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Research Article

Pterocarpus santalinus L. f: A study on phytochemical constituents and biological properties of bark and heartwood extracts

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Abstract

Pterocarpus santalinus L.f Commonly known as "Red Sanders" a highly impressive indigenous deciduous tree, renowned for its characteristic timber of exquisite colour found use in colouring pharmaceutical preparations. The bark and heartwood heal various skin diseases and used in cosmetics and traditional medicines. Hence, phytochemical screening of the bark and heartwood extracted with methanol and ethanol was carried out to understand their medicinal value. Qualitative analysis of bark and heartwood of *P.santalinus* confirmed the presence of alkaloids, flavonoids, tannins, phenols, saponins and terpenoids. Quantitative analysis was also carried out and found that the concentration of phenols and alkaloids were more in heartwood than bark of *P. santalinus*. However, tannins and flavonoids were found to be more in bark than heartwood. Antioxidant activity was carried out by 2, 2-Diphenyl-1-Picryl Hydrazyl (DPPH) assay to determine the scavenging activity and showed 50 percent scavenging activity in both methanol and ethanol extracts of bark and heartwood of *P.santalinus*. Antimicrobial activity against human pathogens viz., *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed that the ethanol extracts of bark found to have antagonistic activity while the ethanol extract of heartwood observed to have synergetic activity against these tested human pathogens.

Keywords: Calyx Yield, Economic Advantage, Inorganic Fertilizer, Organic Fertilizer, Roselle *Pterocarpus santalinus*, phytochemical, antimicrobial activity, antioxidant activity, red sanders.

INTRODUCTION

Plants are the source of first medicines and all plant species are considered as potential resource for humanity. The natural plant based remedies are as old as humankind which was evidenced through pre-historic sites and written records. Plant based natural medicines are the most widely used medicines for both acute and chronic health issues. Over the past 100 years, the development and bulk manufacture of chemically synthesized medicines have revolutionized health care in most parts of the word. In developing countries large population still rely on traditional practitioners and herbal medicines for their primary health care. Use of traditional medicine is not only limited to developing countries, during the past two decades public interest in natural therapies has increased greatly in industrialized countries, with expanding use of ethno botanicals.

The history of medicinal plants used for treating diseases and ailments is probably dates back to the beginning of human civilization. World Health Organization predicted that 80 percent of people worldwide rely on herbal medicines and around 21,000 plant species have the potential for being used as medicinal plants (nhp.gov.in). The forest in India has been a rich repository of large quantities of medicinal plants and home to more than 50,000 species of plants. The growing demand towards medicinal plant products has renewed attention in the production of herbal health care

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formulations, herbal-based cosmetic products, and herbal nutritional supplements. Hence, there is a demand to promote natural products to save the human lives.

There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants because of the worldwide trend toward the use of natural additives in foods, beverages and cosmetics. The increasing demand for plant based antioxidants has increased in recent years due to the growing concern among consumers about these synthetic antioxidants because of their potential toxicological effects (Nunez de Gonzalez et al., 2008). However, antioxidant supplements help the human body from the oxidative damage (Kuhnan, 1976). Oxidative damage plays a significant pathological role in human diseases such as cancer, inflammation arthritis, diabetes and atherosclerosis (Halliwell, 1991).

The alarming increase in the rate of infection by antibiotic resistant microorganism and the microbial infections pose a health problem throughout the world (Davies, 1994). The antimicrobial activity of plants is associated with the defence mechanism against microorganism and plants promise a vital source of natural antimicrobial agents (Jancic et al., 1995). The increasing resistance of most synthetically derived antimicrobial agents is of utmost concern. In recent years, there has been renewed interest in the treatment against different diseases as herbal drugs are generally known to be nontoxic (Rao & Rao, 2001). In general medicinal plants offer polyvalent action through multiple active constituents and are more effective than single compound. (Pullaiah et al., 2019) reported that *P. santalinus* is one such tree species in which the bioactive compounds present in it accounts for its potential health benefits.

