



Antibacterial activity of *Aegle marmelos* On Uropathogenic *Klebsiella pneumoniae*

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ABSTRACT:

Urinary tract infections (UTIs) are common and frequently occurring diseases after respiratory and blood stream infections. The enteric bacterium *Klebsiella pneumoniae* causes the range of infections in humans including meningitis, blood stream infections, pneumonia and urinary tract infections. In the present study antibacterial activity of the methanol, ethanol and acetone extracts of the leaves and fruit pulp of *Aegle marmelos* was studied using agar well diffusion method against uropathogenic *Klebsiella pneumoniae* isolated from urinary tract infected patient. The acetone and methanol extracts of leaves showed significant inhibition of *Klebsiella pneumoniae* at 10mg/ml concentration. Based on the studies it can be concluded that *Aegle marmelos* leaves can be used as a potential source of natural bioactive products in drug screening programs against antibiotic resistant bacterial pathogens.

Keywords: Antimicrobial activity, *Aegle marmelos*, Leaf, Pulp, *Klebsiella pneumoniae*.

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INTRODUCTION:

Urinary tract infections (UTIs) are most common and leading type of bacterial infections affecting humans in all aged groups including neonates, young adults and elderly people (1) (2). The symptoms of infection may be mild to severe depending on part of urinary tract affected and may cover kidneys bladder and urethra. It is estimated that more than 8 to 10 million persons per year get infected with urinary tract and seeks healthcare providers to get treatment. Most frequent causes of UTI are *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa* (3). The increased incidence of infections are also found in diabetics and the people with abnormal urinary system. In case of recurrent urinary tract infections the onset of infection is repeatedly observed where low dose antibiotics are re-prescribed for treatment. In this case chances of gaining drug resistance are high and may cause complications in the treatment and led to greater use of broad-spectrum antibiotics. This intensifies the need to search an effective and potent bioactive agent against drug resistant bacterial pathogens. Medicinal plants are rich in pharmacologically active biomolecules since from ancient times they have proved their potential in treating various ailments and medical conditions. Medicinal plants are considered as best sources of bioactive molecules (WHO 2000) and needs through investigation to understand mechanism and nature of their target molecules (4) (5). *Aegle marmelos* an Indian sacred plant member of Rutaceae is known to have immense medicinal importance as antibacterial, anti-diarrheal, anti-diabetic, anti-dysentery and antiviral agent in addition to use in treating stomach pain, snake bite and food poisoning (6). Considering medicinal importance of *Aegle marmelos* in the present study leaf and fruit pulp extracts were evaluated to check their effect against uropathogenic *Klebsiella pneumoniae*.

MATERIALS AND METHODS:

Plant material and preparation of extracts: The fresh leaves and fruit of *Aegle marmelos* were collected from Shelgaon village in Latur District Maharashtra and the plant parts were identified and authenticated in School of Life Sciences, SRTMU, Nanded. Fresh leaves and fruits of *Aegle marmelos* were cleaned with water and the pulp was separated from fruit. The pulp and leaves were dried in shade for one week. After drying leaves and fruit pulp were pulverized separately into fine powder by using grinding machine and stored in airtight container. This powder was used to prepare methanol, ethanol and acetone extracts. To the 5gm of dried leaf and pulp powder 50ml of ethanol, methanol and acetone were

added individually and the flasks were kept in shaker at 120rpm and 37° C for 24 hrs. The extracts were filtered using Whatman filter paper and dried in evaporation plates. Residues remained on the filter paper were then subjected to secondary extraction as mentioned before. The yield of methanol, ethanol and acetone extracts of leaves and fruit pulp of *Aegle marmelos* were to find out extraction efficiency of solvents.

Test organism:

Klebsiella Pneumoniae used in the present study was isolated from urine sample of urinary tract infected female patient from urocare hospital of Nanded. The isolate was identified based on morphological, biochemical and molecular characteristics in School of Life Sciences, S.R.T.M. university Nanded. The 16s rRNA sequence of isolate is deposited in Genbank with accession number is LC720890.

