

ANTIBACTERIAL ACTIVITY OF AEGLE MARMELLOS AGAINST HUMAN PATHOGENIC MICROBIAL STRAINS

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ABSTRACT

Aegle Marmelos has a number of phytoconstituents which reveals its use of various therapeutic purposes. The main object of the study was to evaluate of antimicrobial potential of *Aegle marmelos* against human pathogenic microbial strains. The antimicrobial activity of ethanolic and methanolic extract of *Aegle marmelos* was tested against 10 human pathogens *Klebsiella aerogenes* ATCC 9621, *Klebsiella pneumonia* ATCC 31488, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 118, *B. subtilis* ATCC 128263, *Pseudomonas aeruginosa* ATCC 13525, *Streptococcus faecium* ATCC 8043, *E. Coli* ATCC 632, *Staphylococcus aureus* ATCC 12600, *Proteus mirabilis* ATCC 29246 and the result indicate that *Aegle marmelos* possesses potential broad spectrum antimicrobial activity.

KEYWORDS: phytoconstituents, Antibacterial activity, Human pathogenic microbial strains, *Aegle marmelos*.

INTRODUCTION

Medicinal plants are the backbone of Traditional medicine in the world (Farnsworth, 1994). Over the years interest in natural products has acquired a cyclic phenomenon. In many countries, including India Japan and China, thousands of tribal communities still use medicinal plants for the cure of various diseases. The great interest in the use and importance of medicinal plants in many developing countries has led to intensified efforts on the documentation of ethno- medical data of medicinal plants Dharet *al.*, 1968 and Waller, 1993).

Aegle marmelos important medicinal plant available in TamilNadu, India and are reported to have various medicinal properties in Traditional medical systems. *Aegle marmelos* is common medicinal plant available in South India and is used as medicine in Siddha and Ayurveda. The plants are distributed throughout India, cultivated as well as grow in wild. In TamilNadu it is located in the river belt of Vattaru and Cauvery and it is also present in most of the Shiva temple of TamilNadu.

The essential oil obtained from the leaves has shown a broad spectrum of anti-bacterial and anti-fungal activities (Benerji and Kumar, 1980; Pattnaik *et al.*, 1996 and Rana *et al.*, 1997). The aqueous decoction of the leaves has been reported to have a significant hypoglycemic effect (Karunanayake *et al.*, 1984 and Ponnachan *et al.*, 1993). The main aim of the present research work was to determine and evaluate the antibacterial potential of *Aegle marmelos* against several standard pathogenic bacterial strains.

MATERIAL AND METHODS

Plant material

Aegle marmelos leaves were collected from G.B. Pant University of Ag. & Technology, Pantnagar, U.K.

Antimicrobial activity

The bacterial strains cultures for the work were collected from the IMTEC, Chandigarh in the form of dry culture.

- a) *Klebsiella aerogenes* ATCC 9621
- b) *Klebsiella pneumonia* ATCC 31488
- c) *Enterococcus faecalis* ATCC 29212
- d) *Escherichia coli* ATCC 118
- e) *B. subtilis* ATCC 128263
- f) *Pseudomonas aeruginosa* ATCC 13525
- g) *Streptococcus faecium* ATCC 8043
- h) *E. Coli* ATCC 632
- i) *Staphylococcus aureus* ATCC 12600
- j) *Proteus mirabilis* ATCC 29246

These spices were extracted by ethanolic extraction by soaking 20 g of plant part in 50 mL of 90% ethanol for overnight at ambient temperature. The mixtures were then filtered. The

filtrate was concentrated on a rotary evaporator at 45°C for ethanol elimination and the extracts were kept in sterile bottles under refrigerated conditions until use. Agar diffusion method was used to screen all the isolates. This was done by dispensing 1ml (2mg/ml) of the plant extract into 19mls of Mueller Hinton agar.

Preparation of solvent extractions

Twenty five gram of shade dried, powder of plant materials (fruit) were filled separately in the thimble and extracted successively with 150 ml each of ethanol and methanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4°C in airtight bottles until further use. 1.0 g of solvent residue was dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay.

Anti-bacterial activity assay

Antibacterial activity of solvent extracts; methanol and ethanol was determined by disc diffusion method on nutrient agar medium (molten Mueller Hinton agar) (Anonymous, 1996). Antibacterial assay of the crude extracts of methanol and ethanol of plant were performed on nutrient agar plate well enriched with various concentrations (25µl, 50µl, 75µl, 100µl) of extracts. The plates were incubated for 24 - 48 h at 37°C. The antibacterial activity was measured as the zone of inhibition. The well enriched with sterilized antibiotic (tetracycline) was used as a standard control. Each treatment consists of three replicates and repeated at least twice. Zone of inhibition (ZOI), Plant extracts inhibiting the growth of the organism, was determined based on the readings.

