

## *In vitro* Antimicrobial Activity of Fruit Extract of *Aegle marmelos* (Bael)

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### ABSTRACT

*Aegle marmelos* is commonly known as Bael, is a species of tree native to India. The tree has religious importance, The tree belongs to family rutaceae. Various parts of tree such as leaves, fruit, seeds, bark and roots has medicinal properties. The present study aimed with evaluation of methanolic fruit extract for phytochemicals and antimicrobial activity, Phytochemical screening indicates the presence of flavonoids, phenols, saponins and other phytochemicals. Extract has low to moderate antimicrobial activity against gram positive, gram negative bacteria and fungus.

**Key words:** *Aegle marmelos*, Bael, Medicinal properties, Phytochemicals, Antimicrobial activity.

### INTRODUCTION

Traditionally used medicinal plant product has known for their therapeutic properties. Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds<sup>9</sup>. The importance of plants in medicine remains even of greater global shift to obtain drugs, as a result of which attention has been given to the medicinal value of herbal remedies for safety efficacy and economy<sup>3</sup>.

It is reported by various researcher that antimicrobial properties of various medicinal plants are being increasingly from different parts of world<sup>13</sup>. Fruits are important sources of minerals, fibers and vitamins which provides essential nutrients for the human health. Plants are sources of phytochemicals

with strong antioxidant activity have attracted a great deal of attention in recent years. Antioxidants not only inhibit the oxidation of food, but also for defense of living system against oxidative stress<sup>1</sup>.

The increasing public interest and awareness of natural medicine led that pharmaceutical industry and academic researchers to pay more attention to medicinal plant<sup>12</sup>. The trend from western to herbal medicine is due to the fact that synthetic drugs have always shown adverse reaction. This led to believe those natural products are safer because they are more suitable with biological system. The use of medicinal plants for treatment is the oldest method of coping with illness. Therefore phytotherapy has been entered into all system of traditional medicine.

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In recent decades the use of herbal products has increased in developed countries. However due to the popularity and global market expansion, the safety of herbal products has become a major concern in public healthcare<sup>6</sup>.

The search for biologically active compounds from plant extracts has always a huge interest to researcher to find out the new source of drugs. The medicinal value of plants lies in phytochemicals that produce a definite physiological action. These plant constituents are alkaloids, tannins, flavonoids, and phenolic compounds<sup>16</sup>. It is reported that near about all synthetic drugs are resistance to human pathogens. Synthetically number of antibiotics has developed by various pharmacological industries

## MATERIALS AND METHODS

**Sample collection--** Fruits of *Aegle marmelos* (Bael) were collected from rural area of Jintur located in Parbhani district of Maharashtra in India. The fruits were washed thoroughly to remove the dirt in running tap water for 5 min and rinsed with sterile distilled water. The fruit sample was cut into pieces and dried under shade at  $25 \pm 2^\circ \text{C}$  for about 10 days. The dried plant samples were grind well into fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The fruit powder was stored in air sealed polythene bags at room temperature before extraction

Other chemicals including methanol, nutrient agar, nutrient broth, DMSO, procured from sigma company. Ferric chloride, Wagner's reagent, Mayer's reagent, ammonium hydroxide, sodium hydroxide, of fisher scientific Ltd. In present study two bacterial strains and one fungal strain was used. Bacterial strains was Gram positive *Staphylococcus aureus*, Gram negative *Escherichia coli* and fungal strain *Aspergillus niger* was used.

**Preparation of leaf extract-** For extraction methanol was used as solvent. 25 grams of dried *Aegle marmelos* (Bael) fruit powder was packed in a whatman filter paper no.1 and was

extracted in a soxhlet apparatus using 200ml of methanol. After repetition of cycles the concentrate were collected<sup>20</sup>.

**Phytochemical screening-** The concentrate extract of *Aegle marmelos* (Bael) were screened for phytochemical/secondary metabolites analysis. Plant constituents has several therapeutic value. Plant material contain alkaloids, flavonoids, phenols, carbohydrates, amino acids, glycosides, tannins and saponins etc. Alkaloid test was performed using Mayer's test. Flavonoids was screened with Shinoda test. Phenol and tannin was analysed with ferric chloride test. Saponin was screened with foam test. Amino acids were observed with ninhydrin test. Molisch's test were carried for carbohydrates and Fehling reaction for reducing sugar<sup>14,21,22</sup>.

**Antimicrobial activity --** Antimicrobial activity was conducted using the disc diffusion method. The Whatman no.1 filter paper was cut into 5mm in diameter. Different concentration of Bael extract were prepared. Disc of filter paper dipped into different concentration of *Aegle marmelos* (Bael) fruit extract. The test microorganism were transferred using the cotton swabs on surface of sterile nutrient agar plates. Already prepared disc were impregnated on seeded culture plate using sterile forceps with appropriate labelling. Plate were kept in laminar air flow for 30 minute for pre diffusion of extract to occurred and incubated at  $37^\circ \text{C}$  for bacteria and  $27^\circ \text{C}$  for fungus for 24 hrs. Resulting zone of inhibition was measured<sup>4,19</sup>.

## RESULTS

The present study carried out by taking *Aegle marmelos* (Bael) methanolic fruit extract. The study involve the phytochemical screening and antimicrobial activity of fruit extract. The extract showed strong reaction for various phytochemical test. Result of active constituents indicated in presence and absence format in observation table 1.

