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Antimicrobial Activity of Methanolic and Ethanolic Extracts of Leaves of Plumeria alba L.

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Abstract

The study was focused on the antimicrobial activity of leaves of Plumeria alba L. In this research, antibacterial and antifungal activities of the plant material was assessed. Plumeria alba L. belongs to Apocynaceae family. Apocynaceae family members have maximum number of alkaloids, which are highly toxic in nature. For antimicrobial activity Agar Well Diffusion method was used. E-coli culture was selected to assess antibacterial activity and Fusarium oxysporum fungus was selected to assess antifungal activity. Different concentrations of methanolic and ethanolic leaves extracts were used for the activities. Both the plant extracts showed positive results against E-coli and Fusarium oxysporum. Ethanolic extracts showed better positive results as compare to methanolic extracts. So, it can be concluded that Plumeria alba L. leaves has antibacterial and antifungal activities, which can be used in different pesticide preparation or else for preparation in different medicines.

Keywords: ***Plumeria alba L., Agar Well Diffusion Method, E-coli, Fusarium oxysporum.***

INTRODUCTION

Plumeria alba L. belongs to Apocynaceae family. It is commonly known as caterpillar tree, pagoda tree, pigeon wood, Champo, white frangipani. Native of the plant is Central America, Mexico. It is cultivated in the tropical regions of the world and is perhaps naturalised in some parts of India. Plumeria alba L. is a small rounded deciduous tree of the dogbane family that grows in a vase-shape to 15-25' tall. It features fragrant white flowers with yellow canters. Upright branches are thick but weak and have a milky sap. This species is native to Puerto Rico and the Lesser Antilles but has been introduced into a number of tropical areas around the world. Very fragrant 5-petaled flowers (to 3" wide) bloom in terminal clusters at the branch tips from spring to fall. Flowers are white with yellow centers. Oblong-lanceolate green leaves (to 12" long) are spirally clustered at the stem ends. Fruits are cylindrical pods (to 8") that are rarely formed in cultivation (Shinde et al, 2014).

Systematic position of Plumeria alba L.:

Kingdom: Plantae (Plants)
Division: Angiosperms
Class: Dicotyledon
Sub-class: Gamopetalae
Series: Bicarpellatae
Order: Gentianales
Family: Apocynaceae (Dogbane family)
Genus: Plumeria
Species: alba
(According to Bentham and Hooker: 1862-1883).

MATERIALS AND METHODOLOGY

Collection and Drying of Plant Material:

The leaves of Plumeria alba L. were collected from Gujarat University, Ahmedabad, Gujarat. The leaves were washed with distilled water and air dried for 30 days and crushed and then stored in paper bags at room temperature.

Extract Preparation Method:

Eight conical flasks were selected and weighed leaves powder transferred into these conical flasks. Solvents Methanol and Ethanol were added (In 1:10 amount, so if 1 gm powder material was there, we have to add 10 ml of Ethanol or Methanol). These processes done for all eight flasks four methanolic extracts and four ethanolic extracts. Cover the flasks with aluminium foil. These all sets were kept on shaker for 24hrs. After that, all extracts separately filtered with the help of Whatman filter paper no.1. After filtration, transferred it into petri plates and allow it open for 24 hrs for solvent evaporation. After 24 hrs all the extracts were ready.

Preparation of Liquid Extracts Series for Anti-microbial tests:

Crude extracts were weighed with the help of weighing balance (mg) and solvent were added in appropriate proportions i.e., 5 mg extract/2 ml Solvent, 10 mg/2 ml solvent, 15 mg/2ml solvent, 20 mg/2ml solvent. Here two solvents namely Ethanol and Methanol were used for Liquid extract preparation. Hence ethanol alone was used as control for ethanolic extract series and methanol alone was tested as control for methanolic extract series.

Antibacterial Activity:

Agar Well Diffusion method for antibacterial activity was selected for study. 25-30ml of nutrient agar media was poured in sterilized petri-plates and allowed it to solidify at room temperature. 24 hrs broth culture of test bacteria was used as inoculums under sterile conditions. The flashy prepared 100µl or 0.1ml (1×10^6 cells/ml) of organisms were spreaded with sterile L shaped bent glass-rod. Using cork-borer several wells of 6mm in diameter were punched. 100µl of the extract was poured in each well. The plates were incubated for optimum growth conditions at 35°C and 1 day. Inhibition zone was measured with zone scale of 1mm or more was considered positive inhibition.

