

Antibacterial properties of *Aegle marmelos* leaves, fruits and peels against various pathogens

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Abstract: The present study was designated to evaluate the antibacterial activities of Ethanolic, Methanolic, Ethyl acetate and Hot water extract from leaves, fruits and peels of *Aegle marmelos*. Compare to all parts, the fruits were showing best result. The antibacterial activities of the extracts against bacteria were tested by agar well diffusion assay and the MIC values were determined by broth dilution assay. The methanolic and hot water extracts showed least antibacterial activity as compared to ethanolic and ethyl acetate extracts. The MIC values were obtained 1.98 mg/ml in ethanolic and ethyl acetate extract of fruits against *S. aureus* and 11.90mg/ml in methanolic extract against *P. aeruginosa*. The antibacterial compound mainly found in *Aegle marmelos* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

Keywords: Antibacterial properties, ethanolic and methanolic plant extract, MIC, zone of inhibition.

Introduction:

The value of medicinal plant in today's world is that they become a potential source for bioactive compounds [1]. The chemicals obtained from medicinal plant are known as phytochemicals serve as lead compounds in drug discovery and design [2,3]. Medicinal plants are valuable for getting novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs [4]. World Health Organization pointed out that more than 80% of world's population depends on plants for primary health care needs. In recent years, multiple drug/ chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects [1]. *Aegle marmelos* (L.) Corr., belongs to the family Rutaceae and is popularly known as Bael tree [5,6]. Hindu physicians regard the unripe or half ripe fruit as astringent, digestive and stomachic and prescribe it for diarrhoea and dysentery [7]. The fresh juice of the leaves is taken with honey as a laxative and febrifuge; it is used in asthmatic complaints. The fruit is used as a remedy for diarrhoea. Two tolas of bark juice is given with a cummin in milk to increase the quality of seminal fluid. The tribals in Salem, Dharmapuri, Vellore regions, Tamilnadu, India offer leaves in the month of July/ August, to god to overcome sterility problem and subsequent year the couples are blessed with the child, Therefore, *A. marmelos* is considered as an emblem of fertility. Beverages prepared with fruit pulp are used to relieve body heat. Cologne is obtained by distillation from flowers [8].

The present studies is carried out to check antibacterial activity of *Aegle marmelos* leaves fruits and peels against one gram positive bacteria (*S. aureus*) and two gram negative bacteria (*P. aeruginosa* and *E. coli*), and also to check the phytochemicals present in sample which are responsible for antibacterial activity.

Materials and Methods:

Collection of Pant:

The *Aegle marmelos* leaves, fruits and peels were collected from local area in Gomti Nagar, Lucknow.

Preparation of Plant Extract:

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant. *Aegle marmelos* leaves, fruits and peels were washed with distilled water and kept in incubator at 37°C for 3-4 days and grinded into fine powder. Now plant material was dissolved in 70% ethanol and 80% methanol, Ethyl acetate and Hot water (1:10); 1 g sample should be dissolved in 10 ml of solvent. Mixtures were kept in the dark for 3 days at room temperature in sterilized beakers wrapped with aluminum foil to avoid evaporation and exposure to sunlight was avoided. After 3 days, mixtures were filtered through whatman no.1 filter paper and kept it in incubator at 37°C till all solvents had completely evaporated from mixtures. Now all mixtures were dissolved in DMSO (Dimethyl sulfoxide).

Tested microorganisms:

Bacterial cultures were obtained from IMTECH, Chandigarh. Subcultures were maintained by MRD LifeSciences, Lucknow. One gram positive culture- *Staphylococcus aureus* (MTCC 2940) and two gram

negative cultures- *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC 739) were used.

Antibiogram analysis:

The antimicrobial activity of *Aegle marmelos* was evaluated against bacterial strains in Ethanolic, Methanolic, Ethyl acetate and Hot water extracts by using agar well diffusion method [9]. Nutrient agar plates were prepared for all extracts, 50 μ l inoculums of each selected bacterium was uniformly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. The equal volume (50 μ l) of antibiotic (tetracycline), distilled water and plant extract were poured into the wells. The plates were incubated at 37°C for 24 hrs.

Determination of minimum inhibitory concentration (MIC) of ethanolic, methanolic, ethyl acetate and hot water extract:

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation at 37°C in shaker Incubator [10,11]. MIC of all samples was determined by broth dilution method. A two-fold serial dilution of the methanolic, ethanolic, ethyl acetate and hot water extracts were prepared and optical density was measured at 600 nm [12].

