

Research Article



Phytochemical and Antibacterial Activities of *Santalum Album*

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ABSTRACT

To identify the antibacterial activity and phytochemicals analysis of *Santalum album* and also screening of leaf, bark and seed extracts of plant for its antibacterial activity. Antibacterial activity of *Santalum album* was performed with methanol solubility against various human pathogens viz. *Bacillus subtilis* (*B.subtilis*), *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S.aureus*) and *Pseudomonas aeruginosa* (*P.aeruginosa*). Preliminary phytochemicals analysis of the ethyl acetate, chloroform, methanol and aqueous extracts of *Santalum album* revealed the presence of alkaloids, cardiacglycosides, flavonoids, oxalates, phenols, sterols and tannins. In leaf, bark and seed extracts, flavonoids present in methanol, aqueous extracts of leaf, and chloroform, methanol extracts of seed. Saponins and quinines are completely absent in all extracts, sterols and oxalates present only in methanol leaf extract. The plant extracts showed better inhibitory activity against the tested organisms.

Keywords: *Santalum album*, Human pathogens, ethanol extract, methanol, chloroform, ethyl acetate, phytochemicals.

INTRODUCTION

India is one of the world's twelfth leading biodiversity centers with the presence of over 45,000 different plant species. This has resulted in the use of large number of medicinal plants with creative properties to treat various diseases¹ as well as to impart flavor to food. Indian system of medicine has identified 1,500 medicinal plants of which 500 are commonly used.² Natural products have been an integral part of the ancient traditional medicine system. Plants having chemical components are often referred to as phytochemicals or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests.³ Plant produce these chemicals to protect itself but recent research demonstrate that many phytochemicals can protect human against diseases.

Phytochemicals constituents are the basic sources for the establishment of several pharmaceutical industries the constituents present in the plant which play a significant role in the identification of crude drugs. Phytochemicals screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, phenols, saponins, steroids, tannins, terpenoids, flavonoids etc.⁴

In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region.⁵ Following such leads, plant parts are usually screened for phytochemicals that may be present.

The presence of phytochemicals of interest may lead to its further isolation, purification and characterization, then it can be used as the basis for a new pharmaceutical product. Pharmacognosy is the study of natural products and their application in the improvement of health. The scope of Pharmacognosy has expanded from the

traditional morphological description of plants and other organisms to encompass the most modern aspects of molecular science relating to the exploration of naturally occurring bioactive compounds, their mode of action and ultimately, their application in all economic and social activities. The search for new therapeutics was initially focused on plant species used in traditional medicines.

Santalum album is a medium sized hemi parasite tree commonly called sandal wood belonging to the family santalaceae for the use of *Santalum album* in India is noted in literature for over two thousand years. Sandal wood contains over 90% of santalol, a mixture of sesquiterpenoids and hence is the focus of many investigations. The essential oil yield from a 50 years old matured tree ranges from 2-5 to 6% which is highest among all known 20 *Santalum* species depending on the age of the tree and color of the hard wood.⁶ *Santalum album* is rich in various essential minerals such as sodium, calcium, magnesium, chlorine, phosphorous, potash, nitrogen, sulfur, and nitrate. It also has traces of copper, zinc, iron and manganese. *Santalum album* help full in treating various diseases and ailments such as gastric irritability, dysentery, genital urinary disorders, prickly heat, skin diseases, fever, heating powers. *Santalum album* oil used in aromatherapy, the sandal wood oil is very quite fragrant, relaxing reduces confusion and helps in balancing the mind. It also helps to clear up dry cough and boosts the digestive system. The present study aims to assess the antibacterial unique bio-active compounds against human pathogenic bacteria.

MATERIALS AND METHODS

Plant Material

Plant materials for the proposed study were collected from the campus of Sri Venkateswara University, Tirupati voucher specimen deposited in Herbarium. The collected



plant material were transported in polythene bags and they washed separately under running tap water and distilled water for removing soil and other dust particles and finally washed with sterilized and de ionized water and the plant material were dried at room temperature.

