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IN VITRO ANTIBACTERIAL AND ANTIFUNGAL STUDIES OF STEREOSPERMUM COLAIS LEAF EXTRACTS

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ABSTRACT

Aim: To screen the various extract of leaves of *Stereospermum colais* for antibacterial and antifungal activity against few clinically important pathogenic bacteria and fungi.

Methods: The various successive solvent extracts *viz*., n-hexane, chloroform, ethylacetate, ethanol and water were screened for its antimicrobial activity. The test organisms used for antibacterial study includes fresh clinical strains isolated from pathologic specimens *viz.*, gram (+ve) *Coagulase negative Staphylococcus*, *Entero cocci*, *Staphylo coccus aureus*, and gram (-ve) *Acinetobacter*, *Citrobacter*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aureginosa*, *Salmonella typhi and Salmonella paratyphi*. The antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) and agar disc diffusion method. The antifungal activity was assessed by MIC. For antifungal activity, the fungi studied were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger and Candida albicans.*

Results: Comparison of antibacterial activity was done with standard antibiotic ciprofloxacin (5µg/disc). The ethanol and chloroform extract showed maximum antibacterial activity followed by ethyl acetate, aqueous and n-hexane. The ethanol extract showed inhibitory effect against all fungi except *Aspergillus flavus*. Chloroform extract showed activity against *Candida albicans*. The other extracts showed significant inhibition on the growth of fungi.

Conclusion: The various extracts of *Stereospermum colais* leaves have potential antibacterial and antifungal activity and can be used as new source for antibacterial and antifungal drugs.

Key words: *Stereospermum colais*, successive extracts, antibacterial, antifungal activity.

1. INTRODUCTION

Stereospermum colais (Buch.-Ham.ex Dillw.) is commonly known as Mabberley, belonging to the family of Bignoniaceae. It is a large deciduous tree, 18-30 m high and 2.8 m in girth found through out the moist regions of India. It is known as Padri in Hindi and Pathiri in Tamil. The leaves are useful in otalgia, odantalgia, rheumatalgia, malarial fever and wounds. The juice of the leaves, mixed with lime juice is used in maniacal cases. Decoction of the leaves is used for treating chronic dyspepsia and also has antipyretic properties. The root of the plant is used as an ingredient of the reputed Dasamula an Ayurvedic formulation (1). The roots are bitter, astringent, acrid, anodyne, appetiser, constipating, diuretic, lithotropic, expectorant, cardiotonic, aphrodisiac, anti inflammatory, antibacterial, febrifuge and tonic, anti emetic, antipyretic. Decoction of roots used in asthma and cough. The present study attempts to bring out the hitherto unearthed antibacterial and antifungal potentials of the plant.

2. MATERIALS AND METHODS

2.1. Plant material

The fresh leaves of *Stereospermum colais,* Family: Bignoniaceae, were collected from Nellivasal nadu region present in Javadhi hills, Vellore district, Tamil Nadu in May 2008 and were authenticated by botanist. The leaves were air dried, powdered and passed through a sieve No.22 and stored in air tight container.

2.2. Successive solvent extraction: The powdered leaves of the plant material were extracted successively with n-hexane, chloroform, ethylacetate, ethanol in a soxhlet apparatus for 20 hrs and finally macerated with

water for 48 hrs. Each extract was concentrated by distillation of the solvent and then evaporated to dryness on water bath.

2.3. Preliminary phytochemical evaluation

The various extracts are subjected to qualitative tests for the identification of various plant constituents (2-4).

2.3.1. Antibacterial activity

The various extracts of *Stereospermum colais* were screened for antibacterial activity against few clinically important pathogenic organisms.

2.3.2. Test microorganisms

The test organisms used for this invitro study includes fresh clinical strains isolated from pathologic specimens viz, Gram (+ve) bacteria, *Coagulase negative Staphylococcus*, *Entero cocci, staphylococcus aureus*, and Gram (-ve)bacteria *Acinetobacter, Citrobacter, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aureginosa, salmonella typhi, salmonella paratyphi A.* The selection of clinical microorganisms depended on their availability. The organisms were maintained on nutrient agar slopes after confirmation by biochemical tests. They were stored at 4^{o}c.

