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EVALUATION OF ANTIOXIDANT NATURE OF METHANOL EXTRACTS FROM LEAF, BARK AND WOOD OF *PTEROCARPUS SANTALINUS* L

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ABSTRACT

The present study was designed to investigate the free radical scavenging activity of methanol extracts of leaf, bark and wood of *Pterocarpus santalinus* L. DPPH (Diphenyl picryl hydrazine) assay was used to determine the antioxidant property. Free radicals damage the tissue 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 methanol extracts of leaf, bark and wood of *Pterocarpus santalinus*. The methanol extract of leaves, bark and wood of *Pterocarpus santalinus* showed significant DPPH radical inhibition of 61.7%, 52.7% and 68.7% respectively at 10 mg/ml concentration. Methanol extracts of wood revealed high scavenging activity than the leaves and bark. The study confirmed the potency of *Pterocarpus santalinus* as an antioxidant source.

KEYWORDS: Antioxidant, DPPH assay, *Pterocarpus santalinus*, Free radicals.

INTRODUCTION

Free radicals initiate many diseases, inflammation, cancer, liver damage and cardiovascular

diseases (Liao & Yin, 2000). Synthetic antioxidants are commercially available chemical compounds that have been suspected to cause negative health effects or side effects. Butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters are commercially available antioxidants. Hence, there is a strong restriction that has been placed on their application. Hence there is a need to substitute them with naturally occurring phytochemicals as antioxidants. Since from ancient times, plants are used in many aspects, such as nutrition, medicine, flavoring, beverages and cosmetics etc. Intake of fresh fruits and vegetables, rich in natural antioxidants have been associated with prevention of cancer and cardiovascular diseases. The higher intake of plant foods correlates with lower risk of mortality from these diseases. Approximately 60 % of the commercially available anti-tumor and anti-infective agents are from natural origin (Charvet-Faury *et al*., 1998). Recently medicinal plants are used as a therapeutic agent as antioxidants to reduce tissue injury induced by free radicals. Many plant extracts and their phytochemicals have shown free radical scavenging properties (Larson, 1988; Koleva *et al*., 2002) but still there is a demand to find out more information regarding antioxidant potentiality of many medicinally important plants. *Pterocarpus santalinus* L (Red sanders) belongs to the family Fabaceae and it is mostly confined to south India in Kadapa and Chittoor on the Tamil Nadu and Andhra Pradesh border. Traditionally parts of the plant are widely used as an ayurvedic and folk medicine to cure the skin disorders, fever, boils, improve sight, headache, gastric ulcers and scorpion sting (Chopra and Nayar *et al* 1956). Literature study showed Leaves, wood and bark of this plant contains phytochemical constituents like alkaloids, phenols, glycosides, triterpenoids, cardioglycosides, tannins, steroids, and santalin. (Krishnaveni & Srinivasa Rao, 2000). It is reported that the wood, leaves and bark of this plant has more biological activity like antibacterial, antifungal, anti dermatophytic, anti diabetic, anti inflammatory, antioxidant, antitumor, analgesic, antiseptic and antihelmenthic properties (Manjunatha BK 2006). Antioxidant property of leaves was evaluated and reported by (Arokyaraj *et al* 2008*)*.In this present study we are reporting the antioxidant properties of leaf, wood and bark of *Pterocarpus santalinus* L.

MATERIALS AND METHODS

Extraction of Plant Material: The healthy and disease free leaves bark and wood of *Pterocarpus santalinus* L plant material was collected from Chittoor of Andhra Pradesh, India in the month of February, 2012. The collected plant material was washed thoroughly in tap water, shade dried in open air separately. Powder of the leaf, bark and wood is obtained

by grinding them mechanically, About 100 gm of each dried powder of the plant were soaked separately in 100 ml of methanol in conical flasks and then subjected to agitation on a rotary magnetic shaker for about 3 days. After 72 hours the plant extracts are filtered with No 42 whatman filter paper separately. Concentrated extracts were preserved in sterilized air tight labeled bottles in refrigerator at 4˚c until required for further use. The extract were filtered under reduced pressure using rotary flash evaporator and subjected for further tests.

