Original Research Article

PTEROCARPUS SANTALINUS Pterocarpus santalinus ETHANOLIC EXTRACT PREPARATION AND ITS FREE RADICAL SCAVENGING ACTIVITY

Running title : *Pterocarpus santalinus* ethanolic extract preparation and its free radical scavenging activity

ABSTRACT

Introduction

Pterocarpus santalinus commonly known as Red sanders, belongs to the family Fabaceae. It is endemic to India and considered globally endangered, with illegal harvest being a key threat. The plant is renowned for its characteristic timber of exquisite color, beauty, and superlative technical qualities. The red wood yields a natural dye santalin, which is used in coloring pharmaceutical preparations and foodstuffs. In the traditional system of medicine, the decoction prepared from the heartwood is attributed various medicinal properties. It has been used in inducing vomiting and treating eye diseases, mental aberrations, and ulcers.

Aim

This study aimed to find the free radical scavenging activity of *Pterocarpus santalinus* ethanolic extract preparation.

Materials and Methods

DPPH and H202 Assay was used to test the antioxidant activity of plant extract. *Pterocarpus santalinus* were purchased commercially from an herbal health centre,in Chennai. The obtained powder *Pterocarpus santalinus* stored in an airtight container. 5 gram of powder is mixed with 50 ml of ethanol and kept in the orbital shaker for 72 hours, after it has boiled in a heating mantle at 62-70℃ for 5-10 min. The extract is filtered using whatman filter paper 1.The filter extract again contracted using heating mantle.

Results

The extract shows very good antioxidant activity for the *P.santalinus* extract by using DPPH and H202 Assay.

Conclusion

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Comment [G2]: Ethanolic extract preparation of Pterocarpus santalinus and its free radical scavenging activity

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The free radical scavenging activity of *Pterocarpus santalinus* ethanolic extract preparation was effective.

Keywords
Pterocarpus santalinus,ethanolic extract,pharmaceutical preparations, traditional medicine.

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INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Arokiyaraj, 2008). Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems (Omotayo *et al.*, 2017).

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Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxide to non-radical forms and function as natural antioxidants in the human body. Due to depletion of the immune system's natural antioxidants in different maladies, consumption of antioxidants as free radical scavengers may be necessary (Habu and Ibeh, 2015). Antioxidants are the substances that reduce, neutralize and prevent the damage done to the body by free radicals. Antibacterial substances present in tissues of higher plants have long been regarded as important factors in the resistance of higher plants to various bacteria. Hence researchers have always felt the need for scientifically screening the plants, which may help the pharmacologists and phytochemicals (Gopinath, Gowri and Arumugam, 2013).

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DPPH (Diphenyl picryl hydrazine) assay was used to determine the antioxidant property. Free radicals damage the tissue and can initiate cancer, heart diseases and liver damage. Commercially available chemical antioxidants have been suspected to cause negative health effects or side effects. Phenols, flavonoids are the type of Phytochemicals that act as an antioxidant agent and can scavenge the free radical without any side effects. The present investigation deals with the antioxidant nature of ethanol extracts of leaf, bark and wood of *Pterocarpus santalinus* (V et al., 2020).

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Bioassay screening of natural origin has been the source of innumerable therapeutic agents. In the area of drug discovery, random screening is a tool in discovering new biologically active molecules and has been most productive('Pterocarpus santalinus Linn. f', no date). For a successful outcome the main requirement is access to a large number of compounds/extracts that must be well screened. *Pterocarpus santalinus* L.f (Red sanders) belongs to the family Fabaceae, traditionally used in treatment for headache, skin diseases, fever, boils, scorpion-sting and to improve sight (Nariya, Shukla and Acharya, 2012). Previous chemical constituents revealed the presence of triterpenes, isoflavone glucosides, savinin and calocedrin. There is no previous report on free radical scavenging activity. Our team has extensive knowledge and research experience that has translate into high quality publications (Rajeshkumar *et al.*, 2018; Nandhini, Rajeshkumar and Mythili, 2019; Veerasamy *et al.*, 2021)(Vairavel, Devaraj and Shanmugam, 2020) (M. Gomathi *et al.*, 2020)(Rajasekaran *et al.*, 2020)(Santhoshkumar *et al.*, 2019) (R *et al.*, 2020) (Saravanan *et al.*, 2018) (Gheena and Ezhilarasan, 2019) (Ezhilarasan, Sokal and Najimi, 2018) (Ezhilarasan, 2018)(A. C. Gomathi *et al.*, 2020)(Dua *et al.*, 2020) (Ramesh *et al.*, 2018)(Arumugam, George and Jayaseelan, 2021)(Joseph and Prasanth, 2021)(Ezhilarasan, Apoorva and Vardhan, 2018)(Duraisamy *et al.*, 2019)(Gnanavel, Roopan and Rajeshkumar, 2019)(Markov *et al.*, 2021). The present study aims to evaluate the free radical Scavenging activity of *Pterocarpus santalinus* ethanolic extract preparation.

