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Research Article

EVALUATION OF ANTIMICROBIAL POTENTIALITY OF 50% AQUEOUS ETHANOLIC LEAF EXTRACT OF *Acacia nilotica Willd*.

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ABSTRACT

Objectives: To study the antimicrobial property of 50% aqueous ethanolic leaf extract of *Acacia nilotica* (L.) *Willd.* against few micro organisms.
Method: The leaves of *Acacia nilotica* (L.) *Willd.* were sequentially soaked in petroleum ether (60-80^0 C), chloroform, benzene and 50% aqueous ethanol, extracts were collected, filtered and concentrated. Antimicrobial potentiality of the extracts were tested against few micro-organisms.
Result: *Acacia nilotica* (L.) *Willd.* exhibited antifungal effect against *Rhizoctonia solani.*
Conclusion: The plant leaf extract can be used as antimicrobial agent against *Rhizoctonia solani*

Keywords: phytochemical products, antimicrobial property, bioassay.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable fungal and bacterial infections and adds urgency to the search for new infection-fighting strategies.

Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases.

Acacia is the most significant genus of family: Leguminosae, first of all described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide, about two-third of them native to Australia and rest of spread around tropical and subtropical regions of the world [1], [2]. There have been reports of more than 40 species of this genus in India in his 'Flora of Madras Presidency.' *Acacia* species are commonly known as 'Babool' in India and ethno medicinally have long been used for the treatment of skin, sexual, stomach and tooth problems. *Acacia nilotica* (L.) *Willd.* (Mimosaceae), commonly known as babul, kikar or Indian gum Arabic tree has been recognized worldwide as a multipurpose tree. It is widely distributed throughout arid and semi-arid zones of the world.

It serves as the source of polyphenols. The plant contains a profile of a variety of bioactive components. [3,4]. Phytochemical investigations of *A. nilotica* (L.) *Willd.* found that phenolic compounds are presents in plant's extracts. The plant contains flavonoids, sterols, triterpenoids, alkaloids and phenolics which possess various health benefits. The isolation and characterization of quercetin, gallic acid, (+)-catechin, (-)-epicatechin, (-)-dicatechin, and (+)-leucocyanidin gallate from the acetone extract of *Acacia arabica*, is reported [5]. The seeds of *Acacia* sp. contain 5.2% oil. Physico-chemical constants and fatty acid composition of the refined seed oil were estimated. The oil was rich in linoleic acid, oleic acid and trace quantities of epoxy and hydroxy fatty acids. Acacia arabica bark is reported to contain catechin, epicatechin, dicatechin, quercetin, gallic acid, leucocyanidin gallate, sucrose and catechin 5-gallate [6].

A. nilotica (L.) *Willd.* has been proved as effective medicine in treatment of malaria; sore throat (aerial part) and toothache (bark) [7],[8],[9],[10],[11],[12]. A number of medicinal properties have acute diarrhea [13]. The bark of plant is used extensively for colds, bronchitis, diarrhea, bleeding piles and leucoderma [14].

The anti-fertility activity of *A. nilotica* (L.) *Willd.* pods and nuts had been tested. The fresh plant parts of this species have been reported to be most active against Hepatitis C virus [15]. It is an important multipurpose tree that has been used extensively for the treatment of various diseases, e.g. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma [16]. The part of the tree finds use in diabetes, skin diseases and leucorrhoea. These are also used as an antidiarrhoeal, antidysenteric, antidiabetic agent. The stem bark is astringent, demulcent, used in diarrhoea, dysentery, diabetes as astringent, antihelmentic, in skin disease, cough and piles, gonorrhoea [17] and as an antiasthmatic [18]. The tender twigs are used as toothbrushes while the thorns are used for joints pains. The gum is used in diarrhoea, dysentery and diabetes [19], dry cough in amoebic dysentery, as a tonic, antiasthmatic analgesic and in oral cavity lesions [20]. Pharmacologically, GA has been claimed to act as an anti-oxidant, and to protect against experimental hepatic-, renal and cardiac toxicities in rats. These reports could not be confirmed by others. The flowers are reported to reduce the body temperature [21]. These are also used in earache and as a tonic, antidiarrhoeal, antidysentary. The fruits are found to be useful in diarrhoea, dysentery and diabetes. The pods are use for impotency, urino-genital disorder and in dry cough. The seeds and leaves extracts are used for general body vigour. The leaves are used in diarrhoea [22], dysentery [23], in headaches; eczema [24], abscess [25] and opthalmic disorder [18]. The root is used for wound healing and for burning sensation. The Italian Africa uses the bark concoction in treating small pox. In Ethiopia, *Acacia nilotica* (booni) is used as a lactogogue (increase milk supply).