Antimicrobial activity of *Aegle marmelos* leaf and pulp extract:

Agar well diffusion assay was used for the evaluation of antimicrobial activity of leaf and pulp extract. Muller Hinton agar plates were prepared and 0.1ml of diluted inoculum of *Klebsiella pneumoniae* (cell density approaching 0.5 Macfarland standard) was added at the center of plate by using micropipette and then spreaded by glass spreader. Six wells were prepared in each plate by using cork borer. 50 microliter of leaf and pulp extract was added individually in each well. The antibacterial activity of leaf and pulp extracts were checked at various concentrations (2.5mg/ml, 5mg/ml, 7.5mg/ml and 10 mg/ml). The plates were kept in refrigerator for 15 min for diffusion and incubated later at 37°C. for 24 hrs. Antibacterial activity was checked by measuring the zone of inhibition against *Klebsiella pneumoniae*.

Results and Discussion:

Urinary tract infections are leading and common type of bacterial infections affecting a large group of population. The emergence of antibiotic resistant strains of bacteria including *Klebsiella pneumoniae* poses a threat in disease treatment and creates many health issues. To overcome these issues search for novel, safe and protect bioactive molecule is needed and in this respect many studies have reported importance of medicinal plants as a source of natural and effective drug compound. In the present study ethanol, methanol and acetone extracts of leaves and fruit pulp of *Aegle marmelos* have been tested against uropathogenic *Klebsiella pneumoniae* strain. The extraction efficiency of methanol was higher than ethanol and acetone in preparation of both leaf and fruit pulp extracts. Among the leaf and fruit pulp extracts of *Aegle marmelos* antibacterial activity against *Klebsiella pneumoniae* was found only in leaf extracts. In presence of three pulp extracts including ethanol, methanol and acetone extracts the inhibition of *Klebsiella pneumoniae* was either low or completely absent. This showed that the fruit pulp of *Aegle marmelos* does not have target molecules against *Klebsiella pneumoniae*. Methanol and acetone extracts of *Aegle marmelos* leaves showed significant inhibition of *Klebsiella pneumoniae* at higher concentrations. In presence of ethanol extract at all studied concentration the antibacterial effect was very low and non-noticeable. At low concentration of methanol and acetone extracts the activity was low and increased with increasing concentration of extracts. This indicated dose of dependent effect of extracts.

Table: 1. Antibacterial activity of leaves extract of *Aegle marmelos*

Test organism	Extract concentration	Extract	Solvent	Zone of inhibition (mm)
Klebsiella pneumoniae	2.5mg/ml	Leaf	Ethanol	-
			Methanol	+
			Acetone	-
	5mg/ml	Leaf	Ethanol	-
			Methanol	-
			Acetone	+
	7.5mg/ml	Leaf	Ethanol	-
			Methanol	+
			Acetone	+
	10mg/ml	Leaf	Ethanol	-
			Methanol	9mm
			Acetone	8mm

Antibacterial activity of *Aegle marmelos* was evaluated by disc diffusion technique at increasing concentration of petroleum ether, acetone and ethanol. Petroleum ether and acetone show minimum zone of inhibition against *Serratia marcescens*. There is no zone of inhibition in methanol extract of *Aegle marmelos* against *Klebsiella pneumoniae* and *Staphylococcus aureus* (7). Ethanol extracts of fruits showed highest inhibitory activity against multi drug resistant *Escherichia coli* as compare to standard antibiotic 20mm(8).



Fig: 1. Antibacterial activity of *Aegle marmelos* leaf extract

The antibacterial activity of *Aegle marmelos* fruit were checked against different microbial strains *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Ethanol, methanol and petroleum ether extracts showed inhibitory effect against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*(9). The antibacterial activity of methanolic extract with minimum inhibitory concentration of stem bark of bael was checked against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at various concentrations. *Staphylococcus aureus* show best zone in entire minimum inhibitory concentration(10). Methanol extract exhibited a much better antibacterial activity as compared to aqueous extract against *Klebsiella pneumoniae*, *Staphylococcus aureus* at 40mg/ml concentration. (11). The susceptibility of *Klebsiella pneumoniae* varied according to plant part and type of solvent used to prepare extract. Absence of low antibacterial activity in presence of all three extracts of fruit pulp may be due to lower concentration of active principle in fruit pulp or need to use other extraction medium.

CONCLUSION:

The present study highlighted the significance of acetone and methanol extracts of *Aegle marmelos* leaves against uropathogenic *Klebsiella pneumoniae*. Further studies on finding phytoconstituents with active principles of mechanism of action of extracts will be required to recommend the use of these extracts as alternative to routinely used synthetic drug molecules.

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