Phytochemical Tests:

The leaves, peels and fruits extracts were screened for some secondary metabolites like-saponins, tannins, alkaloids, anthraquinones, phlobatannins, flavonoids, terpenoids, reducing sugar and poly phenols [13].

Test for reducing sugar:

Take 1ml or 1gm of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 100°C in a water bath. Brick red precipitate shows a positive result.

Test for tannins:

Take 2gm of aqueous extract in a test tube and add 2 drops of 5% ferric chloride, brown color gives positive result.

Test for phlobatannins:

Take 2ml plant sample in a test tube and add 10ml deionized water and boil at 100°C with few drops of 1%HCl. Deposition of red precipitation gives positive result.

Test for Saponins:

Saponin content is determined by boiling 1ml plant sample in 10 ml deionized water for 15 min. and after cooling the extract was shaken vigorously to record froth formation.

Test for terpenoids:

Take 5ml of aqueous extract add 2ml chloroform followed by addition of 3ml conc. sulfuric acid, observe the reddish brown interface for presence of terpenoids.

Test for alkaloids:

Take 1ml of aqueous extract in test tubes and add 2-3 drops of wagners reagent it gives orange red precipitation.

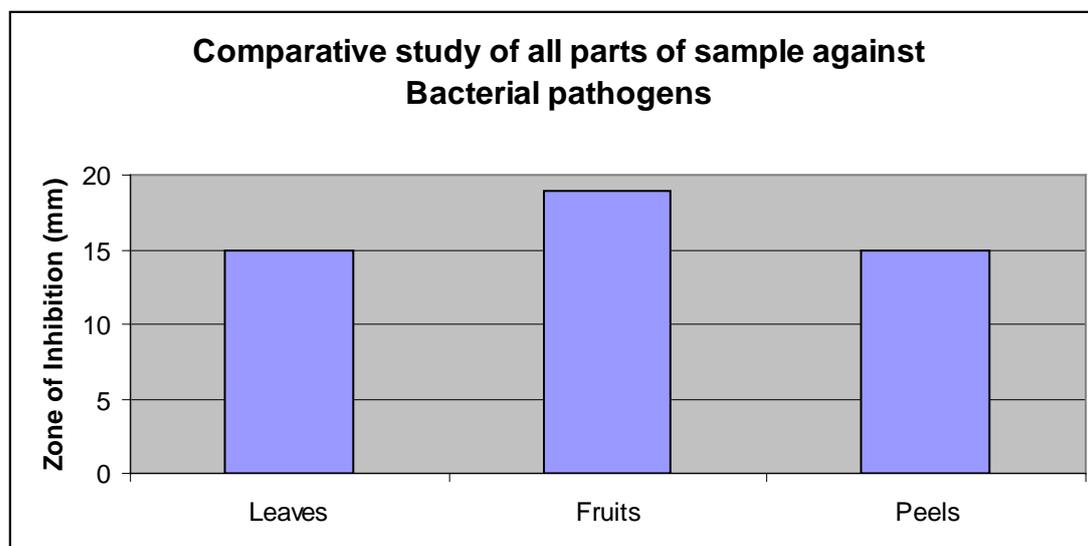
Test for flavonoids:

Add few drops of 1% NH₃ yellow colour observed, showed presence of flavonoids then after this take ethanolic or aqueous extract and add 10ml DMSO then heat it followed by adding Mg (magnesium chloride), add conc. HCl gives red color to confirmed flavonoids.

Test for poly phenols:

Take 2ml ethanolic extract of plant sample and add 1ml folin-ciocalteu reagent and 9ml d/w. between 1-8 min. and add sodium carbonate solution (8ml) vortex to mix then kept test tube in dark take O.D at 760nm.

Results:



Graph 1: Graph showed that *A. marmelos* fruits were having maximum antibacterial activity, compare to leaves and peels.

Graph 2: Graph showed that compare to all solvents, ethanol and ethyl acetate were having maximum antibacterial activity followed by methanol and hot water.

