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Invitro Antimicrobial Activity of *Madhuca Longifolia* Leaf Extract

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----------ABSTRACT----------
The present study was designed to investigate antimicrobial activity of species that is originated from India. The plant Madhuca longifolia has various pharmaceitical and medicinal activities. The plant Madhuca longifolia is treated against two different bacteria namely Escherichia coli and Staphylococcus aureus. The leaf of Madhuca longifolia is analysed for its proficiency of antimicrobial activity. The SEM analysis was accomplished for the leaf extract of the species M.Longifolia against Escherichia coli and Staphylococcus aureus. The SEM results were estimated for its best inhibitory activity.
Key Words*— Indian species, Invitro antibacterial activity, Medicinal plant, SEM analysis.*

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I. INTRODUCTION

Madhuca longifolia is a tree which possess antibiotic activity in all its part of the plant. *M.longifolia* is a large, shady, deciduous tree, wild and cultivated, dotting much of the central, northern and southern Indian landscape. It is commonly called as mahua, mahwa or illupai. It is a fast growing tree that grows upto 20 meters height and possesses evergreen or semi-green foliage. *Madhuca longifolia* belongs to family Sapotaceae. Mahua tree is valued for its flowers, fruits, seeds and timber. Mahua is an economically important fast growing tree throughout the subtropical region of the Indo-Pak subcontinent. The present study was designed with the aim of the antimicrobial activity of leaf extract of *Madhuca longifolia*. The leaves of *Madhuca longifolia* were screened for the antimicrobial activity against the bacteria *Staphylococcus aureus* and *Escherichia coli*. Finally, the results were compared to identify the best antimicrobial activity.

II. MATERIAL AND METHODS

A. Plant Material

The fresh leaves of *Madhuca longifolia* was collected from local area. The collected plant material was free from diseases and there is no any other combination of other plants. It was dried in the shade and powdered to prepare the extract and checked for its antimicrobial.

B. Preparation of extracts

The powdered plant material was soaked in petroleum ether for 48 hours. The purpose to soak in petroleum ether is to remove the fats, oils, terpenes, waxes etc. Then the extract was filtered and then the filtered plant material was soaked in the ethanol(95%). The container is sealed air-tight and kept for a period of seven days, with occasionally shaking and stirring the extract. The whole mixture was filtered with the use of Whattmann filter paper in order to get a pure filtrate. Then the filtrate was evaporated using rotary evaporator under reduced pressure to get in the form of paste by the process of evaporation.

1.1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical screening of the extract was carried out to find an idea of the natural of compounds present in the various extracts of plant. Hence, the presence and absence of compound such as tannins, saponins, flavonoids, etc., are identified by carrying out the phytochemical investigation.

1.2. MIC (Minimum Inhibitory Concentration)

MIC is the minimum inhibitory concentration which is done to identify at which minimum concentration the leaf extract inhibit the bacterial growth effectively. This is done by the protocol of broth dilution method. The dilution is done for the geometric progression of the concentration of the substance in a logarithmic factor. The dilution is done up to 10^{-6} dilutions. For each organism, six separate tubes were used to carry out dilution. The extract with the concentration of 1mg/ml was added. The Muller-Hinton broth was used as a medium. The 5ml of

broth was poured into each test tube and 1ml of extract was poured in the first test tube and from that 1ml of broth was taken and poured into the next test tube. This is repeated up to 6th test tube. Then 0.1ml of culture was added in all the test tubes and was sealed air tight with cotton plugs. Then the tubes were incubated at 37ºC for 24hours. After the period of incubation, the test tubes were observed for turbidity and its minimum inhibition was measured at the absorbance at 600nm.

1.3. BIOFILM FORMATION

In a group of micro organisms, the cells stick to each other on the surface embedded within a self produced matrix of extracellular polymeric substance is known as biofilm. It helps the organisms to communicate and results in the production of virulence factors. This method is done with the use of 96 well microtitre plate. The medium used for biofilm formation is Muller-Hilton broth and 200µl of broth was added to each well and 20µl of culture was added. Then the plant extract was added with two different concentrations of 25µl and 50µl. Then the organism was added in the wells containing plant extract. The wells without plant extract is treated as control. Then it is kept for incubation at 37ºC for 48 hours. After the incubation period the effect of the plant extract on biofilm formation was measured by using plate reader at the absorbance of 495nm.

1.4. SWARMING MOTILITY

The coordinated translocation of the bacterial population across the semi-solid surface of the agar. The translocation depends on two factors such as nutrient composition and the viscosity of the culture. For this study, the agar was added to Luria Broth and then it is used because of its rich nutrients. The method deals with how the plant extract affects the organism's swarming motility. For each organism a medium without extract acts as a control and with plant extract acts as test. Then the medium was poured on the Petri dishes and allowed it to solidify. After solidification, a loop of organism was taken and inoculated on the solidified surface, both in test and control. Then it is allowed for incubation at 37ºC for 24hours. After incubation, the results were observed for swarming motility of the organism.

1.5. SEM ANALYSIS

The samples were analysed using Scanning Electron Microscope for the clarification. This produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the samples produce various signals that can be detected and that contain information about the sample surface topography. Thus with this principle the image of the ruptured cells can be obtained as an image.

