ANTIMICROBIAL ACTIVITY OF ABUTILON INDICUM – A MEDICINAL PLANT

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ABSTRACT
Objective: *Abutilon indicum* is an ethnomedicinal plant that has several medicinal claims and it hasn't been explored thoroughly. Various parts of the plant are used medicinally such as demulcent, aphrodisiac, laxative, diuretic, pulmonary disorders, etc. The study aims to explore the different qualitative, quantitative, and antifungal aspects of *Abutilon indicum*.

Materials and methods: The present study was conducted to evaluate the anti-microbial activity of *Abutilon indicum* extracts against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*E. coli*), and Fungal (*Candida albicans*). The agar well diffusion method was used to test the antimicrobial activity.

Result & Discussion: *Abutilon indicum* extracts exhibited potent antibacterial and antifungal against all the selected bacterial and fungal species. The extracts exhibited the growth inhibitory activity in a dose-dependent manner. Also, the study revels *Abutilon indicum* shows good antimicrobial and anti-fungal activity.

Conclusion: The *Abutilon indicum* plant extracts could be used as an antimicrobial after comprehensive *in-vitro* biological studies.

KEYWORDS: Abutilon indicum, Anti-microbial, *Staphylococcus aureus*, *E. coli*, *Candida albicans*.

INTRODUCTION
Medicinal plants are assuming widespread use in the primary health care of individuals and communities. Plant-derived chemicals are a wide group of chemical compounds that have been found naturally in plants. The extensive existence of these compounds has demonstrated beneficial advantages in terms of antioxidant, antibacterial, and antifungal activities. Although synthetic antimicrobial agents have been already approved in many countries, yet the usage of natural compounds that are derived from microbial, animals, or plants attracts the attention of many researchers. These compounds have exhibited promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens. The plants hold great promise as a source of novel antimicrobial agents. They are readily available, cheap and also, almost; do not have any side effects. Plant derivative compounds including phytochemicals have even been employed to treat various infectious diseases and have shown interesting antimicrobial activity against several human pathogens.
*Abutilon indicum* (Indian Abutilon, Indian Mallow; is a small shrub in the Malvaceae family, native to tropic and subtropical regions and sometimes cultivated as an ornamental. This plant is often used as a medicinal plant and is considered invasive on certain tropical islands.\(^1\) It is extensively grown in Bangladesh, India, Pakistan, Srilanka.\(^2\) In traditional medicine, *A. indicum* is used as a demulcent, aphrodisiac, laxative, diuretic, pulmonary, and sedative (leaves). The bark is astringent and diuretic; laxative, expectorant, and demulcent (seeds); laxative and tonic, anti-inflammatory and anthelmintic (plant); analgesic (fixed oil); diuretic and for leprosy (roots). The previous studies revealed the presence of chemical constituents namely luteolin, chrysoeriol, apigenin 7-O-beta rhamnopyranosyl, quercetin, triacontanoic acid, ursenol, methylstigmasterol, glucopyronoside etc.\(^3\) This present study aimed to evaluate the antimicrobial activity of *Abutilon indicum*, a medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms.

**MATERIALS AND METHODS**

**Sample Collection:** The leaves for the present study from the respective plant *Abutilon indicum* was collected from place allowed to dry under the shade and made into a fine powder.

**Preparation of plant extract:** The powder (100grams) was Soxhlet extracted with methanol and dried under rotavapor at 40-50°C for 3hours. This measure was taken to evaluate the antimicrobial activity.

**Preliminary phytochemical screening**

About 5g of the test drug was macerated with methanol and water separately (100ml) in a closed flask for 24 hours where initial shaking frequently during first 6hrs and kept it for 18 hrs. After 24 hours it was filtered. The filtrate was evaporated with the help of water bath and extract was collected in solid form. These extracts were tested for different constituents.

**Microorganisms:** The bacterial and fungal cultures for the present investigation were obtained from Primer Biotech Research Center, Hyderabad. Two bacteria used for the present study were; *E. coli* and *Staphylococcus aureus* and the fungi used for the present study is *Candida albicans*.

**Procedure**

**In-vitro antimicrobial activity**

**Culture Used:** Two bacteria used for the present study were; *E. coli* and *Staphylococcus aureus*.

**Media used:** Muller Hinton broth

**The reference standard used:** commercially available gentamycin discs were used as a positive control in this study.

**Testing procedure:** The antimicrobial activity of the extract was evaluated by the agar well diffusion method. Bacteria were grown in Muller Hinton broth (HiMedia Laboratories Ltd., India) to match the turbidity of 0.5 McFarland standards to be inoculated on Muller-Hinton agar (HiMedia Laboratories Ltd., India. After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Once wells were formed, they were filled with 100 µL of plant extracts and blanks. Plates were incubated for 24 h at 37 °C to allow leaf extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different leaf extracts against different bacteria were measured in millimeters for further analysis. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagrams\(^4\).

**In-vitro antifungal activity:**

**Culture used:** Candida albicans (ATCC 10231)

**Media used:** Sabroud dextrose agar (SDA) Make: Hi-media

**Reference standard used:** Itraconazole capsule
Culture Preparation: Freshly prepared slants of C. albicans were used and washed the slant by using 10 mL of sterile Normal saline solution.

Media preparation: Sabroud Dextrose Agar was used for determining the activities of, Candida albicans. Media was prepared as per the Manufacturer's Instruction. The media was then autoclaved at 121°C temp. & 15lbs pressure for 20 minutes.

Sample Preparation: Take approximately 100 mg of sample & dissolved it into a 1:1 ratio of Methanol: Dimethyl Sulfoxide. Dissolved the samples by cyclomixture. Filter the samples & use filtrate to evaluate antifungal activity.

