

**SPORE GERMINATION INHIBITION ACTIVITY OF ALKALOID EXTRACTS OF
ALBIZZIA LEBBECK**Prathima Mathias D. A.*¹, Girish M.² and Raghavendra Hegde²¹Associate Professor, Department of Chemistry, I.D.S.G GOVT. COLLEGE, CHIKKAMAGALUR – 577102, Karnataka, India.²Department of Chemistry, I.D.S.G GOVT. COLLEGE, CHIKKAMAGALUR – 577102, Karnataka, India.

Received on: 27/06/2023

Revised on: 17/07/2023

Accepted on: 07/08/2023

*Corresponding Author

Prathima Mathias D. A.

Associate Professor,

Department of Chemistry,

I.D.S.G GOVT. COLLEGE,

CHIKKAMAGALUR –

577102, Karnataka, India.

ABSTRACT

Bark of *Albizzia lebeck* of our campus were collected and shade dried. The dried plant material was extracted with ethanol by Soxhlet extraction method. From the ethanol extract alkaloid fraction was isolated using standard protocol. The alkaloid extract has been tested for absence of other plant metabolites like phenolics, saponins, steroids. Etc. through phytochemical analysis. The extract has been subjected to antifungal activity by spore germination inhibition method. The soil pathogens such as '*Fusarium oxysporum*' and '*Curvularia lunata*' were used in order to find the activity of the extract in field of agriculture. The results indicated that alkaloid extract of *Albizzia lebeck* had good spore germination inhibition activity in comparison to standard.

KEYWORDS: Alkaloid extract, *Albizzia lebeck*, spore germination inhibition, '*Fusarium oxysporum*' and '*Curvularia lunata*'.

1.1 INTRODUCTION AND REVIEW OF LITERATURE

India is blessed with a rich heritage of traditional medicinal systems and biodiversity to complement the herbal needs for treatments administered by these traditional medical systems. The recognized Indian systems of Medicines like Ayurveda, Siddha and Unani use herbs and minerals in the formulations. India has 15 agro – climatic zones, 47,000 plant species of which 15000 are reported to have Medicinal properties with varying degrees.

Albizzia lebeck

The plant *Albizzia lebeck* Benth belongs to family Mimosaceae it is a deciduous tree. In India it is known by various names of different regions, Sirish in Bengal, Begemara in Karnataka and pilo – sarasio in Gujarat.^[1] It is reported to possess anti – asthmatics, anti – inflammatory, anti – fertility and anti – diarrhoeal properties and has been useful in respiratory problem, Snake bite, Scorpion sting and Malarial. It is reported to have antiseptic, anti – dysenteric and ant tubercular properties.^[1]

The bark has acrid taste and is recommended for bronchitis, leprosy, paralysis and helminth infections.^[1] It is also used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea.^[3] Ethanol extract of pods possess antiprotozoal,

hypoglycemic and anticancer properties. *Albizzia lebeck* bark extracts show the immunomodulatory and antimicrobial activity.^[3]

The phytochemical test of the bark shows the presence tannins 7 – 11%, viz D – catechin, Isomer of leucocyanidin [5,7,3¹,4¹- tetrahydroxy flavon – 3,4 diol], melacacidin, leuco – anthrax cyanidin, lebecacidin [8,3¹,4¹- trihydroxy flavon – 3,4- diol], friedelin, β -sitosterol, betulinic acid and its glycosides.^[1]

Saponins were isolated from the bark. The effect of saponin containing n- butanoic fraction (BF) extracted from dried bark of *Albizzia lebeck* was studied on cognitive behavior and anxiety in albino mice.^[1] Shahnaz et al.^[5] in their studies found various plant extracts of *A. Lebeck* to be active against *Rizoctonia solani Kuhn*. *A. lebeck* was able to effectively reduce disease caused by *P.syringae* in green house (P,0.05). Antibacterial activity by bark extract was shown on phytopathogens and speck disease in tomato plant.^[6]

A. lebeck bark extract reduced disease incidence caused by *P. syringae* in green house (P < 0.05). The bark extract has demonstrated antibacterial activity on phytopathogens and could be used against speck disease in tomato plant.^[6]

The main active constituent of bark extract is anthraquinone glycosides. It is active against aerobes and mechanism of action is that glycosides cause the leakage of the cytoplasmic constituents.^[3] The saponins named

Albizzia saponins were isolated from the bark of *Albizzia lebeck*.