Antifungal Activity:

Antifungal activity of the extracts was evaluated using well diffusion method. 25-30 ml of Potato Dextrose Agar media was poured in sterile petri-plates and allowed the media to solidify at room temperature. 4 days old culture prepared in potato dextrose broth of test fungi Fusarium oxysporum was used as inoculums under sterile conditions. The flashy prepared 100µl or 0.1ml (1×10^6 cells/ml) of organism were spread with sterile L shaped bent glass-rod. Wells were punched by using cork-borer having 6mm diameter. 100µl extract was poured in each well. The plates were incubated and inhibition zone were measured.

RESULTS AND DISCUSSION

Antibacterial activity of different extracts of Plumeria alba L. against E-coli:

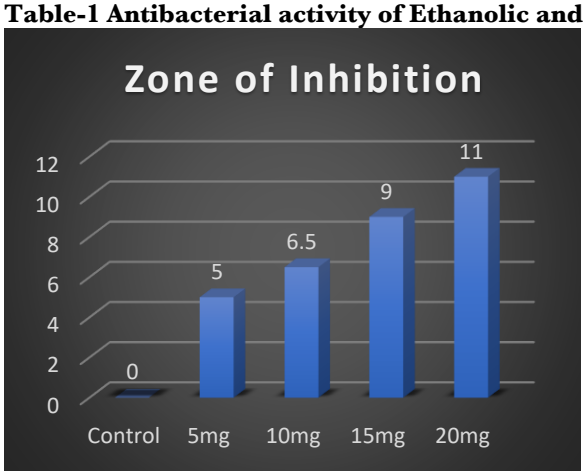
Antibacterial activity of ethanolic extract: The results obtained shows that 5mg concentration of extract results in 5mm zone of inhibition, 10mg concentration of extract results in 6.5mm zone of inhibition, 15mg concentration of extract results in 9mm zone of inhibition and 20mg concentration of extract results in 11mm zone of inhibition.

Antibacterial activity of methanolic extract: The results obtained shows that 5mg concentration of extract results in 4mm zone of inhibition, 10mg concentration of extract results in 7mm zone of inhibition, 15mg concentration of extract results in 8.5mm zone of inhibition and 20mg concentration of extract results in 10mm zone of inhibition.

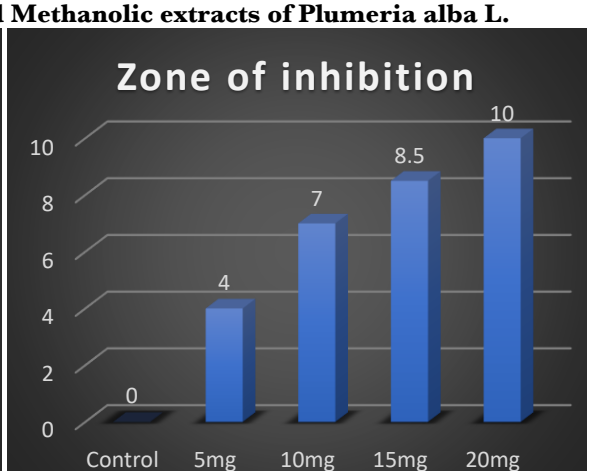
During experiment solvents ethanol and methanol were used as control which showed negative results.

Extracts	**Extract concentration (mg/2ml)**					
	Control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml	mm= milli-meter
Ethanol	0.0mm	5.0mm	6.5mm	9.0mm	11.0mm	
Methanol	0.0mm	4.0mm	7.0mm	8.5mm	10.0mm	

Table-1 Antibacterial activity of Ethanolic and Methanolic extracts of Plumeria alba L.