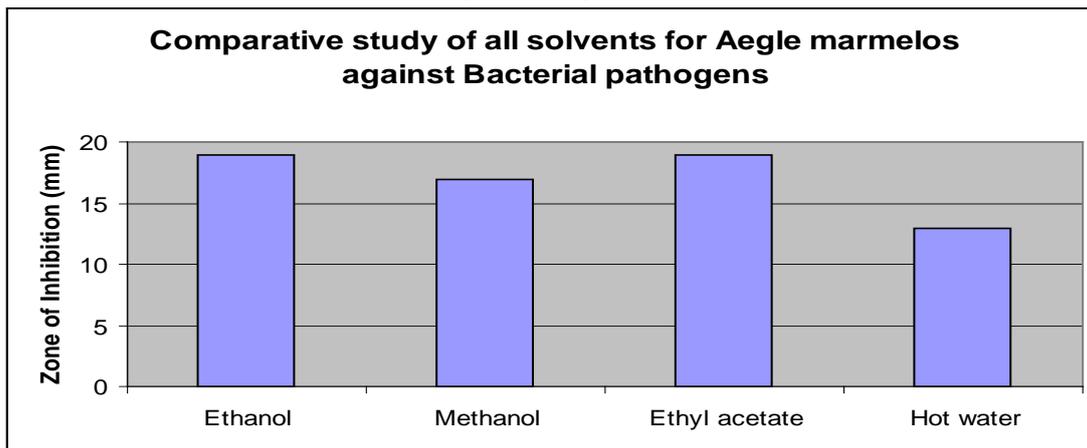
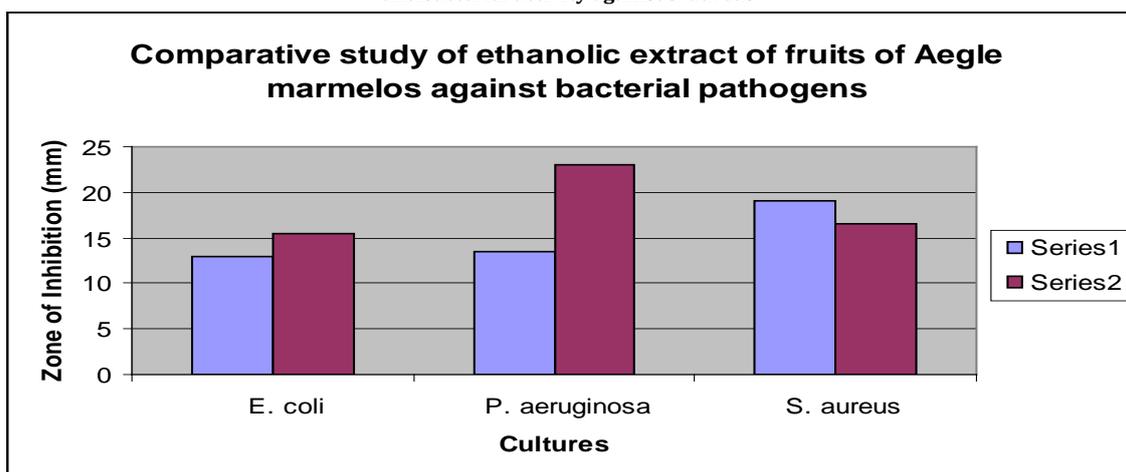


Table 1: Antibiogram of ethanolic extract of *Aegle marmelos* fruits against different bacterial pathogens

Cultures	Zone of inhibition by sample (mm)	Zone of inhibition by Tetracycline (mm)
<i>E. coli</i>	13	15.5
<i>P. aeruginosa</i>	13.5	23
<i>S. aureus</i>	19	16.5

Graph 3: Table 1 and Graph 3 showed that ethanolic extract of *Aegle marmelos* fruits were having maximum antibacterial activity against *S. aureus*

