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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 has 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. aureas-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.[1]

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[2].

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During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [3] 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 [4].

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.[5-6]

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

Material and Methods

The leaves of *Alangium lamarkii* are collected form 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, Maharastra, 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 (10^8-10^9) 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 121^0C 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

Preparation of Media

Sabourand 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 and agar 15g; pH5.6 ± 0.2. 65 gm of SDA was dissolved in 1000ml of distilled water. The medium was sterilized by autoclaving at 121oC 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. 500µgm/ml, 1000 µgm/ml, 1500 µgm/ml, 2000 µgm/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 500µgm/ml).[8-10]

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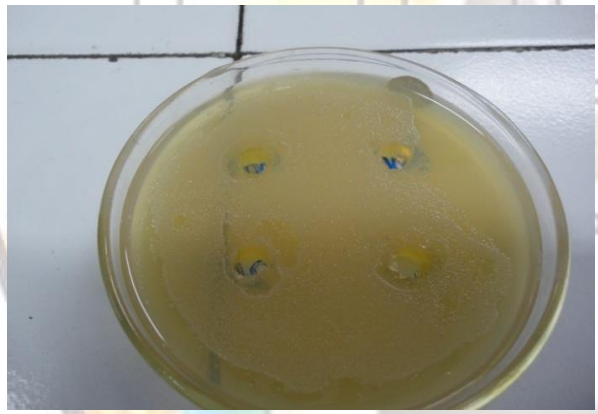
Table 1: Antibacterial Activity of methanolic leaves extract of *A. lamarkii* and comparison with standard antibiotic (Chloramphenicol).

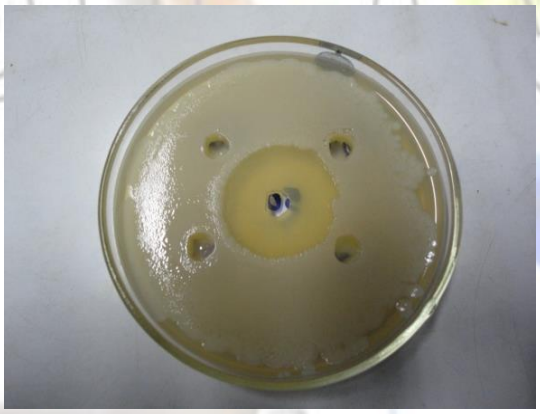
S/No.	Organisms	Zone of inhibition (mm)				
		500 µgm/ml	1000 µgm/ml	1500 µgm/ml	2000 µgm/ml	Standard 50µgm/ml
1.	*Shigella flexneri 4a24*	-	-	-	-	12
2.	*Vibrio cholera 793*	-	-	-	-	10
3.	*Salmonella typhi DI-1*	-	-	-	-	14
4.	*Staphylococcus aureus ML276.*	-	-	7	10	16
5.	*Candida albicans SSKM*	-	-	-	-	13
6.	*Bacillus subtilis US 564.*	-	-	-	-	12

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

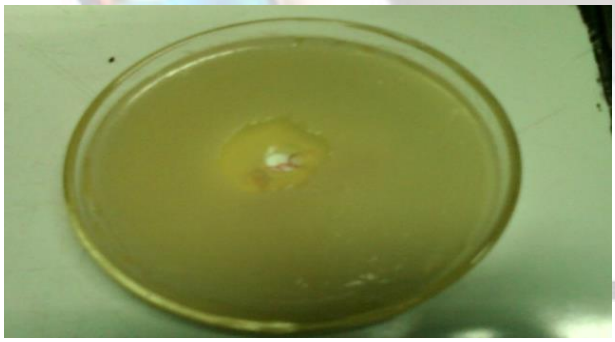
S/ No.	Organism	Concentration of extract in µg/ml and zone of inhibition in mm				Standard drug µg/ml
		500	1000	1500	2000	500
1.	*Candida albicans SSKM*	-	-	-	-	19
2.	*Candida tropicalis ATCC750*	-	-	-	-	20



(A)



(B)



(C)

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 µgm/ml and 2000 µgm/ml)