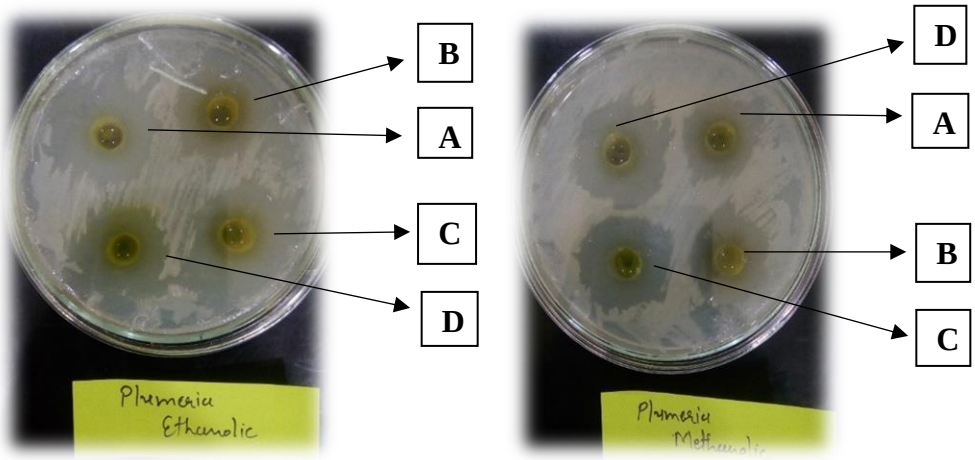


Graph-1 Ethanolic extract effect



Graph-2 Methanolic extract effect

Plates showing Antibacterial activity of Plumeria alba L. against E-coli:



Here, A= 5mg/2ml, B= 10mh/2ml, C= 15mg/2ml and D= 20mg/2ml concentration of extract

Ethanolic extract **Methanolic extract**

Antifungal activity of different extracts of Plumeria alba L. against Fusarium oxysporum

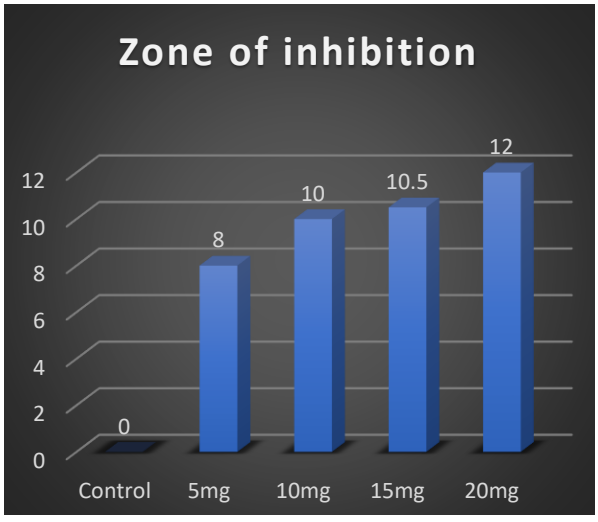
Antifungal activity of ethanolic extracts: The results obtained shows that 5mg concentration of extract results in 8.0mm zone of inhibition, 10mg concentration of extract results in 10.0mm zone of inhibition, 15mg concentration of extract results in 10.5mm zone of inhibition and 20mg concentration of extract results in 12.0mm zone of inhibition.

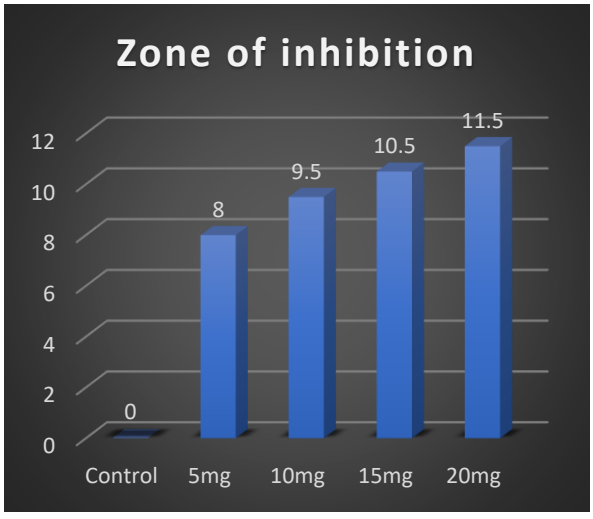
Antifungal activity of methanolic extracts: The results obtained shows that 5mg concentration of extract results in 8.0mm zone of inhibition, 10mg concentration of extract results in 9.5mm zone of inhibition, 15mg concentration of extract results in 10.5mm zone of inhibition and 20mg concentration of extract results in 11.5mm zone of inhibition.

