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Research Article

In-vitro Antibacterial and Antioxidant Activities of Sandalwood (*Santalum Album*)

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Abstract

Santalum album commonly known as Sandalwood is used traditionally for health and wellness. It is an evergreen and hemi-parasitic tree and has a long history in Indian religious rituals and traditional Chinese medicine. Due to its wide application in cosmetics and therapeutics, we have done this study to explore the possibility of using aqueous extract of *S. album* as antibacterial and antioxidant agent. The *S. album* extract was prepared in distilled water. The activity of aqueous extract was evaluated against eight bacterial pathogens including two strains of *Escherichia coli*, one each of *Klebsiella pneumoniae*, *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aeromonas species* and *Klebsiella oxytoca.* The anti-oxidant activity was analyzed by two most common radical scavenging assays of FRAP (ferric reducing antioxidant power) and DPPH (1,1- diphenyl-2-picrylhydrazyl). Results showed that *S. album* had strongest inhibitory activity against *S. aureus* (MTCC 902) i.e. 87% whereas; it showed no inhibition against *E.coli* (ATCC 25922) and *B. subtilis* (MTCC736). The S. album extract showed DPPH radical scavenging activity in a concentration–dependent manner with maximum scavenging of 64% in presence of 500µl of aqueous extract. The FRAP assay also proved antioxidant potential of *S. album* with the highest value of 0.628mM at 200µl of aqueous extract.

Keywords: Antibacterial activity; Antioxidant activity; *Santalum album*; Aqueous extract

Abbreviations

DPPH: 1,1-diphenyl-2-picrylhydrazyl; FRAP: Ferric Reducing Antioxidant Power; mg: Milligram; ml: Milliliter; CFU: Colony Forming Units; OD: Optical Density; TPTZ: 2,4,6-Tripyridyl-s-Triazine

Introduction

In present world of medical and pharmaceutical advancement, microbes have evolved with resistance against the drugs by changing their metabolism and genetic structure [1,2]. These drug resistant microorganisms are more pathogenic with high mortality rate and have become a threat against human race. To overcome microbial drug resistance, scientists are looking forward for the development of alternative and novel drugs [3]. Natural sources such as plants, algae and animals provide an array of natural medicinal compounds for the treatment of various infectious diseases [4-6]. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development [7,8].

Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause various degenerative disorders like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing [9].

Sandalwood, *Santalum album*, has been used since ancient times for religious purposes in incense, in fragrances, and as medicine. Various types of sandalwood trees grow in different countries of the world [10].

The present research has been conducted to study the medicinal properties like antimicrobial and antioxidant potential of aqueous extract of *Santalum album* so that they can be a hope in the field of phytodrugs.

Material and Methodology

Plant material

Sandalwood purchased from Local Ayurvedic Clinic.

Chemicals and reagents

All solvents and chemicals (analytical grade) used for antioxidant and antibacterial assay were purchased from Merck and Himedia. DPPH and TPTZ were purchased from Sigma-Aldrich.

Test microorganism

The following eight clinical isolates of bacteria were used for the study: Two species of *Escherichia coli*, one each of *Klebsiella pneumoniae*, *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aeromonas species* and *Klebsiella oxytoca.* All these cultures were maintained on nutrient agar plates at 4^{O}C.

Methodology

Preparation of aqueous extract

The Sandalwood was ground finely and then strained through muslin cloth. 1 gram of sample was soaked for 2 hours in 20 ml of distilled water (50 mg/ml). The sample was then centrifuged and the supernatant was picked which served as aqueous extract for the further studies.

Antibacterial Assay

Antimicrobial activity of the aqueous extract was tested against three gram-positive bacteria (*Bacillus subtilis, Aeromonas species.* and *Staphylococcus aureus*) and five gram-negative bacteria (two of *Escherichia coli* and one each of *Klebsiella pneumoniae, Klebsiella oxytoca* and *Pseudomonas aeruginosa*). Overnight cultures were prepared in Luria broth (LB) media by inoculation with a single

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colony from agar plates and incubated at 37°C for 12 hrs. Overnight cultures were diluted with fresh LB media to approximately 104 colonies forming units (CFU) and incubated at 37°C for 12-14 hrs in the presence of *S. album* compared to the growth of the control culture where only media and bacterial inoculum was taken. The experiment was repeated twice for the confirmation. The percentage inhibition was calculated by using the formula:

Percentage Inhibition (%) = [(dc - dt)/dc] x 100,

where dc and dt represent OD_{600} of control and treated sample strains respectively.

Antioxidant Activity

DPPH 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. with minor modifications [11]. A range of diluted working solutions of the *S. album* were prepared in distilled water. 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:

DPPH scavenging Activity (%) = [(dc - dt)/dc] x 100,

where dc and dt represent OD_{517} of control and test sample respectively.

FRAP Assay

Antioxidant activity assay was also done following the ferric-reducing antioxidant power (FRAP) method described by Benzie & Strain method with minor modifications [12]. 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 $FeCl_3$ (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 mM Fe^{2+}/ml of aqueous extract. Higher absorbance indicates higher reducing power.

Table 1: Percentage Growth inhibition of various bacterial strains in presence of *S. album*.

