#### **Review Article**

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## Pharmacological Properties and Screening of Antibacterial Activities of Bael (Aegle marmelos L.)

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#### Abstract

Bael (*Aegle marmelos*) used as a medicinal plant in the Ayurveda to treat various gastrointestinal diseases in infants and adults. In India, it is locally known as 'Bael'. Various phytochemical constituents of different parts of bael are responsible for hepatoprotective activity, antioxidant activity, antidiabetic activity, analgesic activity and antibacterial activity. Antibacterial screening of medicinal constituents extracted from plant parts with different solvents had vast therapeutic potential which can provide lesser side effects than with synthetic antibiotics. Among the all parts, the fruits were showing premier results against bacterial strains. Phytochemical screening of aqueous, metholic, and chloroform extract of different parts of *Aegle marmelos* against bacterial strains. The maximum inhibition zones against bacterial strains are dose dependent and extract solvents. The antibacterial activity varied in different parts of bael tree due to the presence of bioactive constituents such as tannins, saponins, terpenoids, alkaloids, skimminine, aegelin, lupeol, cineole, citral, citronellal, cuminaldehyde, eugenol, marmesin, marmelosin, and polyphenols.

#### 1. Introduction

Aegle marmelos (L.) belongs to the family Rutaceae and is commonly known as bael tree in India and golden apple in English (Suja et al., 2017). Tree is a moderate sized, slender, aromatic tree, 6.0-7.5 m in height, and 90 to 120 cm in girth, 3.0-4.5 meter height growing wild throughout the deciduous forests of India. It is grown in tropical and subtropical climate of India, Burma, Pakistan, Bangladesh, Sri Lanka, Northern Malaya, Java and Philippine (Islam et al., 1995) and at an altitude of 1200 meter in the western Himalayas and also occurring in Andaman Island. Various parts of Aegle marmelos (L.) are used in Ayurvedic and Siddha medicines to treat a variety of diseases. Hindus offer prayers to deities Lord Shiva and Parvati. Thus, the tree is also known by the name Shivaduma (the tree of Shiva). On this way Hindus believe that goddess Lakshmi settled in bael leaves (Brijesh et al., 2009; Gupta et al., 2011). However, the tree is largely cultivated and commonly seen near the temples. From the last fifteen years, recent scientific techniques and various medicinal properties reports viz., antibacterial activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, anticancer activity, haemolytic activity, larvicidal activity and anti-inflammatory have been extensively studied (Bhar et al., 2019). Aegle marmelos is one of the most important medicinal plants of India, for its immense low cost medical importance, healthier and harmless than synthetic ones. It was identified that plant extracted substances are used against various diseases, especially the ethnic communities of Jharkhand, who suffer from malnutrition and severe health issues (Dutta et al., 2014). Significant range of therapeutic effects of bael fruit on antibacterial, antiviral, anti-diarrheal, anti-ulcerative colitis, free radical scavenging, antioxidant, hepatoprotective, antidiabetic, cardioprotective, and neuroprotective effects (Baliga et al., 2014). Medicinal plants rich in antibacterial compounds could be an alternative way to reduce bacterial disease and infective gastrointestinal diseases which are the major cause of infant and adult mortality (Sudharameshwari and Radhhika, 2007).

#### 2. Nutritional Aspects of Aegle marmelos

Bael is one of the most valuable Indian medicinal plants. Chemical studies prove that bael fruit is rich in nutritional value (Sawale *et al.*, 2018). Fruit is a rich source of mineral,

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vitamin and fibre. Nutritional value of the Bael fruit (% per 100 g pulp) contains 61.5 g water, 1.8 g protein, 0.39 g fat, 1.7 g minerals, 31.8 g carbohydrates, 55 mg carotene, 0.13 mg thiamine, 1.19 mg riboflavin, 1.1 mg niacin, and 8 mg per 100 g of edible portion vitamin C (Gopalan *et al.*, 1971). No other fruit has such a high content of riboflavin (Mukherjee and Ahmed, 1957). Riboflavin content of ripe fruit is very high. Fruit is highly nutritious with the richest source of riboflavin. Marmelosin a resinous substance is most probably the therapeutically active principal of bael fruits (Roy and Singh, 1979).



Figure 1: Flowering of Aegle marmelos (L.)



Figure 2: Fruit of Aegle marmelos (L.)

