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Antimicrobial Prospective of Extracts From fruit of *Aegle marmelos* in Different Solvents

KEYWORDS: *Antimicrobial activity, Aegle marmelos, Clinical pathogens, Disc diffusion technique, two fold serial dilution method.*

Mayuri C. Rathod
Department of Biotechnology , Veer Narmad South Gujarat University, Surat- 395007, Gujarat - India

Dashrath D. Ghori
Department of Biotechnology , Veer Narmad South Gujarat University, Surat- 395007, Gujarat - India

D. A. Dhale
Department of Botany, SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule-424005 (M.S.) India

ABSTRACT *Aegle Marmelos (L.) Corr. is a medicinal herb belongs to the family Rutaceae, used for antimicrobial screening against selected five pathogenic microorganisms - Escherichia coli MTCC40, Salmonella typhi MTCC3216, Pseudomonas aeruginosa MTCC2581, Proteus vulgaris MTCC428, Staphylococcus aureus MTCC3160. fruits of Aegle marmelos were extracted in ethanol and petroleum ether through cold and hot extraction method. Thus, four extracts were prepared. For control petroleum ether, ethanol, and Ampicilin (in petroleum ether and ethanol) were used. Zone of Inhibition and Minimum Inhibitory Concentration were determined by Disk Diffusion Method and Broth Dilution Method respectively. Results of these two methods showed antimicrobial effect against four microorganisms - Escherichia coli MTCC40, Salmonella typhi MTCC3216, Proteus vulgaris MTCC428, Staphylococcus aureus MTCC3160.*

Introduction
Aegle marmelos is commonly known as Bael and "Bilipatra" in Gujarati. Its Medicinal properties have been described in the ancient medical treatise in Sanskrit, 'Charak-Samhita'(Goyal et al., 2007). The fruit at all stages of maturity has medicinal virtues and has been used as traditional medicine for a long time. Use of traditional medicine is one of the common practices in India due to their wide pharmacological activities (Uma Devi et al 2007). Developed and developing countries use traditional medicine at the primary health care level. The different traditional literatures details number of herbs with significant antimicrobial activity (Jones, 1996; Klocke et al., 1985 and Raveesha et al., 1999). The petroleum ether extracts of *Aegle marmelos* (L.) showed great anti microbial effect against gram positive & gram negative organism followed by ethanol extract, aqueous extract of dried plant powder (Sudharameshwari and Radhika 2007). Now it is aimed to scrutinize scientifically the antimicrobial prospective of extracts from fruit *Aegle marmelos* (L.) Corr. in different solvents (petroleum ether, ethanol) by cold and hot extraction method.

2. Material And Methods
2.1 Collection of Plant: Fresh fruits of *A. Marmelos* (L.) Corr. were collected randomly from the "temple of Bhagvan Shive" at village Bela, Bhavnagar, Gujarat, India. The taxonomic identity of this plant was confirmed by using flora of Gujarat (Amin, 1978). Fresh fruits were collected and washed under running tap water and then dried in oven at 37˚C and then homogenized to fine powder individually.

2.2 Preparation ***Aegle marmelos*** Extracts: For extract preparation, 10gm of fine powder of fruits of *A. Marmelos* were extracted in 250ml of petroleum ether and ethanol separately using Soxhlet apparatus at 70˚C and 80˚C respectively up to four cycles. Solvents were evaporated in oven to make final concentration 40mg/ml. Stock solution of antibiotic-Amphicilin also prepared at same concentration. For cold extraction, 10gm of fine powder of fruits of *A. Marmelos* was extracted in 250ml of petroleum ether and ethanol separately using BOD bottle at room temperature and 150 rpm for 48 hours. Extracts were filtered using Whatman filter paper No.1. Solvent is evaporated in oven to make final concentration 40mg/ml. These stock solutions were stored at 4°C in air tight bottles for further studies.

2.3 Determination of antimicrobial activity: The antimicrobial activity was evaluated on *Escherichia coli* MTCC40, *Salmonella typhi* MTCC3216, *Pseudomonas aeruginosa* MTCC2581, *Proteus vulgaris* MTCC428, *Staphylococcus aureus* MTCC3160 procured from Microbial Type Culture Collection, Chandigarh. The agar disc diffusion technique described by Kirby-Bauer, was used for determining antimicrobial activity (Kirby and et al., 1996). The culture of test organism (optical Density 0.22 at 600nm, approximately 10^5 CFU/ml) was prepared. 0.2 ml of it was spread on nutrient agar plates. Discs prepared by impregnating 5µl of extract/solvent/Amphicilin using micropipette and dried it in oven, were applied on same nutrient agar plate. The plates were kept at 4˚C for 15min for diffusion and then were incubated overnight at 37˚C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

2.4 Determination of minimum inhibitory concentration (MIC): The two fold broth dilution method was used to determine the MIC (Lathia, 2004; Ericsson and Sherris, 1971). Each extracts inhibiting growth of one or more microorganisms were further tested for the MIC. The dilutions were prepared in nutrient broth the decreasing order of concentration (2000µg, 1000µg, 500µg, 250µg, 125µg, 62.50µg, 31.25µg) of extracts/antibiotic/solvent. From the inoculums 10µl of each culture was inoculated separately in each set so that final concentration of microorganism in tubes became 10^6cells/ml. The highest dilution of each extract corresponding to respective test organism showing no visible growth was considered as MIC (the growth was compared with positive as well negative controls).

