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PHYTOCHEMICAL AND ANTI-MICROBIAL STUDY OF *PTEROCARPUS SANTALINUS* LINN HEARTWOOD

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ABSTRACT: In present days many alternative medicines were available for those who cannot be cured by proper medicine. Ayurvedhic medicine was important forms of alternative medicine that was widely available in India. In this work was mainly concerned with the identification of the therapeutic properties of *Pterocarpus santalinus* Linn. The Methanolic extract of *Pterocarpus santalinus* Linn Heartwood was used for its anti-oxidant and antimicrobial activity. *Pterocarpus santalinus* Linn dried bark has very well anti-oxidant and anti-microbial activity. The Methanolic extract of *Pterocarpus santalinus* Linn was checked for anti-microbial activity against pathogenic bacteria such as *E. coli, Staphylococcus aureus, Pseudomonas* etc.

KEYWORDS: *Pterocarpus santalinus Linn*, Antioxidant activity, anti-microbial activity, phytochemical screening.

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1.INTRODUCTION

Pterocarpus Santalinus are found India is endemic to the hills of Andhra Pradesh (Cuddapah, Chittoor, some parts of Nellore), some pockets of Karnataka and Tamil Nadu. The phytochemical analysis of *Pterocarpus santalinus Linn* showed that it contains various components, such as carbohydrates, steroids, anthocyanins, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides and glycerides [1]. Pterocarpus species also contains isoflavonoids, terpenoids and phenolic compounds, β-sitosterol, lupeol, (-)epicatechin [1]. In addition auron glycosides viz., 6-

OH-1-methyl-3',4',5'- trimethoxyaurone-4-O-rhamnoside and 6,4'-dihyroxyaurone-4-O-neohesperidoside, and isoflavone glycoside 4',5-dihydroxy 7-methyl isoflavone 3'-O-beta-D-glucoside are present in *Pterocarpus santalinus* [2]. The heartwood also contains pterocarpol, santalins A and B, pterocarptriol, ispterocarpolone, pterocarpodiolones with β-eudeslol and cryptomeridol [3]. Ether, alkalis and three other crystalline principles santal, pterocarpin, homopterocarpin, small quantity of tannin and kino-tannic acid are also found in the wood [4]. Triterpene has reported to be present in the callus of stem cuttings. The leaves contain methanol and ethanol can be extracted from stem Heartwood [5]. The stem Heartwood extract was shown to contain maximum activity against *Enterobacter aerogenes, Alcaligenes faecalis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus cereus, Bacillus subtilis,* and *Staphylococcus aureus* [6]. Ethanolic stem bark extract is known to possess antihyperglycemic activity. The leaf extract also showed maximum activity against *E. coli, A. faecalis, E. aerogenes*, and *P. Aeruginosa* [8]. This work is about medicinal, phytochemical and pharmacological and medicinal uses of *Pterocarpus santalinus Linn*.

2. MATERIALS AND METHODS

PLANT MATERIAL

From the previous literature studies *Pterocarpus santalinus Linn* (Red Sandalwood) as our Heartwood material for studying anti-diabetic activity in Wistar rats. Thus these Heartwood material was dried and powdered and used for a phytochemical screening. Dried Heartwood of *Pterocarpus santalinus Linn* was selected for further studies.

Physico-chemical Constants

The procedures recommended in Indian Pharmacopoeia and WHO guidelines were followed to calculate the physico-chemical constants [9].

Physicochemical characterization of the Heartwood extract

Physicochemical parameters such as color, consistency, pH and percent yield (% w/w). were determined for all Heartwood extracts

Ash values

Total ash value

The total ash was determined by incinerating 2-3gms of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing, at a temperature not exceeding 450^0C. The ignition was repeated and the percentage of ash with reference to air-dried drug was calculated.

Water soluble ash

The total ash was boiled for 5min with 25 ml of water. The residue was washed with hot water, ignited for 15min at a temperature not exceeding 450^0C, cooled and weighed. This weight was subtracted from the weight of ash, the difference in weight represents the water soluble ash. The

percentage of water soluble ash was calculated with reference to air-dried drug.

Acid insoluble ash

The ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5min and filtered through an ashless filter paper. The residue was washed with hot water, ignited, cooled in a dessiccator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried drug.

Sulphated ash

The sulphated ash was determined by incinerating 1 gm of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing at a temperature not exceeding 450^0C. The residue was moistened with 1 ml of concentrated sulphuric acid, ignited at $80^0 \pm 25^0$C until all black particles have disappeared. It was then cooled, again sulphuric acid was added and ignited. It was cooled and the percentage of sulphated ash was calculated with reference to air dried drug.

