Anti-oxidant, Anti-inflammatory and Anti-Microbial Activity of Pterocarpus santalinus L. f.

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ABSTRACT
Pterocarpus. santalinus L. f., a plant of notable significance, is extensively employed in the treatment of several ailments owing to its abundant therapeutic attributes. Pterocarpus santalinus L. f. family fabaceae known as red sandalwood. It is a deciduous tree of small-to-medium size. In this study in vitro antioxidant, anti-inflammatory, and antimicrobial activity of P. santalinus L. f. leaves, heartwood extracts were investigated. P. santalinus L. f. leaves, heartwood both extracts showed impressive antioxidant activity and when compared to ibuprofen, heartwood extract showed the strongest anti-inflammatory effect. The results of this study also showed significant antimicrobial action against Helicobacter pylori (ATCC 43579), E. coli (ATCC 29181), Staphylococcus aureus (ATCC-6538), and Klebsiella pneumoniae (K36). The highest zone of inhibition was demonstrated by P. santalinus L. f. heartwood extract. Thus, it appears from the available data that P. santalinus L. f. has significant biopotential, and its application as a powerful medicinal agent deserves consideration.

Keywords: Anti-inflammatory, Anti-oxidant, Anti-microbial, Pterocarpus santalinus L. f., Red Sandalwood

INTRODUCTION

Plants, regarded as natural entities, possess significant value as sources of bioactive chemicals and have been extensively utilized for medical purposes throughout many regions globally (Ahad et al., 2021). In recent times, there has been a notable surge in scientific interest surrounding oriental medicine, particularly about the identification of innovative pharmaceuticals for the treatment of diverse ailments such as cancer and diabetes (Natalia et al, 2022).The endorsement of the assessment of the possible therapeutic advantages of plants by the World Health Organisation (WHO) is particularly significant in regions where the availability of secure contemporary medications is limited (Khan and Ahmad, 2019; Ezzat et al., 2019). Traditional plants are extensively utilised by the indigenous population and traditional healers to treat various ailments (Mukherjee et al., 2021).

P. santalinusL. f. known as red sandalwood is a deciduous tree of small-to-medium size that falls within the Fabaceae family (Arbain et al., 2021). The species exhibits a broad geographical distribution, primarily concentrated in tropical regions across the globe, with notable prevalence in India, Sri Lanka, Taiwan, and China.Previous studies have examined the phytochemical composition, pharmacological properties, and traditional medicinal uses of P. santalinus (Azamthulla et al., 2015).According to Ayurveda, an ancient Indian system of medicine, the heartwood of certain plants is utilized as an external remedy for various ailments such as inflammation, diabetes, headaches, skin disorders, jaundice, and wound healing (Bhowmik et al., 2010).The heartwood and leaves extract of the plant P.santalinus L. f. contain bioactive chemicals that exhibit diverse biological actions, indicating the potential of this plant for therapeutic applications in the treatment of several ailments (Dahat et al., 2021). They possess antidiabetic, antibacterial, anti-inflammatory, and hepatoprotective effects in both in vitro and in vivo experiments (Navada andVittal, 2014). But, the use of gel formulations derived from leaves and wood extracts has not yet been thoroughly studied.Therefore,the present research work was conducted to perform a comparative account of therapeutic potential among hydroalcoholic and gel-based formulations of red sandalwood.

Material and Methods

Plants Collection and Authentication: Pterocarpus santalinus L. f. mature plants were collected from Rudeez Agrofarm, Nagaon, Assam in the month of May- June 2023. The wood and leaves of the plant was selected for the proposed work. The plant was authenticated by R. K. Pamarthi Scientist, ICAR-National Bureau of Plant Genetic Resources, Division of Plant Exploration and Germplasm Collection, National Herbarium of Cultivated Plants (NHCP), Pusa Campus, New Delhi 11012, vide reference no, AC-2023/143 dated 19.07.2023. The plant was identified as Pterocarpus santalinus L. f. Family Fabaceae.

