

# Determine the Antimicrobial potential of leaves of *Aegle marmelos* against *Klebsiella pneumoniae*

<sup>1</sup>Tushi Singh, <sup>2</sup>Rajni, <sup>3</sup>Jagriti Sharma

Department of Biotechnology  
R.B.S. College  
Khandari, Agra.

**Abstract-** Bael, *Aegle marmelos* (Linn.) Correa ex Roxb., belonging to family rutaceae is a medium sized tree of Indian origin found upto an altitude of 1200 meter. Various parts of this versatile tree species such as leaves, roots, seed, bark and fruit etc contain a large number of coumarins, alkaloids, sterols and essential oils hence, possess anti-microfilarial, antifungal, analgesic, anti-inflammatory, antipyretic, hypoglycaemic, antidyslipidemic, immunomodulatory, antiproliferative, wound healing, anti-fertility, and insecticidal abilities. The fruits are also used to prepare a large number of by products such as candy, panjiri, toffee, jam etc with help of various post harvest technologies which also helps to reduce the post harvest losses and hence increases the shelf life which further helps in value addition and in generation a good income for a needy farmer.

**Keywords:** Antibacterial activity, aegle Marmelos, distilled water and ethanol extract, klebsiella pneumoniae MTCC109, zone of inhibition.

## INTRODUCTION



*Aegle marmelos* plant leaves and fruit

India widely called as a botanical garden of the largest producer of medicinal plant. Plant as use in medicine rich source pharmacological activity for treatment of various diseases. Natural medicinal plant are pollution free, no side effect, no toxic effect. Herbal medicine are use well liked developing countries. Bael is economical herbal compound. Bael is a one of medicinal tree in thousand of medicinal plant. Bael (*Aegle marmelos*) tree are a usually near by temple of lord shiva. Bael is also a sacred tree to Lord Shiva. The bael fruit is recognized 'RAMAYAN' period. Bael tree is mention in book of 'CHARKA SAMHITA.

*Aegle marmelos* belongs to family Rutaceae and it is considered to have many medicinal and nutraceutical properties. It commonly known as Bael or wood apple plant and is used in various traditional medicines. The medicinal properties of bael indicate that it is a valuable source of variety of bioactive compounds. Plant and the medicines derived from plant are used by humans to treat and to relief physical and mental illness (Dinesh et al., 2011).

Researchers are now aiming to identify and validate the substances from plants to treat various diseases. It has been found that different parts of plant like leaves, fruits, seeds etc. are helpful to human body as they provide health and nutritional promoting compounds (Ganesh et al., 2011). Bael is a medium sized tree (25-30 feet) slender, aromatic tree, and it grows slowly. Plant is having short stem, flaking soft bark and branches are sometimes spiny. Older plants have straight and stiff spines and sharp spikes of about 1 inch length. The leaflets are 4-10 cm long and 2-5 cm in width and are oval are lancet in shape. Leaves are further divided into 3-5 leaflets and mature leaves are having particular fragrance. Fruit if of diameter 2-4 inches and are spherical or oval in shape. Shell of fruit it woody and hard (Dinesh et al., 2011).

It is found that more than 25% of medicines which are used now days are derived indirectly or directly from plants. From the availability and safety point of view, the medicines derived from plants are considered good over the medicines derived from animals and from chemically synthesized medicines. Adverse effects and many sides effects were observed in people using the synthetic drugs but these effects were less in medicines which are derived from plants. In home remedies, plant found in India are considered the main source of active constituents and used to treat many diseases. In India the rural population is more likely to use traditional way of treatment because this kinds of treatment are easily available and are the cheap price. In present time W.H.O is also encouraging the use of herbal medicine which are used traditionally from last many years. Recently, W.H.O conducted a survey in which they found that around 20,000 medicinal plant are used either in pharmaceutical industry or in traditional medicines system. People are using this herbal medicines because they believe that natural medicines are more effective and are safer to use (**Pushendra et al., 2012**).

After distillation of flowers, a drug is yielded which is used against anti- dysenteric, diaphoretic, intestine and stomach diseases. It was found that its leaf extract in able to regenerate cells of pancreas in diabetic rats. For the treatment of intestinal parasites, like *Ascaris lumbricoides* and *Entamoeba histolytica*, powder of unripe fruit is used. Antimicrobial activity against some micro organisms like *Escherichia coli* was found in oil obtained from seeds. Leaf oil is used against cold and some respiratory infections (**Sandeep et al., 2010**).

