. Fractions 58-67 on concentration yielded a pure yellowish homogeneous solid of 25 mg. Preparative thin layer chromatography (stationary phase- silica gel F254, mobile phase - 30 % ethyl acetate in toluene, thickness of plates-0.5 mm) of fractions 58-67 afforded compound 1.

^{1}H-NMR Spectral data of compound 1

^{1}H-NMR spectrum of compound 1 was recorded using Bruker 400 (400 MHz) spectrometer using DMSO-d6 as the solvent and complete assignment of protons are shown in **Table 2**.

Table 2: ^{1}H-NMR data of the Compound 1

H^1	H^2	H^3	H^4	–OH	$–OCH_3$
6.8 ppm (s, ^{1}H)	7.2 ppm (s, ^{1}H)	7.9 ppm (d, ^{1}H)	6.2 ppm (d, ^{1}H)	10.3 ppm (s, ^{1}H)	3.8 ppm (s, ^{3}H)

The ^{1}H-NMR spectrum (400 MHz, DMSO-d6) of isolated compound displayed a singlet peak in the downfield region at 10.3δ which is indicating the phenolic -OH proton. The singlet peak at 3.8δ revealed the presence of methoxy group (-OCH_3) protons. Comparison of the chemical shifts of the hydroxyl and methoxy groups allowed placing these substituents at C-6 and C-7, respectively. The two olefinic protons in the ring A position at C-1 and C-2 appears at 6.8 and 7.2 δ respectively as doublet. The two doublet peak of two olefinic protons in the ring B position at C-3 and C-4 appears at 7.9 and 6.2 ppm respectively. Results are shown in **figure 1**.