RESULT AND DISCUSSION

In this research the antimicrobial activity of *Agelemarmelos* were compared with control drug Tetracyclin against Gram negative organism of *Escherichia coli* ATCC 632, *Pseudomonas aeruginosa* ATCC 13525, *Klebsiella aerogenes* ATCC 9621, *Klebsiella pneumonia* ATCC 31488, *Escherichia coli muroid* ATCC118, *Proteus mirabilis* ATCC 29246 and Gram positive organism of *Staphylococcus aureus* ATCC 12600, *Staphylococcus aureus*, ATCC 12600 and *Streptococcus faecium* ATCC 8043.

In (table 1.1) *Escherichia coli muroid* ATCC118 shows maximum zone of inhibition of about 18 mm, 20 mm, 21mm and 22mm at different concentration of about 25 µl, 50µl, 75 µl and 100 µl against fruit extract of *Agele marmelos* in ethanol, *Proteus mirabilis* ATCC 29246

shows the minimum zone of inhibition at given concentration of about 07mm, 09 mm, 11mm and 13mm.

The observation was found in table 1.2 the same concentration was subjected for extract of *Aegle marmelos* methanol; *Staphylococcus aureus* ATCC 12600 show the maximum activity. The zone of inhibition was observed 16mm, 19mm, 22mm and 26mm.at the given concentration. *Proteus mirabilis* ATCC 29246 shows the minimum zone of inhibition, 05 mm, 07 mm, 10 mm and 12mm.

Anti-microbial activity of different extracts of *A.marmelos* showed positive result against tested microorganism in a variable concentration (Narayan P. Yadav, C. S. Chanotia). The methanol extracts of *Aegle marmelos*, *Salmaliamalabarica*, *Punicagranatum*, *Myristicafragrans*, *Holarrhenaantidysenterica*, *Termineliaarjuna* and Triphala showed strong antimicrobial activity. (Rani P., Khullar N., (2004) *Phytother Res.* 18(8), 670-673).

Table No. 1.1 - Zone of inhibition of extract of *Aegle marmelos* in ethanol with test culture.

S. No.	Name of culture	Concentration of <i>Aegle marmelos</i> (μ l)	Zone of inhibition (mm)
01	<i>Escherichia coli</i> ATCC 632	25	13
		50	14
		75	16
		100	18
02	<i>Pseudomonas aeruginosa</i> ATCC 13525	25	12
		50	14
		75	17
		100	19
03	<i>Klebsellaerogenes</i> ATCC 9621	25	13
		50	15
		75	18
		100	19
04	<i>Klebsellapneumoniae</i> ATCC 31488	25	09
		50	11
		75	12
		100	14
05	<i>Escherichia coli</i> ATCC118	25	18
		50	20
		75	21
		100	22
06	<i>Proteus mirabilis</i> ATCC 29246	25	07
		50	09
		75	11
		100	13

07	<i>Streptococcus faecium</i> ATCC 8043	25	13
		50	16
		75	19
		100	21
08	<i>Enterococcus faecalis</i> ATCC 29212	25	06
		50	08
		75	10
		100	12
09	<i>Staphylococcus aureus</i> ATCC 12600	25	15
		50	17
		75	19
		100	21
10	<i>Bacillus subtilis</i> ATCC 128263	25	14
		50	16
		75	19
		100	22

Table No. 1.2 - Zone of inhibition of extract of *Aegle marmelos* in Methanol with test culture.

S. No.	Name of culture	Concentration of <i>Aegle marmelos</i> (μ l)	Zone of inhibition (mm)
01	<i>Escherichia coli</i> ATCC 632	25	13
		50	15
		75	17
		100	21
02	<i>Pseudomonas aeruginosa</i> ATCC 13525	25	9
		50	10
		75	12
		100	14
03	<i>Klebsella aerogenes</i> ATCC 9621	25	07
		50	09
		75	11
		100	13
04	<i>Klebsella pneumoniae</i> ATCC 31488	25	10
		50	13
		75	16
		100	20
05	<i>Escherichia coli</i> ATCC 118	25	18
		50	21
		75	24
		100	16
06	<i>Proteus mirabilis</i> ATCC 29246	25	05
		50	07
		75	10
		100	12
07	<i>Streptococcus faecium</i> ATCC 8043	25	-
		50	-

		75	-
		100	-
08	<i>Enterococcus faecalis</i> ATCC 29212	25	12
		50	14
		75	17
		100	21
09	<i>Staphylococcus aureus</i> ATCC 12600	25	16
		50	19
		75	22
		100	16
10	<i>Bacillus subtilis</i> ATCC 128263	25	07
		50	09
		75	11
		100	14

- No zone of inhibition

CONCLUSION

In conclusion, there is an evidence to support *Aegle marmelos* have an antimicrobial activity. Hence, they may be useful as therapeutic agents for pathological damage. The methanolic and ethanolic extract of *Aegle marmelos* were found to show the highest inhibitory activity against human pathogens and thereby proves the antimicrobial activity of the plant. It is therefore, the above findings recommended the further phytochemical investigation to identify the mechanism and constituents responsible for the antimicrobial activity.

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