Phytochemical studies and antimicrobial activity of babool seeds

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The antibacterial activity of seed extracts of A. nilotica, P. juliflora and L. leucocephala was determined in vitro using disc diffusion method against different bacterial strains viz. S. aureus, E. coli, P. aeruginosa, K. pneumonia and S. typhi. Dried powder was subjected to successive hot extraction method to obtain various extracts viz. n-hexane, chloroform, acetone, alcohol and water. Assay was performed at a dose of 100 mg/ml, compared to standard Amikacin (30 mg/ml). Maximum inhibition zone of 10 mm was observed by acetone, alcohol and water extract of A. nilotica against E. coli, S. typhi, S. aureus and P. aeruginosa respectively. P. juliflora extracts showed activity (8 mm) against S. aureus and S. typhi only. In L. leucocephala, n-hexane, alcohol and water extractive showed more prominent activity (8 mm). E. coli is neither inhibited by P. juliflora nor by L. does leucocephala extract. Hence a considerable zone of inhibition was observed by all the three babool species, among which A. nilotica exhibit most significant activity against tested strains followed by L. leucocephala and P. juliflora. The study prospects for detailed investigation on isolation and activity guided fractionation of babool seed extracts and their utilization as potential antimicrobials.

Keyword: Antibacterial, Babool, Bacterial strains, Extracts, Seed.

Introduction

Microbial infection is a common health problem worldwide, especially in rural and urban areas having poor sanitation and health facilities. From the time immortal, medicinal plants continues to play an important role for combating different microbial infections and in the recent years there is resurgence of scientific interest to use medicinal plants for newer pharmaco-therapeutics. Thus the need of cost effective and safe phytochemicals with therapeutic potential is urged as alternative sources that can control microbial infections. Babool including commonly known varieties Desi babool (Acacia nilotica), Subabool (Lucenea leucoIcephala) and Vilayati babool (Prosopis juliflora) of family Leguminoseae are one of the oldest existing plant species having various therapeutic, biological and ethano botanical claims and has diverse medicinal properties viz. antispasmodic, anti dysenteric, anti diarrhoeal, anti inflammatory, antiplatelet, spasmodenic, vasoconstrictor actions, antihypertensive, cytotoxic and antioxidant activity1-8. However very little work has been carried out on the antimicrobial potential of the seeds of the babool species, reports are available on the antibacterial activity of leaf & bark extracts5,6,7. Investigation on water and methanolic extracts of leaf, stem and bark of Acacia nilotica showed antimicrobial potential & activity against wide range of microbes and some fungal strains9. Prosopis juliflora has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in healing of wounds10. Decoction prepared from leaf and seed extracts are used in wound healing, as disinfectant and also to treat scurvy11. Strong antibacterial effect was showed by alkaloid rich fraction on gram negative & gram positive like E. coli, S. aureus, B. cereus, P. putida, Klebsiella, Salmonella, Acinetobacter and Alcaligen12. With the literature available, it is evident that majority of studies are focused on aerial parts of babool species and seeds are discarded as waste material, which may be therapeutically effective. However, there is a report on antibacterial activity of the L.leucocephala seed oils13 and although phytochemical characterization of these species had been carried out earlier14 but no literature was available in reference to anti microbial activity. Hence the study evaluates In vitro anti microbial potential of various fractions of Acacia nilotica, Prosopis juliflora and Lucenea leucocephala seeds. Hence the study is done for investigation of phytochemicals & evaluation of in-vitro anti microbial potential of

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various fractions of Acacia nilotica, Prosopis juliflora and Lucenea leucocephala seeds.

Materials & Methods

Plant material
Seeds of Acacia nilotica, Prosopis juliflora and Lucenea leucocephala were procured from local market of Lucknow, specimen prepared and matched with authenticated sample of institute’s herbarium with LWG of NBRS-B1, NBRS-B2, NBRS-B3 for Acacia nilotica, Prosopis juliflora and Lucenea leucocephala respectively.

Test Organism
Five strains of bacteria as test organism viz. Staphylococcus aureus MTCC 737, Escherichia coli MTCC 1687, Pseudomonas aeruginosa MTCC 424, Klebsiella pneumonia MTCC 501 and Salmonella typhi MTCC 1024 were procured from Institute Microbial Technology, Chandigarh, India. Inoculums were prepared by transferring the test organisms in to sterile tubes containing 10 ml of nutrient broth by aseptic loop and then incubated for 48 hr at 36-38°C. The concentration of inoculums is monitored by measuring transmittance (20%) at 530 nm by spectrophotometer.