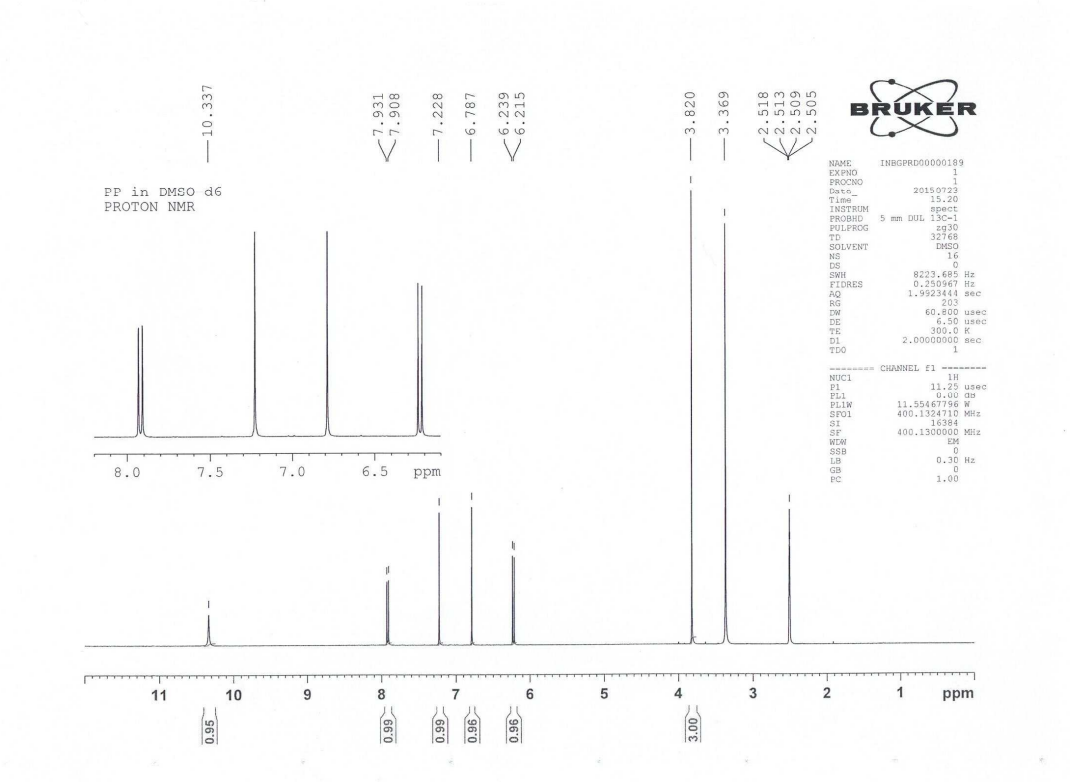


Fig 1: ^{1}H-NMR data of the Compound 1

^{13}C-NMR spectral data of compound 1

^{13}C-NMR spectrum of compound 1 was recorded using Bruker 400 (100 MHz) spectrometer (Plate 2) using DMSO-d6 as the solvent and the complete assignment of carbon are given in **Table 3**.

Table 3: ^{13}C-NMR data of the compound 1

C	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
ppm	145.19	102.72	144.42	151.08	109.52	111.64	149.45	110.49	160.62	55.94

**Solvent peak appeared around 39.467*

^{13}C-NMR was recorded using DMSO-d6 as solvent. The peak at 55.94 ppm shows the presence of C-10 of ring A. The signals at 102.72, 109.52, 110.49 and 111.64 illustrate the existence of C-2 & C-5 of A and C-8 & C-6 of B. The peaks observed in the region of 144.42, 145.19, 149.45 & 151.08 ppm are due to the C-3, C-1 of ring A and C-7 & C-4 of ring B. The peak in the region of 160.62 ppm confirms the presence of C-9 of B. Solvent evolved as a multiple around 39.467. Results showed in **figure 2**.

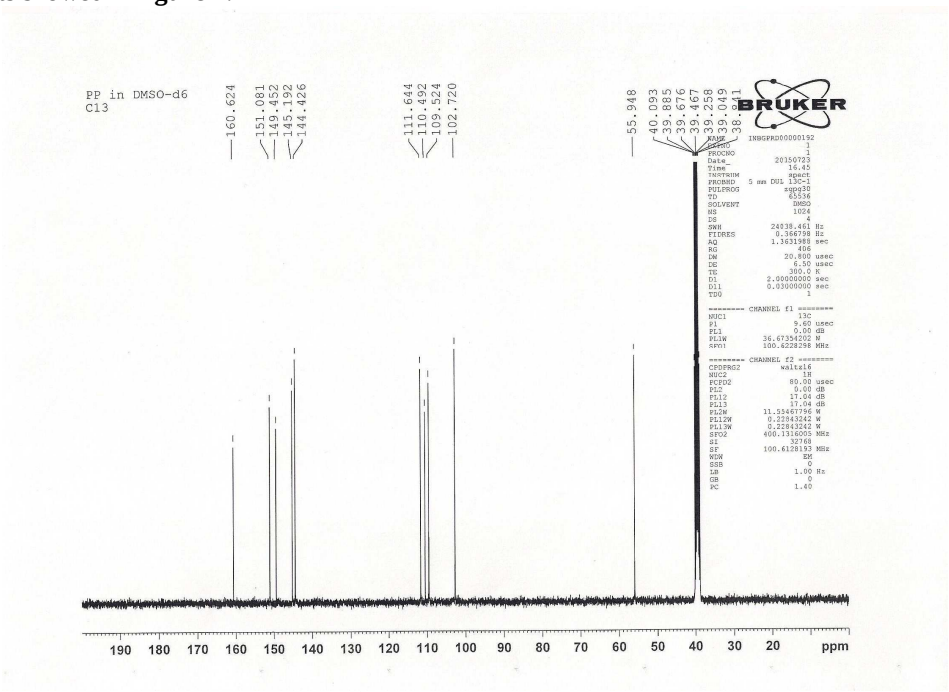


Fig 2: ^{13}C-NMR data of the compound 1

LC-MS study of compound 1

AGILENT 1100 series/MSDL spectrum (Agilent) of compound 1 (Plate 3) was taken and it gave peak m/z: (M+) 193. The prominent peak evolved in the region of 193.0 m/z corresponds to the molecular ion peak of the compound. **Results are shown in figure 3**