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the methanol extract of leaf, bark and wood and standard reference compound was analyzed by the DPPH assay as described by Sanchez-Moreno *et al* 1998 and (Arokyaraj *et al* 2008) with minor modification. This method is based on the reduction of DPPH in methanol solution in the presence of hydrogen donating antioxidant due to the formation of non radical from DPPH- H, the transformation results in the formation of color change from purple to yellow. In this assay, 1 ml of varying concentrations (5, 10, 15, 20 and 25 mg/ml) of methanol extract of leaf, bark and wood of *Pterocarpus santalinus* are dissolved in 1 ml of methanol solution of DPPH (0.2 mM). There is a need to prepare fresh DPPH 0.2 mM and set OD value to 0.8. If the O.D is less than 0.8 add DPPH or more than 0.8 add methanol. The mixture was thoroughly mixed and incubated for 30 min. The optical density of the solution was then measured at 517 nm using Hitachi 2010 spectrophotometer. BHA (µg/ml) has been used as standard reference. The DPPH radical scavenging activity was calculated from the absorption according to the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD Control} - \text{OD Sample}}{\text{OD control}} \times 100$$

Table 1: Antioxidant activity of methanol leaf extracts of *P. santalinus* Data are reported as mean ± SD,

Concentration	% Inhibition of DPPH free radical	
	BHA (µg/ml) standard drug	Methanol Leaf extracts PS (mg/ml)
5	62.2 ± 1.9	42.0 ± 2.2
10	80.6 ± 2.6	61.7 ± 3.5
15	93.3 ± 2.4	75.9 ± 2.7
20	95.6 ± 1.8	82.1 ± 2.3
25	96.4 ± 1.4	83.4 ± 2.5

n = 3. Scavenging activity is expressed as percentage of inhibition of DPPH free radical.

Methanol extract of leaves of *P. santalinus* showed significant free radical scavenging activity generated by DPPH. Scavenging activity was observed from 5 mg/ml to 25 mg/ml (61.7%, 75.9%, 82.1% and 83.4%).

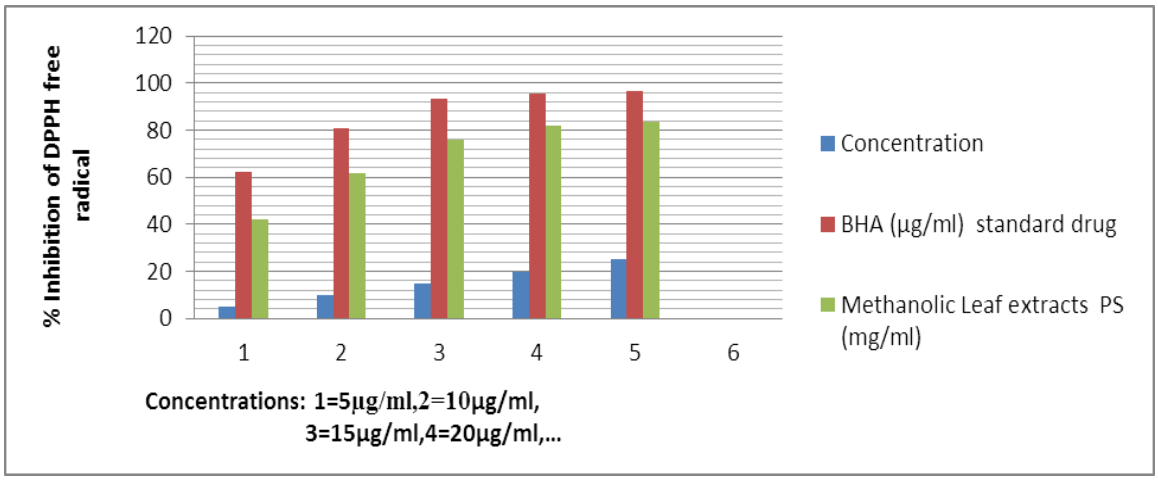

Table 2: Antioxidant activity of methanol bark extracts of *P. santalinus* Data is reported as mean ± SD, n = 3. Scavenging activity is expressed as percentage of inhibition of DPPH free radical.