Extraction
Procured seeds were shade dried, crushed, powdered and sieved to 60 mesh size. Extracts were prepared by successive soxhlet extraction method\textsuperscript{15, 16}. Powdered drug (25 g) was extracted with solvents viz. n-hexane, chloroform, acetone, alcohol and water respectively. Solvent from the extracts were distilled, lyophilized (-40°C, under high vacuum) and percentage yield was calculated.

Phytochemical studies
Phytochemical screening for carbohydrates, proteins, alkaloids flavanoids, tannins, saponins, steroids, anthraquinones and glycosides were performed as per standard procedures\textsuperscript{17, 18}.

Preparation of standard and test samples
A standard preparation is an authentic sample of the appropriate antibiotic for which the potency has been precisely determined by reference to the appropriate international standard. Susceptibility test disc of Amikacin (30µg/disc) was used as standard preparation for present work. Sample solutions (n-hexane, chloroform, acetone, alcohol and water extracts of A. nilotica, P. juliflora and L. leucocephala respectively) of concentration 100 mg/ml were prepared in DMSO (Dimethyl sulfoxide), a whatman no.1 sterile filter paper disc (6 mm in diameter) were impregnated with 10 µl of sample solution individually (100 µg/disc) followed by air drying and then used as test samples.

Antimicrobial Assay
The antibacterial activity of A. nilotica, P. juliflora and L. leucocephala seed extracts i.e. n-hexane, chloroform, acetone, alcohol and water extractives were determined at concentration of 100 mg/ml against a gram positive and a series of gram negative bacterial strains using disc diffusion method\textsuperscript{19} when compared with standard antibiotic, Amikacin (30 mg/ml). The agar plates were inoculated with 0.8 ml of standardized inoculums and spread properly. Various test samples (extracts) of A. nilotica, P. juliflora and L. leucocephala (100 µg/disc) and Amikacin (30µg/disc) were placed on agar surface. Allowed the plate to stand at room temperature for 30 minutes (Pre diffusion time). Inoculated petri dishes were incubated at 37°C, overnight and the antibacterial activity was evaluated by recording the zone of inhibition in millimeter (mm) against the tested strains. Each assay was repeated thrice.

Results and Discussion
Successive solvent extractives of A. nilotica, P. juliflora and L. leucocephala were illustrated in Table 1. The total extracts of n-hexane, chloroform, acetone, alcohol and water in Acacia nilotica was estimated to be 8.30, 1.58, 0.29, 5.76 and 12.15%: 4.85, 1.73, 0.32, 2.06 and 11.53% in Prosopis juliflora and 7.53, 1.24, 0.21, 1.53 and 7.03% respectively. Phytochemical screening reveals the presence of various secondary metabolites viz. steroids, triterpenoids, sugars, tannin, phenolic, flavanoids, alkaloids, glycoside and saponins in different extracts of three babool species, although protein was altogether absent in all the three species and is summarized in Table 2.

Table 1—Successive solvent extracts of A. nilotica, P. juliflora and L. leucocephala seeds.