Pterocarpus santalinus L.f (Red Sanders) belongs to the family Fabaceae is endangered globally and endemic to India. In India, the natural range of *P. santalinus* is limited to 15,540 km in the South eastern Ghats and endemic to Andhra Pradesh (Sarma, 1993), (Raju & Nagaraju, 1999), (Prakash et al., 2006), (Balaraju et al., 2011). The red sandalwood tree is renowned for its exquisite colourand fragrance of heartwood derived from santalinus while the pleasant aroma is caused by the presence of terpenoids (Kumar et al., 1974). It is a precious timber which is highly valued for its pretty and attractive exceptional technical qualitiesmakes it popular in the furniture industry (Prakash et al., 2006), (Arunakumar et al., 2011), (Arunkumar & Joshi 2014), (Azamthulla et al., 2015). *P. santalinus* is used in traditional herbal medicine and a wide array of biological activities including antioxidative, antidiabetic, antimicrobial, anticancer and anti-inflammatory properties (Arunakumara et al., 2011), (Pullaiah et al., 2019). Stem, bark and leaf extracts of *P.santalinus* showed maximum antimicrobial activity (Manjunatha, 2006), (Mohandass et al., 2010). Considering its various health benefits, the present study focused on the evaluation of phytochemical constituentsof the bark and heartwood of *P.santalinus* responsible for its antioxidant and antimicrobial activities. Evaluation of the therapeutic efficacy may pass the development of a health care product.

MATERIALS AND METHODS

Plant material

The bark and heartwood of *Pterocarpus santalinus* were collected from Vilar highways of Kollangarai village, Thanjavur district, Tamilnadu situated between 10°41'23.3"N latitude and 79°10'12.3"E longitude. The bark and heartwood samples were brought to the laboratory, washed under running tap water, shade dried at room temperature and ground into fine powder for experimental analysis.

Preparation of the extracts

The powdered plant samples of *P.santalinus* (20 g) were extracted with 350 ml of methanol and ethanol each using Soxhlet apparatus at 65-80°C for 8-10 h. The solvents of the respective extracts were reduced in rotary vacuum evaporator until complete removal of solvents and stored at 4°C for further use. The stored plant extracts were then dissolved in respective solvents while experimentation to get the solution of 10 mg/10 mL for each extract which has been subjected to *in-vitro* antioxidant and antimicrobial assays.

Qualitative Phytochemical Analysis

Qualitative phytochemical screening was carried out to identify the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins, sterols, phenols, glycosides, and terpenoids in the methanol and ethanol extracts of bark and heartwood of *P. santalinus* using standard methods (Harborne, 1967).

Estimation of alkaloid compounds

1 mg of the extract was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to separating funnel and washed with 10 ml chloroform. The pH of phosphate buffer solution was adjusted to neutral with 0.1N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of bromocresol solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm (Karawya et al., 1971).

Estimation of phenolic compounds

The total phenolic compound present in the bark and heartwood of *P.santalinus* was estimated Folin-Ciocalteau

reagent method described by (Malick & Singh 1980). One gram of bark and heartwood extract of *P. santalinus* was mixed with each 80 mL of ethanol and incubated overnight at room temperature. Centrifuged the homogenate at 10,000 rpm for 20 min and saved the supernatant. Re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness and dissolved the residue in a known volume of distilled water (5 ml). For total phenolic content determination, 0.2-2.0 mL of each sample was taken separately and the volume was made up to 3 ml with distilled water and added 0.5ml of Folin-Ciocalteu reagent. After 3 min, added 2 ml of 20% of Na_2CO_3 solution to each tube and mixed thoroughly. Placed the tube in boiling water bath exactly for 1min, cooled and measured the absorbance at 650 nm. A calibration curve of the standard catechol was constructed and the total phenolics content in the extracts were expressed as mg of catechol equivalent (mg/g extract) by using the standard curve.

Estimation of Tannins compounds

The tannin content was estimated using the procedure described by (Robert, 1971) using vanillin hydrochloride reagent method. 1 ml of the extracts of bark and heartwood of *P. santalinus* were mixed with 5ml of vanillin hydrochloride reagent. The mixture was incubated for 20 min at room temperature. The tannin content of the supernatant was measured at 500 nm.

Estimation of flavonoids compounds

The flavonoid content of bark and heartwood extracts of *P.santalinus* was estimated by the method of (Harborne, 1967). An aliquot of the extracts were evaporated to dryness and added 4 ml of vanillin reagent. The solutions were heated for 15 minutes in a boiling water bath. The flavonoids content present in the sample was measured at 415 nm. Concentration of flavonoids was calculated and the values were expressed as µg flavonoids/g sample.

