Free radical scavenging activity and HPTLC finger print of *Pterocarpus santalinus* L. – an *in vitro* study

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Abstract: Methanol extract of *Pterocarpus santalinus* (leaves) was evaluated for HPTLC finger print, phytochemical analysis and antioxidant activity. Preliminary phytochemical screening (HPTLC fingerprint) revealed the presence of terpenoids, steroids, flavonoids and carbohydrates. DPPH assay was used to determine the antioxidant property. The methanol extract of *Pterocarpus santalinus* showed significant DPPH radical inhibition (83.4% at 25 mg/ml concentration). The study reveals the potency of *Pterocarpus santalinus* as antioxidant source.

Keywords: antioxidant, DPPH assay, *Pterocarpus santalinus*, secondary metabolites

Introduction

Free radicals can initiate or propagate many diseases, such as inflammation, cancer, liver injury and cardiovascular disease (Liao & Yin, 2000). Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free-radical-induced tissue injury. Many plant extracts and phytochemicals have shown to have free radical scavenging properties (Larson, 1988; Koleva *et al*., 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species.

Pterocarpus santalinus L.f (Red sanders) belongs to the family *Fabaceae* and is restricted to part of Andrapradesh. Traditionally it has been used in treatment of headache, skin diseases, fever, boils, scorpion-sting and to improve sight (Chopra *et al.,* 1956). Previous chemical constituents revealed the presence of triterpene, isoflavone glucosides, savinin and calocedrin (Krishnaveni & Srinivasa, 2000). Literature study showed other biological activity but antioxidant activity of this plant, has not been reported. Based on this we have worked on the leaves of *Pterocarpus santalinus* to find out antioxidant activity, phytochemical analysis and HPTLC finger print.

Materials and methods

Plant collection and extraction

Healthy, disease free mature leaves of *Pterocarpus santalinus* were collected from Chittor, Andrapradesh, India. A specimen was deposited at the department herbarium, Loyola College, Chennai. Collected materials were washed thoroughly, shade dried in open air and grounded into powder. The powder was extracted by maceration in methanol (40.4 g) for 72 hr. The plant extract was concentrated using rotary flash evaporator and preserved at 4^0C in air tight bottle until assay.

HPTLC profile (High Performance Thin Layer Chromatography)

Chromatography was performed on 3x10 cm HPTLC plates coated with 0.25 mm layer of silica gel 60 F_{254} (Merck, Germany). Before using, the plates were washed with methanol and activated at 110^oC for 5 min. Samples were applied as 4 mm wide bands and 6 mm apart by using a Camag (Muttenz, Switzerland) Linomat IV sample applicator equipped with 100 µl syringer. A constant application rate of 5 µl/S was used. Mobile phase was ethylacetate: methanol (4:6) and chromatograms were monitored at 254 nm (Srinivas Reddy *et al*., 2008).

Phytochemical analysis

The presence of phytochemicals alkaloids (Draggendorff's), flavonoids (Shibat'as reaction), saponins (Frothing test), tannins (5% ferric chloride), terpenoids (2,4-dinitro-phenyl hydrazine), glycosides (Fehling's solution), steroids (Liebermann's Burchard test) were evaluated according to the methods described by Edeogal *et al*. (2005).

In Vitro DPPH Radical Scavenging activity

The free radical scavenging activity of the leaf extract and standard reference compound was analyzed by the DPPH assay as described by Sanchez-Moreno *et al*. (1998) with minor modification. In this assay, 1 ml of varying

concentrations (5, 10, 15, 20 and 25 mg/ml) of methanol extract of *Pterocarpus santalinus*, dissolved in 1 ml of methanol were mixed with 1 ml of methanol solution of DPPH (0.2 mM). The mixture was vortexed and incubated for 30 min. The optical density of the solution was the 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:

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

Statistical Analysis

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).

Results and discussion

Phytochemical analysis

The phytochemical analysis of methanol extract had showed the presence of flavonoids, terpenoids, steroids, and carbohydrate. 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 anti-inflammatory action (Frankel, 1995). The presence of polyphenolic compound in *Pterocarpus santalinus* prompted us to study the free radical scavenging activity.

HPTLC profile

In the HPTLC fingerprinting of methanol extract, gave eight spots at the following Rf values: 0.06 (18.38%), 0.33 (4.62%), 0.39 (12.50%), 0.54 (6.51%), 0.67 (14.25%), 0.72 (19.23%), 0.83 (13.44%), 0.85 (11.07%). Purity of the sample extract was confirmed by comparing the absorption spectra at start, middle and end position of the band. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. The corresponding HPTLC chromatograms are presented in Fig. 1.

Fig.1. HPTLC finger print of Pterocarpus santalinus

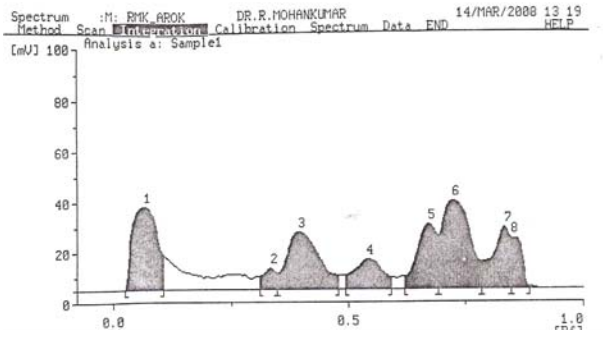

Table 1. Radical scavenging activity of BHA and Pterocarpus santalinus leaves on DPPH free radical. Data are reported as mean ± SD, n = 3. Scavenging activity is expressed as percentage of inhibition of DPPH free radical. 50% and above inhibition DPPH radical is considered as significant for scavenging activity.

Concentration	% Inhibition of DPPH free radical	
	BHA (µg/ml)	PS (mg/ml)
5	62.2 ± 1.9	42 ± 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

BHA- Butylated hydroxy anisole; PS- Pterocarpus santalinus

In Vitro DPPH Radical Scavenging activity

DPPH is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. The present 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. Methanol 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 (Table 1). Similarly 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 (Narayan *et al.*, 2007).

DPPH is characterized as stable free radicals by virtue of the delocalization of the spare electron where the molecule as a whole, so that the molecule 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 H donor, it gets reduced into non-radical state (Diphenyl picryl hydrazine). Hence, the significant decrease in free radical can be attributed to the scavenging ability of *Pterocarpus santalinus* leaves.

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