



Evaluation of the Antibacterial Activity of *Aegle Marmelos* at different Concentrations and temperatures

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Abstract:

Aegle marmelos (Bael plant) is one of the most common ayurvedic plant traditionally used in medicine for the treatment of infectious diseases. The present study was emphasized on the antibacterial activity of the *Agale marmeols* leaves extract on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeuroginosa*, *Salmonella typhimurium*, and Methicillin Resistant *Staphylococcus aureus* (MRSA). Methanolic extract was found to be effective against all organisms. Dual extracts were also found to be effective in our study against the test organisms. Various concentrations of the extracts (25µl-125µl) as well as the Effect of temperature (25 °C-100 °C) on the antibacterial activity was also studied. The highest antibacterial activity was found to be around 1.2cm reported in *Bacillus subtilis* and the lowest antibacterial activity was found to be around 0.3cm reported in *Pseudomonas aeuroginosa*. Our results suggest that Bael leaves can be used in treating infections caused by test organisms.

Keywords: *Aegle marmelos*, *Bacillus subtilis*, MRSA, antibacterial activity, Effect of temperature.

Introduction:

Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of modern medicine. It is said that Ayurveda, the science of medicinal plants has taken its origin from India. Ancient scriptures written by saints explained the important values of medicinal plants as a cure for dreadful diseases [1, 2]. In recent years, multiple drug and chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs. Plants are found to possess many biological active components which played a major role against various diseases. Plants belonging to the family Rutaceae are one of the oldest Indian medicinal plants, one such plant *Aegle marmelos*, commonly referred to as Bilva, Bael and Golden Apple takes its origin from Eastern Ghats and Middle India and is commonly found in dry parts of our country including Himalayas. Bael is said to

Play an important role in Bioremediation. Bael is said to possess properties such as anti-diarrheal, anti-dysenteric, anti-pyretic, anti-inflammatory, anti-lipidemic [3], anti-fungal activity and acts as an astringent. The ripe fruit is used as a laxative [4, 5, 6], root bark is used as a remedy in hypochondriasis, melancholia and palpitation of the heart. Leaves act as a remedy for asthmatic treatments [7, 8]. As the plant supports many medicinal properties, it is mainly used in the treatment of many metabolic and inflammatory disorders. The present study was aimed to evaluate the antibacterial activity of the *Aegle marmelos* leaves against pathogenic microorganisms along with studying the phytochemical properties of the plant.

Materials and methods:

Identification of Plant

Bael is a medium sized deciduous tree bearing strong axillary thorns. Leaves with 3 or 5 leaflets. The flowers are bisexual nearly 2 cm wide, borne in clusters, sweet scented and greenish white. Seeds are numerous and arranged in the cells surrounded by slimy transparent mucilage. Seeds have wooly hairs. The bark is shallowly furrowed and corky. Leaves, fruit, stem and roots of this tree at all stages of maturity are used as ethno medicine against various human ailments.

Figure 1: The leaves and plant of Bael for morphological identification



Collection of leaves:

The leaves were collected from the plant in a sterile polythene cover and dried for 2 weeks under shade and were grounded to fine powder in an electric blender and sieved to get a particle size of 50-150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction.

Preparation of Extract:

The extraction procedure was done by soaking the leaf sample weighed (25-50g) in aqueous extracts such as⁴: Methanol, Hexane, Ethyl acetate, Acetone, Chloroform. Dual extracts (combinations) of Ethyl acetate: Methanol (EA+M) and Hexane: Acetone (H+A) were prepared and kept sterile in a conical flask and kept in a shaker incubator for 48 hours. Further, samples were filtered using Whatmann no.1 filter paper. These extracts were concentrated using the concentrator (1 µg/ µL).

Phytochemical Screening:

The extracts were screened for the presence of phytochemical components such as alkaloids, tannins, flavonoids, carbohydrates, and phenol compounds using Kemp (1986) and Sofowara (1982) method^[9, 10].