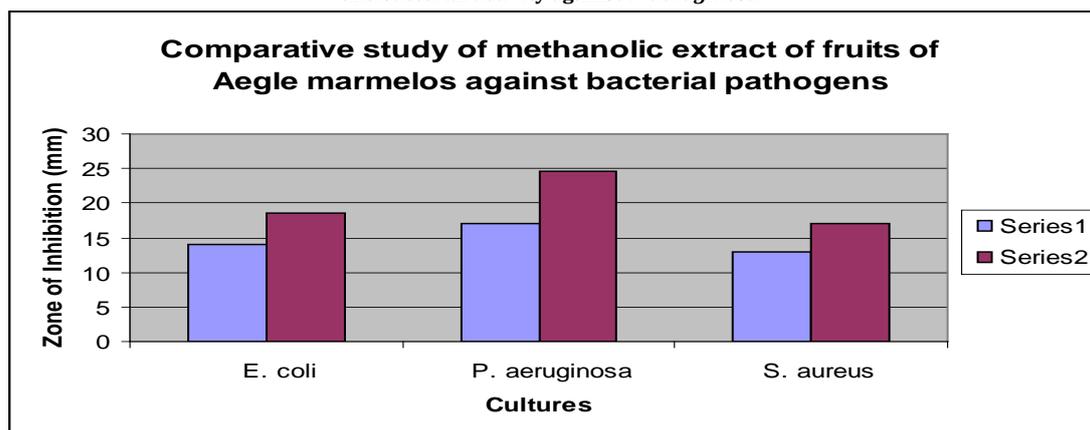


Series 1= Sample, Series 2= Tetracycline

Table 2: Antibiogram of methanolic extract of *Aegle marmelos* fruits against different bacterial pathogens

Cultures	Zone of inhibition by sample (mm)	Zone of inhibition by Tetracycline (mm)
<i>E. coli</i>	14	18.5
<i>P. aeruginosa</i>	17	24.5
<i>S. aureus</i>	13	17

Graph 4: showed that methanolic extract of *Aegle marmelos* fruits were having maximum antibacterial activity against *P. aeruginosa*.

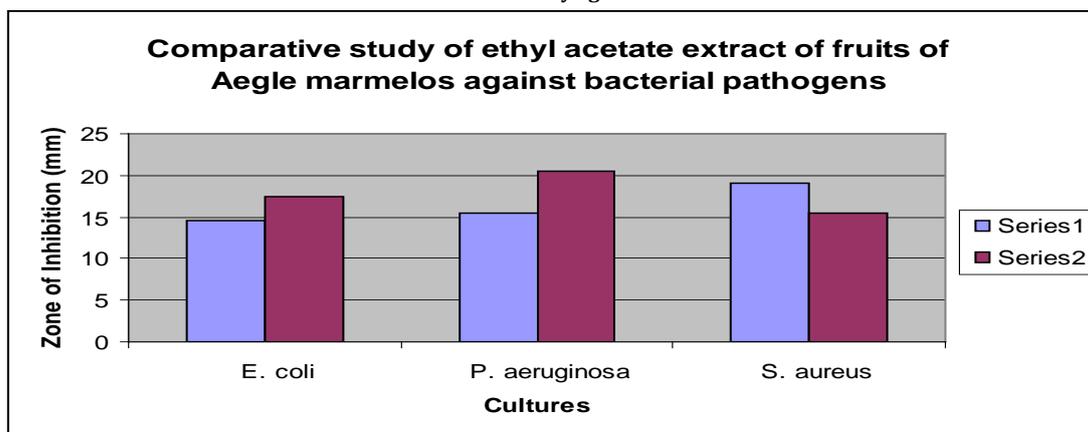


Series 1= Sample, Series 2= Tetracycline

Table 3: Antibiogram of ethyl acetate extract of *Aegle marmelos* fruits against different bacterial pathogens:

Cultures	Zone of inhibition by sample (mm)	Zone of inhibition by Tetracycline (mm)
<i>E. coli</i>	14.5	17.5
<i>P. aeruginosa</i>	15.5	20.5
<i>S. aureus</i>	19	15.5

Graph 5: Table 3 and Graph 5 showed that ethanolic extract of *Aegle marmelos* fruits were having maximum antibacterial activity against *S. aureus*

