

ANTIBACTERIAL ACTIVITY OF AEGLE MARMELLOS LEAF EXTRACTS

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Abstract- Since ancient times, medicinal plants are well known for antimicrobial activity due to the presence of various bioactive constituents; therefore they become important sources of antimicrobial drugs. The antibacterial activity of acetone, chloroform and benzene leaf extracts of *Aegle marmelos* was evaluated by well diffusion method. During the analysis *Pseudomonas aeruginosa* and *Staphylococcus aureus* both showed significant sensitivity for all the extracts and inhibition was measured as 18mm zone of inhibition for both bacteria at the concentration of 100µg/ml test sample. The minimum activity was reported against *Escherichia coli*. The overall study concluded that plant *Aegle marmelos* have potential antimicrobial activities which help in the development of new antimicrobial drugs against resistant micro-organisms.

Keywords- *Aegle marmelos*, Antimicrobial activity, Minimum inhibitory concentration, *Pseudomonas aeruginosa*

I. INTRODUCTION

During last two decades, it has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover still more potent plant drugs. About 250,000 living plant species contain a much greater diversity of bioactive compounds than any chemical library made by humans but only few plant species have been systematically investigated for the presence of bioactive compounds (Wakdikar, 2004). So, a large number of medicinal plants still remain to be investigated for their possible pharmacological values. But systematic efforts were not made to test the efficacy and toxicity of folklore herbs on scientific basis or to standardize their evaluation procedure. *Aegle marmelos*, commonly known as bael also called Bengal quince, golden apple, Japanese bitter orange, stone apple, or wood apple, is a species of tree native to India and Bangladesh. It is present throughout Southeast Asia as a naturalized species. It is sacred tree and used to worship Lord Shiva. The bael leaf considered as the three eyes or trident weapon of Lord Shiva. The bael fruit sometimes referred as Shreephal and worshiped in many hindu houses. The leaves are used for a diabetic. The infusion of leaves can be used against peptic ulcer. Leaves are also useful for the treatment of Jaundice, leucorrhea, wounds, deafness, conjunctivitis. Raw leaves can be used to cure gastric problems and irritation in the bowel. Oil prepared from leaves is proved to stop insect infestation. The leaves are also used in pediatric disorder. Extract from leaves is used in the anti-fungal activity. Aqueous extract of *Aegle marmelos* leaves, was evaluated for hypoglycemic and antioxidant effect by Upadhyaya et al., (2004). Maheshwari et al., (2009) studied on ethnolic extract of dried fruit pulp of *Aegle marmelos* against various intestinal pathogens i.e. *Shigella boydii*, *S. sonnei* and *S. flexneri* and proposed that certain phytochemicals including phenols, tannins and flavonoids were effective against all. It was also confirmed by Kaur et al., (2009) by getting treat *E. coli* with *Aegle marmelos* fruit extract. Citarasu et al, (2003) also experimented *Aegle marmelos* on certain pathogenic bacteria like *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophyla* and *Vibrio sp.*, and concluded its positive bactericidal effects. The present investigation was undertaken to test the antimicrobial activity of leaf extract of bael plant against selected pathogens.

II. Materials and Methods

Plant Material

The leaves of *Aegle marmelos* were collected from local area of Shahjahanpur district of Uttar Pradesh (India) and were authenticated by Dr. Zafar Abbas, Head of Botany Department, Gandhi Faiz-e-Aam College, Shahjahanpur and a voucher specimen BT/18 has been submitted to the herbarium of department for reference sample.

Bacterial Cultures

Staphylococcus aureus (NCIM-2079), *Pseudomonas aeruginosa* (NCIM-5210) and *Escherichia coli* (NCIM-2064).

Solvents and Media

Chloroform, Benzene and Acetone solvents for extraction, Nutrient Agar

Preparation of extract

10 gms of powdered leaves were used for solvent extraction via Soxhlet apparatus following standard protocol (Nag et al,2012). After the complete process, the collected extracts were subjected for evaporation at room temperature. The dried extracts were stored at 4°C for future analysis.

Minimum inhibitory concentration (MIC), Well diffusion method

Extracts were tested for the anti-bacterial potential by Agar well diffusion method (Irshad et al, 2012). Initially, autoclaved nutrient media were poured in the Petri plates under laminar air flow and after solidification of media the bacterial suspension (24 hrs old) swab over the media. The wells were prepared using cork borer. Test sample was dissolved in DMSO in different concentrations such as 25, 50, 100 µg/ml and 40 µl dissolved test sample from each concentration was loaded to the wells and incubated for 24 hrs at 37°C. DMSO (Di Methyl Sulfoxide) was used as a negative control whereas antibiotic amoxicillin disc (10µg) used as positive control.

III. Results and Discussion

In present study, the antimicrobial activity of leaf extracts of *Aegle marmelos* was carried out. Table-1 shown the antimicrobial activity of leaf extracted in acetone, chloroform and benzene solvents against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The maximum zone of inhibition was observed against *Pseudomonas aeruginosa* (18mm) and *Staphylococcus aureus* (18mm) at 100 µg/ml of chloroform and acetone respectively. Zone of inhibition was followed by 50 µg/ml chloroform extract against *Pseudomonas aeruginosa* (16mm). 100µg/ml benzene extract also showed 16mm zone of inhibition with 100µg/ml chloroform extract against *Staphylococcus aureus*. The observed results suggest that *Aegle marmelos* have significant antimicrobial activity. In the present study of *Aegle marmelos*, antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was found significantly maximum by all the extract. Antimicrobial activity of extracts increases as the concentration increases. The observation revealed that among all the test organisms *E. coli* was least sensitive for all three extracts. The data supports the hypothesis that *Aegle marmelos* leaves has an inhibitory effect on the growth of certain pathogens and may be used effectively against various microbial infections and may be employed as a source to develop new antimicrobial agents. This may be due to the presence of phenols, alkaloids, anthocyanin, xanthoproteins, flavanoids, carboxylic acids, coumarins and sterols in the extract. In this sense traditional herbal medicines can benefits of modern science and technology to serves further global needs. The drugs derived from herbs may have the possibility of using in medicine because of its potential antibacterial activity.

Table 1: Effect of pepper extract on growth of bacteria *in vitro*.

Bacteria	Concentrations of Plant Extracts (µg/ml)									DMSO (Negative control)	Amoxycillin (Positive control)
	Benzene			Chloroform			Acetone				
	25	50	100	25	50	100	25	50	100		
	Zone of Inhibition (in mm)										
<i>Staphylococcus aureus</i>	10	12	16	5	10	16	10	15	18	-	20
<i>Pseudomonas aeruginosa</i>	5	5	15	10	16	18	5	5	14	-	16
<i>Escherichia coli</i>	-	5	11	5	11	11	-	5	10	-	6

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Figure 1: Inhibition zone photographs of *Staphylococcus aureus* for acetone extract of *Aegle marmelos*.



Figure 2: Inhibition zone photographs of *Pseudomonas aeruginosa* for chloroform extract of *Aegle marmelos*.