Extract Preparation

Preparation of plant extracts were prepared by the method of Alade PI⁷ with minor modification. 100 g of each powdered plant material were soaked in 100 ml of 70% alcohol for 72 h. and the mixture was stirred every 24 h using a sterile glass rod. At the end of extraction each extract was passed through Whatman filter paper No.1 (Whatman,UK) and The alcoholic filtrates obtained was concentrated in vacuous at 30°C and stored at 4°C until further use.

Preliminary Phytochemical Screening

The stock solution was prepared from leaf and bark crude extracts and was dissolved in its own mother solvents. The obtained stock solutions was subjected to preliminary phytochemicals screening.^{8,9}

Antibacterial Assay

The following bacterial strains were used in this study viz., *Bacillus subtilis* (*B.subtilis*), *Escherichia coli* (*E.coli*), *Staphylococcus aureus* (*S.aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). Disc diffusion assay method was carried out by using standard protocol.¹⁰ Overnight bacterial culture (100µL) was spread over Muller Hinton Agar (Hi Media Laboratories private limited Mumbai, India) plates with a sterile glass L-rod. 100µL of the each extracts were applied to each filter paper disc What man No.1 (5mm dia) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate and plates were inoculated at 37°C for 24 hrs. After incubation, the diameter of inhibition zones was measured.

RESULTS AND DISCUSSION

The qualitative phytochemicals estimation were showed that the presence of alkaloids, carbohydrates, cardiacglycosides, flavonoids, oxalates, phenols, tannins are present in ethyl acetate, chloroform, methanol and aqueous extracts of leaf, bark and seed and absence of proteins and amino acids, quinons, saponins, are completely absent in all extracts. Terpenoids, flavonoids, are present in ethyl acetate and chloroform leaf extracts, sterols are present only in methanol leaf extracts completely absent in ethyl acetate, chloroform and aqueous extracts of leaf. Alkaloids, carbohydrates, cardiacglycosides, flavonoids, Proteins and amino acids, tannins were present in methanol bark extract but oxalates, phenols, quinons, saponins, sterols and terpenoids are absent as shown in Table 1.

The results of the methanol leaf, bark and seed exhibited antibacterial activity against all the tested strain viz. *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas*

aeruginosa and *Escherichia coli* as shown in Table 2. The zones of inhibitions were produced by methanol extracts against all the test organisms. The zones of inhibition were ranging from 4-8mm in diameter. The maximum zone of inhibitions (8mm) noted in 2% methanol leaf extract against *P.aeruginosa* and minimum zone of inhibition (4mm) was observed in *staphylococci*.

The results of the antibacterial activity suggested that, the zone of inhibition of *Santalum album* was observed in leaf, bark and seed methanol extract against *B.subtilis*, *S.aureus*, *P. aeruginosa*, *E.coli* and minimum zone of inhibition was observed in 2% and 5% concentrations of *Santalum album* leaf extract against *S.aureus* additionally *B.subtilis*, maximum zone of inhibition was observed in both *P.aeruginosa* and *E.coli* in 2% methanolic leaf extracts compared to 2% methanolic bark extract as shown in Table 2.

Maximum zone of inhibition was observed in 5% methanol extract in *P.aeruginosa*, minimum zone of inhibition was observed in *Santalum album* methanolic seed extract at a concentration of 2% and 5% was not showing zone of inhibition against the organisms [Table 2].