Concentration (mg/ml)	% Inhibition of DPPH free radical	
	BHA (µg/ml) standard drug	Methanolic extract of bark PS (mg/ml)
5	42.8 ± 1.17	32.0 ± 2.20
10	76.6 ± 2.12	52.7 ± 2.11
15	85.3 ± 2.40	65.0 ± 1.50
20	98.6 ± 1.18	78.8 ± 1.40
25	87.4 ± 1.60	82.2± 2.80

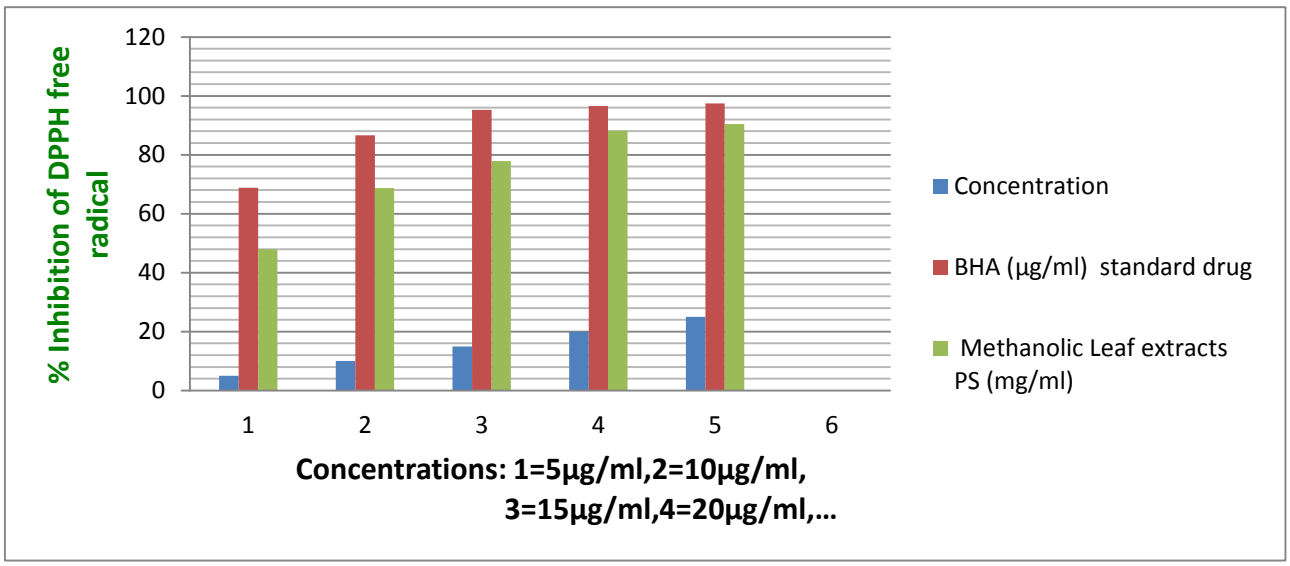

Fig 2: Graphical representation of free radical scavenging activity of bark methanol extracts *P. santalinus*.

Table 3: Antioxidant activity of methanol wood extracts of *P. santalinus* Data are reported as mean ± SD, n = 3. Scavenging activity is expressed as percentage of inhibition of DPPH free radical.

Concentration	% Inhibition of DPPH free radical	
	BHA (µg/ml) standard drug	Methanol Wood extracts PS (mg/ml)
5	68.8 ± 1.17	48.0 ± 2.80
10	86.6 ± 2.12	68.7 ± 3.13
15	95.3 ± 2.40	77.9 ± 2.70
20	96.6 ± 1.18	88.1 ± 2.30
25	97.4 ±1.60	90.4 ± 2.80