2.3.3. Minimum Inhibitory Concentration (MIC) determination

The plant extracts namely n-Hexane, Chloroform, Ethyl acetate, Ethanol and aqueous were dissolved in DMF. The extracts were introduced aseptically into sterilized Petri dishes to get final concentration ranging from 1000 µg/15 ml, 2000 µg/15 ml, 4000 µg/15 ml, and 8000 µg/15 ml, so that the concentrations were 66.67 µg/ml, 133.33 µg/ml, 266.67 µg/ml, and 533.33µg/ml. The volume was made up to 15ml by adding MH media and the Petri dishes were swirled until the agar begins to set (5). The plates with different extracts of various dilutions were inoculated with a loopful of culture at the labeled place. The plates were incubated at 37ºc for 24 hrs. The results were read by the presence or absence of growth of the organisms

(6). MIC for each organism was taken as the lowest conc. of the extract in the nutrient agar that inhibited the visible growth of the organism after 24 hr of incubation at 37 ^{0}C (7).

2.3.4. Agar disc diffusion method

The discs of 6 mm diameter were prepared from Whatman filter paper no.1 and were sterilized in a hot air oven at 160 ºC for 1 hr. The discs were saturated with the extract and solvent DMF and allowed to dry (8). Ciprofloxacin discs (5µg) were used as standard.

The pathogenic strains were then seeded on the MH agar media in a petridish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry and then Ciprofloxacin, Extracts and DMF discs were introduced on the upper layer of the seeded agar plates and maintained at 4ºc for 30 mts to allow perfusion of drugs being tested. The plates were then incubated at 37ºc for 24 hrs. The results were read by measuring the zone of inhibition (8, 9).

2.4. Anti fungal activity

The n-Hexane, Chloroform, Ethylacetate, ethanol and aqueous extracts of the leaves of *Stereospermum colais* were subjected to antifungal studies. The fungi studied were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Candida albicans.* The fungi were maintained on Sabouraud's Agar Slopes and stored at 4 °C.

2.4.1. Minimum Inhibitory Concentration (MIC) determination

The extract were introduced aseptically into sterilized tubes to get final concentration of 500 µg, 1000 µg, 2000 µg (0.5 ml, 1 ml and 1.5 ml) and made up to 3 ml with SDA and the slopes prepared by slanting the tubes.

The different fungi were inoculated into Sabouraud's dextrose agar slant. All the slants were incubated at 37^0C in an incubator for 1 to 4 weeks. The results were read by noting the presence or absence of growth in the slant (10).

3. RESULTS AND DISCUSSIONS

The preliminary phytochemical investigation revealed the presence of carbohydrates, proteins, fats & fixed oils, steroids, glycosides, tannins, flavonoids and terpenoids (Table 1).

Table 1. Qualitative phytochemical evaluation of various extracts of the leaves of *Stereospermum colais*

Phytoconstituents	**n-Hexane**	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Aqueous**
Carbohydrates	-	-	-	+	+
Gums and Mucilage	-	-	-	-	+
Proteins	-	-	-	+	+
Fats and Oils	+	-	-	-	-
Steroids	+	+	-	-	-
Glycosides					
a. Cardiac	-	-	+	+	-
b. Anthraquinones	-	-	-	-	-
c. Saponin	-	-	-	+	+
e. Iridoid	-	-	+	+	+
f. Flavonoids	-	-	+	+	+
Alkaloids	-	-	-	-	-
Tannins & Phenols	-	-	+	+	+
Terpenoids	+	+	+	-	-

\+ denotes the presence of the respective phytoconstituent
\- denotes the absence of the respective phytoconstituent

3.1. Antibacterial studies

All the clinical strains of the different microorganisms screened were susceptible to the extracts of *Stereospermum colais* at the concentration of 66.57 to 533.32 µg/ml. The results of MIC are presented in Table 2. Microbial growth was determined by measuring the diameter of zone of inhibition. The zone of inhibition was found between the range of 8-40 mm. Comparison of antibacterial activity of various extracts was done with standard antibiotic ciprofloxacin. Along with standard, DMSO as negative control was maintained. The results of zone of inhibition are presented in Table 3.