Extraction of plant material

The P. santalinus L. f. heartwood and leaves were washed with water to remove dust and sand particles. The P. santalinus L. f.heartwood and leaves were dried and cut into small pieces. P. santalinus L. f. Heartwood and leaves powder were extracted using successive soxhletion using solvents viz., petroleum ether, ethyl acetate, chloroform and 50% ethanol (hydro alcoholic mixture) at 60°C, in soxhlet apparatus. The petroleum

ether, ethyl acetate, chloroform and 50% ethanol (hydro alcoholic mixture) heartwood and leaves extracts of P. santalinusL. f. were then filtered and concentrated using rotary vacuum evaporator. The dried leaves extracts yield were calculated and stored in amber colored wide mouth bottles in dessicator. These extracts were further used to evaluate their antioxidant, antimicrobial, and anti-inflammatory properties (Halim and Mishra, 2011; Singh and Sharma, 2014; Singh and Devi 2021).

Preliminary phytochemical investigations of the P. santalinus L. f. heartwood extracts

The heartwood and leaves extract of P. santalinus L. f. were analysed qualitatively to determine the presence of different phytochemical components. Standard protocols of the Association of Analytical Chemists were employed to conduct phytochemical screening of the concentrated extracts, to identify their contents (Morsy, 2014; Singh and Devi 2018; Singh and Devi 2021).

Antioxidant assay

DPPH Radical Scavenging Activity

The DPPH radical scavenging experiment was performed using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Analytical-grade ethanol was used to prepare six plant extract concentrations. As a typical antioxidant, L-ascorbic acid was produced in equal proportions. One milliliter of each extract was carefully placed in a sterile test tube and 0.5 ml of 0.3 mM DPPH in methanol was added. Stirred, the mixture rested for 15 minutes at room temperature in a lightless atmosphere.The negative control was 2.5 ml DPPH and 1 ml ethanol. L-ascorbic acid was utilized as the positive control at the same doses as the extracts. A spectrophotometer measured absorbance at 517 nm after incubation without light. The experiments were duplicated.The DPPH radical scavenging activity was estimated using following formula: % Radical scavenging activity = (Ac – As)/ Ac × 100

As- Absorbance of the sample, and Ac - Absorbance of the control (Bondet et al., 1997; Singh and Devi 2018)

Ferric Reducing Antioxidant Power Assay

Six different hydroalcoholic extracts (100, 300, 500, 700, 900 and 1100 µg/ml) were mixed with L-ascorbic acid at equal doses in this investigation. Add these combinations to a solution of 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml 1% potassium ferricyanide ($K_3Fe(CN)_6$). The solution was incubated at 50°C for 20 minutes. Then, 2 millilitres of 10% trichloroacetic acid (TCA) solution was added to the mixture and centrifuged at 1000 rpm for 10 minutes. Suction was used to remove 2 ml of liquid, which was then mixed with 2 ml of distilled water and 1 ml of a 0.1% ferric chloride solution. The experiment was repeated three times each case. Spectrophotometric measurements of absorbances were taken at 700 nm using a UV-vis spectrophotometer and recorded (Benzie et al., 1999).

Antimicrobial activity

To evaluate the antimicrobial effect, a disc diffusion test was conducted on an MHA medium plated with three pathogens. Fresh cultures of the isolates were centrifuged (8000 rpm, 15 min), and the supernatants were removed. Blank discs were inoculated with 40 µl supernatant of each isolate and were placed on separate MHA medium inoculated with Staphylococcus aureus (ATCC-6538), Klebsiella pneumoniae (K36), E. coli (ATCC 29181), Helicobacter pylori (ATCC 43579)**.** After incubation of all agar media at 37°C for 24 hours, the growth inhibition zones of pathogens and isolated lactobacilli inhibitory ability were assessed. (Zaidan et al., 2005; Singh and Devi 2018)

In-vitro Anti-inflammatory Activity

Anti-inflammatory action was assessed by stabilizing human red blood cell membranes. An equal volume of sterilized Alsever medium (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride in water) was combined with the blood. After centrifuging blood at 3000 rpm for 10 min, packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% (v/v) solution was prepared. The assay combination included the plant preparations, 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL hyposaline (0.36%), and 0.5 mL HRBC suspension. Reference medication was ibuprofen. 2 mL pure water was utilized as the control. The assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 10 minutes. A UV-Visible spectrophotometer at 560 nm was used to quantify hemoglobin levels in the supernatant (Shailesh et al., 2011; Singh and Devi 2020).

