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## An *in vitro* study of Antioxidant effect of *Santalum album*, the Indian sandalwood

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**Abstract**

The antioxidant potential of Indian sandalwood, *Santalum album*, was investigated in this study, by DPPH scavenging, FRAP and hydroxyl radical scavenging *in vitro* assays. Aqueous extract of sandalwood callus, at concentrations of 25, 50, 75 and 100 micrograms/ml was used. The *in vitro* studies demonstrated significant antioxidant ability of sandalwood, which was concentration-dependent. At a concentration of 100 micrograms/ml, sandalwood extract showed 48.7% DPPH scavenging, 1 micromoles of ferrous iron/ml (by FRAP assay) and 53.2: scavenging of hydroxyl radicals. Further studies with multiple concentrations and comparison with standard antioxidants are required.

**Keywords:** antioxidant, free radicals, hydroxyl radicals, reducing ability, sandalwood

**Introduction**

Many herbal oils and extracts have beneficial health effects exerted through their anti-inflammatory, antioxidant, anti microbial actions. The Indian sandalwood, *Santalum album* L., referred to as 'royal tree' in India, is one such valuable tree of immense potential as health-promoting agent. The wood of this root hemiparasitic tree is highly aromatic and is known for its medicinal properties since ancient times. Sandal wood has anti-inflammatory, anticancer, antioxidant, antimicrobial and antiseptic properties. Various molecular mechanisms have been proposed in beneficial effects of sandalwood<sup>[1, 2]</sup>.

Sandalwood oil has been shown to contain compounds such as  $\alpha$ - and  $\beta$ -santalol, cedrol, esters, aldehydes, phytosterols, and Squalene<sup>[2]</sup>. Previous pre clinical studies suggest increased activity of detoxifying enzymes in animals fed sandalwood oil<sup>[3]</sup>. While *in vitro* studies have reported free radical-scavenging effects of sandalwood oil, aqueous extract of sandalwood and the compound alpha-santol present in sandalwood<sup>[4, 5]</sup>.

There is a need for assessing the antioxidant effects of sandalwood aqueous extract which is used for religious and medicinal purpose in India. We attempted to analyze the antioxidant effects by assaying ferric ion reducing, DPPH scavenging, hydroxyl radical scavenging and lipid peroxidation-inhibiting actions *in vitro*.

**Materials and Methods**

The present *in vitro* study was done at Medical College Research Centre involving the Departments of Biochemistry, and Research and Development.

**Materials****Preparation of Sandalwood extract**

For the study purpose, sandal wood which is used for religious purposes, was procured from the local market. The sandal wood was ground finely and strained through muslin cloth. One gram of the sample obtained was soaked for 2 hours in distilled water (50 mg/ml). The sample was then centrifuged and the supernatant was collected, and it served as aqueous extract for the further studies.

The chemicals required for the assays were procured from Sigma Aldrich.

**Methods:** All the assays were run in triplicate, and three concentrations of sandalwood extract (25, 50, 75, 100  $\mu$ g/ml) were used.

**1. DPPH Scavenging assay:** The antioxidant activity of *S. album* and the standard was checked on the basis of the free radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) by the method of Braca *et al.*<sup>[6]</sup>.

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Ascorbic acid (1 mg/ml) in distilled water was used as standard. 0.1mM DPPH was prepared in ethanol and 500µl of this solution was mixed with 500µl of working sample solutions and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. 0.1mM DPPH solution was used as control. The range of diluted aqueous extracts was taken as blank. The optical density were recorded and DPPH scavenging was calculated using the formula given below:

$$\text{DPPH scavenging Activity (\%)} = [(dc - dt)/dc] \times 100,$$

Where dc and dt represent OD<sub>517</sub> of control and test sample respectively.

**2. FRAP Assay:** Antioxidant activity assay was also done following the ferric-reducing antioxidant power (FRAP) method described by Benzie & Strain [7]. The FRAP reagents was freshly prepared by mixing 10 ml acetate buffer (300mM, pH 3.6), 1 ml 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution (10mM TPTZ in 40mM/L HCl) and 1 ml FeCl<sub>3</sub> (20mM) water solution. A range of diluted working solutions of the *S. album* were prepared in distilled water. Each sample (200 µl) was added in 1.5 ml of freshly prepared FRAP reagent and mixed and after 5 min, absorbance was measured at 593 nm, using FRAP working solution as blank. Ascorbic acid was used as standard. The results were expressed in micromoles of Fe<sup>2+</sup>/ml of aqueous extract. Higher absorbance indicates higher reducing power.