During the practical work solvents ethanol and methanol were used as control which showed negative results against Fusarium oxysporum

Extract		Extract concentration (mg/2ml)				mm= milli-meter
	Control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml	
Ethanol	0.0mm	8.0mm	10.0mm	10.5mm	12.0mm	
Methanol	0.0mm	8.0mm	9.5mm	10.5mm	11.5mm	

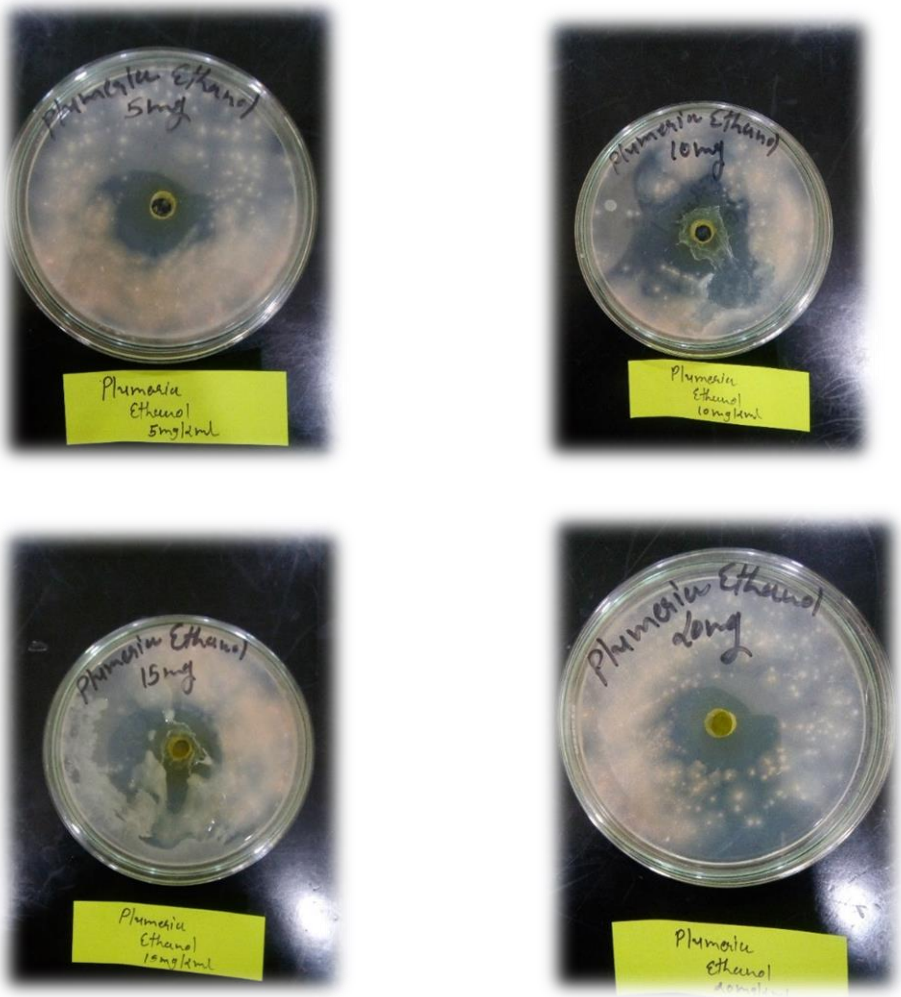
Table:2 Showing antifungal activity of Ethanolic and Methanolic Extracts of Plumeria alba L.





Graph-3 Ethanolic extract effect **Graph-4 Methanolic extract effect**

Plates showing Antifungal activity of ethanolic extracts of Plumeria alba L. against Fusarium oxysporum



Mohmmad S. Afifi et al., 2006 described antibacterial actvity of Plumeria alba L. Bark and Leaves agaist Staphylococcus auerus, Bacillus subtilis and E-coli. They showed zone of inhibition up to 8mm with bark extracts, here in this research antibacterial as well as antifungal activities were analysed and it showed up to 10-11mm zone of inhibition for up to 20mg/2ml leaves extracts concentration. In future different strains of bacteria and fungus cultures can be tried to assess antibacterial and antifungal activities.

CONCLUSION

Plumeria alba L. is an ornamental plant but it has so many phytochemical constituents and because of the presesnce of phytovhemicals it as so many pharmacological activities. It has antibacterial and antifungal activities agaist E-coli bacteria and Fusarium oxysporum. Fusarium oxysporum is plant parasitic fungus which causes the disease on so many economic important crops. So, Plumeria alba L. leaves extract can be used for the presparation of herbal fungicide.

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