Equation for hemolysis percentage:

Protection(%)=100−[O.D test sample/O.D control)]×100

Hemolysis(%)=(O.D test sample/O.D control)×100

(O.D- optical density)

Statistical analysis

All measurements will be performed in triplicate and will determine its standard deviation (SD). Means will be compared using one-way analysis of variance analysis (ANOVA) and Duncan's test ($p < 0.05$).

RESULTS and DISCUSSION

The percentage yield of P. santalinus L. f. hydroalcoholic extracts of heartwood and leaves were found to be 13.35±0.5% w/w and 11.57±0.55% w/w

Preliminary phytochemical analysis of P. santalinus L. f. heartwood extract

The hydroalcoholic heartwood extract of P. santalinus L. f. revealed the presence of glycosides, flavonoids, alkaloids, tannins, phenols, saponins and sterols (Table 1).

Table 1: Preliminary phytochemical analysis of P. santalinus L. f. hydroalcoholic heartwood extract

S.No.	Constituents	Test	Petroleum ether Extract	Chloroform Extract	Hydroalcholic extract
1.	Alkaloids	a) Mayer's reagent b) Dragendorff's reagent c) Hager reagent	+++ +++ +++	+ + +	+++ +++ +++
2.	Steroids	a) Libermann'sburchard test b) 5% KOH	++ ++	++ ++	+++ +++
3.	Phenols	a) Ferric Chloride	++	++	+++
4.	Tannins	a) 10% Lead acetate solution b) Aqueous bromine solution	+++ +++	++ ++	+++ +++
5.	Flavonoids	a) Amyl alcohol + Sodium acetate+ Ferric chloride b) Conc. H_2SO_4 c) Magnesium turning test	+++ +++ +++	+++ +++ +++	+++ +++ +++
6.	Glycosides	Glacial acetic acid + Ferric chloride + Conc.H_2SO_4	+++	+++	+++
7.	Saponins	Foam test	+++	+++	+++

+ shows traces present; ++ shows present; - shows absent

Preliminary phytochemical analysis of P. santalinusL. f. hydroalcoholic leaves extract

The qualitative phytochemical analysis of the hydroalcoholic leaves extracts of P. santalinusL. f. showed the presence of alkaloids, tannins and sterols in extracts.

Table 2: Preliminary phytochemical analysis of P. santalinusL. f. hydroalcoholic leaves extract

S.No.	Constituents	Test	Petroleum ether Extract	Chloroform Extract	Hydroalcholic extract
1.	Alkaloids	a) Mayer's reagent b) Dragendorff's reagent c) Hager reagent	+++ +++ +++	+ + +	+++ +++ +++
2.	Steroids	a) Libermann'sburchard test b) 5% KOH	++ ++	++ ++	+++ +++
3.	Phenols	a) Ferric Chloride	–++	++	+++
4.	Tannins	a) 10% Lead acetate solution b) Aqueous bromine solution	+++ +++	++ ++	+++ +++
5.	Flavonoids	a) Amyl alcohol + Sodium acetate+ Ferric chloride b) Conc. H_2SO_4 c) Magnesium turning test	+++ +++ +++	+++ +++ +++	+++ +++ +++
6.	Glycosides	Glacial acetic acid + Ferric chloride + Conc.H_2SO_4	+++	+++	+++
7.	Saponins	Foam test	–	–	–

+ shows traces present; ++ shows present; - shows absent

Antioxidant Activity

Excessive formation of free radicals, electrically charged molecules, is connected to certain human disorders. Reactive oxygen species (ROS) like hydroxyl radicals harm cell structure and function, oxidize lipids, proteins, and DNA, and contribute to disease development. Dietary phytochemical antioxidants have been demonstrated to remove free radicals in animal experiments. Phenolic and polyphenolic substances, like flavonoids in food, have strong antioxidant properties (Singh and Devi 2018; Martemucci et al., 2022).In the current study ferric reducing antioxidant power and DPPH assay were performed for evaluating various preparations of red sandalwood.

