Study of antimicrobial activity of methanolic extract of *Alangium lamarkii* leaves against selected microbial species

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**Abstract**

*Alangium lamarkii* is one of the most popular medicinal plant in India, commonly known as Ankora. Traditionally the leaves of the plant have been used in inflammations, blood disorders, burning sensation, spermatorrhoea, gleet, acute fever and lumbago but its antimicrobial activity is yet to be reported. The antimicrobial activity of crude methanolic extract of *Alangium lamarkii* leaves were tested by cup plate method against 08 human microbial pathogens. Crude methanolic extract of leaves showed zone of inhibition in *S. aureus*-ML152 while it was not active against other organism such as *Shigella flexneri* 4a24, *Vibrio cholera* 793, *Bacillus subtilis* US 564, *Salmonella typhi* DI-1, *Candida albicans* SSKM and *Candida tropicalis* ATCC750 while comparing with standard drugs.

Key-Words: Antimicrobial activity, Methanolic leaves extract, Human pathogens, Zone of Inhibition, *Alangium lamarkii*.

**Introduction**

The term antibiotic, as used in the official compendia, designated as a medicinal preparation containing a significant quantity of a chemical substance that is produced naturally by a microorganism or artificially by synthesis and that has the capacity to inhibit the growth or kill the micro-organism(s) in dilute solution. Standards of potency and purity for antibiotics are established by the FDA in the form of regulation published from time to time in the Federal Register. As all the recognized antibiotics are subject to the provisions of the regulations, this determines the official standards. The federal regulations governing all aspects of antibiotic testing are extremely detailed and are subject to periodic amendment, they should be consulted with regard to prescribed methods for the assay of individual antibiotics and their preparations. Despite of tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. There impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance.

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages. Since generations, in India, people are using the extracts and leachates of different herbs in order to stimulate and promote the growth of specific herbs. The leaves are useful in treatment of inflammations, blood disorders, burning sensation, spermatorrhoea, gleet, acute fever and lumbago. It is very effective in treatment of migraine. In case of intense pain due to gout, the patients are advised by the healers to apply the Ankol leaves in affected parts. The leaves are also used in treatment of asthma. The leaves are dried and put on fire. The patients are advised to inhale the fumes. According to Ayurveda, the root is acrid, pungent, heatproducing and useful in treatment of biliousness, inflammations etc. The juice is emetic and alexipharmic and useful in treatment of pain, blood disorders, hydrophobia, rat-bite, lumbago, dysentery and diarrhoea whereas the seeds are cooling, aphrodisiac, indigestible and tonic. The root bark is used in piles whereas fruits are considered as purgative, expectorant and carminative.

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The present investigations were, therefore, proposed to evaluate the efficacy of the methanolic extract of *Alangium lamarkii* against microbes.

**Material and Methods**

The leaves of *Alangium lamarkii* are collected from Bankura and Asansol, West Bengal, India. A herbarium sheet was prepared and it was identified and authenticated by the Botanical Survey of India, Howrah, West Bengal, India (CNH/35/2011/TECH.II/446). The leaves were dried in shade or under controlled condition to avoid too many chemical changes occurring and made into a coarse powder.

### Preparation of Methanolic Extract of *Alangium Lamarkii*

The powdered leaves of *Alangium lamarkii* were subjected to soxhlet extraction using Methanol as solvent.

### Bacterial Strains

The various organisms used in the present study include *Staphylococcus aureus* ML276, *Shigella flexneri* 4a24, *Vibrio cholera 793*, *Bacillus subtilis* US 564, *Salmonella typhi* DI-1 were collected from National collection of industrial microorganisms (NCIM), National Chemical Laboratory, Pune, Maharashtra, India. These organisms were maintained on nutrient agar slopes and the organisms were confirmed by biochemical test.

### Preparation of Standard Bacterial Suspensions

The average number of viable *Staphylococcus aureus* ML276, *Shigella flexneri* 4a24, *Vibrio cholera 793*, *Bacillus subtilis* US 564, *Salmonella typhi* DI-1 organisms per ml of the stock suspensions was determined by means of the surface viable counting technique. About \(10^5-10^7\) colony forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

### Preparations of Media for Bacterial strain

Nutrient Agar was used as a media preparation. For 1000 ml Nutrient Agar preparation, Peptone-10gm, Sodium Chloride-5.036, Beef Extract-5.036, and Agar-15.225 was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.3-7.4 which was sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and was used for sensitivity tests.

### Preparation of Standard Solution for Antibacterial activity

Chloramphenicol eye drop was taken as a Standard antimicrobial solution which contains 0.5% w/v of Chloramphenicol, considered as a stock solution. From this stock solution 10µl was taken and dilute into 40µl water to make the concentration 1000µg/ml which pour each bore of the plate.

### Anti-Fungal Activity

Sapourand Dextrose Agar (Hi-media) media (SDA) was used for cultivation of fungi and particularly pathogenic fungi associated with skin infections.