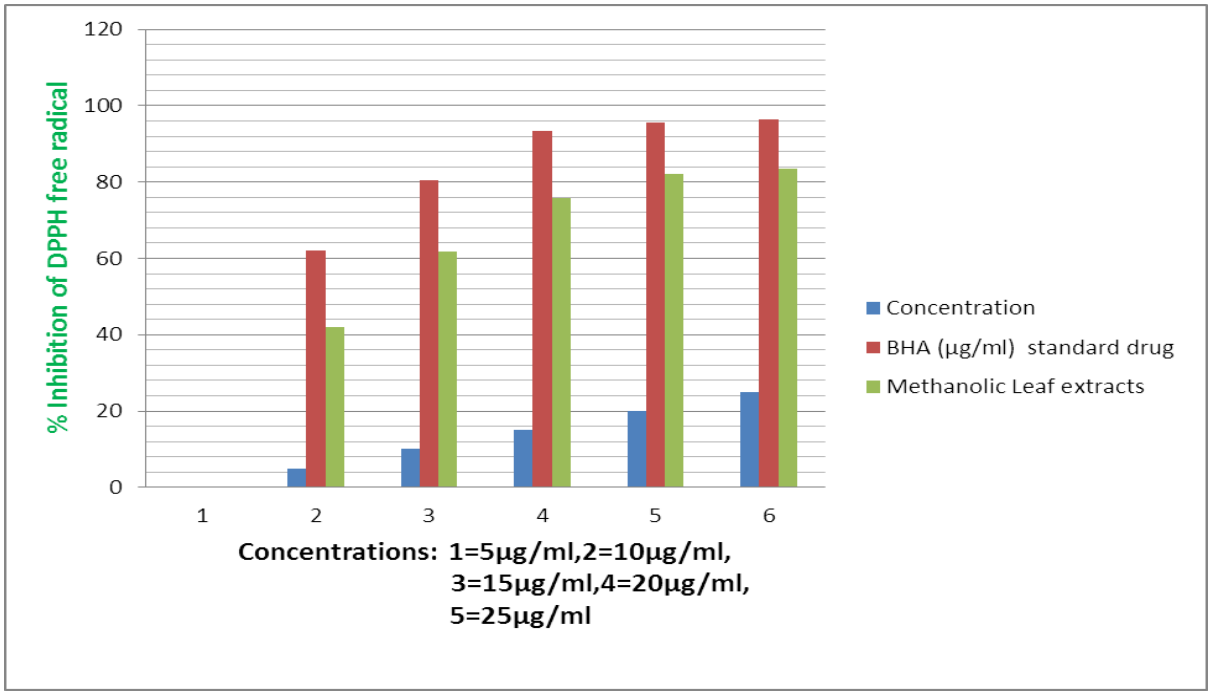

Fig 3: Graphical representation of free radical scavenging activity of wood methanolic extracts *P. santalinus*

RESULTS

From the tables 1, 2, 3 and graphs fig: 1,2,3 methanol leaf, bark and wood extracts of *P. santalinus* showed significant free radical scavenging activity observed from 10 mg/ml to 25 mg/ml generated by DPPH. More than 50% of DPPH radical inhibition is consider to be significant, the inhibition was observed from 10 mg/ml. Leaf, bark and wood methanol extract of *Pterocarpus santalinus* showed significant free radical scavenging activity of 61.7%, 52.7% and 68.7% respectively at 10 mg/ml DPPH. From these studies it can be predicted that the wood extracts showed more antioxidant property than the leaf and bark.

DISCUSSION AND CONCLUSION

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 methanol solution. When a solution of DPPH is mixed with a substance of Hydrogen ion donor, it gets reduced to non 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* and can be read at 517 nm. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Cook & Samman, 1996). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and antiinflammatory action (Frankel, 1995). All data are expressed as mean ± S.D. 50% and above inhibition of DPPH radical is considered as significant for scavenging activity (Omisore *et al*., 2005). Methanol leaf, bark and wood extracts of *P. santalinus* showed significant free radical scavenging activity observed from 10 mg/ml to 25 mg/ml generated by DPPH. More than 50% of DPPH radical inhibition is consider to be significant, the inhibition was observed from 10 mg/ml. Leaf, bark and wood methanol extract of *Pterocarpus santalinus* showed significant free radical scavenging activity of 68.7%, 52.7% and 61.7% respectively at 10 mg/ml DPPH. From these studies we conclude that the methanol wood extracts showed more antioxidant property than the leaf and bark methanol extracts. The presence of flavonoids, phenols and polyphenolic compound in the leaves, bark and wood of *Pterocarpus santalinus* prompted us to study the free radical scavenging activity. From this study it can be considered that the leaves, bark and wood of *Pterocarpus santalinus* as a promising source of naturally occurring antioxidant for medicinal and commercial uses.

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