#### 3. Phytoconstituents of Aegle marmelos

The importance of the bael plants and chemicals they produce assist in overcoming disease. Over 25% of all prescriptions drugs still use plants as their principal ingredients (Patel *et al.*, 2012). George *et al.*, (2016)

reported that the root and fruits of bael contain a number of coumarins, alkaloids, steroids, and essential oils. Different types of carotenoids have been reported in the Aegle *marmelos* these are responsible for imparting yellow pale colour to fruit. Marmelosin, skimmianine and umbelliferone are therapeutically active principles of bael plant (Dhankhar et al., 2011). Minor constituents are like ascorbic acid, sitosterol, crude fibers, tannins,  $\alpha$ -amyrin, carotenoids, and crude proteins are also present hundreds of chemical compounds have been isolated from bael some of them have been proved to be biologically active against various major and minor disease such as lupeol, cineole, citral, citronellal, skimminine, aegelin, cuminaldehyde, eugenol, marmesin, marmelosin, luvangetin, fagarine, marmin, aurapten, psoralen, marmalade, and tannins (Asghar et al., 2018). The list of chemical constituents present in different parts of Aegle marmelos is summarised in Table 1.

Table 1: Chemical constituents present in different parts of	
Aegle marmelos	

SI. No.	Plant Part	Chemical constituents
1	Leaf	Skimmianine, Aeglin, rutin, alpha-si- tosterole, B-sitosterol, Flavone, Lupeol, Cineol, Citral, Glycoside, O-Isopentenyl, Hallordiol, Mameline, Citronellal, Cuuminaldehyde phenylethyle cinna- mamides, Euginol, Marmesin, Aegelin, Glycoside
2	Fruit	Marmelosin, Luvangetin, Aurapten, psoralen, marmalade, Tannin, Phenol
3	Bark	Fagarine, Marmin, Furoquinoline, alkaloids
4	Seed	Essential oil-D-limonene, A-D-phel- landrene, Cineol, Citronellal, Citral, P-cymene, Cumin aldehyde
5	Root	Alkaloid, Halopine, Coumarins, Terpenes

Source: Patel et al., (2012).

#### 4. Pharmacological Properties of the Bael

#### 4.1 Wound Healing Activity

Wound healing is the process of repair of tissue injury followed by inflammatory response occurs and the cells below the dermis begin to increase collagen production. Later, the epithelial tissue is regenerated (Garg and Paliwal, 2011). *Aegle marmelos* seems to improve wound contraction and epithelialization 20 days after application of fruit pulp extract (200 mg/kg) with decrease in free radicals and myeloperoxidase (Gautam *et al.*, 2014). Wound healing effect of methanolic extract of bael applied with two rates (5 and 10%) in incision and excision wound model in rats. Both types of tests exhibited the significant response by its wound contacting ability and increased the tensile strength as compared to nitrofurazone (Jaswanth *et al.*, 2001).

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#### 4.2 Analgesic Activity

Relieve of pain has always remained a dominant concern of medicine. Numbers of anti-inflammatory and analgesic drugs like aspirin, indomethacin, phenylbutazone etc. are in practice, but because of their side effects. Aegle marmelos used mostly frequent in the traditional systems of medicine and in religious rituals. It has analgesic, anti-inflammatory, antipyretic, antidiabetic, antioxidant, hepatoprotective and many other activities (Ghodki et al., 2016). Ethanolic leaf and fruit extracts at doses of 200 mg/kg have shown significant analgesic activity in hot plate model in rats. Various phytochemical constituents of extracts of leaf and fruit pulp is responsible for its analgesic action. The probable mechanisms for its central analgesic property may be similar to that of opioids which is mainly by increasing the pain threshold and release of endorphins compounds (Krishnakanth et al., 2017). The serial extracts of the leaves of Aegle marmelos (Corr.) were investigated for analgesic and antipyretic properties. The analgesic activity produced by extract products to reduce the early and late phases of paw licking in mice. A significant reduction in hyperpyrexia in rats was also produced by the most of the extracts (Arul et al., 2005). Methanolic extract of dry ripe fruits of bael was investigated on central and peripheral analgesics by two tests, tail flick test and acetic acid induced writhing test at 100, 250 and 500 mg/kg doses in animal model. Out of these tests, tail flick test showed significant increase in latency period i.e. 65.38%, 395.37% and 459.25% with respective doses. Diclofenac drug at the dose of 5 mg/Kg increased the latency period consciously but less significantly as compared to induced writhing test at the dose of 100 mg/kg i.e. 89.83% (Atiq-ur-Rahman et al., 2015). Dose levels of 200 and 300 mg/kg of methanol extract of Aegle marmelos seems to be significant in analgesic activity in mice (Shankarannath et al., 2007).