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MATERIALS AND METHODS

Chemicals and Reagents:

The chemicals DPPH(1,1-diphenyl-2-picylhydrazyl),and butylated hydroxyanisole (BHA) were obtained.

Collection and preparation of plants:

Pterocarpus santalinus were purchased commercially from an herbal health centre,in Chennai. The obtained powder *Pterocarpus santalinus* stored in an airtight container. 5 gram of powder is mixed with 50 ml of ethanol and kept in the orbital shaker for 72 hours, after it has boiled in a heating mantle at 62-70℃ for 5-10 min. The extract is filtered using whatman filter paper 1.The filter extract again contracted using heating mantle.The extracts were filtered and concentrated under reduced pressure using a rotary evaporator to get completely dried extracts (PSE Ext). The yield of the leaf crude extract was about 80 g.

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DPPH assay:

The antioxidant activity of *Pterocarpus santalinus* ethanolic extract was tested by adopting the DPPH method(Rajeshkumar *et al.*, 2019) . Butylated hydroxyanisole was used as a positive control, which was diluted with ethanol to prepare sample solution equivalent control to 5, 10, 25, 50 and 100 mg of sample/ml solution. 1 mL of 0.25% DPPH was pipetted into a clean tube followed by various concentrations of the sample solution. The reaction mixture was incubated

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for 30 minute at 37°C. The absorbance of the reaction mixture was measured at 517 nm in a UV Visible double bean spectrophotometer was used. The experiment was done in triplicates. Free radical scavenging activity was calculated by the following equation.

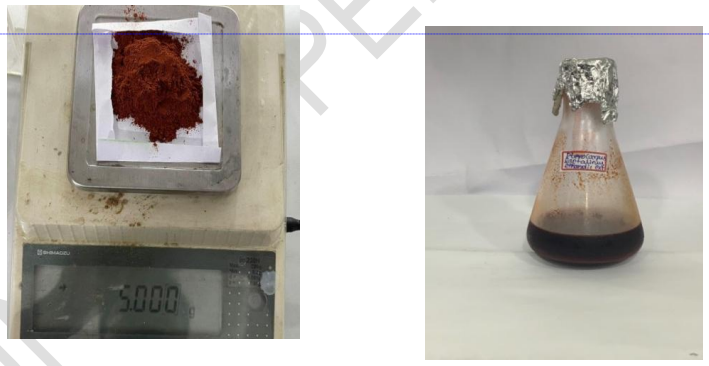
$$\text{Inhibition \%} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}$$

Hydrogen peroxide scavenging assay:

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined. *P.santalinus* of 0.1 mL of extracts (10-50 µg/mL) was transferred into the eppendorf tubes and their volume was made up to 0.4 mL with 50 mm phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mm). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

$$\text{\% inhibition} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULTS



Iamge 1: Image showing the Synthesis *Pterocarpus santalinus* ethanolic extract

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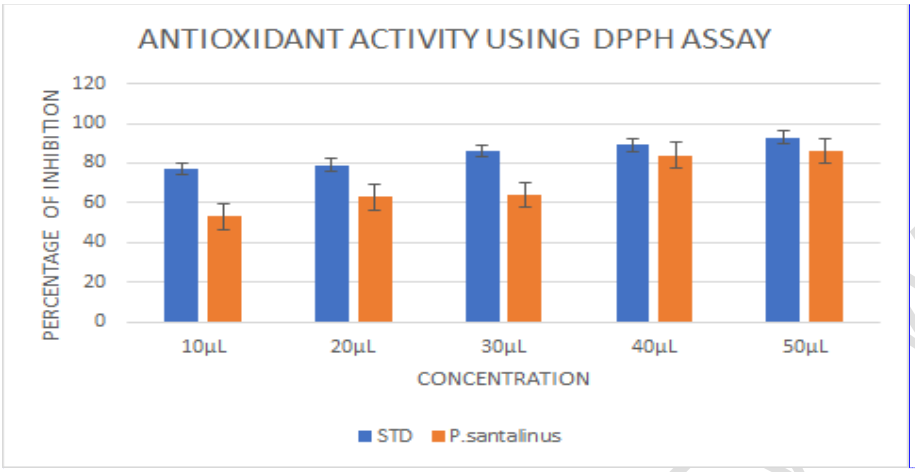

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Figure 1: The above graph depicts the antioxidant activity with an increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Pterocarpus santalinus*.

