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ANTIBACTERIAL ACTIVITY OF *Aegle marmelos* AGAINST BACTERIAL STRAINS

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ABSTRACT

The study was undertaken to evaluate the in *vitro* antibacterial activity of *Aegle marmelos* fruit, leaf and stem plant extracts in methanol, aqueous and petroleum ether solvents against test bacterial strains by Agar well diffusion method. The antibacterial activity by plant extracts was measured by measuring zone of inhibition (ZOI). All plant extract possessed antibacterial activity. Fruit extract in methanol solvent was found to be most sensitive and effective plant extract against *Excherichia coli, Bacillus subtilis and Salmonella typhii*. The zone of inhibition was compared with standard antibiotic Tetracycline. The study suggest that the plant is promising for development of phytomedicine and have antimicrobial properties.

KEYWORDS : Zone of Inhibition, Antibacterial, Phytomedicine, Extract

Infectious diseases are disorders caused by pathogenic micro-organisms like bacteria, virus, fungi and protozoa. Pathogenic bacteria thrive in the body emitting toxins which damage cells and tissues that result in bacterial diseases. To cure these diseases, medicinal plants represent a rich source of antibacterial agents, Jeeshna et.al., 2009 and Thomas and Ponnamal, 2006. This is because green medicines are more harmless, healthier and safer then synthetic ones. Therefore trade value of Indian medicinal plants is expected to increase several times in years to come, Jit, 1995.

Aegle marmelos (L.) belongs to family Rutaceae, commonly known as Bael (Hindi) and Golden Apple (English). It has been used for time immemorial in traditional systems of medicine for relieving constipation, diarrhea, dysentery and respiratory infections. There are reports available on antimicrobial activity of plant extracts of many members of Rutaceae family viz. *Murraya paniculata*, (Sundaram et. al., 2011; *Citrus aurantifolia*, Taiwo et. al., 2007 and *Vepris lanceolata*, Fawzia et.al., 2004). Present study was aimed to focus antibacterial activity of *Aegle marmelos* in which fruit, leaf and stem extract in aqueous, methanol and petroleum ether solvent were tested against test bacterial strains (*Escherichia coli* MTCC 1652, *Pseudomonas aeruginos*a MTCC 647, *Bacillus subtilis* MTCC 441, *Salmonella tyhphii* MTCC 733).

MATERIALS AND METHODS

Plant material was collected from Keoladeo National Park, Bharatpur and certified as *Aegle marmelos* by Botanical survey of India, Jodhpur. Bacterial strains were collected from IMTECH, Chandigarh (Institute of Microbial technology).

Plant extracts were prepared in methanol and petroleum ether solvent by Soxhlet apparatus. 100mg of plant extract was redissolved in 10 ml of DMSO (Dimethyl Sulphoxide) to get 100mg/10ml of stock concentration of plant extract. Aqueous plant extract was prepared by dissolving 100 ml of sterile distilled water in 25 gm of powdered plant material and was kept at 37^0C for two days. After that solution was filtered and water was evaporated at room temperature. After evaporation, plant residue was dissolved in DMSO to make stock concentration.

Agar well diffusion method, Perez et.al., 1990 was used for antibacterial activity against test bacterial strains. In this method, 50 µl of bacterial inoculums was spread on agar plates and wells were filled with 25µl, 50 µl, 75µl and 100 µl concentration of plant extract different solvents. Tetracycline was used as control. Nutrient agar plates were incubated at 37^0C for 48 to 72 hours. After incubation, the diameter of zone of inhibition was measured. Mean of three replicates was recorded.

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Table 1: Antibacterial Activity of Plant Extracts Fruit, Leaf and Stem of *Aegle marmelos* in Different Solvents Against *Escherichia coli* MTCC 1652

S. No.	Type of Solvent	Concentration of plant extract in	Zone of Inhibition (mm)			
			Fruit	Leaf	Stem	Control
1.	Methanol	25		9±0.66	4±0.33	9±0.33
		50	15±0.33	12±0.33	7±0.57	12±0.66
		75	17±0.66	15±0.57	10±0.88	15±0.66
		100	20±0.57	18±0.00	12±0.57	17±0.57
2.	Aqueous	25	10±0.88	7±0.57	4±0.00	9±0.33
		50	13±0.57	9±0.57	6±0.57	12±0.66
		75	16±0.33	11±0.00	8±0.33	15±0.66
		100	19±0.33	14±0.33	10±0.57	17±0.57
3.	Petroleum ether	25	4±0.66	9±0.33		9±0.66
		50	6±0.88	11±0.66	…….	12±0.66
		75	8±0.66	14±0.33	6±0.66	15±0.33
		100	11±0.57	16±0.57	9±0.33	17±0.57

Table 2: Antibacterial Activity of Plant Extracts Fruit, Leaf and Stem of *Aegle marmelos* in Different Solvents Against *Pseudomonas Aeruginosa* MTCC 647