Antibacterial activity:

Microbial cultures:

The test organisms used were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and Methicillin Resistant *Staphylococcus aureus* (MRSA). The inoculum for each microorganism was prepared from broth cultures (10^5 CFU/ml) and was maintained on Nutrient agar plates at 37°C in an incubator^[11]

Determination of Antibacterial activity:

The agar diffusion method was used to detect the antibacterial activities of the extracts. Inoculum density of each bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 McFarland standard, resulting in a suspension containing approximately $1-2 \times 10^5$ CFU/mL. Nutrient agar plates were inoculated by streaking the swab over the entire sterile agar surface. The inoculum was allowed to dry at room temperature. Four equidistant holes were made in the agar using sterile cork borers and 15µL of extract was added to each well^[12, 13]

Antibacterial activity at various concentrations:

Various extracts prepared were added to the plate at different concentrations ranging from 25µl-150µl (25 µl, 75 µl, 100 µl, 150 µl). Further these plates were incubated at 37°C in an incubator for overnight.

Antibacterial activity at various temperatures:

Extracts were exposed to various temperatures ranging from 25°C-125°C. Immediately after exposure they were added to plates. Further these plates were incubated at 37°C in an incubator for overnight. This was performed in order to understand the activity of extract with respect to temperature and its effect on various microorganisms⁶.

Results and Discussion:

Antibacterial Activity:

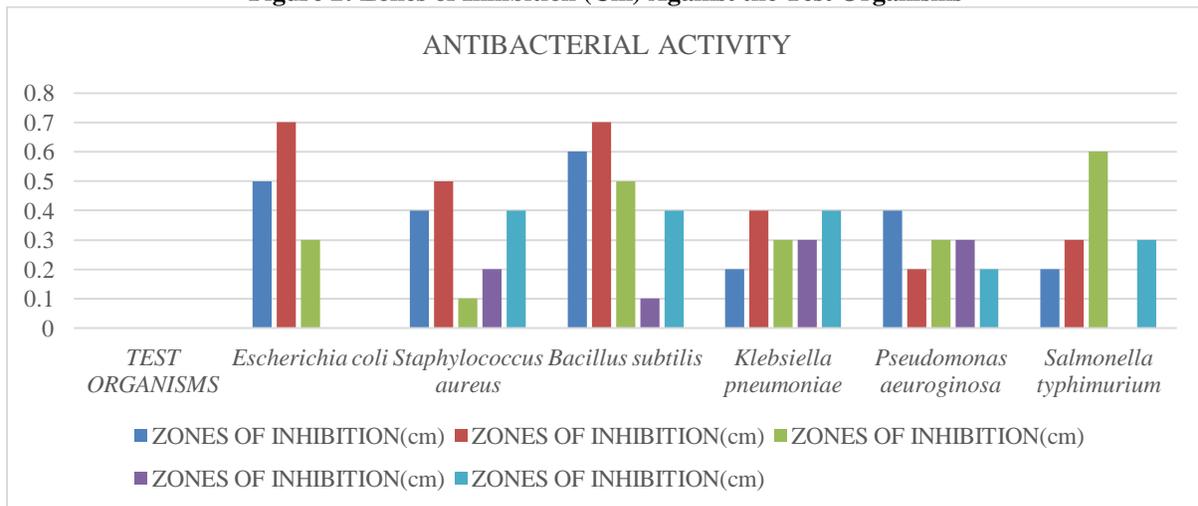
From our work, Bael leaf extracts were found to be effective against bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium* and MRSA. There were significant zones of inhibition observed with different bacterial species using various extracts. Methanolic extract was found to be very effective against all organisms. The zones were found to be highest in *Bacillus subtilis* and the least was observed in MRSA. Whereas, there was no effect on *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The zones of inhibition were ranging between 0.3-1.2cm. Ethyl acetate was found to be effective against *Salmonella typhimurium*. The zones of inhibition formed were 0.3-0.8cm. Acetone extract has not shown any activity on *Staphylococcus aureus* and *Pseudomonas aeruginosa* but has relatively expressed its action on other organisms, the zones of inhibition ranges from 0.3-0.7cm. Chloroform extract of bael has shown highest antibacterial activity against *Staphylococcus aureus* which was found to be 1cm. The extracts used such as methanol, ethyl acetate, acetone and chloroform were found to be very diffusive when added to the well. Similar results were reported by^[14, 15] controls studied were the Aqueous extracts which did not show any effect against bacterial strains. As these extracts were found to be effective we had planned using dual extracts which is mixing two extracts like Ethyl Acetate: Methanol and Hexane: Acetone. Dual extracts was also simultaneously tested for antibacterial activity. This was performed for the first time using Bael leaf. Maximum zones were observed in the combination of EA+M and the zone of inhibition was highest for *Bacillus subtilis*. H+A has not shown any antibacterial activity in all strains tested. The results