#### 4.3 Anti-Diabetic Activity

Aegle marmelos leaf extract has been used in Ayurveda as a medicine for diabetes. The present study examined the action of Aegle marmelos against experimental diabetes as well as the antioxidant potential of the drug. It has been reported that alloxan induced oxidative stress which causes damage to the pancreatic  $\beta$ -cell and produces hyperglycemia in rats. A methanolic extract of Aegle marmelos was found to reduce blood sugar in alloxan diabetic rats. Reduction in blood sugar could be seen from the 6<sup>th</sup> day after continuous administration of the extract and on the 12<sup>th</sup> day sugar levels were found to be reduced by 54%. It was evident that significant decrease in lipid peroxidation, conjugated diene and hydroperoxide levels in serum and liver induced by alloxan. Aegle marmelos extract effectively controlled the oxidative stress induced by alloxan and produced a reduction in blood sugar (Sabu et al. 2004). Leaf explant of bael has a potential for application in diabetes management compared to the ordinary plant material. Treatment using extracts from both leaf and callus produced significant decreases in blood sugar level in streptozotozin diabetic rabbits. Among the

various extracts, the methanol extracts of the leaf and callus brought about the maximum anti-diabetic effect. The study revealed that the *in vitro* callus culture of *bael* has as much potential in diabetes management as the original leaf extract (Brijyog et al., 2019). Bhavani (2014) reported that alcoholic extract of A. marmelos found to be reducing the blood sugar in alloxan induced diabetic rats. Sugar levels in blood were reduced by 54% and could be seen from the 7th day after continuous use of the extract till the 28<sup>th</sup> day. Arumugama et al. (2008) studied the effect of different organic solvent extracts of leaf and callus of Aegle marmelos. Extracts from both leaf and callus produced significant decreases in blood sugar level in streptozotozin diabetic rabbits. Among the various extracts, the methanol extracts of the leaf and callus brought about the maximum anti-diabetic effect shown in Table 2.

Table 2: Effect of different organic solvent extracts of leaf and callus of *Aegle marmelos* on blood sugar levels of solution of streptozotozin (STZ)-diabetic rats

Treatment	Blood sugar level (mg/dl)				
	(Mean ± SD)	Callus extract treated			
Control	36.1 ± 2.8	360.9 ± 3.1			
Petroleum ether Ex- tract	236.5 ± 2.5	256.6 ± 1.5			
Benzene Extract	222.5 ± 2.6	251.6 ± 1.6			
Chloroform extract	226.1 ± 2.0	225 ± 1.8			
Methanol Extract	207.1 ± 2.0	210.1 ± 2.0			

#### 4.4 Antioxidants and Hepatoprotective Activity

Antioxidants are significant in the prevention of human illness and may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation (Andlauer and Furst, 1998). Bael exerts hepatoprotective activity through its antioxidant and anti-inflammatory properties which was enhanced by piperine (Ratheea et al., 2018). The alcoholic extracts of the leaves of Aegle marmelos possess hepatoprotective activity, anti stress, anti oxidant properties that may be due to cytoprotective mechanism (Ramamurthy and Gowri, 2015). Hepatoprotective activity of an effective drug lies in its ability to inhibit aromatase activity of cytochrome P-450. This in turn is believed to activate the process of liver regeneration. Therefore, it is suggested that flavonoids in *Aegle marmelos* could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase. In addition, it has been pointed out that hesperidin present in the fruits reduces the signs of hypovitaminosis C in experimental animals which in turn may play a major role in detoxification and thus offering hepato protection against CCl, induced toxicity (Rajasekaran et al., 2009).

The antioxidant activity observed in Aegle marmelos may



be affiliated with their main phytochemical like phenols, flavonoids, and tannins. Bael fruit can be used as a natural antioxidant to treat free radical induced cellular damages and can also be used with other drugs to give two-way effects (Gupta et al., 2018). The scavenging activity of bael fruit powder in vacuum drying ranged from 65.36% to 81.33% of the concentration 200  $\mu$ g/ml to 1000  $\mu$ g/ml. Vacuum dried fruit powders of bael have radical scavenging activity. Therefore vacuum drying can be recommended as the most effective drying method to protect chemical characteristics and retention of antioxidant properties of fruit powders (Wijewardana et al., 2016). Tagad et al., (2018) studied the antioxidant activity of extracts in different solvent systems (methanol, ethanol and distilled water). In methanol extract the phenolics and flavonoid content of Aegle marmelos fruit was 81.46 mg tannic acid equivalents per g fresh weight (TAE/g fw) and 21.68 mg rutin equivalents per g fresh weight (RE/g fw) respectively. The effect of different solvents on the antioxidant activity of Aegle marmelos was measured, methanol extract exhibited highest free radical scavenging activity (2.256 ± 0.14%), Ferric reducing 0.026 ± 0.12 Mg/ AAE/g fw and ferrous ion chelating  $(59.60 \pm 0.09 \%)$  activities.

