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Phytochemical Screening and Antibacterial Activity of Aqueous Leaf Extract of *Santalum album* Linn

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Original Research Article

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Abstract: *Santalum album Linn* is one of the important medicinal plants belonging to the family Santalaceae. Ayurveda and Unani medicinal systems use it for the treatment of several ailments. *Santalum album Linn* has Anti pyretic, Anti helmintic, Anti-microbial, Hepato protective and Anti-cancer activities. As per World Health Organization many people are suffering from microbial infections. The present study was designed to evaluate the anti-microbial activity of *Santalum album Linn.* The preliminary phytochemical studies determine the various secondary metabolites like Carbohydrates, Glycosides, Alkaloids, Phenols and Tannins are present. These aqueous extract was screened for their antimicrobial activity against *B.Subtilis* pathogen bacteria by agar cup-plate method. These aqueous extract shows significant activity against at 10, 20 and 40µgm/ml. The Activity Index is 0.21, 0.45, 0.86 (*B. subtilus*) and compared with a standard drug Ofloxacin. This study gives the way for further studies to elucidate the other properties of *Santalum album*.

Keywords: *Santalum album*, Anti-microbial activity, Ofloxacin, phytochemical screening.

INTRODUCTION

In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, the undesirable side effect of certain antibiotics, and the emergence of previously uncommon infections, has forced scientists to look for new antimicrobial substitutions from various sources such as medicinal plants [1, 2]. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents [3-6].

Santalum album commonly known as White or East Indian sandalwood, Chandan, Indian sandalwood belonging to the family Santalaceae. Native to semi-arid areas of the Indian subcontinent. It is now planted in India, China, Sri Lanka, Indonesia, Malaysia, the Philippines and Northern Australia. And it is known by various names in different regions Sandal, Sandal tree, sandalwood, White sandal tree in English, chandan, srikhanda in Bengali, Chandanamu in Telugu, Arishta-phalam in Sanskrit [7].

Uses

- The plant extract is used in the treatment of diabetis.
- Leaves rubbed against the temple can relieve headaches [7-10].
- To stop bleeding from a shallow cut, apply a poultice of a fresh leaves.
- It is used as anti-bacterial & anti-inflammatory effect when applied to wounds or insect bites.
- Leaf tea treats gastric ulcer and diarrhoea.
- Leaves treat fevers, bronchitis, eye and ear infections, and inflammations of the mucus membrane [11-12].

The powder ground from the seeds also used in the treatment of scurvy skin diseases

MATERIALS AND METHODS

Collection of plant materials

The plant *Santalum album Linn* was collected from the College Medicinal garden area of Sathupalli.

Extraction of drug

The dried powdered plant material of *Santalum album* was extracted with water using Soxhlation extraction method. After exhaustive extraction the collected aqueous extract was subjected to evaporation to obtain the pure drug of extract.

Phytochemical screening [**13**]

The presence of various chemical constituents in plant extracts was determined by preliminary phytochemical screening. To perform the phytochemical screening the weighed amount (1gm) of powdered form of leaf of *Santalum album* were taken.The leaves were Soxhlated for 12 hrs by using water as a solvent. After 12 hrs they were filtered and the filterates were obtained.

These filtrates were further concentrated and isolated in ethyl acetate and ethanol and the drug was used to perform the phytochemical screening. The aqueous extract was screened for the presence of various phyto- constituents using the different chemicals and reagents.

Selection of bacterial strains

Medically important bacterial strains used in this study were Bacillus subtilis, procured from market, India. These bacteria served as test pathogens for antibacterial activity.

Standard reference antibiotic

The reference antibiotic used is Ofloxacin obtained from Market.

Preparation of broth culture

For the preparation broth culture of used bacteria, the liquid media was prepared as per given composition for broth culture. After the sterilization of media the bacterial strains were inoculated under laminar air flow. The incubation of inoculated media was carried out at 37^0C for 48 hours.

Table-1: Preparation of broth culture

S.NO	Name of the Components	Weight
1	Beef extract	1gms
2	Peptone	1gms
3	Salt(Sodium Chloride)	0.5gms
4	P^H	7.4±0.2

Preparation and sterilization of the media

The media agar media was prepared as per the formula given. The nutrient agar media was prepared as for the formula given .the nutrient agar was taken in boiling tubes at 20ml quantities. These tubes were plugged with non-absorbed cotton and kept in autoclave (120°C, 301b/sq. inch) to sterilize the media for an hour.

Table-1: Preparation and sterilization of the media

S.No	Name of the component	Weight
1	Peptone	5 gms
2	Beef extract	10 gms
3	Sodium Chloride	10 gms
4	Agar	20 gms
5	Distilled water	1000ml

Plating the media

Molten media was poured on to the Petri dish (pre-sterilized in oven for 3 hours at 110° in order to avoid contamination). The plated Petri dishes were kept on a plane surface to avoid non uniform solidification of medium. All these operations were performed on a sterile room which was fitted with laminar air flow.

Bacterial culture preparation

Bacterial culture slants were grown on stationary phase in nutrient agar. A small portion of the culture was introduced into 7-8ml of peptone containing (l% w/v) sterile water. A day old culture was used for the testing and for determination of each extract.

Assay procedure

The assay procedure was carried out by Cup plate diffusion method. The sterilized molten agar media was poured in to petridishes, and kept aside. Then 1ml of broth culture of bacterial solution was spread over the solid agar plate. By the use of sterile borer small bores were made over the plate and it was filled with test solution, Standard solution and diluting solution respectively for each bacterial plate.Then the plates were kept under incubation for 48 hours at 37^0C. The Zone was measured using scale in millimeter.