were tabulated in Table: 1. Antibacterial activity for all the organisms were demonstrated in the Figure 2.

Table: 1 Antibacterial Activity of the Bael Leaf Extracts

EXTRACTS	ZONES OF INHIBITION(cm)				
	METHANOL	ETHYL ACETATE	ACETONE	CHLOROFORM	ETHYL ACETATE+METHANOL
TEST ORGANISMS					
<i>Escherichia coli</i>	0.75	0.2	0.6	0.5	0.3
<i>Staphylococcus aureus</i>	0.6	0.7	0	1	0.5
<i>Bacillus subtilis</i>	1.2	0.3	0.7	0.5	0.6
<i>Klebsiella pneumoniae</i>	0.2	0.4	0	0	0.2
<i>Pseudomonas aeruginosa</i>	0.3	0	0	0.2	0
<i>Salmonella typhimurium</i>	0.7	0.8	0.3	0.2	0.2
<i>MRSA</i>	0.3	0	0.4	0	0

Figure 2: Zones of Inhibition (Cm) Against the Test Organisms



Antibacterial Activity at Various Concentrations:

In order to study at which concentration there would be maximum activity of the extracts; we have tested various concentrations ranging from 25µl -150µl for all the extracts. The highest was reported in Methanol extract. Methanolic extract has shown its activity on all organisms except *Klebsiella pneumoniae* and the zones of inhibition were ranging from 0.3-0.7cm at the concentrations of 25µl -100µl. Ethyl Acetate has shown its activity on *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Salmonella typhimurium* at the concentrations of 50µl-100µl and the zones were ranging from 0.3-0.5cm. Acetone extract has shown its antibacterial activity at the concentration of 50 µl in *E.coli*, *MRSA* and *Salmonella typhimurium* and the zones of inhibition were found to be in the range of 0.3-0.6cm. Chloroform extract has shown its antibacterial activity at a concentration of 50µl -100µl on all organisms except *MRSA* and *Klebsiella pneumoniae*. Results of zones of inhibition for the concentrations against all bacterial species were tabulated in table 2.

Figure 3 demonstrates the antibacterial activity at various concentrations.

Effect of Temperature on Antibacterial Activity:

The role of temperature on the antibacterial activity was previously studied. But, in order to understand the effect of temperature on various extracts and role of those extracts on the various organisms, all the extracts prepared were exposed to different temperatures ranging from 25⁰C-125⁰C. Methanolic extract has shown maximum activity at the temperature ranging from 25⁰C-50⁰C in all organisms. Zones of inhibition were found to be 0.2-0.8cm. Hexane extract has shown its activity at the temperature range of 25⁰C-75⁰C in *Escherichia coli* and *Bacillus subtilis* and the zones of inhibition was found to be 0.1-0.8cm. Ethyl acetate extract and Chloroform extract’s activity was seen only at 25⁰C, but in different organisms, Ethyl acetate activity was observed in *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumonia*, whereas chloroform extract activity was seen in *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas*

aeruginosa. Zones of inhibition were found to be 0.2-0.6cm Acetone extract activity was seen at temperatures 25^oC-50^oC in *Escherichia coli*, MRSA and *Salmonella typhimurium*. Zones of Inhibition

were in detail tabulated in table 3. Figure 4 demonstrates the effect of temperature on the antibacterial activity.