The world health organization (WHO) with the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest. Some plant extract and phytochemicals constituents in the plant sample are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer.^{11,12} All secondary metabolite components displayed antibacterial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant extracts.¹³ The biochemical screening of methanol crude extracts of *Santalum album* used in this study revealed that the crude extracts contained alkaloids, flavonoids, saponins and tannins (Table 1). The most effective bioactive compounds alkaloids and flavonoids were found in both methanol leaf and bark extracts. Tannins are another active compounds found to be present in both leaf and bark extracts, therefore, the detected different bioactive compounds in different crude extracts from leaf and bark extracts of *Santalum album* may be responsible for antibacterial activities several reports are available on flavonoids groups which exhibited high potential biological activities such as anti-oxidant and anti-inflammatory, anti-microbial, anti-analgesic, anti-cancer and anti-allergic reactions.¹⁴⁻¹⁷

The antimicrobial activity of the leaf and bark crude extracts was estimated using standard conventional methods against *S.aureus*, *E.coli*, *P.aeruginosa* and *B.subtilis*. The methanol crude extracts of *Santalum album* and its fractions revealed comparatively small antibacterial potential against gram-positive and gram negative bacteria at the concentrations of 2% and 5%



with their respective zone of inhibition of 4-7 mm (Table 3).

Generally the antimicrobial activity of plant crude extracts depends on the dose and the type of bacterial strains employed. Also this anti bacterial actions could be related to their chemical components in the crude extracts.¹⁸⁻²² The bioactive compounds such as tannins and flavonoids components were present in the crude extracts. However these bio active compounds were inducing the antibacterial activity. The amount of active components

in the crude extract may be diluted or increased their concentrations by fractionation.^{14,18,16,20-22}

Because they have the ability to inactivate microbial activity, enzymes, cell envelope transport proteins and so forth.¹⁸⁻²² Hence the presently studied *Santalum album* plant extracts could be of considerable inferences to the development of new life saving drugs. However further research is required to isolate the bioactive principle of this species as well as further studies on its bio-efficiency against human pathogens.

Table 1: Analysis of phytochemicals in Aqueous, methanol, ethyl acetate, chloroform

S.No	Phytochemicals	Leaf Extract				Bark Extract				Seed Extract			
		Ethyl acetate	Chloroform	Methanol	Aqueous	Ethyl acetate	Chloroform	Methanol	Aqueous	Ethyl acetate	Chloroform	Methanol	Aqueous
1	Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
2	Cardiac glycosides	+	+	+	+	+	-	+	+	-	-	+	-
3	Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	-
4	Flavonoids	+	-	+	+	-	-	-	-	-	+	+	-
5	Oxalates	-	-	+	-	-	-	-	-	-	-	-	-
6	Phenols	+	+	+	+	-	-	-	-	-	-	-	-
7	Phlobatanins	+	-	-	+	+	-	+	+	-	-	-	-
8	Proteins and Amino acids	-	-	-	-	+	-	+	-	-	-	-	-
9	Quinines	-	-	-	-	-	-	-	-	-	-	-	-
10	Saponins	-	-	-	-	-	-	-	-	-	-	-	-
11	Sterols	-	-	+	-	-	-	-	-	-	-	-	-
12	Tannins	+	+	+	+	+	+	+	+	-	-	-	-
13	Terpenoids	+	+	-	-	+	+	+	-	-	+	+	-

+ = high presence of bio active compounds, - = absence of bioactive compounds.

Table 2: Antibacterial activity of methanol extract of *Santalum album*

Bacterial strains	Diameter of zone of inhibition (mm)					
	Leaf		Bark		Seed	
	2%	5%	2%	5%	2%	5%
P.aeruginosia	8	5	5	6	0	0
S.aureus	4	4	4	4	0	0
E.coli	5	6	4	5	0	0
B.subtilis	4	5	5	5	0	0

CONCLUSION

The present antimicrobial study of different crude extracts of *Santalum album* that the methanol crude extract from leaf and bark extracts shows highest activity against the employed bacteria and no activity was observed in seed methanol crude extract.

Phytochemicals screening showed that the antibacterial activities of the crude extracts of *Santalum album* depends on the presence of phytochemicals such as alkaloids, steroids, flavonoids and tannins.

The plant crude extracts could serve as potential sources of new antimicrobial activity.

Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceuticals.

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