S. No.	Type of Solvent	Concentration of Plant Extract in	Zone of Inhibition (mm)			
			Fruit	Leaf	Stem	Control
1.	Methanol	25		13±0.88	10±0.57	8±0.57
		50	14±0.00	16±0.57	12±0.33	10±0.33
		75	17±0.33	19±0.88	14±0.66	13±0.66
		100	20±0.57	22±0.57	16±0.57	16±0.00
2.	Aqueous	25	12±0.88	10±0.33	7±0.57	8±0.57
		50	14±0.66	13±0.33	10±0.66	10±0.33
		75	16±0.57	15±0.66	13±0.33	13±0.00
		100	18±0.87	18±0.66	16±0.33	16±0.00
3.	Petroleum ether	25	7±0.88	8±0.66	……..	8±0.57
		50	10±0.33	11±0.88	5±0.00	10±0.33
		75	12±0.57	13±0.88	8±0.33	13±0.66
		100	14±0.57	16±0.88	11±0.57	16±0.00

…….. No zone of inhibition

RESULTS AND DISCUSSION

Traditional therapies involve the use of plant extracts or their active principles. Plants are used medicinally in different countries and are source of many potent and powerful drugs, Srivastava et. al., 1996.

In present research, all plant extracts were found to show antibacterial activity against test bacterial strains and variability in activity was also found in fruit, leaf and stem extracts in different solvents. Variability in In *vitro* activity of *Limonia acidissima* plant parts was also seen by Thomas and Ponnamal, 2006. In present study, fruit extract in methanol solvent was found to show maximum inhibition against E. coli MTCC 1652, *Bacillus subtilis* MTCC 441 and *Salmonella typhii* MTCC 733 by forming inhibition zone of 20±0.57mm, 22±0.57mm and 21±0.66mm 100 µl concentration (Table 1, 3 and 4) [Figure A, C and D].

Table 3: Antibacterial Activity of Plant Extracts Fruit, Leaf and Stem of *Aegle Marmelos* in Different Solvents Against *Bacillus subtilis* MTCC 441

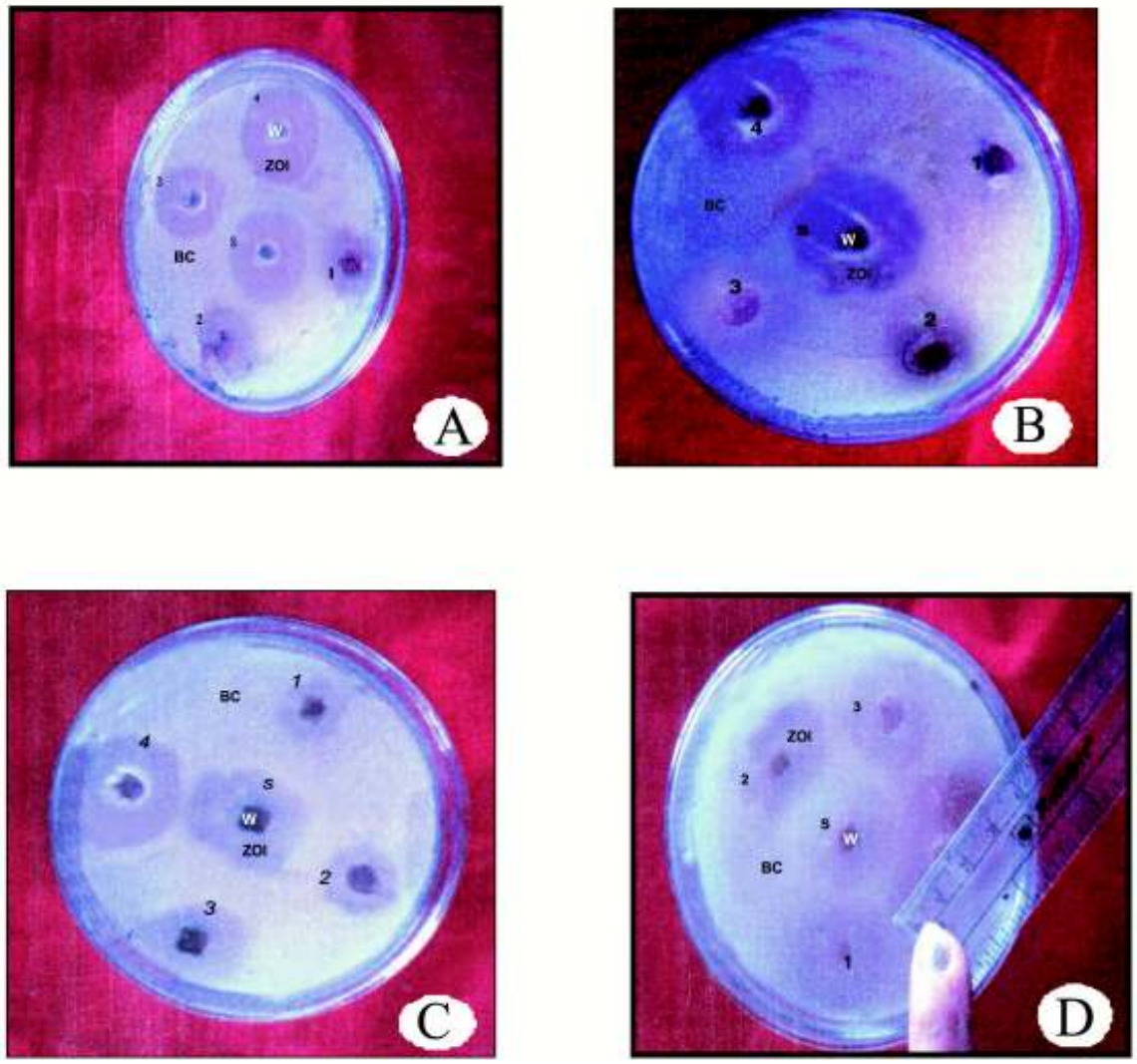
S. No.	Type of Solvent	Concentration of plant extract in solvent (µl)	Zone of Inhibition (mm)			
			Fruit	Leaf	Stem	Control
1.	Methanol	25	13±0.57	7±0.88	10±0.33	10±0.33
		50	15±0.66	9±0.66	12±0.00	13±0.57
		75	18±0.66	11±0.33	15±0.57	16±0.57
		100	22±0.57	13±0.33	17±0.33	19±0.33
2.	Aqueous	25	11±0.88	4±0.33	9±0.66	10±0.33
		50	13±0.57	7±0.66	11±0.33	13±0.57
		75	16±0.33	9±0.66	13±0.57	16±0.57
		100	19±0.33	11±0.33	15±0.33	19±0.33
3.	Petroleum ether	25	9±0.57	……	5±0.33	10±0.33
		50	11±0.66	5±0.57	8±0.33	13±0.57
		75	14±0.00	7±0.66	10±0.57	16±0.57
		100	17±0.57	9±0.57	12±0.33	19±0.33