Antibacterial activity

The antibacterial activities of the extracts were determined by agar cup plate method. Nutrient agar medium was used for the test. Under aseptic conditions in the laminar air flow chamber nutrient agar medium was dispensed into pre sterilized Petri dishes to yield a uniform depth of 4mm. The media was allowed to solidify.

The test microorganisms were seeded into media containing Petri dishes by spread plate method (100µl) with 24 hours cultures of bacteria. The plates

were kept for pre diffusion for 15 minutes before use. Wells were then punched with a sterile cork borer (6mm) diameter and 50µl of the extracts (10, 20, 40µg/ml in DMSO) were placed into each well. A negative control was maintained using 50µl DMSO in a well and 50µl of standard antibiotic (streptomycin at 10µg/ml) was the positive control. Duplicates were maintained for each extracts. Finally the Plates were incubated for 18-24 hrs at 37ºc. The diameter of zone of inhibition (mean of triplicates ± S.D) was indicated by clear area which was devoid of growth of microbes was measured.

Activity Index (A.I.) = Zone of Diameter / Standard Drug Concentration

Minimum Inhibitory Concentrations

The minimum inhibitory concentrations (MIC) for the most active extracts were recorded after 24hrs. The extracts were diluted to get concentration ranging from 0.25, 5, 10, 15, 20mg/ml. After sterilization the media was dispensed into Petri plates and was inoculated with 24 hrs culture of each organism with a sterile corn borer (6mm). Wells were prepared and different crude extracts ranging from 0.25mg-20mg/ml were added to the wells and controls were maintained without plant extract. Inhibitions of organism growth in the plate containing test crude extracts were judged by comparison with growth in blank.

RESULTS AND DISCUSSIONS

Table-3: **Phytochemical screening**

Name of phyto constituent	Aqueous extract
Alkaloids	+ + +
Carbohydrates	+ +
Amino acids	-
Tannins	+
Steroids	-
Saponins	-
Flavanoids	+
Glycosides	+
Mucilages	-
Proteins	-

Preliminary phytochemical screening of the aqueous extract of *Santalum album* reveals the presence of Alkaloids, Carbohydrates, Flavonoids, Tannins and Glycosides. Different doses of the aqueous extracts were screened for their activity mainly due to the presence of alkaloids and Flavonoids respectively.

Table-4: Antibacterial activity

S.no	Name of organism	Conc. µg/ml	Zone of inhibition		ofloxacin
			ZOD (mm)	AI	ZOD (mm)
2	*Bacillus subtilis*	10	5	0.21	23
		20	10	0.45	22
		40	20	0.86	23

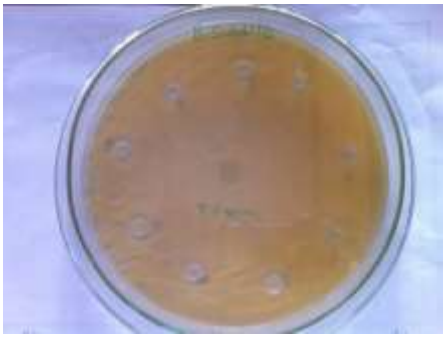

Fig-1: **Zone of inhibition of *bacillus subtilis***

The results of anti-bacterial screening by agar cup plate method indicate the highest antibacterial activity was shown by the aqueous extract of leaf against the *Bacillus subtilis*. Standard antibiotic

ofloxacin was effective against all organisms and showed a zone of inhibition of 22-25mm.The results of the investigation showed that the leaf extract from *Santalum album* have good antibacterial activity (10, 20 and 40µgm/ml) against *Bacillus subtilis* due to presence of alkaloids and flavonoids.

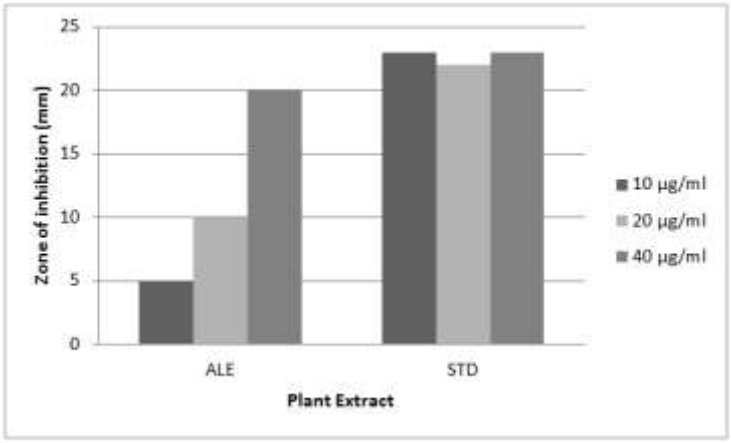

Fig-2: **Antibacterial activity of leaf extract on Bacillus subtilis**

CONCLUSION

The plant *Santalum album* was collected from the Medicianl garden of Mother Teresa pharmacy college, Sathupalli. The wet leaves of the plant can subjected to Soxhlation by using water as a solvent. Then this extraction distilled to get a concentrated mass. Phytochemical investigation was done.

The work states that the presence of alkaloids, carbohydrates, glycosides, Flavonoids and Tannins in the extract of *Santalum album* was responsible for its antibacterial activity. These compounds exhibit a maximum zone of inhibition against *Bacillus subtilis*. It is interesting to observe the results of high antibacterial effect of aqueous extract. This study gives the way for further studies should be undertaking to elucidate the exact mechanism of action by which extract exert their antibacterial effect.

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