In different parts of plant different bioactive compounds are found. But of extraction purpose the solvent should be properly selected. Because some compounds are soluble in one solvent but can not be in other solvents. In other words the yield may also differ with change in solvent. Different part of plant are having various nutritional values. Leaves of bael are used in asthma and laxative for mucus membrane having a free discharge. To reduce or dispel fever decoction of plant leaves is used and also enhances the secretion of mucus from bronchial tube. For inflammation of body parts and severe conjunctival inflammation, hot poultice of leaves is used. Juice of bael leaves is used to treat dropsy and jaundice. Leaf extract of bael is helpful in resting blood glucose, and to keep the body weight to normal values. Leaves of bael works same as insulin works i.e. it promotes the ability to utilize the externally available glucose in body by stimulating glucose uptake. Extract of bael is used to cure some other problems like it helps in lowering blood urea, reduce lipid peroxidation and cholesterol and increased level of super dioxide dismutase, catalase, glutathione peroxidase level in serum and also in liver in experimental diabetic animals (**Sharma et al., 2007**).

Fresh and young leaves of bael are supposed to cause sterility and abortion. Leaves of bael are used to prepare medicated oil which helps to give relief from recurrent cold and respiratory infections. Leaves are used in backpain, abdominal disorders, beriberi, acute bronchitis, child birth, hair tonic, abscess, cut and wounds, nervous disorders, cardio tonic etc. (Gaur et al., 1999). Ripe fruit of bael enhances digestion and also helps to treat inflammation of rectum. Fruit pulp of bael is used to prevent growth of piles. Fresh juice of bael is bitter in taste and helps to lower blood sugar (**Vyas et al., 1979**).

As bael plant contains a lot of antioxidants so it requires to micro propagate the plant using tissue culture techniques. In south India bael is found as red-listed medicinal species. In developing countries about 70% world population is dependent on these treatments for their good health. It was found that seed progeny of bael are not uniform and easily attacked by insects and they have less survival potential.

Vegetative propagation through roots and other conventional method is very slow, difficult and season dependent. So micropropagation technique is used in mass multiplication of bael plant and other fruit species.

The most widely used technique in plant tissue culture is micropropagation. The present study was carried out to develop an efficient in vitro regeneration system via callus phase by using various plant growth regulators and secondary metabolites are extracted from dried powder of leaves and bark several solvents (**Abirami et al., 2013**).

There are some standardized techniques like HPLC, TLC and GCMS etc. to separate and detect which are present in *Aegle marmelos*. From bark of *Aegle marmelos* the bioactive compounds named maremin and faganine are identified. Two pharmacologically active compounds i.e. 1,2- benzenedicarboxylic and Di-n-octyl phthalate are proven to show antimicrobial activity. Terpenes are the phenolic compounds and from bael mono and tri terpenes are also found (**Diana et al., 2014**).

#### PLANT PROFILE

Scientific classification

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliosida

Order: Sapindales

Family: Rutaceae

Subfamily: Aurantioideae

Genus: *Aegle*

Species: *marmelos*

Botanical name: *Aegle marmelos*

**ECOLOGY**-Bael is of Indian origin and can be found in Bangladesh, Egypt, Malaysia, Myanmar, Pakistan, Sri Lanka and Thailand. The tree fills wild in dry woodlands on the slopes and fields of focal and Southern India, just as in Burma, Pakistan, and Bangladesh, just as in blended deciduous and dry timberlands. The soil type is well drained soil, but it has grown well and fruited on the oolitic limestone of Southern Florida. It also grows well in swampy, alkaline or stony soils having pH range from 5 to 8.