In-vitro antioxidant activities - DPPH radical scavenging activity

The ability of the bark and heartwood methanol and ethanol extracts to scavenge the DPPH free radicals was assessed using the method described by (Sharma et al., 2009). 0.1 mM solution of DPPH (1, 1-Dipheny- 2-Picrylhydrazyl) in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (20-100 mg/ml). The solution was mixed thoroughly and incubated for 30 minutes at room temperature. The absorbance was measured at 517 nm using UV-Vis spectrophotometer. A blank was prepared without adding sample extracts. Ascorbic acid at various concentrations (20 to 100 mg/ml) was used as standard. Lower the absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = [(A control– A test) / A control] × 100

Evaluation of antimicrobial property of *P.santalinus* bark and heartwood

The antibacterial potency of both bark and heartwood extracts of *P.santalinus* was evaluated using three bacterial strains infectious to human health. Major bacterial human pathogens that causes a wide variety of clinical manifestations are Klebsiella pneumoniae, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were used for the antimicrobial assay. All the original culture of the bacterial strains were maintained on Müller-Hinton (MH) agar plates at 4°C and grown at 37°C if required. Antimicrobial susceptibility test was performed for all microbial isolates following Kirby Bauer disc diffusion method. To concentrate the bacteria 20 ml of fresh bacterial culture was centrifuged at 4000 x g for 15 min. 100 µL of bacterial inoculum containing 108 CFU/mL was spread over sterile plates containing Mueller Hinton agar medium. Sterile filter paper discs (6 mm in diameter) impregnated with both bark and heartwood extracts concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5µg of Gentamycin was used as positive control. The culture plates were stored at 4°C for 2 h to allow plant extracts diffusion followed by incubation for 24 hrs. at 37°C. The diameters of inhibition zones were measured and considered as indication for antibacterial activity.

RESULTS AND DISCUSSION

The processed bark and heartwood samples subjected to hot extraction using polar solvents to separate the therapeutic compounds. The purpose of all extraction is to separate the soluble plant metabolites (Handa et al., 2008). The *P.santalinus* bark and heartwood showed higher yield in methanol than ethanol and yield was high in bark than heartwood (**Table 1**). Methanol extracted 39% more than ethanol in both bark (11.71 and 8.41%) and heartwood (11.42 and 8.21%) extracts. (Tchamadeu et al., 2011) reported that the yield of hot extract was high in dried bark materials of *Pterocarpus soyauxii*.

Qualitative analysis of bark and heartwood of *P.santalinus*

The phytochemical research approach is considered effective in discovering bioactive profile of plants of therapeutic importance. The ethanol extract of bark and heartwood contains alkaloids, flavonoids, tannins, phenols and terpenoids and the methanol extract of bark and

heartwood contains alkaloids, tannins, saponins, phenols and terpenoids. Both bark and heartwood extracts showed the presence of flavonoid in ethanol extract and saponins in methanol extract only (**Table 2**). (Arunakumar et al., 2011) confirmed the presence of various components, such as carbohydrates, steroids, anthocyanins, saponins, tannins in *P. santalinus*. The presence of flavonoids, glycosides, and phenols were reported in the bark of P.santalinus (Kondeti et al., 2010) and carbohydrates, flavonoids, terpenoids, phenolic compounds, alkaloids, saponins, tannins, and glycosides were reported in the heartwood of red sanders (Kesari et al. 2004). A variety of plant ingredients with diverse structures are capable of promoting health benefits. These secondary metabolites are widely used in human therapy, veterinary, agriculture, scientific research and in countless other area (Kandukuri et al., 2009).

Quantitative analysis of secondary metabolites of *P.santalinus* bark and heartwood

Plant produces vast and diverse assortment of organic compounds have shown their nutritional value in the form of flavors, food addition and as biochemicals having industrial application and health benefits. Chemical examination of Pterocarpus woods started more than a century, yet new compounds are still being discovered and variety of compounds were identified some which are unique to the genus (Seshadri, 1971). Quantitative analysis of phytochemicals in methanol and ethanol extracts of the bark for phenol, alkaloid, tannin and flavonoid showed that tannin was quantified more in both the extracts (12.58 mg/g and 12. 41 mg/g) than other phytochemicals (**Table 3**). In heartwood also tannin was quantified high, but less than bark extracts comparatively. Phenols and alkaloids were estimated more in heartwood (3.60 & 6.62 mg/g) than bark of *P. santalinus* (**Table 3**). Tannin and flavonoids were quantified more in bark than heartwood of red sanders which proved its applicability in dying capability. Compared to heartwood, bark have large amount of tannins, phenols, alkaloids and flavonoids as reported (Wang et al., 2014) also reported that tannins are present in many plants which are also considered as a major compound. Most secondary metabolites that are used in skincare or body care products for therapeutic actions are flavonoids that exhibit antioxidant benefits.

