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Original Article

Comparative GC-MS Profiling of n-Hexane Extracts from the Heartwood and Small Branches of *Pterocarpus santalinus* Linn. f.

Anju Sonkar[1,*], Om Prakash Rout[1], Pravin Kumar Joshi[1], Akhil Kumar Agrawal[1], Arun Kumar Singh Parihar[2], Nagendra Singh Chauhan[2], Chandan Sahu[2]

[1]Department of Dravyaguna Vigyana, Shri Narayan Prasad Awasthi Govt. Ayurveda College, Raipur, Chhattisgarh, INDIA.
[2]Department of Medical Education ,Drugs Testing Laboratory Avam Anusandhan Kendra, Raipur, Chhattisgarh, INDIA.

ABSTRACT

Background: *Pterocarpus santalinus* Linn.f. (Red Sandalwood) is an important medicinal plant known for its diverse bio active compounds. Evaluation of its phytoconstituents is essential for ensuring its therapeutic potential and quality. Chromatographic and mass spectrometric techniques are effective tools for profiling natural products. **Objectives:** The present study aims to comparatively analyze and identify the major phytochemical constituents present in the n-hexane extracts of heartwood and small branches of *Pterocarpus santalinus* using GC-MS. **Materials and Methods:** The n-hexane extracts were prepared using liquid-liquid extraction based on polarity and analyzed through GC-MS technique. **Results:** GC-MS analysis revealed several bioactive compounds, with 19 compounds identified in the heartwood and 8 compounds in the small branches, Four compounds were common to both extracts, resulting in 23 unique compounds. Including lipids, phenolics, flavonoids, alkaloids and terpenoids. Homopterocarpin was identified as the major compound in both extracts with peak areas of 63.78% and 13.26%, respectively. Other compounds such as tetradecanoic acid, 3-hexadecanol and isopropyl myristate were also detected in both samples. Additionally, Pterocarpin (4.56%) was found as a major compound in the heartwood extract. These compounds are associated with antimicrobial, antifungal, antioxidant and anti-inflammatory activities. **Conclusion:** The comparative analysis indicates significant similarity in phytoconstituents between the heartwood and small branches, suggesting that small branches may serve as a potential alternative source to heartwood, subject to further pharmacological validation.

Keywords: Bioactive Compounds, GC-MS Analysis, Heartwood, n-Hexane Extract, *Pterocarpus santalinus*, Small Branches.

Correspondence:
Dr. Anju Sonkar
Post Graduate Scholar, Department of Dravyaguna Vigyana, Shri Narayan Prasad Awasthi Govt. Ayurveda College, Raipur-492010, Chhattisgarh, INDIA.
Email: anjusonkar21@gmail.com
ORCID: 0009-0007-4953-3560

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INTRODUCTION

Medicinal plants are essential for treating and preventing a wide range of disease and they play a significant role in all existing preventative measures. Numerous plant-based medications have already demonstrated their potential safety and effectiveness (Godara *et al*., 2019). Now a days, many medicinal plants are at risk of going extinct due to urbanization and deforestation, however in recent years, there has been a surge in the demand for medicinal plants from a variety of processing industries, including food, cosmetics, pharmaceuticals, and perfumes. This has caused substitution and adulteration in the market for raw drugs (Vasudevan *et al*., 2022). In developing countries, a large proportion of the population relies on traditional medicine for primary healthcare needs. The increasing demand and trade of medicinal plants pose a significant threat to their sustainability, leading to the risk of extinction of several species. Hence, the conservation and proper management of medicinal plant resources have become essential. In India, many commonly used medicinal plants are slow-growing forest species, where parts such as bark, heartwood, and underground portions are predominantly utilized (Srivastava *et al*., 2016).

Pterocarpus santalinus Linn.f. is a small to medium sized deciduous tree, belongs to the "*Fabaceae*" family, there has a dense, spherical crown that can grow up to 10 to 15 meters. It is commonly known as Red Sandalwood (Donga *et al*., 2017), which has about 35 species worldwide and is widely distributed throughout the tropical regions of Africa, Indo-Malayan, North and South America (Mabberley *et al*., 2017). In India it is primarily distributed in South regions, Andhra Pradesh is a native

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place of red sandalwood (Arun *et al.*, 2014). It is distributed in the districts of Kadapa, Chittoor, Nellore, Prakasham and Kurnool. It is endemic to the forest of Seshachalam, Veligonda, Lankamal and Palakonda hill ranges of Andhra Pradesh, India. *Pterocarpus santalinus* Linn.f. was included in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1995 and International Union for Conservation of Nature (IUCN) list of endangered species in 1997 (Ahmedullah, 2021). According to the Medicinal Plant Board, Estimated Annual Trade (MT) of *Pterocarpus santalinus* Linn.f. is 200 to 500 (National Medicinal Plant Board, n.d.). The red sandalwood yields a natural dye, Santalin and wood is well known highly valued for its bioactive chemical, therapeutic applications and appealing appearance (Zhang *et al.*, 2019).