The shade dried powdered Heartwood material of *Pterocarpus santalinus Linn* was weighed and extracted using 50% ethanol (hydro alcoholic mixture) at 60°C, 50% ethl acetate at 50°C and methanol at 50°C in soxhlet apparatus and distilled water (aqueous extract) at 100°C for 18 h by hot reflux extraction method. The aqueous, ethanolic, ethl acetate and methanolic Heartwood extracts of *Pterocarpus santalinus Linn* was then filtered and concentrated using rotary vacuum evaporator. The dried Heartwood extracts were stored in amber colored wide mouth bottles under refrigeration (4°C) and were used for phytochemical and pharmacological investigations.

The preliminary phytochemical investigations were conducted employing various phytochemical tests and the presence of various phytochemical constituents were detected [10]**.**

Test for carbohydrates

A small quantity of aqueous and hydroalcoholic Heartwood extracts were dissolved in 5 ml of distilled water and filtered.

a. **Molisch's test**- The filtrate was tested with alcoholic solution of _ napthol and sulphuric acid. A purple coloured ring indicated the presence of carbohydrates.

b. Fehling's test- The filtrate was treated with equal quantity of Fehling A (Copper sulphate) and Fehling B (Sodium potassium tartarate) and solution was heated. Brick red precipitate indicates the presence of sugars.

c. Barfoed's test- Formation of red colour within 2 min after addition of the reagent indicates the presence of monosaccharides.

d. Benedict's test- The filtrate was heated with this reagent for 2 min. Formation of red precipitate indicates the presence of reducing sugars.

e. Selwinoff's test- The filtrate was heated with this reagent for 1-2 min. The formation of red colour of the solution indicated the presence of ketohexose like fructose.

2. Tests for non-reducing sugars

The aqueous and hydroalcoholic Heartwood extracts which did not give response to Fehling's and Benedict's tests confirmed the presence of non-reducing sugars. The presence of nonreducing sugars was also indicated by positive Fehling's and Benedict's tests by the hydrolysed test solution.

3. Tests for non-reducing polysaccharides

In this test, 3 ml of test solution of extract was mixed with few drops of dilute iodine solution. The blue colour of the solution confirmed the presence of non-reducing polysaccharides.

4. Test for gums and mucilage:

About 1 ml of extract was added slowly to about 25 ml of alcohol with constant stirring. Formation of a precipitate indicates the presence of gums and mucilage

5. Test for resins

The extract was dissolved in alcohol and diluted it 10 times with water, turbidity formed indicated the presence of resins.

6. Test for proteins

Biuret test (General test): To 3 ml extract solution 4% sodium hydroxide and few drops of 1% copper sulfate solution was added. The appearance of violet or pink colour indicated the presence of proteins.

7. Tests for amino acids

a. Ninhydrin test (General test): The extract and 3 drops of 5% ninhydrin solution were heated in a boiling water bath for 10 min. Purple or bluish colour indicated the presence of amino acids.

b. Millon's reagent: The extract was heated with 3 drops of Millon's reagent. The dark red colour solution confirmed the presence of tyrosine.

8. Test for glycosides

A small portion of the extract was hydrolyzed by boiling with dilute hydrochloric acid for few min and hydrolysate was subjected to following tests.

a. Libermann-Burchard test - Chloroform solution of hydrolysate was treated with acetic anhydride and sulphuric acid. Formation of blue or blue–green colour indicated the presence of steroidal saponins whereas red, pink or violet colour indicated the presence of triterpenoids saponins.

b. Legal's test - The hydrolysate was dissolved in pyridine and solution of sodium nitroprusside was added to it and made alkaline. Formation of pink or red colour indicated the presence of cardiac glycosides.

c. Borntrager's test - An organic solvent like ether or chloroform was added to the hydrolysate and the contents were shaken. The organic layer was shaken and treated with solution of ammonia. The development of pink colour indicated the presence of anthraquinone glycosides.

9. Test for Saponin Glycosides

Foam test: About 1 ml of extract was diluted with water to 20 ml and shaken in a graduated cylinder

for 15 min. A 1 cm layer of foam indicates presence of saponins.

10. Test for flavonoids

Shinoda test: A small piece of magnesium ribbon was added to the alcoholic solution of the extract followed by drop wise addition of concentrated hydrochloric acid. The green blue colour indicates the test is positive.