Table 4: Antibacterial Activity of Plant Extracts Fruit, Leaf and Stem of *Aegle marmelos* in Different Solvents Against *Salmonella Typhii* MTCC 733

S. No.	Type of Solvent	Concentration of plant extract in solvent (µl)	Zone of Inhibition (mm)			
			Fruit	Leaf	Stem	Control
1.	Methanol	25	14± 0.57	12±0.66	8±0.33	11±0.66
		50	17±0.33	15±0.57	10±0.33	13±0.66
		75	19±0.66	17±0.33	13±0.57	15±0.33
		100	**21±0.66**	19±0.33	15±0.57	18±0.33
2.	Aqueous	25	11±0.88	9±0.33	7±0.57	11±0.66
		50	14±0.57	11±0.33	9±0.66	13±0.66
		75	16±0.33	14±0.57	12±0.33	15±0.33
		100	18±0.66	16±0.00	14±0.57	18±0.33
3.	Petroleum ether	25	8±0.33	8±0.57	…	11±0.66
		50	11±0.57	10±0.33	…….	13±0.66
		75	14±0.88	13±0.33	7±0.66	15±0.33
		100	17±0.88	15±0.66	10±0.33	18±0.33

Pseudomonas aeruginosa MTCC 647 was inhibited maximum by leaf extract in methanol solvent (ZOI= 22±0.57mm) (Table 2). The essential oil obtained from *Aegle marmelos* leaves exhibited activity against *Pseudomonas* and *Xanthomonas* species, Pandey et.al., 1981. Antibacterial activity of *Aegle marmelos* may be due to presence of Cuminaldehyde and Eugenol because these compounds have shown their activity against some bacterial strains, Duke, 1992. *Glycosmis pentaphylla* family Rutaceae plant parts has also been reported for its significant activity in methanol solvent, Jeeshna et.al., 2009.

In this study, after fruit extract stem extract in methanol solvent was found more susceptible then leaf extract against *Bacillus subtilis* MTCC 441 by forming inhibition zone of 10±0.33mm, 12±0.00mm, 15±0.57mm



Figures A-D : Antibacterial Activity of Plant Extract Fruit, Leaf And Stem In Different Solvents Against Test Bacterial Strains

ZOI = Zone of Inhibition, BC= Bacterial Colony, W=well, S= Standard

Figure A : Activity of fruit extract in methanol solvent *Escherichia coli* MTCC 1652
Figure B : Activity of leaf extract in methanol solvent *Pseudomonas aeruginosa* MTCC 161
Figure C : Activity of fruit extract in methanol solvent *Bacillus subtilis* MTCC 441
Figure D : Activity of fruit extract in methanol solvent *Salmonella tythii* MTCC 733

and 17±0.33mm at 25µl, 50µl, 75µl and 100µl concentration. Stem extract of medicinal plants have been reported for its antimicrobial activity, Jeyachandran (2003).

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