The plant exhibited antidiabetic, antimutagenic, antiproteolytic, antifertility, antioxidant and antimicrobial activity. The methanol extracts of *C. reflexa* is implicated as an antimicrobial agent. Plant extracts of *C. reflexa* growing on different sources (*Acacia arabica* and *Zizyphus jujube*) were exhibited anti microbial potentiality against *Staphylococcus aureus*, *Staphylococcus epidermidis*, gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and fungus *Aspergillus niger*. *Acacia nilotica* (Family: Fabaceae) showed significant antibacterial activity against three phytopathogenic

Xanthomonas pathovars viz*., Xanthomonas axonopodis pv. malvacearum, X. a. pv. phaseoli* and *X. campestris pv. vesicatoria* associated with angular leaf spot of cotton, common blight of bean and bacterial spot of tomato respectively. The antimicrobial activity of the extracts of *Acacia nilotica* was found against *Shigella sonnei. Bacillus subtilis, Pseudomona fluorescence, Staphylococcus aureus, Xanthonomonas axonopodies pv. malvacearum* [26]. The plant exhibited antiviral activity against the Turnip mosaic virus. The aqueous leaf extract of the plant also of *A. senegal* showed nematicidal activity against *Meloidogyne incogenita* as it inhibited its hatching [27].

Thus this plant contains wide varieties of phytochemical constituents. However the claim that safe usage of plant extract in folk medicine is unsubstantiated by scientific studies. Hence, the current study has been undertaken to investigate the antimicrobial property of 50% aqueous ethanolic leaf extract of *A.nilotica* (L.) *Willd.* against few under mentioned microorganisms.

In this study, ethanolic extracts of aerial parts (leafs and fruits) of *Acacia* which had been described in herbal books and folklore medicine of having antimicrobial activity, was screened for its antimicrobial activity.

MATERIALS AND METHODS

Preparation of plant extract:

100 gms of dried leaves of each plant were grounded into fine powder and was sequentially soaked into petroleum ether, chloroform, benzene and 50% aqueous ethanol for 7 days each in room temperature. The extracts were filtered, collected and condensed under reduced pressure. Dark residual solid were collected from each extract which were then subjected to antifungal and antibacterial bioassay.

Preparation of sample solution:

The test solutions were prepared by dissolving the dark residual masses in few drops of propylene glycol and then diluting with sterile water in the concentration of 60 µg/ml. Few drops of propylene glycol diluted with sterile water was used as control. All the dilution was sterilized by filtration using membrane filter (0.02 µ pore size) in the laminar air flow.

Table 1: represents the names and characteristics of the micro organisms used micro organisms used:

Name	Growth medium	Growth Condition	Temp (^{0}C)	Incubation time (hrs)	Subculture (month)	Special features
Bacterial strains						
Serratia marcescens	No. 3	aerobic	30	48	2	-
Erwinia herbicola	No. 74	aerobic	37	24	1	-
Xanthomonas sp.	No. 3	aerobic	30	48	2	-
Arthrobactor chlorophenolicus	No. 3	Aerobic	28	48	1	Type strain, Degrades 4-chlorophenol
Fungal strains						
Botrytis cineria	PDA	aerobic	38	2	3	Produces hyphal mat
Fusarium oxysporum	PDA	aerobic	28	5-7	3	Produces macro and microconidia
Rhizoctonia solani	PDA	aerobic	32	2-3	3	Produces dark brown sclerotia
Aspergillus flavus	PDA	Aerobic	28	1-2	3	Type strain degrades 4-chloro- phenol

Preparation of media:

Growth media no.3:

Beef extract	1.0 gm
Yeast extract	2.0 gm
Peptone	5.0 gm
NaCl	5.0 gm
Agar	15.0 gm
Distilled water	1.0 Lt.