Standard preparation of Itraconazole - Take the weight of the filled capsule. The active content of the capsule i.e. pellets was powdered into mortar-pestle. Took powder equivalent to one capsule weight into 100 ml volumetric flask and make up the volume 100 ml with Dichloromethane. The solution was sonicated and prepared 50 mcg/ml standards solution by dilution method.

Testing Procedure: Cooldown sterile media up to 55°C then measured 15 ml of SDA media by sterile measuring cylinder and transferred into sterile Petri plate. Likewise, prepared 3 plates for evaluation. The plates were allowed to solidify on the smooth surface. In the rest of the media add 5µl fungal culture and mix slowly. Then the media was poured on above SDA containing plates. The plates were solidified and then made required wells in SDA plates labeled as an std. & test, at a proper distance by the sterile borer. Add std. & test samples in respected labeled well. When samples were diffused completely in well, incubate SDA plates into Biological Oxygen Demand (BOD) incubator at 25°C for 72 hours and observe the zone of inhibition.

RESULT
Preliminary phytochemical results showed the presence of Alkaloids, tannin, flavonoids, steroids, and carbohydrate in the extracts of Abutilon indicum. The results are depicted in Table no. 1

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phyto-constituents</th>
<th>Tests</th>
<th>Abutilon indicum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ME</td>
</tr>
<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>Molisch’s</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Reducing sugar</td>
<td>Fehling test</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Proteins</td>
<td>Biuret</td>
<td>_</td>
</tr>
<tr>
<td>4.</td>
<td>Amino acids</td>
<td>Ninhydrin</td>
<td>_</td>
</tr>
<tr>
<td>5.</td>
<td>Alkaloids</td>
<td>Wagnor</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Tannins</td>
<td>Lead Acetate</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Steroids</td>
<td>Salkowski</td>
<td>_</td>
</tr>
<tr>
<td>8.</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Saponin glycoside</td>
<td>Foam</td>
<td>_</td>
</tr>
</tbody>
</table>

The antimicrobial activity of the extract was examined against Gram-positive and Gram-negative bacteria and fungal strains by measuring the zone of inhibition. The antimicrobial activity was performed by Agar disc diffusion method at concentration level of 25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml respectively. Gentamycin (antibacterial), Itraconazole (antifungal) as standard drug. Antibacterial and antifungal potential of ethanolic extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antimicrobial activity and antifungal are shown in table no. 2.
Table No. 2- Anti-microbial and Antifungal Activity of Extracts of A. indicum

<table>
<thead>
<tr>
<th></th>
<th>25mg/ml</th>
<th>50mg/ml</th>
<th>75mg/ml</th>
<th>100mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli (mm)</td>
<td>14 ± 0.32</td>
<td>16 ± 0.49</td>
<td>18 ±0.56</td>
<td>20 ± 0.70</td>
</tr>
<tr>
<td>Staphylococcus aureus (mm)</td>
<td>14 ± 0.18</td>
<td>16 ± 0.42</td>
<td>17 ±0.45</td>
<td>19 ± 0.43</td>
</tr>
<tr>
<td>Candida albicans (mm)</td>
<td>15 ± 0.25</td>
<td>17 ± 0.32</td>
<td>19 ± 0.49</td>
<td>21 ± 0.35</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study was carried out to evaluate the antibacterial and antifungal activities of the aqueous and ethanolic extracts of *Abutilon indicum* against bacterial and fungal species. *Abutilon indicum* extracts exhibited potent antibacterial and antifungal against all the selected bacterial and fungal species. The extracts exhibited the growth inhibitory activity in a dose-dependent manner. The results show that Abutilon indicum extracts were found to be more effective against all the microbes tested.

Escherichia coli is a gram-negative, rod-shaped micro-organism, facultatively anaerobic, and non-sporeulating organism. Some virulent strains of E.coli can cause several intestinal and extraintestinal infections, mastitis, septicemia, gram-negative pneumonia, gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for the hemolytic-uremic syndrome (HUS), peritonitis. *Streptococcus pyogenes* occurs as a commensal on human skin, particularly the scalp, armpits, and nasopharynx; its primary habitat is the moist squamous epithelium of the anterior nares. It may cause a variety of clinical infections with high morbidity rates; these include wound sepsis, septicemia, osteomyelitis, post-surgical toxic shock syndrome, and septic arthritis. In infants, *S. pyogenes* can cause severe disease. Streptococcal scalded skin syndrome (SSSS).  *Streptococcal* endocarditic (infection of the heart valves) and pneumonia may be fatal. The Differences in antimicrobial activity of medicinal plants are related to differences in their contents of active compounds. Available reports tend to show that alkaloids and flavonoids are the responsible compounds for the antimicrobial activities in higher plants.

CONCLUSION

Plant leaves have been used as an herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid, and phenolic compounds. Their concentrations may vary in different plants which results in unique medicinal properties for a specific plant. The present article successfully evaluated the role of *Abutilon indicum* for its antimicrobial activity. It was already reported that phytochemicals have an excellent ability to act against microorganisms. This could be due to the active chemicals which are present in the *Abutilon indicum* making it a potential antimicrobial activity. We could also accomplish the role of *Abutilon indicum* as an antifungal agent. The results of the present study showed that extract of *Abutilon indicum* Linn. has potent anti-microbial activities. Thus the ethanolic whole plants of *Abutilon indicum* extracts may be attributed to the presence of phenolic compounds and flavonoids etc., Therefore, further investigation is needed to isolate and identify the active compounds present in the plant extract and its efficacy.
REFERENCES