Antioxidant activity of *P.santalinus* bark and heartwood

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants

Table 1. Methanol and ethanol extracts yield of bark and heartwood of *P. santalinus*.

S.No.	Plant sample	Plant extract yield (%)			
		Bark		Heartwood	
		Ethanol	Methanol	Ethanol	Methanol
	Pterocarpus santalinus	8.41 ± 0.002	11.71 ± 0.002	8.21 ± 0.01	11.42 ± 0.02

Note: % - Percentage

Table 2. Phytochemical Screening of methanol and ethanol extracts of *P.santalinus* bark and heart wood.

S. No.	Phytochemicals	Bark		Heartwood	
		Ethanol extract	Methanol extract	Ethanol extract	Methanol extract
1.	Alkaloids	+	+	+	+
2.	Flavonoids	+	-	+	-
3.	Tannins	+	+	+	+
4.	Saponins	-	+	-	+
5.	Steroids	-	-	-	-
6.	Phenol	+	+	+	+
7.	Glycosides	-	-	-	-
8.	Protein	-	-	-	-
9.	Carbohydrates	-	-	-	-
10.	Terpenoid	+	+	+	+

Note: "+" - Present ; "-" – Absent

Table 3. Quantification of secondary metabolites in bark and heartwood of *P. santalinus*.

S. No.	Phytochemicals	Bark		Heartwood	
		Ethanol extract	Methanol extract	Ethanol extract	Methanol extract
1.	Phenols (mg/g)	1.227 ± 0.01	1.221 ± 0.03	3.58 ± 0.01	3.60 ± 0.02
2.	Alkaloids (mg/g)	3.566 ± 0.02	3.602 ± 0.03	6.57 ± 0.01	6.62 ± 0.02
3.	Tannins (mg/g)	12.41 ± 0.02	12.58 ± 0.02	8.27 ± 0.02	8.21 ± 0.02
4.	Flavonoids (mg/g)	4.43 ± 0.01	4.47 ± 0.02	2.47 ± 0.02	2.87 ± 0.01

through their scavenging power are useful for management of that disease (Pourmorad et al., 2006). DPPH is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. Many plant extracts and their bioactive phytochemicals have shown free radical scavenging properties (Larson, 1988), (Koleva et al., 2002) yet there is a high demand to find out more information regarding antioxidant potentiality of many medicinally important plants. Antioxidant property of red sanders leaves was reported by (Arokyaraj et al., 2008). The scavenging activity of ethanol and methanol extracts of bark and heartwood of *P. santalinus* showed more than 50% antioxidant activity. When compared with methanol extract, ethanol extract showed high level of antioxidant activity (Shoba et al., 2004). On comparing bark and heartwood, bark extract showed more absorbance activity of antioxidant (Gnanadesigan et al., 2011) molecules. The ethanol bark and heartwood extract of *P. santalinus* showed significant DPPH radical inhibition of 83.4 and 79% respectively at 1000µg/ml concentration (**Table 4**). Both methanol and ethanol extracts of bark revealed high scavenging activity than the heartwood which confirmed the potency of red sanders bark as antioxidant source (Chaitanya et al., 2014). High level of total phenol and flavonoid contents showed the best antioxidant activity, may be due presence of hydroxyl groups existing in the phenolic and flavonoid compounds (Siddique et al., 2010). It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook & Samman, 1996) and flavonoids a group of polyphenolic compounds (Frankel, 1995). The presence of flavonoids, phenols and polyphenolic compounds in bark and heartwood of *P.santalinus* prompted us to study the free radical scavenging activity which proved that the bark and heartwood of *P. santalinus* are promising sources of naturally occurring antioxidant for medicinal and commercial uses.