**BOTANICAL DESCRIPTION**-It is a slow growing medium to small size tree that can reach a height of 25 to 30 feet. The stem is soft and dense, with a few spiky branches, the lower ones drooping. Young suckers bear many stiff, straight spines. The deciduous, alternate leaves, borne singly or in group, are composed of 3 to 5 oval, pointed and shallowly toothed leaflets, 4-10cm long, 2-5cm wide, the terminal one with a long petiole. More than 100 phytochemical compounds have been isolated from various part of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroid, and tannin. Every part of *A. marmelos* plant such as its fruit, stem, bark, and leaves possess medicinal property and is used for treating various eye and skin infections. The wood of bael plant when freshly cut gives strong aroma and is used for carts, carving tool, knife handles, pestles and comb etc. the fruit of bael is having high nutritional values. Fruit is used to prepare jam, juice, jelly, syrup, toffee and some other products. In reports it was found that pulp of fruit contain water, protein, fat, sugar, fibers, potassium, calcium, iron. These compounds are very well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders.

## MATERIALS AND METHODS

Solvent used during the project work were of high purity obtained. Chemicals used for chemical characterization are ethanol and distilled water and Mueller Hinton Agar (MHA). **Mueller Hinton agar** is a standardized solid medium recommended for the study of the susceptibility of bacteria to antimicrobial agents by the method of diffusion or dilution in agar. It was originally formulated by Mueller or Hinton as a protein free medium for the primary isolation of *Neisseria* species. This non-supplemented medium, initially recommended by Bauer et al, was selected by CLSI, CA-SFM and by EUCAST as the reference medium for several reasons. This culture media is poor in sulfonamides, trimethoprim and tetracycline inhibitors, and allows satisfactory growth of most non-demanding pathogens while demonstrating batch-to-batch reproducibility. Suspend the components, dehydrated powder, in water (38 gm in 1000ml of purified/ distilled water). The medium is boiled for a few seconds until the ingredients are completely dissolved. Sterilize by autoclaving at 121°C (15 lbs) pressure for 15 minutes. Cool to 47°C, mix well before pouring into sterile petri dishes.

**Test organism- Gram negative bacteria-** *Klebsiella pneumonia* (MTCC 109) was used to evaluate the antibacterial activity. Bacterial culture was maintained in NA slants stored at 4°C.

*Klebsiella pneumonia* are bacteria that normally live in your intestines and feces. Experts refer to them as Gram-negative, encapsulated, and nonmobile bacteria. They also have a high tendency to become antibiotic resistant. These bacteria are harmless when they're in your intestines or stool.

## METHODOLOGY-

**Collection of plant material-** Fresh leaves of *Aegle marmelos* were collected from the orchard of Raja Balwant Singh College Khandari, Agra.

**Extract preparation-** The leaves of the plant were properly washed in running tap water and rinsed in distilled water. The rinsed leaves were hot air dried for 3 days.

The dried leaves were pulverized using pestle and mortar to obtain a powdered form which was stored in sterilized air tight glass container at 4°C until used.

The crushed powdered leaves of *Aegle marmelos* were measured by Weighing Balance. The weighing was 15gms of powdered leaves. As shown in the figure.

Extract was prepared by Soxhlet extraction method. A thimble was prepared by using filter paper. About 15 gm of pulverized material was uniformly packed into a thimble and run in Soxhlet extract.

It was extracted with Ethanol (100ml) and Distilled water (100ml) according to the solvents boiling temperature for the period of about 48 hours or 21 cycles or till the solvent in the siphon tube of extractor became colourless. Soxhlet apparatus comprises of extractor, condenser and a round bottom flask. When extract prepared then extract was kept in a refrigerator at 4°C for antibacterial activity.

### **Preparation of Mueller Hinton Agar plates**

Weighing the Mueller Hinton Agar on the Weighing Balance. Suspend 7.5 gm of the medium in 250ml of distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.

Allow to cool at room temperature

### **Antibacterial activity of Aegle marmelos**

Disc diffusion method used to study the antimicrobial activity of plant extracts, agar diffusion method was used against different microorganisms. In this method the pure culture of microorganism is sub cultured nutrient broth and is kept for incubation at 37°C and the stock culture is revived. For preparation of discs (6 mm) Whatman's filter paper was used. These discs were left to dry under laminar air flow in the serial dilution of leaves extracts of Aegle marmelos. These discs were left in different concentration of Ethanol extract and Aqueous extract.

### **Serial dilution**

A serial dilution definition Is a stepwise series of dilutions that are performed to reduce the concentration of a substance in a solution to a more usable concentration. Serial dilutions are made by performing the same dilution multiple times, using certain amounts of previous solutions added to the subsequent dilution. A dilution factor is often used, which indicates the factor by which the stock is diluted.