**Table 1: Phytochemicals screening of *Aegle marmelos* (Bael) fruit extract**

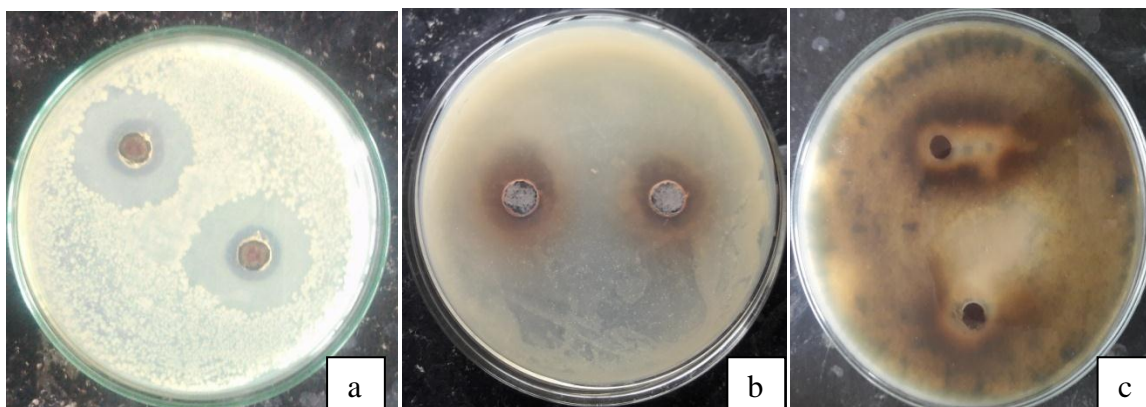
Sr. No.	Active constituents	Test Name	Present/Absent
1	Carbohydrate	Molisch's test	÷
2	Reducing sugar	Fehling test	–
3	Alkaloids	Mayers test	+
4	Tannins	Ferric chloride test	–
5	Proteins	Folin Ciocalteau test	++
6	Flavonoids	Shinoda's test	++
7	Amino acids	Ninhydrin test	+
8	Saponins	Foam test	–
9	Phenols	Ferric chloride test	++
10	Glycosides	Sodium nitroprusside test	–

Antimicrobial activity were performed using disc diffusion method. The fruit concentrate were further dissolved in methanol and used for activity. The activity were completed using different concentrations of fruit extract of *Aegle marmelos*. Antimicrobial activity of fruit extract were analysed against gram positive *Staphylococcus aureus*, gram negative *Escherichia coli* and fungus *Aspergillus niger*. The culture quantity (10µl) of all the

microorganism were kept in equal quantity. The extract showed good antimicrobial activity against all the bacteria and fungus used in study. Comparatively the antimicrobial result indicate that the fruit extract of *Aegle marmelos* (Bael) has significant activity against gram positive microorganism. The result of antibacterial and antifungal activity indicated as zone of inhibition in mm in table 2.

**Table 2: Antimicrobial activity of fruit extract of *Aegle marmelos* (Bael)**

Sr.No.	Microorganism	Amount of culture	Concentration of extract	Zone of inhibition in mm
1	<i>Staphylococcus aureus</i>	10 µl	1mg/ml	5±1
			2mg/ml	8
			3mg/ml	12±2
2	<i>Escherichia coli</i>	10 µl	1mg/ml	3
			2mg/ml	7
			3mg/ml	9±1
3	<i>Aspergillus niger</i>	10 µl	1mg/ml	7
			2mg/ml	7±2
			3mg/ml	9±1

**Antibacterial (a), (b) and antifungal (c) activity of *Aegle marmelos* (Bael) methanolic fruit extract**

## DISCUSSION

Active constituents are the phytoconstituents of plant material used as therapeutic agent in number of infections. Methanolic extracts of the plants showed positive results for many of tests, indicates that methanol is used as a best solvent for extraction of phytochemicals. Herbs have a wide variety of active phytochemicals including flavonoids, terpenoids, lignans, sulphides, polyphenols, carotenoids, coumarins, saponins, plant sterols and phthalides. Several phytoconstituents are used in cancer treatment, several other herbs and their phytoconstituents used in cardiovascular problems enhance the immune system<sup>10</sup>.

The phytochemical screening of fruit extract of *Aegle marmelos* (Bael) showed that they are rich in proteins, flavonoids and phenols. Most of observed phytochemicals showed medicinal activity as well as exhibiting physiological activity. The biological function of alkaloids is very important and is used in analgesic, antispasmodic and bactericidal activities<sup>18</sup>. The secondary metabolites like polyphenols are known as bioactive molecules influenced various activities like reproduction as well as their resistance to parasites and environmental stress. Flavonoids demonstrate the whole spectrum of biological activities<sup>5</sup>. Many polyphenols identified as powerful antioxidant, cardioprotective<sup>7</sup>, anticancer, antithrombotic and antihypertensive<sup>8</sup>.

Antimicrobial activity is the inhibition of growth of bacteria due to activity of phytochemicals<sup>17</sup>. Plant extract used as antimicrobial agents had enormous therapeutic potential as they can serve the purpose for lesser side effects, which often associated with synthetic antimicrobials<sup>11</sup>. The change in chemical composition revealed the change in the antimicrobial activity. The phytochemicals present in any plant material responsible for the antimicrobial activity. Emergence of multidrug resistance in humans and animals to pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin<sup>2</sup>.

The medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicines had been shown to have genuine utility and about 80% of rural population depends on its primary health care over the years. The World Health Organization advocated that countries should interact with traditional medicines with a view to identifying and exploiting aspects that provide safe and effective remedies for ailment of both microbial and non microbial origin<sup>15</sup>.

## CONCLUSION

The active constituents of plants are beneficial for plant itself, involves in defence mechanism. There is no doubt that plants have continued to offer a large range of natural compounds belonging to different molecular families. The fruit extract of *Aegle marmelos* showed various bioactive compounds which are widely used for various activities including traditional medicine. The study required to isolate the active constituents from the fruit extract of *Aegle marmelos*.

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