3. RESULT AND DISSCUSION
The screening of antibacterial activity was carried out by disc diffusion method and determination of MIC values was carried out by two-fold broth dilution method for test organisms such as *Escherichia coli* MTCC40, *Salmonella typhi* MTCC3216, *Pseudomonas aeruginosa* MTCC2581, *Proteus vulgaris* MTCC428, *Staphylococcus aureus* MTCC3160.

3.1 Results of Determination of Zone of Inhibition (ZOI)
The ZOI of extracts in petroleum ether and ethanol of 600 µg/ml concentrations against the test organisms are shown Table 1 provides the measured ZOI of the extracts of fruits of *A. marmelos* with different solvents against five bacteria.

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

Table 1: Results of determination of zone of inhibition of fruits of A. marmelos

SR. NO.	Extracts	Zone of Inhibition (mm)				
		E. coli	S. typhi	S. aureus	P. vulgaris	Ps. arugenosa
1.	Petr. ether	0	0	0	0	0
2.	AP	14	20	14	22	0
3.	FPH	16	22	18	24	0
4.	FPC	16	22	18	24	0
5.	Ethanol	0	0	0	0	0
6.	AE	16	14	12	24	0
7.	FEH	18	24	16	28	0
8.	FEC	18	24	16	28	0

Note: Petr. ether: Petroleum ether; AP: Ampicilin in Petroleum ether; FPC: Fruit in Petroleum ether by Cold extraction; FPH: Fruit extract in Petroleum ether by Hot extraction; AE: Ampicilin in Ethanol; FEH: Fruit extract in Ethanol by Hot extraction; FEC: Fruit extract in Ethanol by Cold extraction.

Each plant extracts showed antibacterial activity against *E. coli*, *S. typhi*, *S. aureus,* and *P. vulgaris*, except *P. aeruginosa* which is resistant to extract of fruits. Each extract was very effective against *P. vulgaris* among all five organisms and it was also seen that ethanol extract was more effective than the petroleum ether extract against *P. vulgaris*. Among four susceptible stains *E. coli* had less susceptibility towards the petroleum ether extracts, while among them *S. aureus* was less susceptible towards ethanol extracts. For *P. vulgaris* and *S. typhi* both extracts (petroleum ether and ethanol) were very effective and less effective in case of *E. coli* and *S. aureus*. It was seen that the all extract has more effect than reference antibiotics and control-petroleum ether and ethanol with respective pathogenic microorganism.

3.2 Results of Determination of MIC

After evaluating the values of ZOI of extracts, each positive extracts was taken for MIC test by two fold broth dilution method. MIC of ethanol extract and petroleum ether extract of fruits were tested against *E. coli*, *P. vulgaris*, *S. aureus, and S. typhi*. The test organisms were inoculated in various concentrations of plant extracts i.e. 2000µg/ml, 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, and 31.25µg/ml.

Table 2: Results of MIC for E. coli MTCC40 of fruits of A. marmelos

Sr. No.	Extracts	Dilution of Extracts (µg/ml)						
		2000	1000	500	250	125	62.50	31.25
1.	Petr. ether	+	+	+	+	+	+	+
2.	AP	-	-	-	+	+	+	+
3.	FPH	-	-	-	+	+	+	+
4.	FPC	-	-	-	+	+	+	+
5.	Ethanol	+	+	+	+	+	+	+
6.	AE	-	-	-	+	+	+	+
7.	FEH	-	-	-	+	+	+	+
8.	FEC	-	-	-	+	+	+	+

Note: Petr. ether: Petroleum ether; AP: Ampicilin in Petroleum ether; FPC: Fruit in Petroleum ether by Cold extraction; FPH: Fruit extract in Petroleum ether by Hot extraction; AE: Ampicilin in Ethanol; FEH: Fruit extract in Ethanol by Hot extraction; FEC: Fruit extract in Ethanol by Cold extraction. '+': presence of growth '-': absence of growth.

Figure 1: Result of MIC using ethanol extraction of fruit of fruits of A. marmelos for E. coli MTCC40.