Antibacterial activity of *Pterocarpus santalinus* bark and heartwood

The antibacterial activity of methanol and ethanol extracts of both bark and heartwood of *P. santalinus* was carried out in human pathogens. Mostly the plant is having medicinal properties and an important source for developing the new chemotherapeutic agents. The first step towards this goal is the in-vitro antibacterial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, antihelmintic, antimolluscal and anti-inflammatory properties of plants. In the present study the ethanol extract of bark and heartwood were subjected to antibacterial study against the human pathogens Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa. The antibiotic (standard) used for the antibacterial study was Amikacin at pH 7. The inhibition rate of Staphylococcus aureus was observed as 14 MM, Klebsiella pneumonia was 11MM and Pseudomonas aeruginosa was 12 MM (**Table 5**). The ethanol extract of red sanders bark showed antagonistic activity against the pathogens used for the study (**Figure 1**). The ethanol extract of bark inhibited the growth of bacteria but in heartwood there was no inhibitory activity was observed against the bacteria. (Chandra Sekhar Challa et al., 2018) was also noticed the antagonistic activity in heartwood of *P.santalinus*. The leaf extract of *P.santalinus* has antibacterial activity (Manjunatha, 2006). The stem bark extract of red sanders showed maximum activity against *Enterobacter aerogenes*, *Alcaligenes faecalis*, *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus* (Manjunatha, 2006). The leaf extract showed maximum activity against *Escherichia coli, Alcaligenes faecalis, Enterobacter aerogenes and Pseudomonas aeruginosa* (Manjunatha, 2006). The bioactive compounds such as

Table 4. Percentage of Antioxidant activity of *P.santalinus.*

Plant samples	Extracts	Percentage of radical scavenging activity (%)				
		Concentration of sample extracts (µg)				
		S1 (200)	S2 (400)	S3 (600)	S4 (800)	S5 (1000)
Bark	Ethanol	56.3	68.1	75.9	82.1	83.4
	Methanol	52.4	60.4	69.0	72.2	80.0
Heartwood	Ethanol	54.0	61.7	72.0	75.3	79.0
	Methanol	51.0	56.4	60.2	68.8	76.6

Note. "%" - Percentage; "µg"- Microgram; "S"- Sample

Table 5. Antibacterial activity of ethanol extract of *P.santalinus* bark.

Sample code	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
	Zone of Inhibition (mm)		
Bark EE	11 ± 0.02	12 ± 0.01	14 ± 0.01
Negative control	Resistant	Resistant	Resistant
Positive control (Amikacin)	15 ± 0.01	15 ± 0.01	17 ± 0.02

Note. mm – Millimeter; EE – Ethanol extract

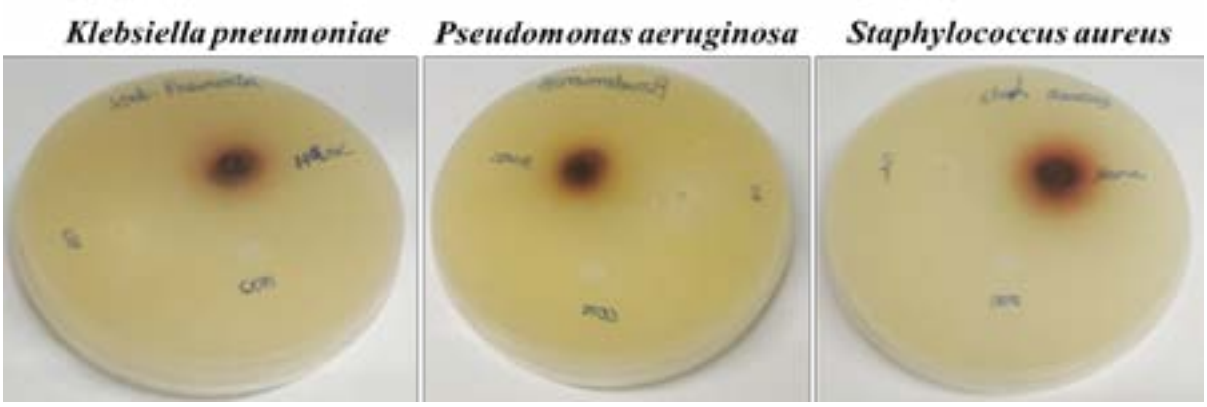

Figure 1. *In-vitro* antibacterial activity of ethanol extract of *P.santalinus* bark.

phenols, flavonoids and tannins reported in the bark and heartwood may be attributed for antibacterial activity. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms (Tsuchiya et al., 1996) and Phenolics and polyphenols present in the plants are known to be toxic to microorganisms (Mason & Wasserman, 1987). The significant antibacterial activity of P.santalinus may be due to the individual or combined action of the active phytochemicals reported upon.

CONCLUSION

Pterocarpus santalinus is a multipurpose high value native tree species of India which contains many potent phyochemicals for use in pharmaceuticals and cosmetics industries in addition to its timber and industrial uses. The presence of flavonoids, phenols and polyphenolic compound in bark and heartwood of *P. santalinus* is promising source of naturally occurring antioxidant for medicinal and commercial uses. The maximum inhibitory activity of *P.santalinus* against human pathogens suggested that the bark and heartwood of this species is considered as one of the valuable natural source of desirable cosmetic and medicinal values.

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