**3. Hydroxyl radical scavenging activity:** This was done by the method of Haliwell *et al.* [8]. Using Fenton reaction (with

Fe<sup>3+</sup>- ascorbate-EDTA-H<sup>2</sup>O<sup>2</sup>), hydroxyl radical was generated and the quantification of 2-deoxy-D-ribose degradation product which reacts with thiobarbituric acid under hot conditions to give a pink coloured product. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard. The reaction mixture contained 0.2 ml of sandalwood extract or standard solution in 0.8 ml of 0.05 M phosphate buffer pH 7.4, 0.2 ml of 1.04 mM EDTA, 0.2 ml of 1 M ferric chloride and 0.2 ml of 28 mM 2-deoxy-D-ribose. Thiobarbituric acid reagent was used for the reaction. The absorbance at 532 nm. Thiourea was used as the standard. The percentage of hydroxyl radical scavenging activity was calculated by following formula:

$$0\% \text{ hydroxyl radical scavenging activity} = [A0 - (A1 - A2) \times 100 / A0]$$

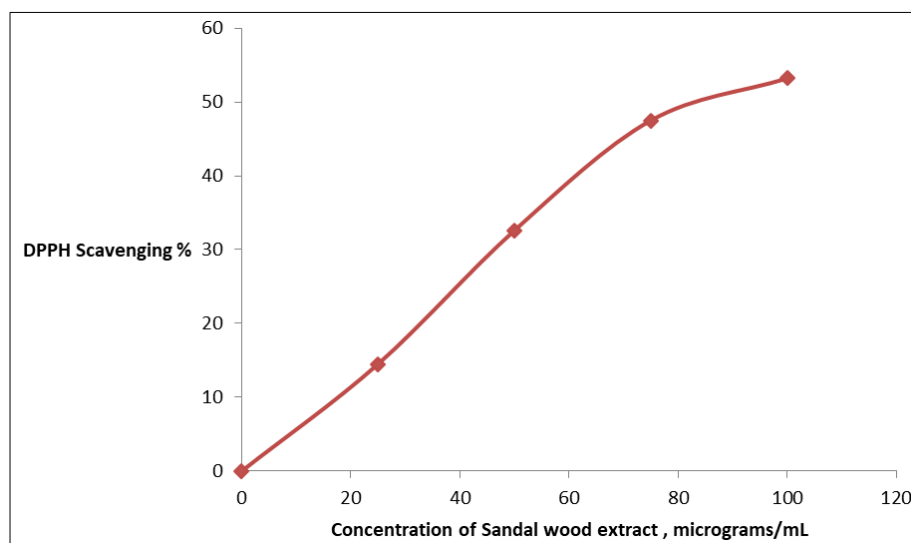
A1: Absorbance of the solution after adding 2-deoxy-D-Ribose; A2: Absorbance of the solution without 2-deoxy-D-Ribose; A0: Absorbance of control (without sample).

## Results

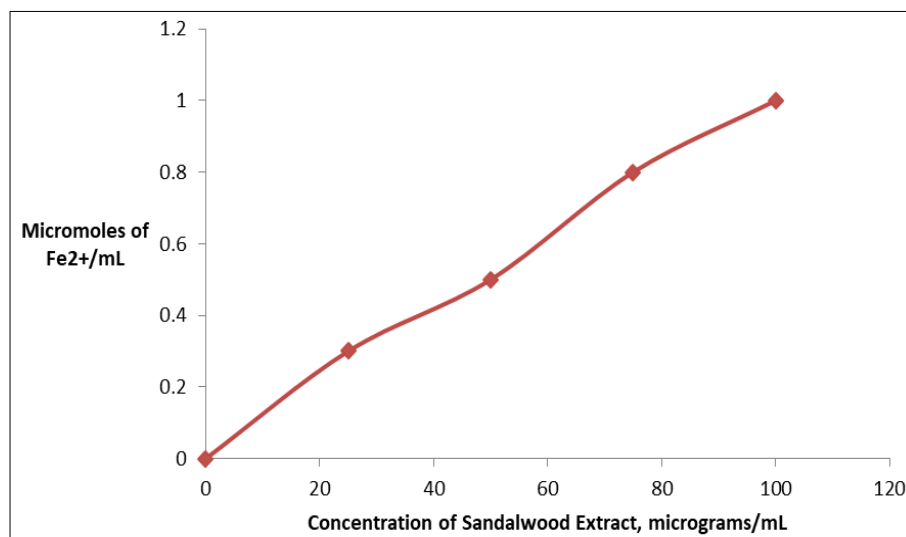
We observed significant free radical scavenging ability of sandalwood extract. The aqueous extract of sandalwood in concentrations of 25, 50, 75 and 100 µg/mL exhibited antioxidant property as evidenced by DPPH scavenging assay. The scavenging % increased with increase in the concentration of sandalwood extract (Table, Figure 1). The FRAP assay showed increase in the reduction of ferric iron (micromoles of ferrous ion/mL) with increase in the concentration of sandalwood extract (Table 1, Figure 2). The hydroxyl radical scavenging % increased with increase in the concentration of sandalwood extract (Table 1, Figure 3).