Many substances with a variety of biological characteristics can be produced by plants in the form of secondary metabolites, which can be used as effective medications to treat a range of illnesses (Gomathi *et al.*, 2015). Phytochemical studies of heartwood, leaf, bark, stem of this tree, have identified many metabolite groups such as terpenoids, triterpenoids, flavonoids, alkaloids, phenols, saponins, glycosides, sterols and tannins. In addition, specific bioactive compounds like santalinus A, B and Y, pterocarptriol, pterocarpol, pterocarpodiolones, beta eudesmol and cryptomeridiol have been determined, all of high medicinal values (Bulle *et al.*, 2016). Different metabolites and their concentration change in different parts of the same plant (Donga *et al.*, 2017). The bioactive compounds are known to show numerous pharmacological activities. for *Pterocarpus santalinus* Linn.f. have been documented potential for wound healing (Biswas *et al.*, 2004), hepato-protective (Manjunatha, 2006a), anti-bacterial properties (Manjunatha, 2006b), anti-Helicobacter pylori effects (Narayan *et al.*, 2007), anticancer (Kwon *et al.*, 2006), antidiabetic properties (Nagaraju *et al.*, 2008), anti-inflammatory, analgesic, and antioxidant properties (Kumar, 2011), cytotoxic properties (Wu *et al.*, 2011), acute and sub chronic oral toxicity (Tchamadeu *et al.*, 2011). For the creation of natural products, these characteristics necessitate a comprehensive phytochemical investigation, assessment, and validation of this herbal medication.

GC (Gas Chromatography) is one of the most popular methods for separating secondary metabolites in plant analysis. It has the innate capacity to use electron capture detectors, which have extremely high sensitivities, to quantify chemicals present at low concentrations (AI Rubaye *et al.*, 2017). A single quadrupole MS detector is connected to the end of the GC system in this apparatus. The two main ionization methods used in GC-MS are electron and chemical ionization. Because there are many mass spectral databases and libraries that match electron ionization-based GC-MS, many analysts choose for the first method (Amirav *et al.*, 2020). Due to its high separation ability, selectivity, robustness, sensitivity, and reproducibility, as well as the abundance of well-established libraries of both trade and "in-house" metabolite databases, Gas Chromatography-Mass Spectrometry (GC-MS) is generally regarded as a versatile analytical technique (Gruber *et al.*, 2020).

To date, most of the research was done on heartwood and bark, only a few works was reported on leaves of the plant. The standardized parameters of small branches of *Pterocarpus santalinus* Linn.f. have not been prepared yet. The objectives of this work were to compare the bioactive compounds their chemical structure and probable pharmacological activities and detect many new or unknown bioactive compounds which were not earlier reported. A qualitative analysis of the phytochemicals in the *n*-hexane extract of heartwood and small branch of *Pterocarpus santalinus* Linn.f. using GCMS for the first time. The work could help to screen and predict the compounds responsible for the common biological activities attributed to *Pterocarpus santalinus* Linn.f. heartwood and small branch also this concept can help to avoid adulteration, conserving rare, endangered plant species.

MATERIALS AND METHODS

Collection of Plant

The heartwood of *Pterocarpus santalinus* Linn.f. was purchased by through website i.e. Kerala Spices Online, Kerala, India. and the fresh small branches of *Pterocarpus santalinus* Linn.f. was manually collected from the natural source i.e. Gandhamardan Hills from Odisha, India. Both plant sample was gathered in compliance with all applicable National, International, and Institutional rules and regulations.

Authentication of Plant Sample

Both the samples were identified, authenticated by Shri B.M.K. Ayurveda Mahavidyalaya, Shahapur, Belagavi, Karnataka, India and were given the CRF code of both samples as CRF/Auth/305/2025-2026 and CRF/Auth/306/2025-2026.

Study Conduction

The present study was conducted at Devansh Testing and Research Laboratory Pvt. Ltd., Roorkee, Uttarakhand, India.

Plant Sample Preparation

The small branches were thoroughly washed with tap water to remove dust particle; both the sample dissected into small pieces and dried under shades at room temperature for 15 to 20 days than ground to a fine powder by using an electrical grinder. Both sample powder was kept in airtight plastic bags till further use.

Extraction Method

The *Pterocarpus santalinus* Linn.f. heartwood and small branch powder sample 0.5 g was mixed separately with 5 mL of water and 5 mL of HPLC grade solvent n-hexane, followed with agitation for 10 min to ensure that all of the compounds were

dissolved. The mixture was then centrifuged at 6000 rpm for 5 min to facilitate phase separation. The organic (hexane) layer was carefully collected and filtered through PPFE 0.22 µM filter paper. A fraction of 0.5 mL of the filtered organic phase was then diluted to 1 mL with n-hexane prior to further analysis.