Serial dilutions are commonly used to avoid having to transfer a very small volume to make a highly diluted liquid. Serial dilution of the leaves of Ethanol and Aqueous extract of Aegle marmelos The dilution blanks are prepared by having tubes filled with an appropriate dilution liquid. To perform serial dilution, 1 ml of the starting sample is added to 9 ml of Dilution Blank Tube 1. This is then followed by the same procedure, where 1 ml from Tube 1 is added to 9 ml of Tube 2, 1 ml from Tube 2 is added to 9 ml from Tube 3, and so on until the desired concentration is reached. So the dilution in these tube 1, 1/10, 1/100 became respectively. 25 micro litre of the suspension were transferred to the discs.

Gentamycin in concentration 0.1 mg/ml was used as standard to check antimicrobial activity.

For negative control the discs were prepared in respective solvent. To check the antimicrobial activity the media used is Muller Hilton agar.

The test organisms that is *Klebsiella pneumoniae* were spread over the solidified Muller Hilton agar plate.

Then the discs already prepared in different extracts were positioned on MH Agar media. Then these plates were kept in microbiological incubator at 37°C for 24 hours. Antimicrobial activity was interpreted by the size of diameter of zone of inhibition i.e, the clear area around the disc.

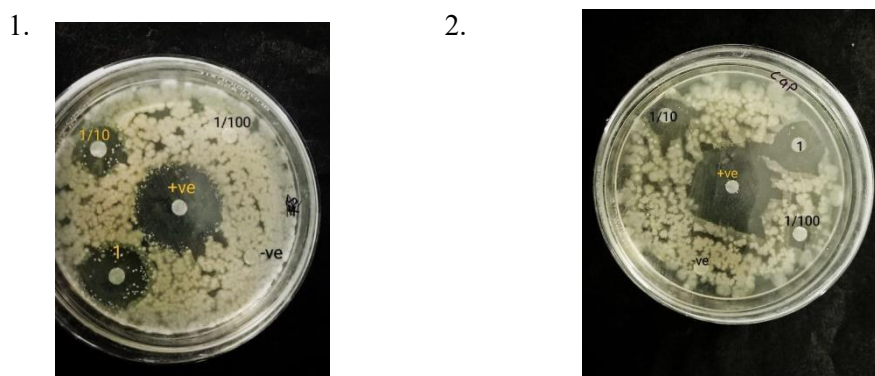
### **RESULT**

In extraction of plant part i.e. leaves, different amount of crude extracts were obtained. It was observed that all the extracts in different solvents are having different yield of crude extract. The amount of crude extract of plant part in different solvents.

It is clear that out of all the extracts, leaf in distilled water had given maximum yield of crude extract. For leaf extraction distilled water is the better solvent. From this it is clear those leaves certain compounds which can be extracted using specific solvent system.

### **Antimicrobial activity-**

The antimicrobial activity of ethanol and distilled water extract of leaf was studied by disc diffusion method. Test organisms for antimicrobial activity are *klebsiella pneumoniae* (MTCC 109). The activity of extract against a particular microorganism is calculated by the diameter of zone of inhibition. Higher diameter of zone of inhibition means the extract is more effective against that microorganism. No zone of inhibition means the microorganism to be tested is resistant against that extract. Gentamycin is an antibiotic which is used as standard to check the antimicrobial activity of plant extract. Effect of different solvent of plant extract on *klebsiella pneumoniae* is shown in figure.



**Fig. effect of leaf extract on klebsiella pneumoniae (1.leaf+distilled water, 2.leaf+ethanol)**

From the studies the ethanol extract showed maximum zone of clearance which indicates that ethanol extract having maximum efficiency against *klebsiella pneumoniae*. Leaf extract in ethanol had shown the antimicrobial effect. In case of distilled water and ethanol extract the zone of inhibition was found to be 13 mm. In Gentamycin the zone of inhibition

was having diameter of 26 mm. when compared with the standard, the distilled water and ethanol extracts had shown 38% inhibition.