11. Test for alkaloids

A small portion of solvent free extract was stirred with few drops of dilute hydroalcoholic acid and filter. The filtrate was tested with following reagents.

a. Dragendrof reagent (Potassium bismuth iodide) - To 2-3 ml filtrate, few drops of the reagent was added. Orange brown precipitate is formed.

b. Mayer's reagent (Mercury potassium iodide) – To 2-3 ml filtrate, few drops of the reagent added gives cream precipitate.

c. Hager's reagent (Saturated picric acid)- With 2-3 ml of filtrate the reagent gives yellow precipitate.

d. Wagner's reagent (Iodine reagent)-With 2-3 ml of filtrate the reagent gives reddish brown precipitate.

12. Test for phenolic compounds and tannins

A small quantity of extract was diluted with water and tested with following reagent. a) Dilute ferric chloride ($FeCl_3$) solution (5%) Intense blue, green, red or purple colour indicates the presence of phenolic compounds. An appearance of violet colour indicates the presence of tannins.

b) Acetic acid solution: Forms red colour solution indicating presence of phenolic compounds

c) Solution of gelatin (1%) containing 10% sodium chloride (NaCl)- Precipitate indicates positive test for tannins.

d) Lead acetate solution (10%)-Gives buff coloured precipitate for phenolic compounds.

Extractive values

Ethanol soluble extractive

5gms of dried coarse powder of Heartwoods were macerated with 100ml of 90% ethanol in a closed flask for 24hrs, shaken frequently during 6 hours and allowed to stand for 18hrs. Filtered immediately taking precautions against loss of ethanol. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 1050C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried drug.

Water soluble extractive

5gms of coarse powder was weighed and dissolved in 100ml of water in a stoppered flask, heated at 800C, shaken well and allowed to stand for 10min. It was cooled, 2gms of kieselghur was added and filtered. 5ml of the filtrate was transferred to a tarred evaporating dish and the solvent was evaporated on a water bath. The percentage of water soluble extractive was calculated with reference

to air dried drug.

Determination of volatile oil in drug

50gms of the drug was boiled with water in a Clavenger's apparatus. The process was continued till no more oil was collected in the graduated tube. The volume of oil was measured and expressed in percentage.

Determination of crude fibre content

About 2gms of the drug was accurately weighed and extracted with ether. Then 200ml of 1.25% sulphuric acid was added and boiled for 30min under reflux. It was filtered and washed with boiling water until free of acid. The entire residue was rinsed back into flask with 200ml of boiling 1.25% sodium hydroxide solution and again boiled under reflux for 30min. The liquid was quickly filtered and the residue was washed with boiling water until neutral, dried at 1100C to constant weight. It was then ignited to 30min at 6000C, cooled and weighed. The percentage of crude fibre content was calculated with reference to the air dried drug.

Determination of loss on drying

Glass stoppered shallow bottle was weighed that had been dried in the same conditions to be employed in the determination. About 1gm of the sample was transferred to the bottle and distributed evenly by gently side wise shaking to a depth not exceeding 10mm. Place the loaded bottle in a drying chamber (the stopper was removed and left in the chamber). The sample was dried to a constant weight and allowed to cool. The bottle along with the content was weighed. The process was repeated until the successive weights differed not more than 0.5mg (drying to constant weight). The percentage loss of weight was calculated with reference to the air dried drug.

Determination of foaming Index

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml of boiling water. The flask was maintained at moderate boiling at 80-900C for about 30min. It was cooled, filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100ml. Ten stoppered test tubes were cleaned (height 16cm, diameter 1-6cm) and marked from 1 to 10. 1, 2, 3ml up to 10ml of the filtrate was measured and transferred to each tube and adjusted the volume of the liquid with water to 10ml. Then the tubes were stoppered and shaken lengthwise for 15sec uniformly, allowed to stand for 15min the length of the foam was measured in each tube. If the height of the foam in each tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the Heartwood material is measured and transferred to a 100ml volumetric flask (V2) and the volume is made to 100ml and followed the same procedure.

Fluorescence Analysis

The fluorescence analysis of the drug powder as well as various extracts were carried out by using the method of Chase and Pratt. The behavior of the powder with different chemical reagents was

also carried out.

In-Vitro Efficacy Evaluation

Dried Heartwood Methanolic extract was dissolved in the respective solvents at the stoc 0.8 ml of concentration of 1 mg/ml. The appropriate dilutions of the stock solutions were made and used for the *in vitro* antioxidant assays [11].