Chromatographic Analysis Conditions and Instrumentation

GC-MS System

In this system, the non-polar and semipolar bioactive compounds of heartwood and small branches of *Pterocarpus santalinus* Linn.f. extracts are identified and separated by the instrument GC-MSMS (Shimadzu-TQ-8040EI), The instrument consisting of MS part of Triple Quadrupole with MS/MS that allow it for the Lower Limit of Detection (LLD), subsequently coupled with GC Unit -2010 Plus and Auto Sampler i.e. AOC-20S. the GC-MS unit has ion source type of Electron Ionization, energy of 70eV with a source temperature of 280°C. Helium gas was used as a carrier gas with a flow rate 1 mL/min. The Column Rxi-5Sil MS (30 M, 0.25 mM ID, 0.25 µM FT) was used for separation. the oven temperature started at 60°C, maintained for 2 min, then raised at a rate of 4°C /min until reaching 280°C, where it was sustained for 5 min. The integrated Mass detectors are Flame Ionization Detector (FID) and Electron Capture Detector (ECD). For the analysis of both sample extract the condition was set up with parameters of 62 min run time, injection volume 1 µL and injector temperature was maintained at 250°C, where spilt less injection mode and split ratio was 10:0. The MS specifications were as follows: Ion source temperature 200°C, interface temp. 290°C, Scan speed 3333, event time 0.3 sec, solvent cut time 2 min. The mass spectrometric data were scanned in the range of 45 to 1000 m/z.

Determination of Bioactive Compounds

Shimadzu's Lab Solution GC-MS Software was used to store and process data as well as monitor all equipment parameters. The National Institute of Standards and Technology (NIST 17) library were used to identify the compounds and a comparison with the literature was reported.

Statistical Analysis

No statistical analysis was performed in this study.

RESULTS

The GC-MS analysis of n-hexane extracts of heartwood of *Pterocarpus santalinus* Linn.f. revealed the presence of nineteen bioactive compounds. The active principles with their retention time, formula, Molecular Weight (MW), peak area (%), and GC-MS chromatogram are presented in Table 1 and Figure 1.

The principal bioactive compounds in *Pterocarpus santalinus* Linn.f. heartwood included, Homopterocarpin (peak 5,63.78%),

Figure 1: GC-MS Chromatogram of Pterocarpus santalinus Heartwood n-Hexane Extract.

4-t-Butyl-2-[4-nitrophenyl]phenol (peak 7, 24.29%), Pterocarpin (peak 8, 4.56%), 2H-I-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)- (peak14,1.35%), Phenol,2-(3,4-dihydro-7-methoxy-2H-I-benzopyran-3-yl)-5-methoxy-(peak11,0.77%), 3-(10-Methyl-10H-acridin-9-ylideneamino)-phenol(peak12,0.71%), Acridin-9-yl (4methoxyphenyl)-amine(peak 10, 0,55%), 2H-1-Benzopyran-7-ol,3,4-dihydro-3- (2-hydroxy-4-methoxyphenyl)-(peak13,0.48%), Isopropyl myristate(peak4,0.46%), 4H-1-Benzopyran4one,5hydroxy3 (4hydroxyphenyl)7methoxy(peak16,0.46%), Dibenz[d,f] cycloheptanone,2,3, 9-trimethoxy-(peak 17,0.46%), 5-Acetoxy-3-(3,4-diacetoxyphenyl) -7-methoxy-4H-chromen-4-one (peak 19, 0.39%), Tetradecanoic acid (peak 2, 0.38%), 2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-(peak15, 0.28%), Durohydroquinone (peak18,0.26%), Trismethoxyresveratrol (peak6,0.23%), 6Ar,12aR) 6a,12aDihydro6H[1,3] dioxolo [4',5',5,6]benzofuro[3,2-c] chromen-3-ol (peak 9, 0.21%), Benzene,1,2,3-trimethoxy-5- (1-propenyl)-,(E)- (peak 1, 0.18%), 3-hexadecanol (peak 3, 0.17%).

The active principles in the n-hexane small branch extract of *Pterocarpus santalinus* Linn.f. indicated the presence of eight compounds. The compounds with their retention time, formula, Molecular Weight (MW), peak area (%) and GC-MS Chromatogram are presented in Table 2 and Figure 2.

The principal bioactive compounds in *Pterocarpus santalinus* Linn.f. small branches included, Tetradecanoic acid (peak 3, 23.98%), Isopropyl myristate (peak 5, 15.70%), Homopterocarpin (peak 8, 13.26%), 1,2,3,5-Cyclohexanetetrol, (1.alpha,2.beta,3. alpha,5.beta) (peak 2, 12.28%), n- Hexadecenoic acid (peak 7, 11.60%), Dibutyl phthalate (peak 6, 9.45%), 4H-Pyran-4-one,2 ,3-dihydroxy-6-methyl- (peak 1, 7.90%), 3- Hexadecanol (peak 4, 5.83%).

The GC-MS analysis of n-Hexane extracts of heartwood and small branch of *Pterocarpus santalinus* Linn.f. detected the presence of nineteen and eight bioactive compounds respectively. Homopterocarpin and Pterocarpin are the major compounds identified in the heartwood extract with the peak area of 63.78% and 4.56%. Homopterocarpin is the major compound identified

Table 1: GC-MS analysis of n-hexane extract of *Pterocarpus santalinus* Heartwood.

