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 microorganisms.

Method: The leaves of Acacia nilotica (L.) Willd. were sequentially soaked in petroleum ether (60-80°C), chloroform, benzene and 50% aqueous ethanol. Extracts were collected, filtered and concentrated. Antimicrobial potentiality of the extracts were tested against few microorganisms.

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 multiresistant strains of bacteria and the recent appearance of strains 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 ‘Baboo’ 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 epyoxy 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 diarrhoea [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 antidiarrheal, antisynergic, antidiabetic agent. The stem bark is astrigent, demulcent, used in diarrhoea, dysentery, diabetes as antisthangling, antihelmintic, 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, antihelmentic 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, antisynergic. 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 ophthalmal 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 lactagogue (increase milk supply).

The plant exhibited antidiabetic, antimutagenic, antiproteolytic, antifertility, antioxidiant 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 col., 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, Pseudomonas fluorescence, Staphylococcus aureus, Xanthomononas axonopodis pv. malvacearum [26]. The plant exhibited antiviral activity against the Turnip mosaic virus.

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 Anilotica (L.) Wild. against few under mentioned microorganisms.

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

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

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

**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 medium 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 1×10⁶ cfu/ml. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about 40-42 °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 1×10⁶ 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.

<table>
<thead>
<tr>
<th>Plant selected</th>
<th>Fractions</th>
<th>Serratia marcescens</th>
<th>Erwinia herbicola</th>
<th>Xanthomonas sp.</th>
<th>Arthrobacter chlorophenolicus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia nilotica</em> (L)</td>
<td>Pet Ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wild.</td>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>50% aq.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fractions</td>
<td>Botrytis cinerea</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fusarium oxysporum</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fungal strains</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>Rhizoctonia solani</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aspergillus flavus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Plant selected** = *Acacia nilotica* (L) Wild.; **Fractions** = Pet Ether, Chloroform, Benzene; 50% aq. ethanol; **Bacterial strains** = *Serratia marcescens*, *Erwinia herbicola*, *Xanthomonas sp.*, *Arthrobacter chlorophenolicus*.

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

*Acacia nilotica* (L) Wild. 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* Wild. 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) Wild. leaf extract, which should be very useful for clinical study of this plant leaf extract.

**ACKNOWLEDGMENTS**

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**REFERENCES**