Table 2: Antibacterial Activity at Various Concentrations

EXTRACTS	ZONES OF INHIBITION(cm)											
	METHANOL			ETHYL ACETATE			ACETONE			CHLOROFORM		
	25	50	100	25	50	100	25	50	100	25	50	100
TEST ORGANISMS												
<i>Escherichia coli</i>	0.5	0.7	0.3	0	0	0	0.3	0.6	0.2	0.2	0.3	0.2
<i>Staphylococcus aureus</i>	0.4	0.5	0.1	0.2	0.4	0.3	0.3	0.2	0.2	0.3	0.3	0.5
<i>Bacillus subtilis</i>	0.6	0.7	0.5	0.1	0.4	0.5	0.3	0.5	0.4	0.2	0.6	0.5
<i>Klebsiella pneumoniae</i>	0.2	0.4	0.3	0.3	0.4	0.7	0.3	0.4	0.6	0.4	0.6	0.4
<i>Pseudomonas aeruginosa</i>	0.4	0.2	0.3	0.3	0.2	0.4	0.2	0.4	0.3	0	0.3	0.3
<i>Salmonella typhimurium</i>	0.2	0.3	0.6	0	0.3	0.5	0	0.4	0	0	0	0
MRSA	0.3	0.6	0	0	0	0	0	0.5	0	0	0	0

Figure 3: Antibacterial Activity at Various Concentrations

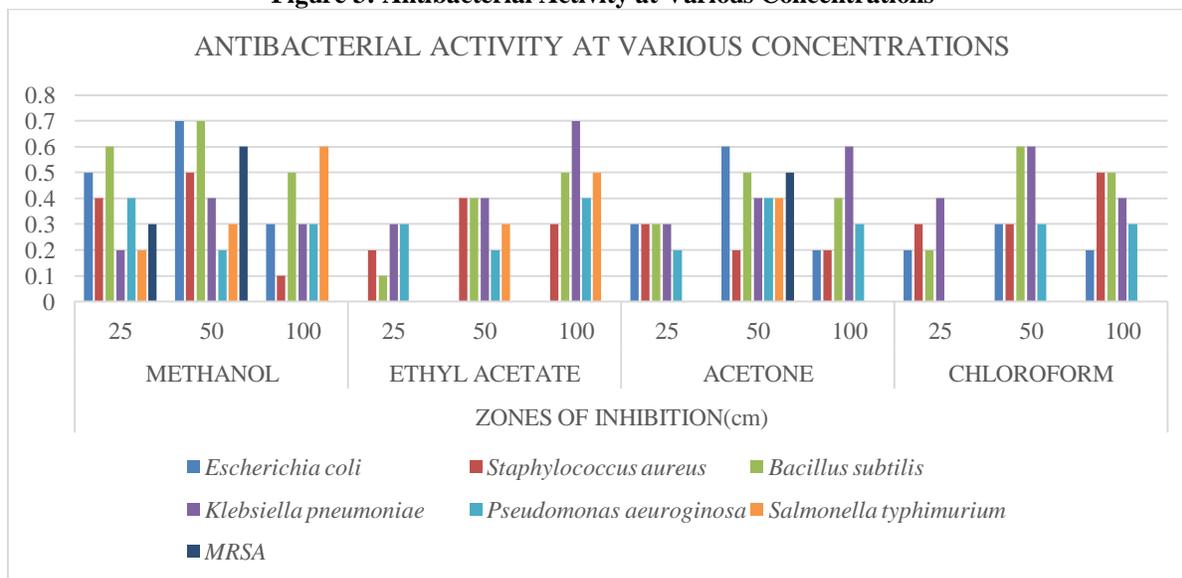
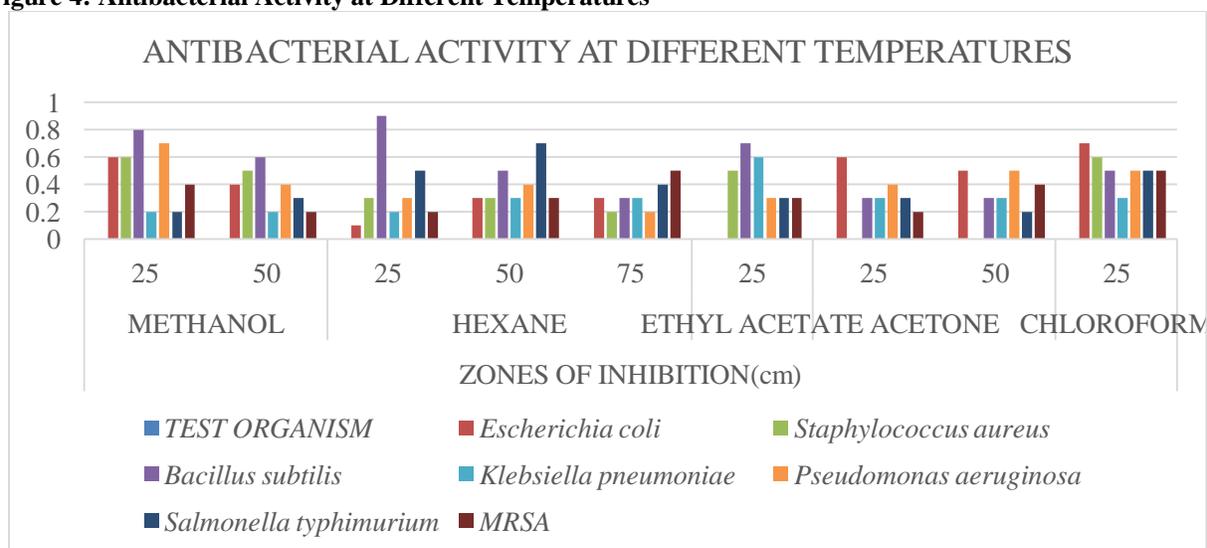