DPPH Radical Scavenging Activity

To 0.1 ml of Methanolic solution of DPPH an equal volume of test compound was added at different concentrations in methanol. Equal volume of methanol was added to control. Above mixture was kept at room temperature for 20 minutes for incubation. Absorbance was recorded at 517 nm. Scavenging capacity was calculated by monitoring the decrease in absorbance at 517 nm. The antioxidant activity of test drug was expressed as IC50.

Percentage Inhibition was measured by using formula;

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Nitric Oxide Radical Scavenging Activity

Nitric oxide from sodium nitroprusside in aqueous solution at pH interacts with oxygen to generate nitrite ions, which were measured by the Griess reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10mM) in phosphate-buffered saline and various concentrations of Aqueous, hydroalcoholic and methanolic extracts at different concentrations were incubated at 25ºC for 150 min. A 0.5-ml aliquot of the incubated sample was removed and 0.5 ml Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% phosphoric acid) was added. The absorbance of purple chromophore formed during diazotization of nitrite along with suphanilamide and subsequent coupling with napthylethelenediamine was measured at 546 nm. Percent inhibition was measured by comparing the absorbance values of test samples as per the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

3. RESULTS AND DISCUSSION

Physicochemical characteristics of the crude drug

Various physicochemical characteristics of the powder drug of Heartwood were carried out.

Ash analysis and moisture contents

In the present study ash analysis for crude powder drug of Heartwood was carried out. It was observed that highest value of total ash was recorded for Heartwood (4.5%). Acid insoluble ash was in the range of 3.25%, Water soluble ash was in the range of 3.34 % (Table 1). Plant species with medicinal properties have been playing a fundamental role in the efforts for drug discovery all over the world. 80% populations in developing countries are dependent on plants for their primary health

care, and in spite of the significant progress in the field of synthetic organic chemistry of the twentieth century, more than 25% of prescribed medicines in developed countries are derived directly or indirectly from plant sources [12].

Table 1. Physico-chemical constants

S.N	Parameters	Percentage (%w/w)
1	pH	6.8±0.5
2	Total ash	4.5±0.3
3	Acid insoluble ash	3.25±0.4
4	Water soluble ash	3.44±0.5
5	Sulphated ash	4.3±0.3
6	Water soluble extractive	12.2±0.4
7	Alcohol soluble extractive	3.5±0.4
8	Crude fibre content	1.25±0.2
9	Loss on drying	4.56±0.6
10	Foaming index	Less than 100

Preliminary phytochemical screening is important and useful for isolation of pharmacologically active compounds present in the plants, [14]. This serves as an important tool for the quality assurance of plant for future studies. Till now almost all investigated plants showed to contain different active constituents of pharmacological importance in the form of secondary metabolites [15]. Qualitative phytochemical screening of the leaves, revealed that Proteins ,carbohydrates and tannins were present in both aqueous and ethanolic extracts of all the parts. Alkaloids were detected in both aqueous and ethanolic extracts of leaf and stem .Flavonoids were present in both type of extracts of all parts except root. Saponins and glycosides were present in all extracts; however Glycosides were not detected in Borntrager test. Phytosterol and tri-terphenoids were present in all the parts, while spot test for fixed oil gave negative result. Volatile oil were only detected in the ethanolic and aqueous extracts. The result showed that these plants rich in bioactive compounds and hence is a potential source of therapeutic properties (Table 2).

Table 2: Phytochemical studies

S.No	Secondary metabolites	Methanol	Ethyl acetate	Ethanolic	Aqueous
1	Steroids	+	+	+	+
2	Triterpenes	++	++	+	-
3	Saponins	+	-	+	+
4	Tri terpinoidal saponins	+	-	++	-
5	Alkaloids	+	+	+	+
6	Carbohydrates	-	+	+	-
7	Flavonoids	++	+	++	+
8	Tannins	++	+	+	+
9	Glycosides	+	+	+	+
10	Polyphenols	+	+	+	+

Fluorescence Analysis

Fluorescence study with UV light is a very useful tool for evaluation of crude drugs. Crude extract or powder drugs, when viewed under UV light give characteristic fluorescence [16]. The fluorescence phenomenon is the emission of light of different wave lengths, under the influence of UV light, due to different chemical constituents, otherwise not observable in ordinary visible light [17]. Fluorescence study helps for authentication and standardization of crude drugs. Saveral Crud drugs of plant origin have been authenticated though the fluorescence study technique e.g., *Morinda umbellatai* [18], *Holoptelea integrifolia* [19], root and stem of *Ichnocarpus frutescens* [20], leaves of *Catunaregum spinosa* , *Hygrophila auriculata* and *Crocus sativus*. Powder drug leaf was such and after treatment with various solvents was subjected to fluorescence analysis. Observations were made under visible light and under UV light of short wave length and long wave length. Results are presented in Table 3. Powder drug of the two plants exhibited fluorescence. Fluorescence study is very useful, quick, easy and unfailing method for the detection of adultrations.