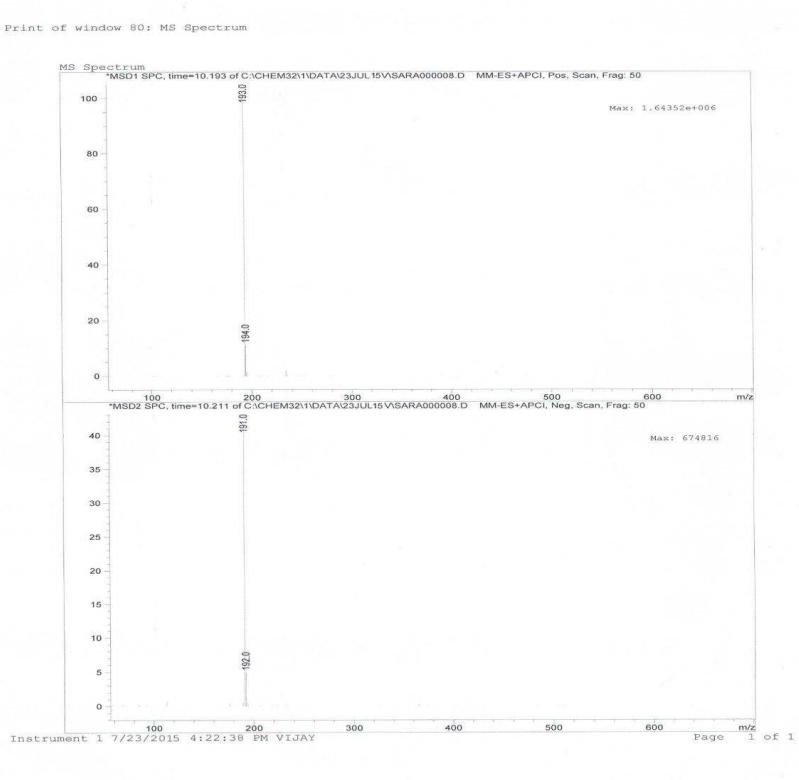
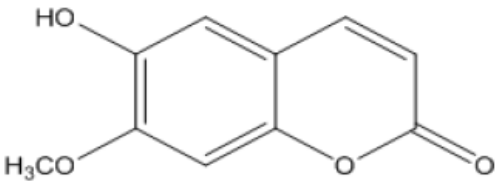


Fig 3: LC-MS study of compound 1

DISCUSSION

Herbs have been playing major role in curing various diseases throughout the world. The use of herbal medicines in most developing countries as therapeutic agents for the maintenance of health has often been reported [3]. *Pterocarpus santalinus* Linn. heartwood powder (500g) was defatted with petroleum ether and successively extracted further with ethanol in a soxhlet extract and blackish brown residue was obtained and percentage yield was found to be 15% w/w. and preliminary phytochemical studies revealed the presence of glycosides, flavonoids, alkaloids, tannins, phenols, saponins and sterols.

Crude extracts was subjected to column chromatography for isolation of compound and purification over silica gel. The structure of the isolated compound was determined by LC-MS, ^{1}H & ^{13}C NMR data analysis and based on spectral data's compound was found to be 6-hydroxy-7-methoxy-2H-chromen-2-one ($C_{10}H_8O_4$) **Figure 4**.



6-hydroxy-7-methoxy-2*H*-chromen-2-one

Chemical Formula: $C_{10}H_8O_4$
Exact Mass: 192.04
Molecular Weight: 192.17
m/z: 192.04 (100.0%), 193.05 (11.1%), 194.05 (1.4%)
Elemental Analysis: C, 62.50; H, 4.20; O, 33.30

Fig 4: Structure of compound 1

CONCLUSION

It is concluded that, proposed structure of the compound was characterized as 6-Hydroxy-7-methoxycoumarin with $C_{10}H_8O_4$ and the melting point 204^oC was confirmed by comparison of its spectral data with reported values.

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