Table 2. Minimum Inhibitory concentration of the extracts of *Stereospermum colais* against bacterial strains.

Micro organisms	**Minimum Inhibitory Concentration (µg/ml) of Extracts**				
	n-Hexane	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Aqueous**
Staphyloccocus aureus	266.67	266.67	533.32	66.67	266.67
Escherichia coli	266.67	66.67	533.32	66.67	266.67
Klebsiella	66.67	133.33	133.33	66.67	266.67
Citrobacter	66.67	66.67	133.33	66.67	133.33
Acinetobacter	133.33	133.33	266.67	66.67	533.32
Pseudomonas	133.33	66.67	133.33	266.67	533.32
Entero cocci	533.32	266.67	266.67	533.32	533.32
Coagulase negative staphylococcus	66.67	133.33	66.67	66.67	266.67
Salmonella typhi	66.67	266.67	133.33	266.67	266.67
Salmonella paratyphi A	66.67	133.33	66.67	66.67	266.67

Table 3. Zone of Inhibition of the Extracts/standard of *Stereospermum colais* against clinical strains.

Extracts	Zone of Inhibition (mm)									
	Microorganisms									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
n- Hexane	20	30	35	11	22	18	31	08	30	25
Chloroform	11	22	35	21	24	27	30	18	30	30
Ethyl acetate	25	34	35	22	25	21	27	10	30	30
Ethanol	29	40	35	25	14	22	31	23	26	29
Aqueous	20	33	35	11	20	30	26	13	25	29
Ciprofloxacin	30	39	35	35	32	33	36	25	32	34

1) *Coagulase negative staphylococcus,* 2) *Entero cocci* 3) *Staphlococcus aureus,* 4) *Acinetobacter,* 5) *Citrobacter,* 6) *Escherichia coli,* 7) *Klebsiella pneumoniae,* 8) *Pseudomonas aureginsa,* 9) *Salmonella typhi,* 10) *Salmonella paratyphi A.*

All the extracts showed prominent antibacterial activity when compared with standard compound. Standard showed maximum zone of inhibition of 39 mm. The ethanol and chloroform extract showed maximum zone of inhibition of 40 mm and 35mm respectively followed by ethyl acetate, aqueous and n-hexane extracts.

3.2. Antifungal studies

The fungi tested were susceptible to the leaf extracts of *Stereospermum colais*. The results of MIC are presented in Table 4. The ethanol extract showed inhibitory effect on all fungi except *A.flavus* where as chloroform extract showed inhibitory effect only against *C.albicans*. The other extracts showed significant inhibition on the growth of fungi.

Table 4. Minimum inhibitory concentration of leaf extracts of *Stereospermum colais* against pathogenic fungi.

Extracts	Minimum Inhibitory Concentration (µg/ml)			
	1	**2**	**3**	**4**
n-Hexane	>500 <1000	>1000 <2000	>1000 <2000	>500 <1000
Chloroform	>1000 <2000	>2000	>2000	>2000
Ethyl acetate	>500 <1000	>500 <1000	>1000 <2000	>1000 <2000
Ethanol	>1000 <2000	>2000	>500 <1000	<500
Aqueous	>1000 <2000	>1000 <2000	>1000 <2000	>1000 <2000

1) *Candida albicans*, 2) *Aspergillus flavus*, 3) *Aspergillus fumigates*, 4) *Aspergillus niger*

4. CONCLUSION

The antimicrobial properties against pathogenic bacteria and fungi may be due to the presence of terpenoids, flavanoids, phenolic compounds and glycosides. The various extracts of *Stereospermum colais* leaves have potential antibacterial and antifungal activity and can be used as new source for antibacterial and antifungal drugs.

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