Extractive values

Extractions of crude powder drug with different solvents gives different extractive values. Extractive value is one of the useful methods for evaluation of crude drugs, and provides guidance about the most suitable solvent to be used for extraction, and also helps in detecting various types of adulteration and exhausted materials i.e. Water and alcohol soluble extractive values can be used for the detection of adulterants, defective processing and poor quality of the drug while petroleum ether soluble extractive value indicates lipid contents present in the crude drugs. The fluorescence analysis of powder with various reagents and extracts are given in the Table 3 and 4.

Table 3: Fluorescence analysis of powder

S.No	Reagents	Day light	Short UV (254nm)	LongUV(365nm)
1	Powdered Heartwood	Red	Dark Red	Red
2	Powder + 1 N HCl Yellow	Light Yellow	Red	Red
3	Powder + 1 N NaOH	Pale Red	Red	Red
4	Powder + 50%HCl	Light Yellow	Fluorescent Red	Fluorescent Red
5	Powder + 50%H_2SO_4	Dark Red	Dark Red	Dark Red
6	Powder +50%HNO_3	Dark Brown	Brown	Reddish brown
7	Powder + Methanol	Red	Fluorescent Red	Light Red
8	Powder + Methanol +1 N NaOH	Red	Brown	Red

Table 4: Fluorescence analysis of various extracts

S.No	Extracts	Day light	Short UV (254nm)	Long UV(365nm)
1	n-Hexane	Red	Red	Dark Red
2	Chloroform	Reddish brown	Brown	Dark Red
3	Ethyl acetate	Yellowish Red	Red	Light Red
4	Methanol	Brownish Red	Orangish Red	Light Red

Evaluation of Invitro Efficacy of Extracts

DPPH Radical Scavenging Activity of Extracts

Different fractions of *Pterocarpus santalinus Linn* for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activities in Table 5. Methanolic extract showed the highest scavenging activity followed by Ethyl acetate. DPPH scavenging activity was significantly correlated with phenolics and flavonoids in different extracts [21-25].

Table 5: Antioxidant activity of Heartwood solvent extracts based on their polarity

Extracts	Concentration of extract in PPM	% of DPPH free radical Scavenging activity
Ethanol	50	45%
	100	58%
	150	60%
	200	65%
	400	68%

Ethyl acetate	50	55%
	100	58%
	150	65%
	200	70%
	400	75%
Methanol	50	65%
	100	68%
	150	70%
	200	75%
	400	82%
Aqueous	50	55%
	100	58%
	150	65%
	200	68%
	400	70%

The antimicrobial activity index of extracts of *Pterocarpus santalinus Linn Heartwood* at different concentrations was also investigated and is detailed in Table 6. Methanolic extract of the Heartwood recorded the highest antimicrobial activity and achieved the highest activity index among all the extracts. The difference in the activity indices may be due to different phytoconstituents present in the individual extracts. This is because different solvents have different degrees of solubility for different phytoconstituents [26-31].

Table 6: Antibacterial Activity of *Pterocarpus santalinus Linn* Heartwood

Extracts No	Gram negative bacteria		Gram positive bacteria	
	E.coli MTCC 443	*P.aeruginosa MTCC 424*	*S.aureus MTCC 96*	*S.pyogenes MTCC 442*
	Zone of inhibition in mm [b]			
Methanolic Extract	15	16	15	13
Control	10	8	7	6

4. CONCLUSION

Phytochemicals present in the different extracts of Heartwood of *Pterocarpus santalinus Linn* was identified. Among the extracts Methanolic extract has highest anti-oxidant property when compared to other extracts. In the present study it was found that *Pterocarpus santalinus Linn* Heartwood Methanolic extract has an excellent antimicrobial activity. The pathogenic bacteria were inhibited in presence of the Heartwood extracts of *Pterocarpus santalinus Linn M*ethanolic extract. Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant

is controlling pathogenic bacteria.

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CONFLICT OF INTEREST

Authors have no conflict of interest

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