Sl. No.	Bioactive Compound	R.Time	Formula	Molecular weight (MW)	Area %
1.	Benzene,1,2,3-trimethoxy-5-(1-propenyl)-, (E)-	28.196	$C_{12}H_{16}O_3$	208	0.18
2.	Tetradecanoic acid	31.625	$C_{14}H_{28}O_2$	228	0.38
3.	3-Hexadecanol	32.730	$C_{16}H_{34}O$	242	0.17
4.	Isopropyl myristate	33.261	$C_{17}H_{34}O_2$	270	0.46
5.	Homopterocarpin	49.359	$C_{17}H_{16}O_4$	284	63.78
6.	Trismethoxyresveratrol	49.501	$C_{17}H_{18}O_3$	270	0.23
7.	4-t-Butyl-2-[4-nitrophenyl] phenol	50.708	$C_{16}H_{17}NO_3$	271	24.29
8.	Pterocarpin	51.511	$C_{17}H_{14}O_5$	298	4.56
9.	(6aR,12aR)-6a,12a-Dihydro-6H- [1,3] dioxolo[4',5',5,6] benzofuro[3,2-c] chromen-3-ol	52.685	$C_{16}H_{12}O_5$	284	0.21
10.	Acridin-9-yl-(4methoxy-phenyl)-amine	52.972	$C_{20}H_{16}N_{2O}$	300	0.55
11.	Phenol,2-(3,4-dihydro-7-methoxy-2H-I -benzopyran-3-yl)-5-methoxy-	53.109	$C_{17}H_{18}O_4$	286	0.77
12.	3-(10-Methyl-10H-acridin-9-ylideneamino)-phenol	53.770	$C_{20}H_{16}N_{2O}$	300	0.71
13.	2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-	54.076	$C_{16}H_{16}O_4$	272	0.48
14.	2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-	54.716	$C_{17}H_{18}O_5$	302	1.35
15.	4H-1-Benzopyran-4-one,7-hydroxy-3-(4-methoxyphenyl)-	55.266	$C_{16}H_{12}O_4$	268	0.28
16.	4H-1-Benzopyran-4-one,5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-	55.785	$C_{16}H_{12}O_5$	284	0.46
17.	Dibenz[d,f]cycloheptanone,2,3,9-trimethoxy-	57.456	$C_{18}H_{18}O_4$	298	0.46
18.	Durohydroquinone	57.877	$C_{10}H_{14}O_2$	166	0.26
19.	5-Acetoxy-3-(3,4-diacetoxyphenyl)-7-methoxy-4H-chromen-4-one	61.108	$C_{22}H_{18}O_9$	426	0.39

in the small branch extract with the peak area of 13.26%. A bioactive compounds Tetradecanoic acid, 3-Hexadecanol, Isopropyl myristate, Homopterocarpin with the peak area was present (Tables 1 and 2) in both n-Hexane extract of *Pterocarpus santalinus* Linn.f. heartwood and small branches.

The Mass Spectra of identified compounds from *Pterocarpus santalinus* Linn.f. heartwood and small branches were presented in Figure 3.

DISCUSSION

Extraction Procedure

Using an appropriate solvent and extraction technique, Active Phyto constituents (secondary metabolites) such flavonoids, phenols glycosides, saponins, alkaloids, terpenes, ester carbohydrate, alcohol, hydrocarbons and steroids are separated from inactive material (Abubakar *et al.*, 2020). The extracted

Table 2: GC-MS analysis of n-hexane extract of *Pterocarpus santalinus* Small Branches.

Sl. No.	Bioactive Compound	R. Time	Formula	Molecular Weight (MW)	Area %
1.	4H-Pyran-4-one,2,3-dihydroxy-6-methyl-	11.624	$C_6H_8O_4$	144	7.90
2.	1,2,3,5-Cyclohexanetetrol, (1.alpha,2.beta,3.alpha,5.beta)	27.026	$C_6H_{12}O_4$	148	12.28
3.	Tetra decanoic acid	31.561	$C_{14}H_{28}O_2$	228	23.98
4.	3-Hexadecanol	32.731	$C_{17}H_{36}O$	256	5.83
5.	Isopropyl myristate	33.263	$C_{17}H_{34}O_2$	270	15.70
6.	Dibutyl phthalate	36.574	$C_{16}H_{22}O_4$	278	9.45
7.	n- Hexadecanoic acid	36.822	$C_{16}H_{32}O_2$	256	11.60
8.	Homopterocarpin	49.138	$C_{17}H_{16}O_4$	284	13.26