### Composition (gm/liter)

- Peptone 10g
- Dextrose 40g
- Agar 15g; pH5.6 ± 0.2.

65 gm of SDA was dissolved in 1000ml of distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15psi pressure [7].

### Preparation of Standard Solution

150 mg Fluconazole Tablet was grinded in a morter pestle and dissolve in 300ml distilled water to make the concentration 500µgm/ml. This concentration was given in each bore for making the zone of inhibition.

### Anti-Microbial Study of *Alangium lamarkii*

**Determination of Zone of Inhibition by Cup Plate Method**

1. The method is based on the diffusion of a standard antibiotic (Chloramphenicol) from a cavity through the solidified agar medium.
2. 100mg of the methanolic extract of *A.lamarkii* was dissolved in 50 ml DMSO to make a stock solution of 2mg/ml.
3. 500µg/ml, 1000 µg/ml, 1500 µg/ml, 2000 µg/ml was taken from the stock solution in different test tubes and from this solution only 200µl was taken and pour into different cavities.
4. A standard Chloramphenicol (50µg/ml) was used as positive control.
5. The antibiotic assay medium was sterilized by autoclaving and the Petri dishes were prepared under laminar air flow. The test microorganisms
were spread on the surface of the plate by spread plate technique.
6. 5 cups were prepared in each plates keeping adequate distance from each others by using flame sterilized cork borer.
7. Standards and test solutions were poured in each labeled cavity of plates.
8. All the plates were transferred in refrigerator for proper diffusion of antibiotics at 4°C for 1-2hrs.
9. All the plates were placed in incubator at 32-35°C for 24-48hrs for proper incubation.
Same method was carried out in antifungal activity study where Fluconazole was used as standard drug (concentration 500µg/ml).3-10

Results and Discussion
The effect of temperature on the CL glow curve of Methanolic Extract of A.lamarkii shows antimicrobial activity against pathogenic bacteria. The Petri dishes were incubated at 37°C for 24 hours. After incubation it was observed that the antimicrobial activity was shown against S. aureus-ML152 while it not active against Staphylococcus aureus ML276, Shigella flexneri 4a24, Vibrio cholera 793, Bacillus subtilis US 564, Salmonella typhi DI-1 when standard antibacterial drug was used as chloramphenicol(Table 1)(Figure 1A-B).
On the other hand, Methanolic Extract of A.lamarkii not shows antifungal activity against Candida albicans SSKM and Candida tropicalis ATCC750 & compare with the standard drug fluconazole(Table 2).11-12
The result shows that the methanolic leaves extract of A. lamarkii has displayed concentration dependant antimicrobial activity, thus indicating antimicrobial activity towards pathogenic bacteria. The antimicrobial activity was shown against S. aureus-ML 276 while it has no activity against Staphylococcus aureus ML276, Shigella flexneri 4a24, Vibrio cholera 793, Bacillus subtilis US 564, Salmonella typhi DI-1, Candida albicans SSKM and Candida tropicalis ATCC750. But the antimicrobial activity was found to be less than the standard agent Chloramphenicol (standard antibacterial agent) and fluconazole (standard antifungal agent), used in the study.

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References
Table 1: Antibacterial Activity of methanolic leaves extract of *A. lamarkii* and comparison with standard antibiotic (Chloramphenicol).

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Organisms</th>
<th>500 µgm/ml</th>
<th>1000 µgm/ml</th>
<th>1500 µgm/ml</th>
<th>2000 µgm/ml</th>
<th>Standard 50µgm/ml</th>
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<tbody>
<tr>
<td>1.</td>
<td><em>Shigella flexneri</em> 4a24</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>2.</td>
<td><em>Vibrio cholera</em> 793</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
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<tr>
<td>3.</td>
<td><em>Salmonella typhi</em> DI-1</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>4.</td>
<td><em>Staphylococcus aureus</em> ML276.</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>10</td>
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</tr>
<tr>
<td>5.</td>
<td><em>Candida albicans</em> SSKM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
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<tr>
<td>6.</td>
<td><em>Bacillus subtilis</em> US 564.</td>
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</tbody>
</table>

Table 2: Antifungal activity of methanolic leaves extract of *A. lamarkii* comparing against standard drug.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Organism</th>
<th>Concentration of extract in µg/ml and zone of inhibition in mm</th>
<th>Standard drug µg/ml</th>
</tr>
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<tr>
<td></td>
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<td>500 1000 1500 2000</td>
<td>500</td>
</tr>
<tr>
<td>1.</td>
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<td>-    -    -    -</td>
<td>19</td>
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<td>2.</td>
<td><em>Candida tropicalis</em> ATCC750</td>
<td>-    -    -    -</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig. 1: (A) Plate showing Zone of inhibition with Extract. (B) Plate showing Zone of inhibition with Standard drug. (C) Plate showing Zone of inhibition with *Staphylococcus aureus* ML276 (1500 µg/ml and 2000 µg/ml)