Growth media no.74:

Tryptone	10.0 gm
Yeast extract	5.0 gm
NaCl	10.0 gm
Distilled water	1.0 Lt.

Potato Dextrose Agar (PDA) medium:

Peeled potato	250 gm
Agar	20 gm
Dextrose	20 gm
Distilled water	1 lt., pH 6.8-7

Determination of antimicrobial activity of the crude leaf extracts of the plant by bioassay method:

Antibacterial assay by cup diffusion method: [28]:

The bactericidal assay was done with the above prepared test solution following agar cup diffusion method of with certain modification.

Concentration of the bacterial culture used in the bioassay experiment was adjusted to 1×10^6 cfu/ml. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about 40-42 ^{0}C. The tubes were inoculated with 0.1 ml of the appropriate culture suspension of each bacterium, mixed gently and poured onto previously solidified nutrient agar plates. Wells were bored in each plate (9 cm diameter) with an aseptic cork borer of 7 mm when the seeded plates had solidified. With the aid of a micropipette each of the wells was filled with 0.2 ml of test solution and allowed to diffuse. A control plate was maintained by adding propylene glycol diluted with sterile water in the well only. The petridishes were sealed with a strip of parafilm and incubated at the required temperature and duration.

Antifungal assay by cup diffusion method: [29]:

4-5 days old cultures of the fungal sps. were used for the bioassay experiments. Fungal suspension was prepared in such a way that the fungal concentration would be approximately 1×10^6 cfu/ml. An overnight broth culture were used as inoculums on sterile molten PDA medium. Small wells were bored in each plate (9 cm diameter) with an aseptic cork borer of 7 mm when the seeded plates had solidified. With the aid of a micropipette each of the wells was filled with 0.2 ml of test solution and allowed to diffuse. A control plate was maintained by adding propylene glycol diluted with sterile water in the well only. The petridishes were sealed with a strip of parafilm and incubated at the required temperature and duration.

Measurement

After incubation the diameter of the zone of inhibition around the well was measured in cm. Antimicrobial studies were done in triplicates and diameters of inhibition zones (cm) were expressed as means and standard errors of means.

RESULT AND DISCUSSION

Table 2: Screening of antimicrobial activity of different solvent fractions collected from *Acacia nilotica Willd.* against the microbial strains selected:

Plant selected	Fractions	Bacterial strains			
		Serratia marcescens	*Erwinia herbicola*	*Xanthomonas sp.*	*Arthrobactor chlorophenolicus*
Acacia nilotica(L.) *Willd.*.	Pet. Ether	-	-	-	-
	Chloroform	-	-	-	-
	Benzene	-	-	-	-
	50% aq. ethanol	-	-	-	-
	Fractions	Fungal strains			
		Botrytis cinera	*Fusarium oxysporum ciceri*	*Rhizoctonia solani*	*Aspergillus flavus*
Acacia nilotica (L.) *Willd.*	Pet. Ether	-	-	-	-
	Chloroform	-	-	-	-
	Benzene	-	-	-	-
	50% aq. ethanol	-	-	+ (2.12 cm)	-

Pet. Ether = petroleum ether, 50% aq. Ethanol = 50% aqueous Ethanolic extract.

Acacia nilotica (L.) *Willd.* is a plant rich in wide range of phytochemical compounds. The data on table 2 exhibited that 50% aqueous ethanolic leaf extract of *Acacia nilotica Willd.* possesses antifungal property against *Rhizoctonia solani.*

CONCLUSION

It showed most promising antifungal and antibacterial effect against. This study presents valuable data on antimicrobial property of *A. nilotica* (L.) Willd. leaf extract, which should be very useful for clinical study of this plant leaf extract.

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