For our present investigation, the shade dried, coarsely powdered bark of the *Albizzia lebeck* was subjected to Soxhlet extraction and crude extract was obtained by solvent evaporation. A small portion of crude extract was subjected to alkaloid isolation. Both the crude extract and crude alkaloid extracts were investigated for Phytochemical and antibacterial test.

1.2 MATERIALS AND METHOD

1.2. A Collection

Bark of *Albizzia lebeck* of our campus, I.D.S.G. Government college, Chickmagalur district were collected in the month of February, and shade dried. The dried plant material was extracted with ethanol by sohxlet extraction method.

1.2.B Extraction

Extraction was carried out by Soxhlet extraction method. In this method, the finely ground crude solid plant material containing some desired compound is extracted using ethanol and Soxhlet extraction apparatus. The extract obtained was later dried after solvent recovery.

Table 2: Phytochemical investigation of crude ethanol extract.

Sl. No	Test for	Ethanol extract	Alkaloid extract
01	Alkaloids	+	+
02	Steroids	+	-
03	Amino acids	-	-
04	Saponins	+	-
05	Flavones	-	-
06	Carbohydrates	+	-
07	Phenolics and Tannins	-	-
08	Lactones	-	-

Key: - Indicates absent and + Indicates present

1.2.e. Spore Germination Inhibition Assay

The fungal spores accumulate sufficient energy and therefore germinate on artificial nutrient medium. Spores start the process of germination with the formation of germ tube which arises from the surface. A cavity slide technique is used for this study. Agar slants of fungal culture were prepared and incubated for five days at 25 ± 1 °C. Suspension of culture was prepared (1 drop ~ 1 x

Table 1: Extract details.

Part of Plant Used	Bark
Weight taken (g)	194.2
Nature of the extract	Reddish brown
Alcohol extract (g)	34.84
Alkaloid extract (g)	10.98

1.2.C. Alkaloid Extraction From Crude Ethanol Extract

The alcohol extract was subjected to chloroform extraction in order to remove water soluble non basic organic materials and filtered. The residue was dissolved in water and acidified to P^H2 and then steam distilled. The solution of the rest of the alkaloid salts were extracted with ether and the ether layer obtained after this extraction was evaporated to give crude alkaloids.

1.2.D. Phytochemical Screening

The crude extracts obtained after sohxlet extraction were subjected to Phytochemical screening. Standard procedure were followed for the testing and the results obtained follows.

10^4 spores/ml) and 1 μ l of test and 1 μ l of spore suspension was placed in cavity and incubated at 25 ± 1 °C for 12 hrs.

The spores were observed at 12 hrs, under microscope for presence of germ tubes. Effect of crude extract of *Albizzia lebeck* on spore germination of fungi are as shown in Table-3.

Table 3: Spore germination inhibition activity of extracts from *Albizzia lebeck*.

Test fungi	Tested concentration = 0.05 g Percentage spore germination inhibition assay			
	Alcohol extract	Alkaloid extract	Control-1(Distilled water)	Control-2(DMF)
Fusarium oxysporum NCIM 1281	96.93% \pm 0.02	95.23% \pm 0.04	0%	0%
Curvularia lunata NCIM 1131	78.62% \pm 0.04	84.46% \pm 0.03	0%	0%
Standard	98% \pm 0.05	97% \pm 0.02	-	-

Key: P <0.05 – significant

1.3 CONCLUSION

During the course of studies conducted by several workers to find out alternatives to synthetic chemical fungicides, a number of chemical compounds isolated from plants were found to be antifungal. There are several alkaloids known to be antifungal. Our present investigation showed that the crude ethanol and alkaloid extracts were highly antifungal in nature. The alcohol extract showed 96.93 % and its alkaloid extract showed 95.23 % inhibition against *Fusarium oxysporum*. The alcohol extract showed 78.62 % and its alkaloid extract showed 84.46 % inhibition against *Curvularia lunata*. Results were promising in comparison to the standard antifungals. Testing these extracts under field conditions against some fungal plant diseases would be interesting and thus find broad scope.

1.4 ACKNOWLEDGMENTS

The authors are thankful to the principal I.D.S.G Government College, chickmagalur for providing infrastructural facilities.

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