<table>
<thead>
<tr>
<th>S No.</th>
<th>Solvents</th>
<th>Acacia nilotica</th>
<th>Prosopis juliflora</th>
<th>Lucenea leucocephala</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>n-hexane</td>
<td>8.30</td>
<td>4.85</td>
<td>7.53</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>1.58</td>
<td>1.73</td>
<td>1.24</td>
</tr>
<tr>
<td>3.</td>
<td>Acetone</td>
<td>0.29</td>
<td>0.32</td>
<td>0.21</td>
</tr>
<tr>
<td>4.</td>
<td>Alcohol</td>
<td>5.79</td>
<td>2.06</td>
<td>1.53</td>
</tr>
<tr>
<td>5.</td>
<td>Water</td>
<td>12.51</td>
<td>11.63</td>
<td>7.06</td>
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</tbody>
</table>
Antibacterial assay of babool species shows that the diameter of inhibition zone for bacterial growth in various extracts at a dose of 100 mg/ml and standard at 30 mg/ml. Acetone, alcohol and water extract of A. nilotica exhibit maximum zone of inhibition in comparison to various extracts of other two species also i.e. 10 mm against E. coli, S. typhi, S. aureus, and P. aeruginosa. While n-hexane and chloroform extracts showed inhibition zone of 8 mm against P. aeruginosa and E. coli. Gram negative strain K. pneumonia was inhibited by chloroform, acetone, alcoholic and water extracts of A. nilotica (8 mm), similarly a minimum inhibition diameter of 6 mm was shown in E. coli by n-hexane, alcoholic and water extracts. S. aureus is inhibited by all the four successive extracts of A. nilotica, except water extract with diameter of 6 mm. P. aeruginosa is inhibited by chloroform, acetone and alcoholic extract, while both K. pneumonia shows the inhibition zone of 6 mm by n-hexane and S. typhi by n-hexane, chloroform and water extracts respectively (Figure 1). Maximum inhibition of 8 mm was shown by alcoholic extract of P. juliflora against S. aureus and by n-hexane and water extract against S. typhi respectively. E. coli, P. aeruginosa, and K. pneumonia strains were not inhibited by P. juliflora extracts, which is noteworthy. Further S. aureus is inhibited by n-hexane, chloroform, acetone and water extract although S. typhi inhibited by chloroform, acetone and alcohol extract with inhibition zone of 6 mm (Figure 2). E. coli was not inhibited by any of the extracts of L. leucocephala. n-hexane, alcohol and water extracts of L. leucocephala possess maximum inhibition within the specie, of 8 mm against P. aeruginosa, K. pneumonia and S. typhi, whereas alcohol and water extracts showed inhibition against P. aeruginosa, K. pneumonia and P. aeruginosa, K. pneumonia, S. typhi respectively having inhibition diameter of 8 mm. S. aureus was the strain which was inhibited by all the five successive extracts in same range, having zone of inhibition of 6 mm, which is similar for

### Table 2—Phytochemical screening in A. nilotica, P. juliflora and L. leucocephala seeds extracts.

<table>
<thead>
<tr>
<th>Babool seed</th>
<th>Phytochemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. nilotica</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>B</td>
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<td></td>
<td>C</td>
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<tr>
<td></td>
<td>D</td>
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<tr>
<td></td>
<td>E</td>
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<tr>
<td>P. juliflora</td>
<td>A</td>
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<td></td>
<td>B</td>
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<td></td>
<td>C</td>
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<td></td>
<td>D</td>
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<td></td>
<td>E</td>
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<tr>
<td>L. leucocephala</td>
<td>A</td>
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<td>C</td>
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St: Steroid, Tr: Triterpenoid, Pr: Protein, Car: Carbohydrate, Ta: Tannin, Ph: Phenolic,
Fl: Flavanoid, Al: Alkaloid, Gl: Glycoside, Sa: Saponin

**Fig 1**—Antibacterial activity of A. nilotica seed extracts against microbial strains. B1: Escherichia coli, B2: Staphylococcus aureus, B3: Pseudomonas aeruginosa, B4: Klebsiella pneumonia, B5: Salmonella typhi. Values represents the zone of inhibition in mm. Experiment was repeated three times and the values represent the average diameter.
P. aeruginosa, K. pneumonia and S. typhi by chloroform & acetone extract and later strain was inhibited by alcoholic extract also (Figure 3). Zone of inhibition (diameter) shown by standard Amikacin is 24 mm.

Conclusion

The seed extracts investigated, showed promising anti microbial activity on organisms viz., E. coli, S. aureus, P. aeruginosa, K. pneumoniae, and S. typhi, as these are pathogenic and can be fatal if not evaluated and cured. Assay revealed that among three species screened A. nilotica shows most significant results against all the tested strains, which is followed by L. leucocephala and P. juliflora species respectively. It also supports that within the extracts of each species, water extract exhibit the most promising activity as compared to n-hexane, chloroform, acetone and alcohol. This may be due to polar phyto chemicals as polysaccharides, gums, tannin’s etc, but this needs extensive studies on activity guided isolation of phyto-molecules. The study thus prospects the babool seeds to be explored for potential plant based antimicrobials.

Acknowledgement

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References


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