Table 3: Results of MIC for S. typhi MTCC3216 of fruits of A. marmelos

Sr. No.	Extracts	Dilution of Extracts (µg/ml)						
		2000	1000	500	250	125	62.50	31.25
1.	Petr. ether	+	+	+	+	+	+	+
2.	AP	-	-	-	+	+	+	+
3.	FPH	-	-	-	-	+	+	+
4.	FPC	-	-	-	-	+	+	+
5.	Ethanol	+	+	+	+	+	+	+
6.	AE	-	-	-	-	+	+	+
7.	FEH	-	-	-	-	+	+	+
8.	FEC	-	-	-	-	+	+	+

Note: Petr. ether: Petroleum ether; AP: Ampicilin in Petroleum ether; FPC: Fruit extract in Petroleum ether by Cold extraction; FPH: Fruit extract in Petroleum ether by Hot extraction; AE: Ampicilin in Ethanol; FEH: Fruit extract in Ethanol by Hot extraction; FEC: Fruit extract in Ethanol by Cold extraction. '+': presence of growth '-': absence of growth.

Figure 2: Result of MIC using ethanol extraction of fruit of A. marmelos for S. typhi MTCC3216.

Table 4: Results of MIC of A. marmelos for S. aureus MTCC2581.

Sr. No.	Extracts	Dilution of Extracts (µg/ml)						
		2000	1000	500	250	125	62.50	31.25
1.	Petr. ether	+	+	+	+	+	+	+
2.	AP	-	-	+	+	+	+	+
3.	FPH	-	-	-	+	+	+	+
4.	FPC	-	-	-	+	+	+	+
5.	Ethanol	+	+	+	+	+	+	+
6.	AE	-	-	-	+	+	+	+
7.	FEH	-	-	-	+	+	+	+
8.	FEC	-	-	-	+	+	+	+

Note: Petr. ether: Petroleum ether; AP: Ampicilin in Petroleum ether; FPC: Fruit extract in Petroleum ether by Cold extraction; FPH: Fruit extract in Petroleum ether by Hot extraction; AE: Ampicilin in Ethanol; FEH:Fruit extract in Ethanol by Hot extraction; FEC: Fruit extract in Ethanol by Cold extraction. '+': presence of growth '-': absence of growth.

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

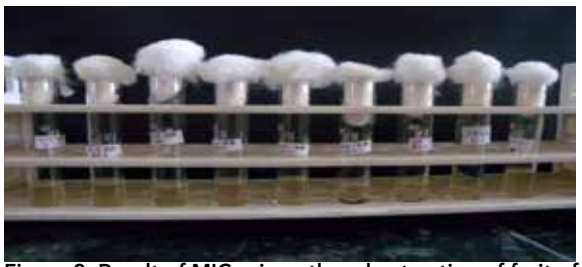

Figure 3: Result of MIC using ethanol extraction of fruit of *A. marmelos* for *S. aureus* MTCC2581.

Table 5: Results of MIC of *A. marmelos* for *P. vulgaris* MTCC428.

Sr. No.	Extracts	Dilution of Extracts (µg/ml)						
		2000	1000	500	250	125	62.50	31.25
1.	Petr. ether	+	+	+	+	+	+	+
2.	AP	-	-	-	+	+	+	+
3.	FPH	-	-	-	-	+	+	+
4.	FPC	-	-	-	-	+	+	+
5.	Ethanol	+	+	+	+	+	+	+
6.	AE	-	-	-	+	+	+	+
7.	FEH	-	-	-	-	-	+	+
8.	FEC	-	-	-	-	-	+	+

Note: Petr. ether: Petroleum ether; AP: Ampicilin in Petroleum ether; FPC: Fruit extract in Petroleum ether by Cold extraction; FPH: Fruit extract in Petroleum ether by Hot extraction; AE: Ampicilin in Ethanol; FEH:Fruit extract in Ethanol by Hot extraction; FEC: Fruit extract in Ethanol by Cold extraction. '+': presence of growth '-': absence of growth.

Figure 4: Result of MIC using ethanol extraction of fruit of A. marmelos for P. vulgaris MTCC428.

The growth of *E. coli* MTCC40 in fruit's extract of *A. marmelos* in petroleum ether and ethanol, Ampicilin was seen below the concentrations of 500µg/ml concentrations. So, 500µg/ml is MIC of fruit's extract of *A. marmelos* in petroleum ether and ethanol (Table 2, Fig.1).

Similarly, the growth of *S. typhi* MTCC3216 in fruit's extract of *A. marmelos* in petroleum ether and ethanol was seen below the concentrations of 250µg/ml concentration. So, 250µg/ml is MIC of fruit extract of *A. marmelos* in petroleum ether and ethanol (Table 3, Fig. 2).

In the same way MIC of each extracts for *S. aureus* is 500µg/ml (Table 4, Fig. 3). While 250µg/ml is MIC of fruit extract of *A. marmelos* in petroleum ether for *P. vulgaris* & 125µg/ml is MIC of fruit extract in ethanol (Table 5, Fig.4).

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