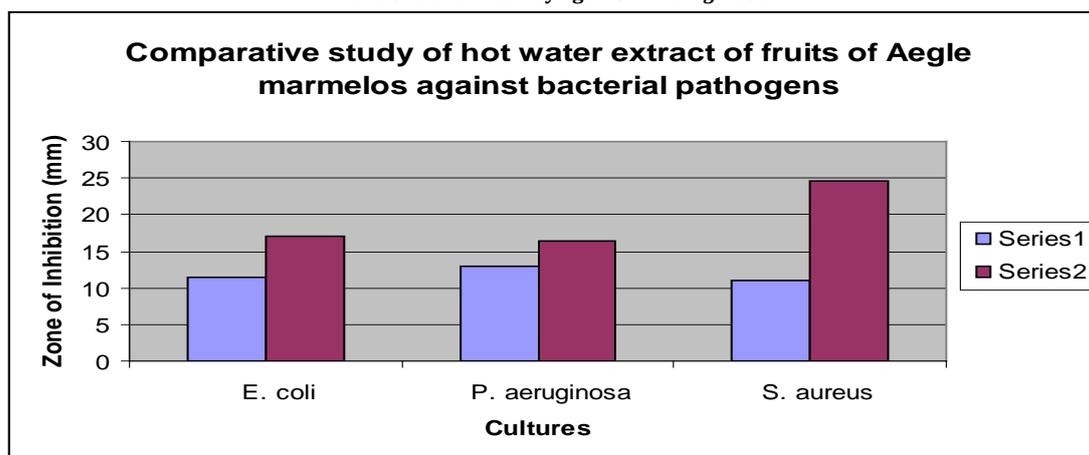


Series 1= Sample, Series 2= Tetracycline

Table 4: Antibiogram of hot water extract of *Aegle marmelos* fruit against different bacterial pathogens:

Cultures	Zone of inhibition by sample (mm)	Zone of inhibition by Tetracycline (mm)
<i>E. coli</i>	11.5	17
<i>P. aeruginosa</i>	13	16.5
<i>S. aureus</i>	11	24.5

Graph 6: Table 4 and Graph 6 showed that methanolic extract of *Aegle marmelos* fruits were having maximum antibacterial activity against *P. aeruginosa*

**Table 5: MIC value of fruits against bacterial pathogens for solvents**

Test tube	Conc. of extracts (mg/ml)	Ethanolic extract of fruits O.D against <i>S. aureus</i> (600nm)	Ethyl acetate extract of fruits O.D against <i>E. coli</i> (600nm)	Methanolic extracts of fruits O.D against <i>P. aeruginosa</i> (600nm)
1	71.92	0.85	0.65	0.38
2	11.90	0.62	0.62	0.20
3	1.98	0.50	0.28	0.53
4	0.33	0.70	0.82	0.40
5	0.05	0.49	0.81	0.51
6	0.009	0.51	0.59	0.41

Table showed that the least concentration were obtained 1.98 mg/ml in ethanolic and ethyl acetate extract of fruits against *S. aureus* and 11.90mg/ml in methanolic extract against *P. aeruginosa*

Table 6: Phytochemicals Analysis

Tests	Leaves	Fruits	Peels
Reducing sugar	-	+	+
Tannins	+	+	+
Phlobatannins	+	+	+
Saponins	+	+	+
Terpenoids	+	+	+
Alkaloids	+	-	-
Flavonoids	-	-	-
Poly phenols	+	+	+

Table showed that the Phytochemicals mainly found in *Aegle marmelos* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

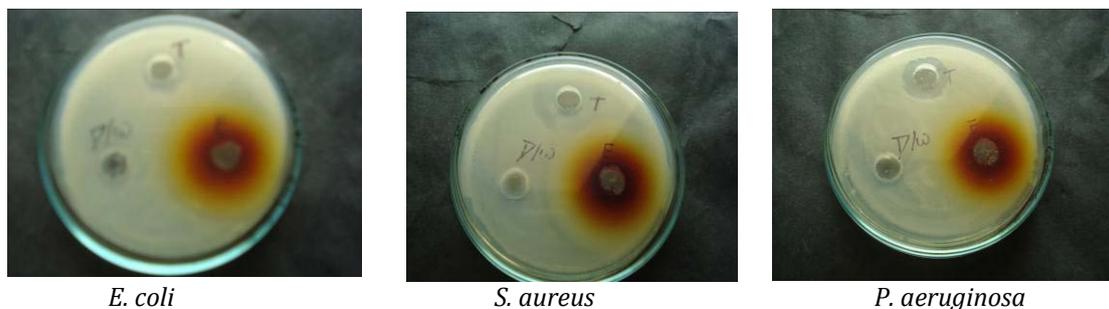


Figure 1: Antibacterial activity of ethanolic extract of fruits:

Figure showed that *S. aureus* was having maximum zone of inhibition compare to *E. coli* and *P. aeruginosa*.