In vitro DPPH scavenging activities

The technique of DPPH radical scavenging has been commonly used to evaluate the antioxidant efficacy of various substances over time. This method involves testing molecules for their ability to scavenge and reduce DPPH free radicals in vitro. The result of DPPH radical scavenging is a change in color from blue to yellow, which can be measured at 517 nm. In the present study, P. santalinus L. f. hydroalcoholic extract of heartwood as well as leaves showed remarkable in vitro DPPH radical scavenging activity in a dose-dependent manner. However, the standard ascorbic acid exhibited significantly higher DPPH radical scavenging activity than P. santalinus L. f. leaves and heartwood extract.

Table 3: In vitro DPPH scavenging activities of P. santalinus L. f.

Conc(µg/ml)	Ascorbic Acid	HPE	HCE	HHE	LPE	LCE	LHE
100	59.09 ±0.02	48.63 ±0.03	45.03±0.02	50.01 ±0.02	47.98 ±0.02	44.91 ±0.01	49.89 ±0.03
200	69.89±0.02	56.54±0.01	55.87±0.02	59.99±0.02	55.77±0.01	54.07±0.01	58.97±0.01
300	77.39 ±0.03	62.08 ±0.02	59.43 ±0.03	65.45 ±0.03	64.68 ±0.02	61.08 ±0.02	66.48 ±0.01
400	80.11 ±0.01	68.19 ±0.03	65.11 ±0.01	71.02 ±0.03	68.11 ±0.01	65.01 ±0.02	70.01 ±0.01
500	83.31 ±0.03	71.01 ±0.03	71.23 ±0.03	78.87 ±0.03	73.23 ±0.02	70.34 ±0.03	75.66 ±0.01
600	85.67 ±0.01	78.50 ±0.02	66.89 ±0.01	80.77 ±0.02	77.79 ±0.03	74.77 ±0.02	79.98 ±0.03
IC_{50}	0.06	0.22	0.24	0.12	0.25	0.28	0.18

The values are expressed as mean ± SEM. ($P > 0.05$, one-way ANOVA followed by Duncan's test). HPE- Heartwood Petroleum extract, HCE- Heartwood chloroform extract; HHE- Heartwood Hydroalcoholic extract; LPE- Leaves Petroleum extract, LCE- Leaves chloroform extract; LHE- Leaves Hydroalcoholic extract

In vitro ferric reducing antioxidant power activities of P. santalinus L. f.

The study used the ferric-reducing antioxidant power (FRAP) assay. This method measures the ability of the sample to reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The amount of Fe^{2+} formed can be detected by measuring the absorbance at 700 nm. The leaf and heartwood extracts of the plant showed a concentration-dependent increase in absorbance values at 700 nm. The heartwood beads had significantly higher absorbance values compared to other extracts and bead preparations. However, the standard, ascorbic acid, had significantly higher absorbance values than the petroleum ether, chloroform and hydroalcoholicheartwood and leaves extracts (Enemali et al., 2019). Our results also support those of Halim and Misra who showed that P. santalinus L. f. heartwood extracts reduced levels of MDA, the end products of lipid peroxidation, in diabetic subjects (Halim and Misra, 2011). This reduction can be attributed to an increase in superoxide dismutase activity, which prevents free radical activity.

Table 4: In vitro ferric reducing antioxidant power activities of P. santalinus L. f.

Conc (µg/ml)	Ascorbic Acid	HPE	HCE	HHE	LPE	LCE	LHE
100	1.99 ±0.01	1.86 ±0.01	1.79 ±0.02	1.90 ±0.01	1.85 ±0.03	1.76 ±0.02	1.89 ±0.03
300	2.37 ±0.02	2.11 ±0.01	2.05 ±0.02	2.28 ±0.01	2.09 ±0.02	2.01 ±0.01	2.19 ±0.02
500	2.55 ±0.01	2.31±0.01	2.35 ±0.01	2.50 ±0.01	2.30 ±0.03	2.30 ±0.02	2.48 ±0.02
700	2.75 ±0.01	2.52 ±0.02	2.62 ±0.03	2.73 ±0.02	2.55 ±0.01	2.63 ±0.03	2.70 ±0.01