#### 5. Screening of Antibacterial Activity of Aegle marmelos

Antimicrobial activity retards the growth of bacteria due to activity of phytochemicals. Antimicrobial agents extracted

from plant parts had enormous therauptic potential as they can provide lesser side effects than associated with synthetic antimicrobials (Nandedkar *et al.*, 2017). Leaf and fruit extracts of bael fruit have been used for antimicrobial activity against gram positive and gram negative microorganisms. Maximum zone of inhibition was observed against *Roultella plantikola* with leaf extract 11 mm and fruit extract 9 mm. The plant extract showed a zone of inhibition 18 mm activity against *Pencillium chrysogenum* and minimum against *Candida albican* (Meena *et al.*, 2016). The efficacy of extract of bael was evaluated against bacterial strains. The highest inhibitory activity was observed with aqueous extract against *S. epidermidis* whereas methanolic extract was powerful against *S. aureus* at 40 mg/ml concentration (Mujeeb *et al.*, 2014).

Methanolic leaf extract showed better antibacterial activity against *Pseudomonas aeruginosa* (15 mm) at 100 mg/ml concentration followed in descending order by *E. coli* (14 mm), *Bacillus subtilis* and *Staphylococcus aureus* (9 mm) and *Klebsiella pneumoniae* (7.4 mm) (Table 3). Methanolic leaf extract at higher concentration and the commercial antibiotic, cefotaxime were found to be equally effective against *P. aeruginosa*. They also found the methanolic extract of *A. marmelos* more active in *P.* aeruginosa and *E. coli* (Surender *et al.*, 2015).

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lable 3: Antibacterial	activity	of Aegle	marmeios	methanolic	extract

Zone of inhibition (mm)										
Test organism	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.125 mg/ml	Control			
P. aeruginosa	15.0 ± 1.6	13.5 ± 2	12.0 ± 1	9.2 ± 0.9	$6.3 \pm 0.4$	$6.0 \pm 0.4$	23.0 ± 1			
B. subtilis	9.3 ± 1.6	8.2 ± 0.9	8.3 ± 1	6.5 ± 1.1	6.2 + 0.4	6.2 ± 0.9	24 ± 1.3			
E. coli	14.0 ± 2.4	13.0 ± 2.9	$12.0 \pm 1.3$	$10.0 \pm 0.6$	6.5 + 0.9	6.3 ± 0.6	15 ± 1.0			
K. pneumoni	$7.4 \pm 0.4$	$6.4 \pm 1.1$	6.3 ± 1.0	6.3 ± 1.0	$6.2 \pm 0.6$	6.0 ± 1.0	$29.0 \pm 1.0$			
S. aureus	9.0 ± 0.9	8.2 ± 0.9	8.3 ± 0.7	6.5 + 0.7	6.3 ± 0.9	6.2 ± 1.0	30 ± 1.6			

NB: Values are given in Mean ± SD (n=3); Control (Cefocaxime).

Three extracts of leaves of *Aegle marmelos* has been screened for its potential against five bacterial strains: *Lactobacillus, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi, Escherichia coli.* Chloroform extract showed good antibacterial against *E. coli.* The antibacterial

activity of chloroform extract showed the highest activity against *E. coli* by forming an inhibition zone of 15 mm. Ethyl acetate leaf extract was found to be moderate against *Pseudomonas aeruginosa* forming inhibition zone of 13 mm presented in Table 4 (Gangai-Abirami *et al.*, 2014).

Table 4: Antibacterial activity of Aegle Marmelos

Bacterial strains	Zone of inhibition (mm)								
	Ethyl acetate			Petroleum ether			Chloroform		
	25 µl	50 µl	75 μl	25 µl	50 µl	75 µl	25 μl	50 µl	75 µl
Pseudomonas aeruginosa	11	12	13	9	10	11	10	12	13
Staphylococcus aureus	8	9	10	9	11	12	7	8	9
Escherichia coli	7	8	11	9	12	13	10	12	15
Lactobacillus	7	8	9	10	12	13	9	11	12
Salmonella typhi	8	12	13	7	9	11	11	13	14





Figure 3: Graph showed that *A. marmelos* fruits were having maximum antibacterial activity, compared to leaves and peels



(a) *E. Coli* (b) *S. aureus* (c) *P. aeruginosa* Figure 4: Antibacterial activity of Ethanolic extract of fruits