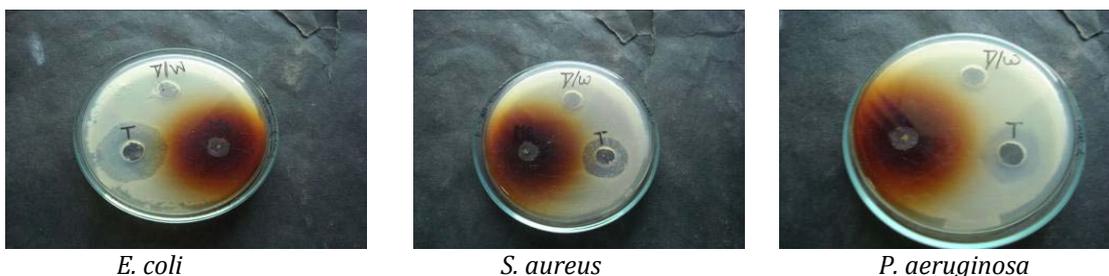


Figure 2: Antibacterial activity of methanolic extract of fruits:

Figure showed that *P. aeruginosa* was having maximum zone of inhibition compare to *E. coli* and *S. aureus*.

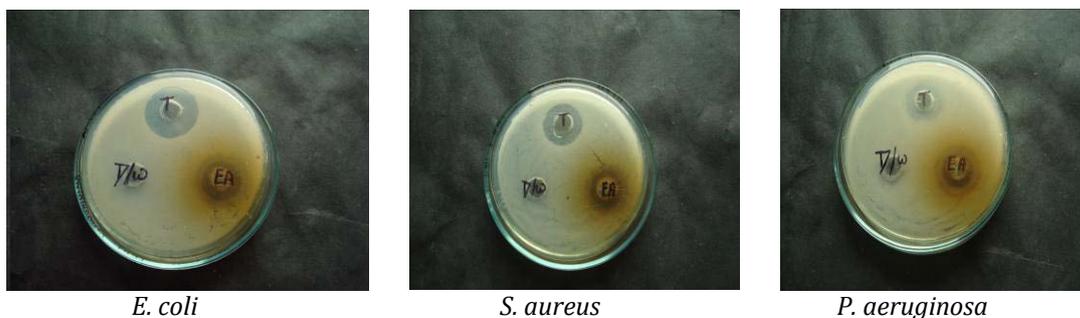


Figure 3: Antibacterial activity of ethyl acetate extract of fruits:

Figure showed that *S. aureus* was having maximum zone of inhibition compare to *E. coli* and *P. aeruginosa*.

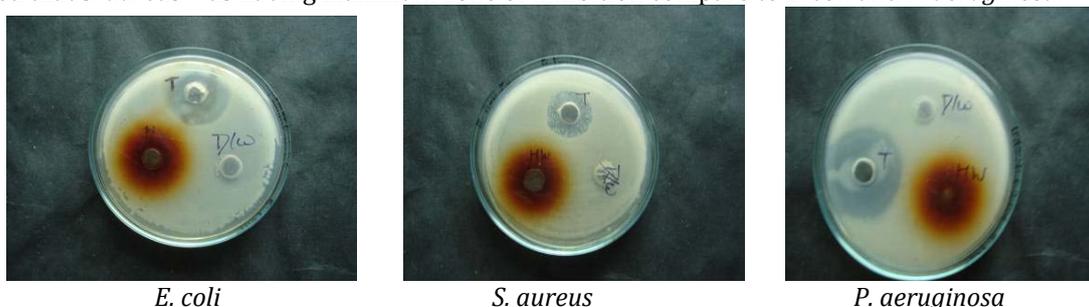


Figure 4: Antibacterial activity of hot water extract of fruits:

Figure showed that *P. aeruginosa* was having maximum zone of inhibition compare to *E. coli* and *S. aureus*.

Discussion:

Plants are known to have beneficial therapeutic effects in traditional Indian system of medicine. Much work has been done on ethnomedicinal plants in India. It has been suggested that phytochemical extracts from plants are used in allopathic medicine as they are potential sources of antiviral, antitumor and antimicrobial agents [14,15]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Plants have been reported to possess antimicrobial, antifungal and other activities. This has been elucidated by various workers [16,17,18]. In this present study the antibacterial activity was found to be best in fruits of the *aegle marmelos* against bacterial pathogens compare to all peels and leaves. Also compare to all solvents the ethanolic and ethyl acetate extracts of *Aegle marmelos* fruits were

showing best result while the methanolic and hot water extract were showing minimum inhibition. The Antibiogram analysis showed that zone of inhibition was observed 19 mm against *S. aureus* for ethyl acetate extract. The MIC values were obtained 1.98 mg/ml in ethanolic and ethyl acetate extract of fruits against *S. aureus* and 11.90mg/ml in methanolic extract against *P. aeruginosa*. The antibacterial compound mainly found in *Aegle marmelos* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

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