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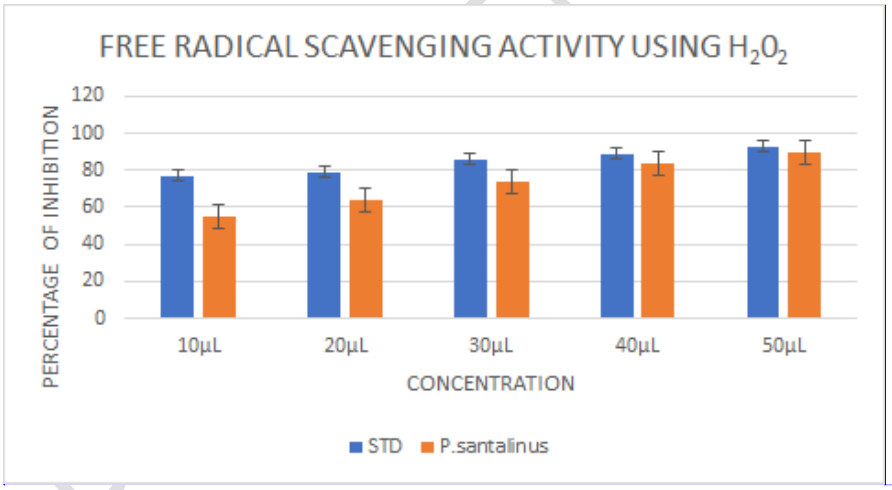

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Figure 2 : The above graph depicts the Scavenging activity of *Pterocarpus santalinus* ethanolic extract on Hydrogen peroxide increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Pterocarpus santalinus*.

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The results of antioxidant activity and free radical scavenging were depicted in (Figures 1-2). In the present study, the total antioxidant of *Pterocarpus Santalinus* ethanolic extract (PSE Ext) was determined using the DPPH Assay method. PSE Ext showed antioxidant property in a concentration dependent manner. The result indicated that the PSE Ext significantly (<0.05) inhibited Hydrogen peroxidation. DPPH is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. The present study investigated the scavenging activity of PSE Ext, and expressed the inhibition of DPPH free radicals using BHA as standard reference.

To investigate the effect of different doses of vitamin C (5, 10, 15, 20, 25, 30, 35, 40 mg ascorbic acid) on antioxidant potential, One of the vital roles of ascorbic acid (vitamin C) is to act as an antioxidant to protect cellular components from free radical damage. Ascorbic acid has been shown to scavenge free radicals directly in the aqueous phases of cells and the circulatory system.Ascorbic acid and its esters function as antioxidants with some substrates by protecting double bonds and scavenging oxygen. Ascorbic acid also lowers the oxidation state of many metals and valence may thus affect oxidation catalysis.

In our study the antioxidant activity with an increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Pterocarpus santalinus*. In **DPPH Assay** the percentage of inhibition are 10µL,20µL,30µL,40µL,50µL. The Standard - 77,79,86,89,93.The *P.santalinus* - 53,63,64,84,86 (Figure 1).In the present study the Scavenging activity of *Pterocarpus santalinus* ethanolic extract on Hydrogen peroxide increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Pterocarpus santalinus*. In Hydrogen peroxide Assay the percentage of inhibition are 10µL,20µL,30µL,40µL,50µL. The Standard - 77,79,86,89,93.The *P.Santalinus* - 55,64,74,84,90.

DISCUSSION

The free radical scavenging activity of ethanolic extract of *P. santalinus* and also that of ascorbic acid was evaluated through its ability to quench the synthetic DPPH radical. There are many methods for evaluating the antioxidant activity of both natural and artificial compounds. The DPPH and Hydrogen peroxide assay constitutes a rapid and low cost method that has frequently been used for evaluation of the antioxidative potential of various natural products (R, Sudha and Anusha, 2018). The radical scavenging reaction of ascorbic acid with DPPH was essentially instantaneous; the reaction of DPPH with *P. santalinus* was also fast but slower compared to that ~~with~~ of ascorbic acid. It is usually noticeable as discoloration of ethanolic extract of plant samples from purple to yellow; hence, DPPH is widely used to evaluate the free radical scavenging capacity of antioxidants. Therefore, in the present study, *P. santalinus* was screened for its possible antioxidant and radical scavenging activity by DPPH and H_2O_2 (Kumar, 2011).