**Table: Effect of different solvents on *k. pneumoniae* bacteria.**

S.No.	Organism	Solvent	Zone of inhibition (mm) diameter				
			+ve	-ve	1	1/10	1/100
1.	klebsiella pneumoniae (MTCC109)	Distilled Water	30mm	---	13mm	10mm	---
		Antibiotic- (Gentamycin)					
		Ethanol	30mm	---	18mm	15mm	10mm
		Antibiotic- (Gentamycin)					

Antimicrobial activity against klebsiella pneumoniae was checked using same method. It was found that distilled water and ethanol extract of leaves had shown maximum activity against klebsiella pneumoniae. The diameter of zone of inhibition in case of d. water and ethanol extract of leaves was 13/18mm. The diameter of zone inhibition in standard was 30mm.

## CONCLUSION

Aegle marmelos contains different classes of secondary metabolites are further used as herbal and ayurvedic medicines. The study was conducted to check Antimicrobial activity of Aegle marmelos on bacteria. With this the total of 2 types of extracts were prepared from leaves of Aegle marmelos i.e. aqueous and ethanol. To determine the Antimicrobial properties disc diffusion method: kirby-Bauer method was being used. The results obtained from the present study demonstrated that the bacteria are sensitive against Bael i.e. it inhibits their growth. Conclusion this study, these results definitely portrait Bael Aegle marmelos bacteriostatic agent. Apart from it's antimicrobial potential. Bael is well known health benefits so there is no harm to use bael plant for medicinal properties for a better health. Finally, we can conclude that bael possess antimicrobial properties and could serve as source for future researches. Further studies will be done for new properties for the promised future of mankind.

The conversational methods for plant regeneration are slow and it was found that leaves thus produce are no much viable so researchers are now trying to increase yield of secondary metabolites, production and to make the plant resistant to adverse environmental conditions. In antibacterial activity the extract had shown their inhibitory effect. So further study can be done to know about the unknown compound shown by the peaks of antibacterial activity. By doing this we will be having a wide range of bioactive compounds. Further these bioactive compounds can be isolated and used in preparation of traditional medicines.

## REFERENCES:

1. **Abirami H. and Kumar P.S. (2013).** In Vitro Regeneration and Extraction of Secondary Metabolites In Aegle marmelos (L.) Correa Asian Journal of Plant Science and Research, 3(2): 99-106.
2. **Dinesh S. and Anprung P. (2011).** Bioactive compounds and volatile compounds of Thai bael fruit (Aegle marmelos (L.) Correa) as a valuable source for functional food ingredients. International Food Research Journal, 15(3): 287-295.
3. **Diana S., and Ruhil S., Balhara M., Dhankhar S. And Chhillar A.K. (2014).** Aegle marmelos (Linn.) Correa : A potential Source of Phytomedicine. Journal of Medicinal Plants Research, 5 (9) : 149-150.
4. **Gaur U.K., Varadhrajan V. and Krishnamurthy V. (1999).** Physicochemical evaluation, Phytochemical screening and chromatographic fingerprint profile of Aegle marmelos (L.) Leaf extract. World Journal of Pharmaceutical Research, 1 (3); 813-837.
5. **Ganesh M., Mandal M., Paralakoti Somaiah M. and Murthy S. (2011).** Comparative analysis of antioxidant and antibacterial properties of Aegle marmelos, Coriandrum sativum and Trigonella foenum graecum. Research Article, Acta Biologica Indica, 1 (1); 105-108.
6. **Pushpendra P.K., Sahu J., Sahu L., Prajapati N. K. and Dubey B.K. (2012).** Aegle marmelos: A Review on Properties. International Journal of Phytopharmacological Research, 1(5); 332-341.
7. **Sharma PC, Bhatia V, Bansal N and Sharma A (2007).** A review on Bael Tree. Natural Product Radiance 6(2): 171-178. Ind J Nat Prod Resour, 6(2): 171-178.

8. **Sandeep K., Tamil Selvi S., Bharathidasan R., Rajkumar K., Ilakkiya R. and Prabakaran M. (2010).** Evaluation of phenolic content and antioxidant activity of *Aegle marmelos* (L.) Corr. Serr. Journal of Natural product and Plant resources, 3(4); 24-28.
9. **Vyas R.S., Singh S. and Deswal, R.P.S. 1979.** Effect of micronutrient, plant growth regulators and soil amendment on fruit drop, cracking yield and quality of Bael (*Aegle marmelos* Correa) under rainfed conditions, Indian Journal of Horticulture, 61, (2), 175-176.