900	2.99 ±0.02	2.75 ±0.03	2.71 ±0.02	2.95 ±0.01	2.61 ±0.01	2.69 ±0.02	2.90 ±0.01
1100	3.08 ±0.01	2.90 ±0.02	2.83 ±0.01	3.03 ±0.01	2.90 ±0.01	2.81 ±0.01	3.00 ±0.02
EC_{50}	0.14	0.18	0.17	0.16	0.19	0.18	0.17

The values are expressed as mean ± SEM. ($P > 0.05$, one-way ANOVA followed by Duncan's test). HPE- Heartwood Petroleum extract, HCE- Heartwood chloroform extract; HHE- Heartwood Hydroalcoholic extract; LPE- Leaves Petroleum extract, LCE- Leaves chloroform extract; LHE- Leaves Hydroalcoholic extract

In-vitro antimicrobial activity

The regular use of these pharmaceuticals by humans has led to the development of drug resistance in human pathogens, making it extremely difficult for researchers to create safe and efficient treatments for infectious diseases. Numerous research teams have investigated the antibacterial and radical-scavenging properties of plant extracts, demonstrating the extracts' potent antibacterial and radical-scavenging properties without any negative side effects.The previous findings suggested that the leaves and heartwood extracts of P. santalinus L. f. when tested for antibacterial activity against Gram-positive bacteria, including Staphylococcus aureus, S. epidermidis, B. subtilis, E. coli,and P. aeruginosa. Heartwood extract, exhibited higher inhibitory activity than leaves extract (Manjunatha, 2006). Furthermore, among various timber-yielding plants examined for antibacterial action against uropathogenic bacteria, P. santalinus showed the highest inhibition (Mishra and Padhy, 2013).Moreover, a comparative data indicated that methanolic extract of P. santalinus leaves extracts exhibits more inhibitory action than the aqueous extract (Dey et al., 2014). The Other studies suggest that lignans in P. santalinus may have antimicrobial effects against Helicobacter pylori (Narayan et al., 2007).

In the current study, with a dose-dependent rise in inhibitory zones, the plant preparations demonstrated outstanding effectiveness against Staphylococcus aureus (ATCC-6538), Klebsiella pneumoniae (K36), E. coli (ATCC 29181), and Helicobacter pylori (ATCC 43579). The hydroalcoholic heartwood extract showed a rise in the inhibitory zone that was dosage dependent and ranged from 10.3 to 16.88 mm for Helicobacter pylori, 9.99 to 15.45 mm for E. Coli, and 7.88 to 17mm for Staphylococcus aureus (Table 5). Maximum inhibition was exhibited in the range of 8.65 to 17.5 mm for Klebsiella pneumoniae. Leaf Hydroalcoholic extract was also found to cause a dose-dependent increase in the inhibitory zone, with values for Staphylococcus aureus, E. Coli, and Helicobacter pylori ranging from 7 to 16.3 mm, 9.5 to 15.03 mm, and 9.8 to 16 mm, respectively (Table 6). LHE showed remarkable inhibition against Klebsiella pneumoniae again in dose dose-dependent manner with values ranging from 8 to 16.8.

Table 5: Growth inhibition zone diameter (mm) of pathogens at various concentrations of Heartwood Hydroalcoholic extract (HHE)

Bacterial species	**Zone of inhibition in (mm) at various Concentration of HHE** (µg/ml)						
	100	**300**	**500**	**700**	**900**	**1100**	**Standard**
Staphylococcus aureus (ATCC-6538)	7.88	9	11	14.77	14.9	17	18.5
Klebsiella pneumoniae (K36)	8.65	9.89	10.8	15	16.1	17.5	19
E.coli (ATCC 29181)	9.99	10.87	12.06	15	15.3	15.45	17
Helicobacter pylori (ATCC 43579)	10.3	11.08	13.98	16	16.45	16.88	19.02

Table 6: Growth inhibition zone diameter (mm) of pathogens at various concentrations of Leaves Hydroalcoholic Extract (LHE)