C. Results

1.1. Preliminary Phytochemical Analysis

BIOACTIVE COMPOUNDS	OBSERVATION	RESULT
Tannins	Deep blue to black colour	+
Saponins	Formation of foam	+
Flavonoids	Pink colour	+
Alkaloids	Yellow precipitate	+
Cardiac glycosides	Blue colour	+
Steroids	Red colour and green fluorescence	+

1.2. MIC

The minimum inhibition concentration was studied using broth micro dilution method. After the period of incubation is completed it shows effective inhibition in the minimum concentration of 0.35 mg for leaf extract. This shows that the leaf extract has the potential to inhibit the bacterial growth even it is less concentration.

1.3. BIO FILM FORMATION

This method was performed on the 96 well plate. After the incubation is completed the plate was observed for turbidity. As there is no turbidity was observed, so the broth was opaque. Hence it is proved that the leaf extract inhibits the biofilm formation and for conformation each well were read at plate reader with the absorbance at 495nm.

The results obtained for plant extract,

	Escherichia coli		*Staphylococcus aureus*	
	1	2	1	2
A (Control)	2.657		2.875	
B (25µl)	0.390	0.495	0.324	0.478
C(50 µl)	0.657	0.768	0.766	0.817

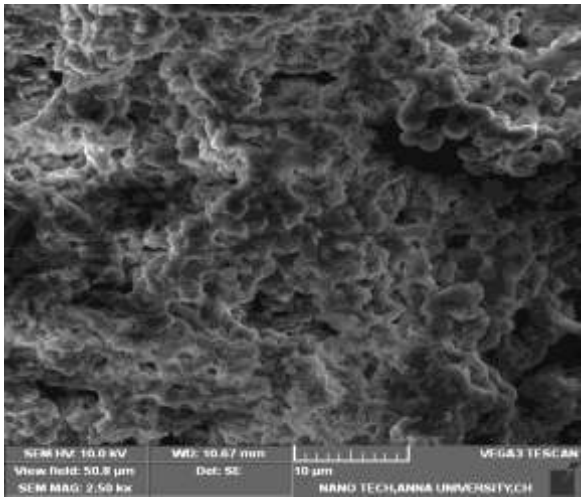
1.4. SWARMING MOTILITY

The swarming motility test was done to control the motility of the organisms. In this method, the control which has the absence of leaf extract shows motility on the agar surface and on the test which was treated with the extract has no swarming motility was observed. This shows that the leaf extract has the ability to inhibit swarming motility of the organisms effectively.

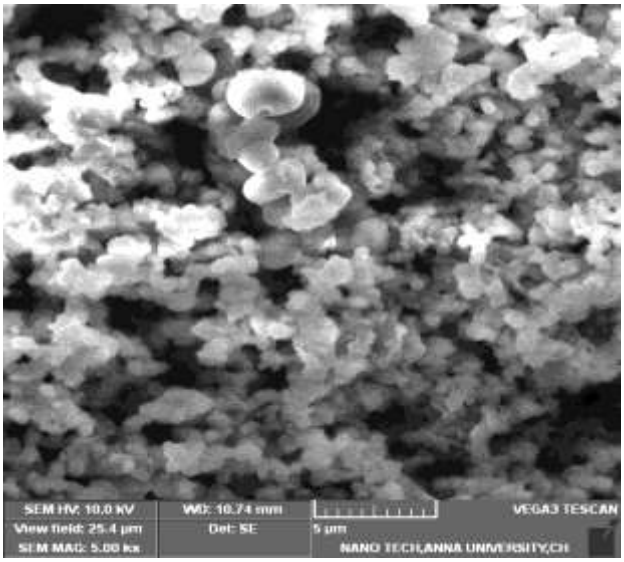
D.Figures

1.1. Fig. of Images of Preliminary Phytochemical Analyses

1.2. Image of SEM analysis of *Escherichia coli*



1.3. Image of SEM analysis of *Staphylococcus aureus*



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E. Abbreviations and Acronyms

MIC – Minimum Inhibition Concentration
Mg – milligram
µl – micro liter
ml –milliliter

III. CONCLUSION

In the present study the plant Madhuca longifolia was chosen for the research study. The antimicrobial activity is determined for the leaf extract. The bacteria which is inhibited by the plant extract was determined by growth curve analysis, for different time intervals. The antimicrobial activity was done for the plant extract against bacteria. Thus all the studies and research done on *Madhuca longifolia* has shown that this plant is an ideal source for the further development of drugs for treating different kinds of diseases. Thus, the bacterial infections can be treated with this herbal plant extract.

The leaves extract showed positive result in the inhibition of bacteria. This was confirmed with images of SEM analysis. Hence, this study proved that this plant is an ideal source for the further development of drugs for the disease caused by *Escherichia coli* and *Staphylococcus aureus*. It can be used as a drug to treat various bacterial and has high effective results for antibacterial activity.

The future studies are also carried for the anti-inflammatory activity with the plant extract. The antimicrobial activity is also checked for the seed oil of *Madhuca longifolia.*

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