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In the previous study DPPH is a stable free radical by potency of the delocalization of the spare electron where the molecule as a whole, do not dimerise, as would be the case with most other free radicals. The delocalization gives rise to the deep violet color, characterized by an absorption band (517 nm) in ethanol solution. When a solution of DPPH is mixed with a substance of Hydrogen ion donor, it gets reduced to a radical state (Diphenyl picryl hydrazine) and gives yellow color. Hence, the significant decrease in free radical can be attributed to the scavenging ability of *Pterocarpus santalinus* and can be read at 517 nm. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Kumar, 2011; Djouonzo *et al.*, 2016). In our study *P. santalinus* was screened for its possible antioxidant and radical scavenging activity by DPPH(MacLachlan and Gasson, 2010).

In other study Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and antiinflammatory action (A. *et al.*, 2018). The present study also indicates the Flavonoids also include the free radical scavenging properties (Subramania *et al.*, 2006).

In the present study DPPH is an easy, rapid and sensitive method for the antioxidant screening of plant extracts (Modi *et al.*, 2018). The previous study investigated the scavenging activity of methanol extract of *Pterocarpus santalinus* leaves, and expressed in percentage of inhibition of DPPH free radicals using BHA as standard reference compound. Ethanol extract of Pterocarpus santalinus showed significant free radical scavenging activity generated by DPPH. Scavenging activity was observed from 10 mg/ml to 25 mg/ml (61.7%, 75.9%, 82.1 % and 83.4%). Since more than 50% of DPPH radical inhibition is considered to be significant, the inhibition was observed from 10 mg/ml. BHA showed strong free radical scavenging activity at all concentrations(Nikolova, Petrova and Zayova, 2013).

In Similar studies, *P.santalinus* ~~the~~ stem bark extract was reported to contain maximum activity against Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Bacillus cereus, Bacillus subtilis and Staphylococcus aureus (Manjunatha, 2006). The ethanol extract of *Pterocarpus santalinus* at dose of 50-250 mg/kg showed gastroprotective effect in reserpine induced, pyloric-ligated experimental rats (Kalaivani and Prasanna, 2016). Our present study showed that *P.santalinus* ethanolic extract has the gastroprotective effect and antibacterial activity (P.S.Tresina and Tresina, 2012).

In the previous study PSE Ext showed significant free radical scavenging activity generated by DPPH. Scavenging activity was observed at the concentrations of 25, 50 and 100mg/ml with 52%, 68%, and 75% of activity respectively. Since more than 50% of DPPH radical inhibition is considered to be significant, the inhibition was observed from 25mg/ml.Significant scavenging activity was observed Ext (Gao *et al.*, 2018). In the present study Antioxidants play a vital role in inhibiting and scavenging radicals, thus providing protection to humans against infections and

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degenerative diseases. Numerous plant constituents have proven to show free radical scavenging activity. Flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (Bidchol *et al.*, 2009).

In other studies Antibacterial study of *Pterocarpus santalinus* inhibited the growth of gram positive bacteria and gram negative bacterium. Maximum inhibitory activity was observed against Bacillus subtilis (0.312 mg/ml), however no activity was found against Pseudomonas aeruginosa and Klebsiella pneumonia. Phytochemical analysis showed the presence of flavonoids, terpenoids and steroids, these constituents have diverse pharmacological properties including antioxidant and antimicrobial activity (Pal *et al.*, 2011). In the present study Antioxidant activity of *Pterocarpus santalinus* may be due to the synergetic effect of two or more chemical constituents of the plant extract (Sinnathambi *et al.*, 2007).

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CONCLUSION

As our knowledge of the mechanisms of human diseases has increased, particularly metabolic diseases such as diabetes, liver disease and hypertension, the role played by highly reactive oxygen species such as free radicals has become increasingly relevant. Research on medicinal plants for natural antioxidants is also increasing. The present study indicates that the powder of *P. santalinus* possess antioxidant properties and could serve as free radical inhibitors, scavengers or primary antioxidants. A significant relationship between the antioxidant capacity and ascorbic acid content was found. With this kind of investigation it would be easier to treat and prevent the human damages occurring due to the free radical. Therefore, further research is needed for the isolation and identification of the active components in the extracts.

Based on our observations, it was confirmed that *Pterocarpus santalinus* (Leaf) showed a strong free radical scavenging effect in the cell free system. Phytochemical research is needed to identify the active principles responsible for this biological activity of this medicinal plant. Further studies are aimed at the isolation and identification of bio-active molecules from the ethanolic extract of *Pterocarpus santalinus*.

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