S.No.	Bacterial strain	Percentage Inhibition
	E.coli (ATCC 25922)	0
	K. pneumoniae (ATCC 700603)	69.2
	S. aureus (MTCC 902)	87
	B. subtilis (MTCC 736)	0
	E.coli (MTCC 443)	78.1
	P. aeruginosa (MTCC 2453)	65.5
	Aeromonas spp. (A10 MDR)	76.8
	K. oxytoca (A13 MDR)	77.4

Results

Antibacterial Assay

Antimicrobial assay of the aqueous extract was examined against various bacterial strains by accessing the percentage inhibition in presence of *S. album* compared to the control where only media and cultures were added. The results suggested that *S. album* exhibits bactericidal property *in-vitro* i.e. the growth of microorganisms was inhibited in its presence as shown in Table 1.

It was found that *S. album* had strongest inhibitory activity against *S. aureus* (MTCC 902) i.e. 87% whereas, it showed no inhibition against *E.coli* (ATCC 25922) and *B. subtilis* (MTCC736) as shown in Figure 1.

Antioxidant Activity

DPPH assay

DPPH radical scavenging assay is the most widely used method for screening antioxidant activity, since it can accommodate many samples in a short period and detect active ingredients at low concentration. The decrease in the absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as the colour changes from purple to yellow. *S. album* showed DPPH radical scavenging activity in a concentration–dependent manner as shown in the figure (Figure 2).

FRAP Assay

The ferric reducing antioxidant power of *S. album* is presented in figure (Figure 3). The results showed that FRAP value of *S. album* increase in the concentration-dependent manner. The highest absorbance of FRAP was observed in *S. album* at 200 µl and the lowest was that in at 20µl i.e 0.628 and 0.078 as compared to standard i.e. 0.210 and 0.965 respectively. These concentrations were effective to react with ferric tripyridyltriazine (Fe^{III}-TPTZ) complex and produce a blue colored ferrous tripyridyltriazine (Fe^{II}-TPTZ). From the observations, it is clear that *S. album* showed fair antioxidant activity comparable to ascorbic acid.

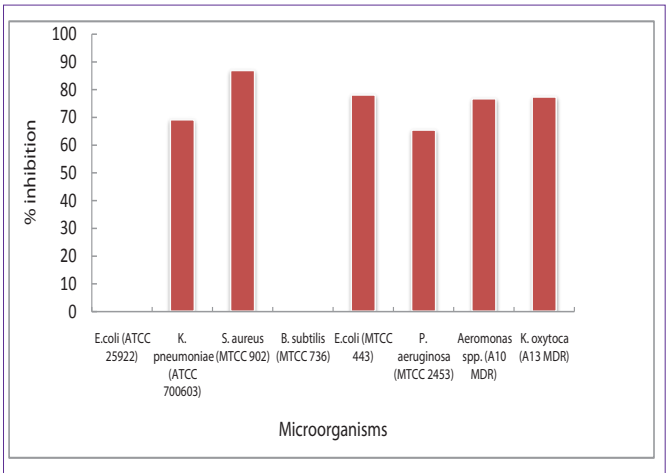

Figure 1: Graphical representation of % inhibition of Antibacterial potential of *S. album*.

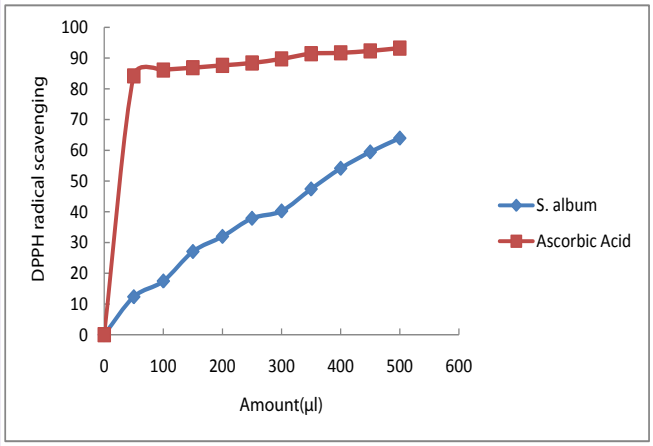

Figure 2: DPPH scavenging assay of *S. album* in comparison with Ascorbic acid.

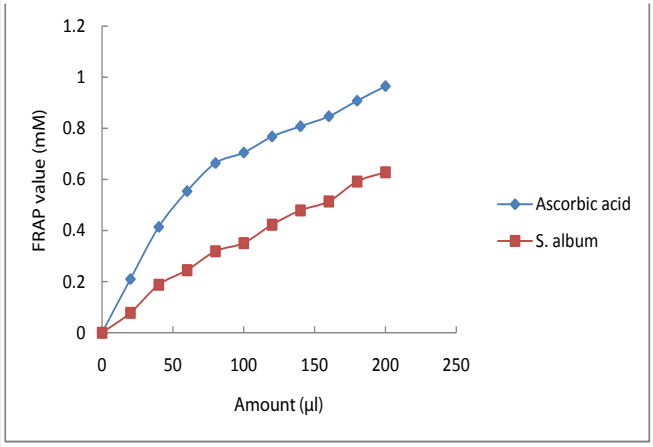

Figure 3: FRAP values of *S. album* in comparison with Ascorbic acid.

Discussion

Antioxidant and antimicrobial properties possessed by various plant extracts have recently been of great interest in both research and food industry, due to their possible use as natural additives which emerged from a growing tendency to replace synthetic antioxidants with natural ones. Owing to the antioxidant and antibacterial activities exhibited by the plant extracts, we have done this study to find out their possible role in food and pharmaceutical industries. Also, results show that aqueous extract of *S. album* possesses the potent antioxidant & antimicrobial substances which may be responsible for its anti-tumor, anti-carcinogenic activity and remedy for hepatitis B viral infection mechanism as well as justify the basis of using this plant's extract as traditional remedies.

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