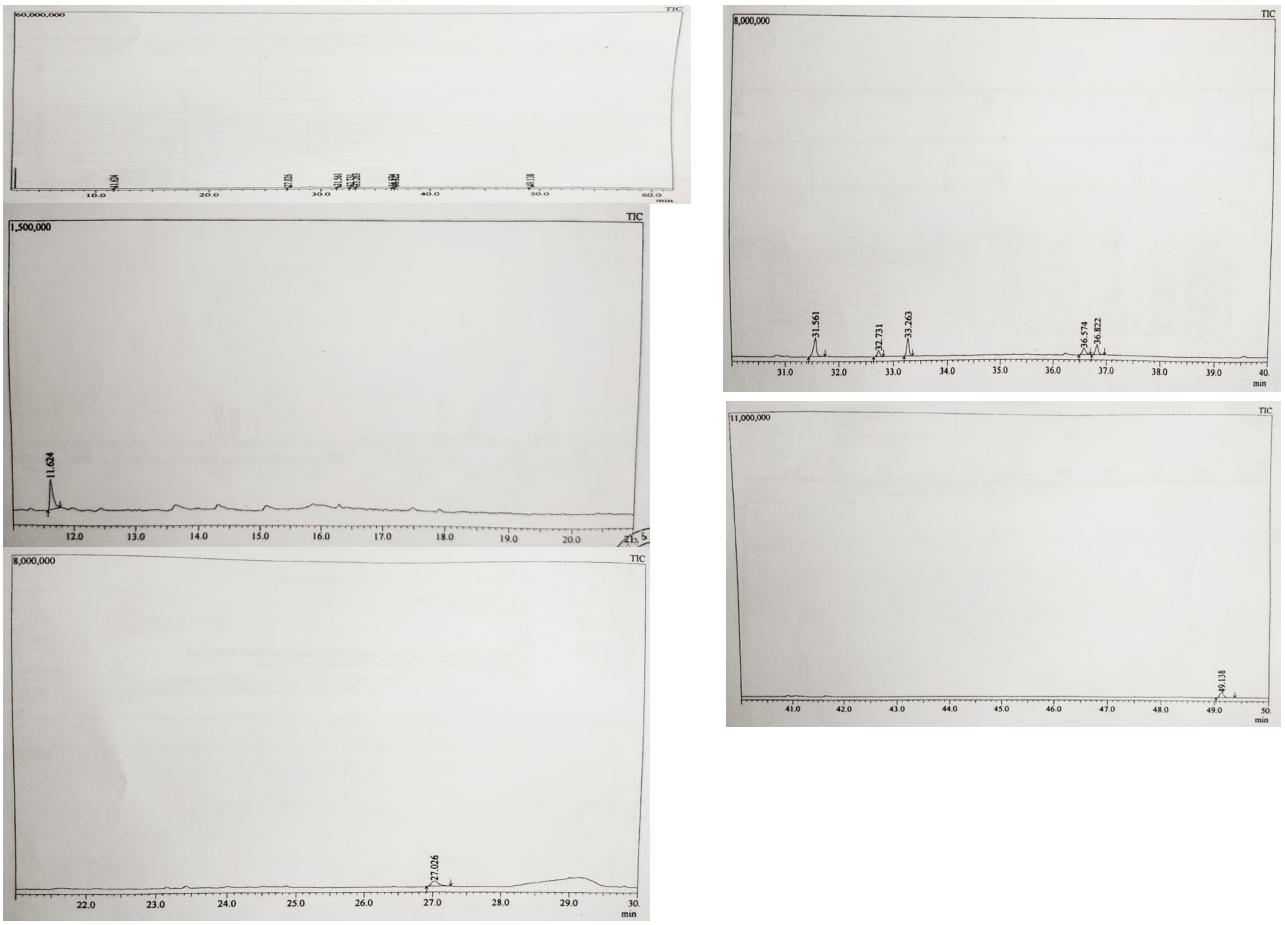

Figure 2: GC-MS Chromatogram of Pterocarpus santalinus Small Branches n-Hexane Extract.

physiologically active chemicals from plants are influenced by the extraction solvent. Polar compounds are often extracted using polar solvents like water, methanol, and ethanol, while non-polar or moderately polar compounds are typically extracted using non-polar solvents like hexane and dichloromethane (Altemimi *et al.*, 2017; AI Rifai *et al.*, 2018). On the other hand, a number of variables, such as the solvent used, temperature, the kind of plant material, and the solvent-to-sample ratio, influence the selection of an appropriate extraction technique (Oubannin *et al.*, 2024). A common and simple method for cleaning up samples is Liquid-Liquid Extraction (LLE), which involves separating the analytical compounds between two immiscible solvents. The potential to use a large sample volume, consistent extracted matrix background, cost-effectiveness, simplicity and repeatability, ease of technique transfer, and relatively quick method development time are some benefits of Liquid-Liquid Extraction (Silvestre *et. al.*, 2009). Plant extract components are separated according to their polarity via liquid-liquid extraction, with hexane being the least polar and n-butanol being the most