Pandey and Mishra (2011) studied antibacterial activities of ethanolic, methanolic, ethyl acetate and hot water extract from leaves, fruits and peels of *Aegle marmelos*. Compared to all parts, the fruits were showing the best result. The methanolic and hot water extracts showed least antibacterial activity as compared to ethanolic and ethyl acetate extracts. The minimum inhibitory concentration (MIC) values were obtained 1.98 mg/ml in ethanolic and ethyl acetate extract of fruits against *S. aureus* and 11.90 mg/ml in methanolic extract against *P. aeruginosa*. The antibacterial compound mainly found in *Aegle marmelos* were tannins, phlobatannins, saponins, terpenoids, alkaloids and polyphenols showed in (Figure 1 and 2). It also showed that *S. aureus* having maximum zone of inhibition compare to *E. Coli* and *P. aeruginosa*.

Antimicrobial screening on *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus* sp. and *Pseudomonas aeruginosa*. Methanolic extract of bark of bael was screened for antimicrobial activity. The extract was found to have an activity on *Escherichia coli*, *Staphylococcus* aureus, *Enterococcus* sp. and *Pseudomonas aeruginosa*. The results of the antimicrobial activity by the agar disc diffusion method are presented in the Table 5. The inhibition distance ranged

from 0-19 mm in disc diffusion method. In case of gram positive bacteria, (Staphylococcus aureus and Enterococcus sp.) inhibition distance ranged approx 0-9 mm but for gram negative (Escherichia coli) it was 0-10 mm. Among the gram positives, Staphylococcus aureus (ID = 9 mm) was most sensitive and among gram negatives, Escherichia coli (ID = 10 mm) was most sensitive. Therefore, these values are more sensitive compared to those of the standard reference antibiotic (Gentamicin ID upto 15 mm) (Sumita et al., 2015). In other studies the aqueous and ethanolic extracts from the leaves of Aegle marmelos were screened against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and *Bacillus subtilis* by using disc diffusion test technique. Bacillus subtilis exhibit about 22 mm inhibition zone were considered resistant compared with the standard antibiotics such as Penicillin (Venkatesan, 2009). The antibacterial activity of the methanol, chloroform and aqueous extracts from the leaves, bark and fruit of A. marmelos was studied against Bacillus subtilis, Staphylococcus aureus (Gram Positive), Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Salmonella paratyphi A and Salmonella paratyphi B (Gram Negative) by using disc diffusion method. Results revealed that the methanolic extract has significant antibacterial activity against tested bacteria (Poonkothai and Saravanan, 2008). Antibacterial activity of the Agale marmelos leaves extract on Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhimurium, and Methicillin Resistant Staphylococcus aureus (MRSA). Methanolic extract was found to be effective against all organisms. Various concentrations of the extracts (25-125 µl) as well as the effect of temperature (25-100 °C) on the antibacterial activity was found to be around 1.2 cm reported in Bacillus subtilis and the lowest antibacterial activity was found to be around 0.3 cm reported in Pseudomonas aeuroginosa (Yesaswini, 2014). Dahiya et al., (2018) analyzed Aegle marmelos bark extracts against selected microbial strains like Escherichia coli (MTCC-443), Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus brasiliensis and Candida albicans. Extracts of acetone and ethanol 70% showed highest activity against Escherichia coli whereas methanol

Table 5: Inhibition values obtained against four test organisms in different concentrations of extract for disc diffusion method								
Organisms	Standard Genta- micin (10 mg)	Different concentration of bael extract						
		25 µg/ml	50 µg/ml	100 µg/ml	10 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
				Inhib	ition Distan	ce (ID)		
Staphylococcus aureus	19 mm	0.8 mm	0.8 mm	0.7 mm	9.0 mm	0.8 mm	0.8 mm	0.9 mm
Escherichia coli	22 mm	0.7 mm	10.0 mm	0.5 mm	8.0 mm	0.6 mm	0.8 mm	10.0 mm
Enterococcus sp.	21 mm	0.85 mm	0.7 mm	0.0 mm	0.0 mm	-	-	-
Pseudomonas aeruginosa	23 mm	0.65 mm	0.6 mm	0.7 mm	0.0 mm	-	-	-

Source: Sumita et al., (2015).



and chloroform extracts showed significant activity against *Bacillus subtilis*.

#### 6. Conclusion

From this review, *Aegle marmelos* can be used for various therapeutic purposes. The various parts of this tree have great potential to overcome the various diseases without producing any side effect. The human disease such as diabetes, liver toxicity, microbial infection, inflammation of intestine, and analgesic can be treated with *Aegle marmelos*. Extensive research has been done on screening of bael extracts against bacterial strains play a very important role in preventing various diseases due to the presence of bioactive compounds.

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