Bacterial species	Zone of inhibition in (mm) at various Concentration of LHE (µg/ml)						
	100	**300**	**500**	**700**	**900**	**1100**	**Standard**
Staphylococcus aureus (ATCC-6538)	7.0	7.0	10.8	13.2	14.5	16.3	17.98
Klebsiella pneumoniae (K36)	8.0	10.8	11.7	14.7	15	16.8	17.08
E.coli (ATCC 29181)	9.5	10.9	13	14.0	14.65	15.03	16.88
Helicobacter pylori (ATCC 43579)	9.8	11	13.	15	15.6	16	17

In vitro anti-inflammatory activity

Inflammation is critical for the onset and progression of disorders. Anti-inflammatory and cytotoxic properties are the main therapeutic effects of most phytomedicines. T lymphocytes significantly increase chronic inflammation by stimulating inflammatory cells such mast cells, eosinophils, neutrophils, and macrophages, leading to increased production of chemical mediators and pro-inflammatory cytokines. The anti-inflammatory properties of lignan components from P. santalinus L. f. heartwood were examined had strong anti-inflammatory action with an IC50 of 0.19 µg/mL (Wu, 2011). Kumar found that the heartwood extract (500µg/mL) significantly inhibits carrageenan-induced inflammation in paw edema (Kumar, 2011; Singh and Devi 2018; Singh and Devi 2020).

In the present study anti-inflammatory study of various P. santalinus leaves and Heartwood hydroalcoholic extract showed significant stabilization at various concentrations (100, 300, 500,700,900 and 1100µg/ml) towards Human Red Blood Cell membranes (HRBC). Ibuprofen being a standard, showed lowest Haemolytic effect (11.81) and highest percentage protection (98.99), indicating excellent anti-inflammatory effect.Among different plant preparations P. santalinus HHE at 1100 (µg/ml) exhibited maximum anti-inflammatory activity which is indicated by minimum Haemolytic effect (28.09%) and maximum Protection percentage (90.89 %). The results were tabulated in table 7 and table 8.

Table 7: Haemolytic effect (%) of P. santalinus L. f. heartwood and leaves hydroalcoholic extract

Concentration (µg/ml)	Ibuprofen	LHE	HHE
100	32.89±0.01	43.33±0.02	46.76±0.01
300	30.21±0.02	41.76±0.03	44.02±0.02
500	28.09 ±0.01	39.18 ±0.02	41.08 ±0.02
700	25.01±0.02	34.11±0.01	37.65±0.01
900	18.06 ±0.01	30.01 ±0.01	32.02 ±0.01
1100	11.81 ±0.01	26.78 ±0.02	28.09 ±0.02

Ibuprofen was used as the control. The values are expressed as mean ± SEM. ($P > 0.05$, one-way ANOVA followed by Duncan's test); HHE- Heartwood hydroalcoholic extract, LHE- Leaves hydroalcoholic extract

Table 8: Protection (%) of various red sandalwood preparations

Concentration (µg/ml)	Ibuprofen	LHE	HHE
100	75.33±0.03	49.34±0.01	65.73±0.01
300	78.32±0.01	50.32±0.01	69.65±0.03
500	80.88 ±0.01	53.07 ±0.03	71.11 ±0.02
700	85.08±0.02	61.97±0.01	79.06±0.01
900	89.03 ±0.01	69.17 ±0.01	83.80 ±0.01
1100	98.99 ±0.01	76.11 ±0.02	90.89 ±0.02

Ibuprofen was used as the control. The values are expressed as mean ± SEM. ($P > 0.05$, one-way ANOVA followed by Duncan's test); HHE- Heartwood hydroalcoholic extract, LHE- Leaves hydroalcoholic extract

CONCLUSION

Based on the obtained data, it was determined that P. santalinus L. f. leaves and heartwood extracts, have significant antibacterial, anti-inflammatory, and antioxidant properties because of their phytochemicals. When compared to several standards, P. santalinus L. f. heartwood extract showed exceptional antioxidant, anti-inflammatory, and antibacterial properties among all the preparations. Last but not least, the current study offers the proof for other researchers to employ P. santalinus L. f. as a successful natural medication. It is advised to conduct more research to fully assess P. santalinus L. f. safety and therapeutic efficacy.

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