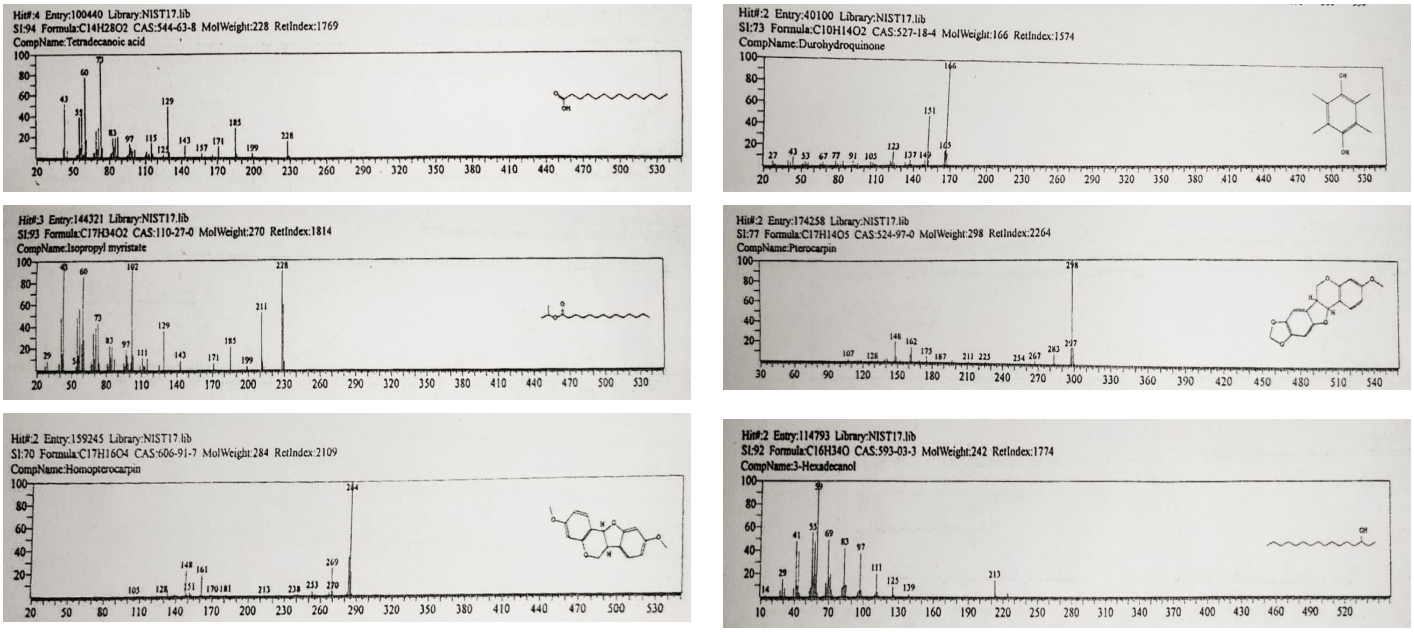

Figure 3: Mass Spectra of identified compounds from n-Hexane extract of Heartwood and Small Branches of *Pterocarpus santalinus* Linn.f.

Table 3: Pharmacological Activities of Compounds Identified in the Heartwood and Small Branches of *Pterocarpus santalinus*

Sl. No.	Compound Name	Specific Classification	Plant Part	Pharmacological Activity
1.	Benzene,1,2,3-trimethoxy -5-(1-propenyl)-,(E)-	Methoxy-substituted phenylpropene	Hw	Antioxidant, Antimicrobial (Cowan,1999)
2.	Tetradecanoic acid	Saturated fatty acid	Hw, Sb	Antimicrobial, Anti-inflammatory (Desbois *et al.*, 2010)
3.	3-Hexadecanol	Fatty alcohol (long -chain aliphatic alcohol)	Hw, Sb	Antimicrobial, Emollient (Togashi *et al.*, 2007)
4.	Isopropyl myristate	Fatty acid ester	Hw, Sb	Skin permeation enhancer, Antimicrobial (Moiseev *et al.*, 2019)
5.	Homopterocarpin	Iso Flavonoid (pterocarpans)	Hw, Sb	Antimicrobial, Antifungal, Antioxidant (Krishnaveni *et al.*, 2000)
6.	Trismethoxyresveratrol	Stilbenoid (resveratrol derivative)	Hw	Antioxidant, Anticancer, Anti-inflammatory (Koc *et al.*, 2024)
7.	4-t-Butyl-2-[4-nitrophenyl] phenol	Substituted phenol	Hw	Antioxidant, Antimicrobial (Srivastava *et al.*, 2023)

Sl. No.	Compound Name	Specific Classification	Plant Part	Pharmacological Activity
8.	Pterocarpin	Isoflavonoid (pterocarpans)	Hw	Antimicrobial, Anti-inflammatory (Ayena *et al.*, 2021)
9.	(6aR,12aR)-6a,12a-Dihydro-6H-[1,3]dioxolo[4',5',5,6]benzofuro[3,2-c] chromen-3-ol	Isoflavonoid (pterocarpan derivative)	Hw	Antifungal, Antibacterial (Ko *et al.*, 2004)
10.	Acridin-9-yl-(4methoxy-phenyl)-amine	Acridine Alkaloid	Hw	Antimicrobial, Anticancer (Sondhi *et al.*, 2010)
11.	Phenol,2-(3,4-dihydro-7-methoxy-2H-I -benzopyran-3-yl)-5-methoxy-	Flavon (flavonoids derivative)	Hw	Antioxidant (Dias *et al.*, 2021)
12.	3-(10-Methyl-10H-acridin-9-ylid eneamino)-phenol	Acridine Alkaloid derivative	Hw	Antimicrobial, Cytotoxic (AI -Marzook *et al.*, 2017)
13.	2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-	Flavanol	Hw	Antioxidant, Cardioprotective (Qin *et al.*, 2011)
14.	2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-	Flavanol	Hw	Antioxidant, Cardioprotective (Singh *et al.*, 2014)
15.	4H-1-Benzopyran-4-one,7-hydroxy-3-(4-methoxyphenyl)-	Flavone	Hw	Anti-inflammatory, Antioxidant (Singh *et al.*, 2014)
16.	4H-1-Benzopyran-4-one,5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-	Flavone	Hw	Antiinflammatory, Antioxidant (Singh *et al.*, 2014)
17.	Dibenz[d,f]cycloheptanone,2,3,9-trimethoxy-	Dibenzocycloheptanone derivative	Hw	CNS Activity (Porre *et al.*, 2022)
18.	Durohydroquinone	Hydroquinone derivatives	Hw	Antioxidant (Giner *et al.*, 2022)
19.	5-Acetoxy-3-(3,4-diacetoxyphenyl)-7-methoxy-4H-chromen-4-one	Flavone (acetylated derivative)	Hw	Antioxidant, Antimicrobial (Gumisiriza *et al.*, 2024)
20.	4H-Pyran-4-one,2,3-dihydroxy-6-methyl-	Oxygenated heterocyclic compound (pyranone derivative)	Sb	Antioxidant, Metal chelating Antimicrobial (Almalki, 2023)
21.	1,2,3,5-Cyclohexanetetrol, (1.alpha,2.beta,3.alpha,5.beta)	Cyclitol (polyol)	Sb	Antioxidant, Osmo protective (Owczarczyk *et al.*, 2018)
22.	Dibutyl phthalate	Phthalate Ester	Sb	Not reported Endocrine disruption (Wowkonowicz, 2023)
23.	n- Hexadecanoic acid	Saturated fatty acid	Sb	Antioxidant, Antimicrobial, Anti-inflammatory (Martinez *et al.*, (n.d.))