Table 3: Antibacterial Activity at Different Temperatures

EXTRACTS	ZONES OF INHIBITION(cm)								
	METHANOL		HEXANE			ETHYL ACETATE	ACETONE		CHLOROFORM
TEMPERATURE	25	50	25	50	75	25	25	50	25
TEST ORGANISM									
<i>Escherichia coli</i>	0.6	0.4	0.1	0.3	0.3	0	0.6	0.5	0.7
<i>Staphylococcus aureus</i>	0.6	0.5	0.3	0.3	0.2	0.5	0	0	0.6
<i>Bacillus subtilis</i>	0.8	0.6	0.9	0.5	0.3	0.7	0.3	0.3	0.5
<i>Klebsiella pneumoniae</i>	0.2	0.2	0.2	0.3	0.3	0.6	0.3	0.3	0.3
<i>Pseudomonas aeruginosa</i>	0.7	0.4	0.3	0.4	0.2	0.3	0.4	0.5	0.5
<i>Salmonella typhimurium</i>	0.2	0.3	0.5	0.7	0.4	0.3	0.3	0.2	0.5
MRSA	0.4	0.2	0.2	0.3	0.5	0.3	0.2	0.4	0.5

Figure 4: Antibacterial Activity at Different Temperatures



Phytochemical Analysis:

All the phytochemical screening was performed using the standard protocols and the results obtained were tabulated in table 4. Further studies have to be performed in order to understand the role of these phytochemical constituents in the use of traditional medicine.

Anthocyanins	+
Carboxylic Acids	+

Table 4: Phtochemical Analysis

Phytochemical tests	Result
Carbohydrates	+
Amino acids	+
Alkaloids	+
Flavanoids	+
Xanthoproteins	+
Steroids	+
Phytosteroids	+
Coumarins	+

CONCLUSION:

The results from our study conclude that *Agele marmeols* (Bael plant) possess significant antibacterial activity against gram positive and gram negative bacteria. Phytochemical constituents of the plant are one among the contributing elements for the antibacterial activity. Further isolation and purification of these constituents would establish the role of the active components in traditional medicine. The role of these components needs to be further characterised and the detail pharmacological properties have to be studied in order to determine the side effects. Clinical trials have to be performed to authenticate the antibacterial activity of the Bael plant. The other parts of the plant such as root and

stem also have to be studied to confirm its use in medicine for the treatment of infectious diseases.

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