**Table 1:** Antioxidant Activity of Sandalwood Extract as Demonstrated by DPPH scavenging, FRAP and hydroxyl radical Scavenging. Average of the three assays is shown here

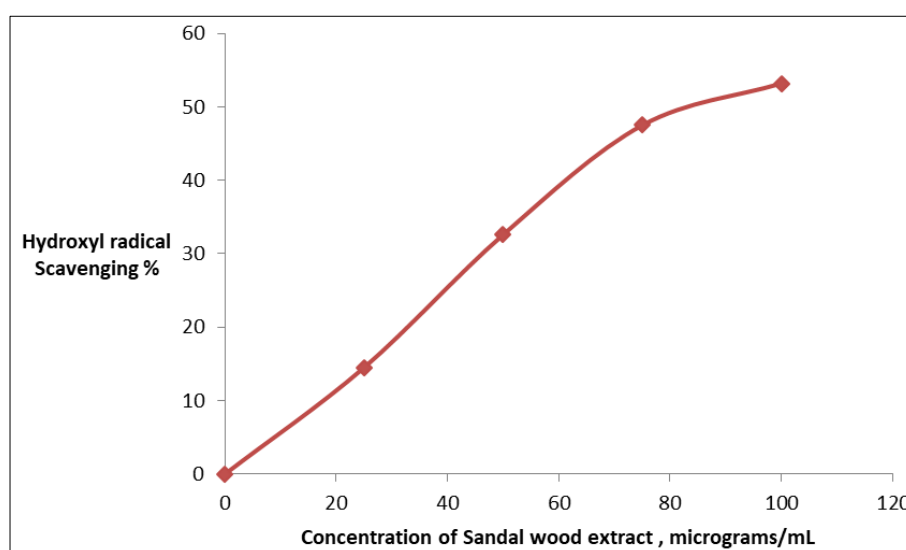
	25 µg/ml sandalwood extract	50 µg/ml sandalwood extract	75 µg/ml sandalwood extract	100 µg/ml sandalwood extract
DPPH Scavenging, %	11.2	21.7	35.4	48.7
FRAP assay, micromoles of Fe <sup>2+</sup> /ml	0.3	0.5	0.8	1.0
hydroxyl radical scavenging activity, %	14.5	32.6	47.5	53.2



**Fig 1:** DPPH Scavenging (%) by Sandalwood extract



**Fig 2:** FRAP Assay measuring Ferric ion reducing ability of Sandalwood extract



**Fig 3:** Hydroxyl radical scavenging (%) by Sandalwood extract

## Discussion

Sandalwood is considered as a “treasure” in India due to its religious and medicinal uses. Sandal wood is a rich source of antioxidants such as tannins, Proanthocyanidin, terpenoids and saponins [13]. In this study, we used the extract from the callus to assess the antioxidant capacity of sandalwood. We observed significant scavenging of free radicals and reduction of iron by sandalwood extract. The aqueous extract used at concentrations from 25 to 100 micrograms/mL showed significant antioxidant property which was proportional to the concentration (Table 1, Figures 1 to 3).

The DPPH scavenging assay, developed by Blois determines the antioxidant activity by using a stable free radical  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) and it is based on the scavenging ability of the antioxidants [12]. The concentration-dependent increase in scavenging of DPPH by sandalwood extract indicates its antioxidant potential. We observed DPPH scavenging ability of sandalwood proportional to the concentration. The concentration of 100 micrograms/ml aqueous extract of sandalwood showed 48.7% DPPH scavenging. Previously, Shamsi *et al.* showed the concentration-dependent DPPH scavenging ability of sandalwood extract (50 mg/ml) with maximum scavenging of 64% in presence of 500 $\mu$ l of aqueous extract [4]. Sandalwood callus extract (in dichloromethane: methanol) and oil showed

comparable DPPH scavenging ability which was more than the scavenging ability of quercetin [13].

The ferric reducing antioxidant power (FRAP) assay is a typical ET-based method that measures the reduction of ferric ion ( $\text{Fe}^{3+}$ )- ligand complex to the intensely blue-colored ferrous ( $\text{Fe}^{2+}$ ) complex by antioxidants in an acidic medium. We observed concentration-dependent ferric ion reducing ability of sandalwood, and highest ability at concentration of 100 micrograms/ml. The findings in concordance with those of previous studies. Shamsi *et al.* Reported significant ferric ion reducing ability of sandalwood aqueous extract comparable to ascorbic acid [4]. Misra and Dey observed that the FRAP value for sandalwood callus extract was comparable to that of  $\alpha$ -tocopherol, and much lower than sandalwood oil, thus indicating higher efficacy of sandalwood oil as reducing agent [13].

We observed significant antioxidant capacity of sandalwood extract evident by scavenging of hydroxyl radicals at concentrations of 25 to 100 micrograms /ml, and highest ability at 100 micrograms/ml with scavenging of 53.2 %. As per the observations of Misra and Dey sandalwood callus extract and oil showed comparable and significantly higher hydroxyl radical reducing ability at a concentration of 100 micrograms/ml, when compared to gallic acid [13].

## Conclusions

Sandalwood callus extract showed significant antioxidant capacity evident by DPPH scavenging, FRAP and hydroxyl radical scavenging assays. Further studies employing various concentrations of sandalwood extract, and comparison of its antioxidant ability with standard antioxidants such as tocopherol, quercetin, gallic acid and silymarin are required to establish its antioxidant potential. Pre clinical studies with experimental animals are required for elucidation of the mechanisms of antioxidant ability of sandalwood and its therapeutic potential.

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**Conflict of interest:** None

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