(Abbreviation - Hw: Heartwood, Sb: Small Branch).

polar. In this study, n-hexane has been used as a solvent in the liquid -liquid extraction technique.

GC-MS System Condition Optimization

One of the best techniques for identifying a number of secondary metabolites found in plant extract is GC-MS (Thamer *et al.*, 2023). By analyzing the impact of the carrier gas flow speed, temperature program, and detection conditions and considering the best possible separation of the compounds, the GC-MS parameters were optimized in the analysis of the constituents of the non-polar and semi-polar constituents of *Pterocarpus santalinus* Linn.f. heartwood and small branch extract. Each spectrum was compared to spectra from the main source of MS spectral libraries, NIST, in order to identify peaks (AI Suliman *et al.*, (n.d.)).

Analysis of Phytoconstituents of *Pterocarpus santalinus* Linn.f

Pterocarpus santalinus Linn.f. became the most sought-after tree in scientific circles, Due to its high commercial worth and

Table 4: Common phytochemicals detected from *Pterocarpus santalinus*.

Sl. No.	Heartwood & Small Branches
1.	Homopterocarpin
2.	3- Hexadecanol
3.	Isopropyl myristate
4.	Tetradecanoic acid

therapeutic qualities (Hanuma *et al.*, 2021). Previous reviews explored *Pterocarpus santalinus* Linn.f. phytochemistry, pharmacology, and ethnomedical uses (Arunakumara *et al.*, 2011; Navada *et al.*, 2014). The plant's heartwood contains bioactive chemicals that have been shown to have a diversity of biological actions, indicating that *Pterocarpus santalinus* Linn.f. may be utilized to treat a number of disorders (Arunakumara *et al.*, 2011; Manjunatha, 2006). It is widely used in conventional medicine and is abundant in phenols and flavonoids (Hanuma *et al.*, 2021).

The plant contained carbohydrates, flavonoids, terpenoids, phenolic compounds, alkaloids, saponins, tannins, and glycosides, as reported in a phytochemical characterization. Furthermore, research has shown that heartwood powder contains a number of specific components, including pterocarpol, santalin A, B, and Y, pterocarptriol, isopterocarpalone, pterocarpodiolones with β-eudesmol, and cryptomeridiol (Krishnamoorthy *et al.*, 2011), as well as a number of other specific compounds like isoflavones, isoflavonoid glucosides, triterpenes, sesquiterpenes, and related phenolic compounds like β-sitosterol, lupeol, epicatechin, and pterostilbenes (Kumar *et al.*, 1975; Krishnaveni *et al.*, 2000; Krishnaveni *et al.*, 2000b; Krishnaveni *et al.*, 2000c; Cho *et al.*, 2001; Kesari *et al.*, 2004).

This study compares the different phytochemical constituents isolated from heartwood and small branches through the standard GC-MS [Gas Chromatography Mass Spectrometry] technique. The comparative GC-MS analysis of both plant parts identified various classes of bioactive compounds that include, lipids, isoflavonoids alkaloids, stilbenes, fatty acids and their esters, alcoholic and phenolic compounds, esters. The presence of these compounds indicates that the extract possesses significant pharmacological potential, which may be attributed to the combined effect of primary and secondary metabolites.

The bioactive compounds reported in heartwood of *Pterocarpus santalinus* Linn.f. through the present study are from groups of Lipids (Tetradecanoic acid, Isopropyl myristate, 3-Hexadecanol), Phenolics (Durohydroquinone, 4-t-Butyl-2-[4-nitrophenyl] phenol), Flavonoids (Homopterocarpin, Pterocarpin, (6aR,12aR)-6a,12a-Dihydro-6H-[1,3]dioxolo[4',5',5,6] benzofuro[3,2-c] chromen-3-ol), 2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-4-methoxyphenyl)-, 2H-1-Benzopyran-7-ol,3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-,4H-1-Benzopyran-4-one,7-hydroxy-3-(4-methoxyphenyl)-, 4H-1-Benzopyran-4-one, 5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-,5-Acetoxy-3-(3,4-diacetoxyphenyl)-7-methoxy-4H-chromen-4-one, Phenol,2-(3,4-dihydro-7-methoxy-2H-I-benzopyran-3-yl)-5-methoxy-),Phenol,2-(3,4-dihydro-7-methoxy-2H-benzopyran-3-yl)-5-methoxy, 5-Acetoxy-3-(3,4-diacetoxyphenyl)-7-methoxy-4H-chromen-4-one), Polyphenols (Trismethoxyresveratrol), Alkaloids (Acridin-9-yl-(4methoxy-phenyl)amine, 3-(10-Methyl-10H-acridin-9-ylideneamino)-phenol), Phenylpropanoids (Benzene,1,2,3-trimethoxy-5-(1-propenyl)-,(E)-), Terpenoids / aromatics (Dibenz[d,f]cycloheptanone,2,3,9-trimethoxy-).

The bioactive compounds analyzed and reported for the first time in small branches of *Pterocarpus santalinus* Linn.f. through the present study are from groups of Phenolics (4H-Pyran-4-one,2,3-dihydroxy-6-methyl), Carbohydrate derivative (1,2,3,5-Cyclohexanetetrol, (1.alpha,2.beta,3.alpha,5.beta), Lipids (Tetradecanoic acid, n - Hexadecenoic acid, 3- Hexadecanol, Isopropyl myristate), Phthalate ester (Dibutyl phthalate), Flavonoids (Homopterocarpin).

The pharmacological activities of the phytochemical compounds identified from the heartwood and small branches of Pterocarpus santalinus L.f. are summarized in Table 3.

CONCLUSION

The conventional therapeutic plant species *Pterocarpus santalinus Linn.f.*, also known as Rad sanders, has been documented to be a rich source of bioactive phytochemicals with a wide range of biological activities. This study presented a comprehensive GC-MS analysis of n-hexane extract of heartwood and small branches. The findings of the analysis provided a detailed comparison of various classes of bioactive compounds found both plant parts. A comparative analysis of total 23 different bioactive compounds have been done and further discussed in details on the basis of potential biological role as well as medicinal importance.

The present study identified potential bioactive compounds in the heartwood and small branches of Pterocarpus santalinus L.f., including Homopterocarpin, Pterocarpin, Durohydroquinone, Trimethoxyresveratrol, Isopropyl myristate etc. which may be due to tissue specific functions and differences in physiological role of the plant parts.at the same time, both parts contain four common bioactive component (Table 4) indicating a certain level of similarity in their chemical composition and metabolic pathway. The presence of this shared phytochemical suggests that both parts may possess similar biological and pharmacological properties. This compound might play and important role in the plant defense mechanism and could contribute to its medicinal value. Overall, the biological activity of the extract may be attributed to the synergistic interaction between phenolic compounds and lipid-based constituents. Such interactions are known to enhance the stability and efficacy of bioactive molecules. The predominance of antioxidant and antimicrobial compounds suggests that the extract could serve as a potential source of

natural therapeutic agents. The identification of common phytochemicals in small branches suggests that they could be used as an alternative source of heartwood after the comparison and confirmation of experimental studies, including bioassay-guided fractionation and mechanistic evaluations, are necessary to confirm the pharmacological activities and to identify the most active constituents responsible for the observed effects. The study provides the base for further research may provide future prospects to explore the medicinal characterization, isolation and biological of these compounds which will help sustainable utilization of plant resources.

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ABBREVIATIONS

GC-MS: Gas Chromatography Mass Spectrometry; **GC-MSMS:** Gas Chromatography-Tandem Mass Spectrometry; **LLE:** Liquid-Liquid Extraction; **FID:** Flame Ionization Detector; **ECD:** Electron Capture Detector; m/z: Mass-to-charge ratio; **MW:** Molecular Weight; **R.Time:** Retention Time; **NIST:** National Institute of Standards and Technology; **CCRAS:** Central Council for Research in Ayurvedic Sciences.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Dr. Anju Sonkar: Contributed to the research concept and design, data collection, and manuscript writing. **Dr. Om Prakash Rout, Dr. Pravin Kumar Joshi, Dr. Akhil Kumar Agrawal, Arun Kumar Singh Parihar, and Chandan Sahu:** Contributed to critical revision and final approval of the article. **Dr. Nagendra Singh Chauhan:** contributed to data analysis, interpretation, as well as critical revision and final approval of the article.

SUMMARY

The present study highlights *Pterocarpus santalinus* Linn.f. as a rich source of bioactive phytochemicals, as revealed through GC-MS analysis of heartwood and small branches. A total of 23 compounds were identified, including several therapeutically important constituents such as Homopterocarpin and Pterocarpin. The presence of both unique and common phytochemicals indicates tissue-specific variations along with similarities in metabolic pathways, suggesting comparable pharmacological potential. The predominance of antioxidant and antimicrobial compounds underscores its medicinal value. Notably, the detection of common bioactive constituents in small branches suggests their potential as a sustainable alternative to heartwood. Overall, this study provides a foundation for future pharmacological validation and supports the sustainable utilization of this medicinal plant.

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