Phytochemical and Pharmacological Profile of Leaves of *Aegle Marmelos* Linn

Narayan P. Yadav^{1*}, C. S. Chanotia²

Abstract: Aegle marmelos (Linn) correa, commonly known as bael (or bel), belonging to the family Rutaceae, is a moderatesized, slender and aromatic tree. A number of chemical constituents and various therapeutic effects of leaves of A. marmelos have been reported by different workers. Extensive investigations have been carried out on different parts of Aegle marmelos and as a consequence, varied classes of compound viz., alkaloids, coumarins, terpenoids, fatty acids and aminoacids have been isolated from its different parts. Broadly, Aegle marmelos leaves contain alkaloids, Phenylpropanoids, terpenoids and other miscellaneous compounds whereas potential pharmacological activity of the leaves are hypoglycemic, anti-inflammatory, antimicrobial, anticancer, radioprotective, chemopreventive and anti-oxidative activity. Anhydroaegeline can be used as marker to standardize the plant material with respective to its potential anti diabetic activity.

Introduction

Aegle marmelos (Linn) correa, commonly known as bael (or bel), belonging to the family Rutaceae, is a moderate-sized, slender and aromatic tree. It is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India. It is extensively planted near Hindu temples for its wood and leaves which are generally used for worship. Its branches are armed with sharp straight spines. The bark is soft, light grey and exfoliating in irregular flakes. The bright green leaves are alternate and trifoliolate (rarely five-foliolate). The flowers are greenish white and sweet-scented, fruits are yellowish grey and globose with woody rind and seeds are numerous, oblong and compressed. The roots are fairly large, woody and often curved.^{1,2} Fresh leaf juice is used in asthmatic complaints and jaundice. The Chinese used the leaves and young fruits to adulterate Opium. In Bengal it is used for dysentery. In Konkan, small and unripe fruits are used for piles. The juice of bark is a remedy for poverty of seminal fluid.3

The plant has been used in the Indian traditional medicines from time immemorial. It is associated with various important medicinal properties. Chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites. Some of the compounds have been screened for bioactivity.

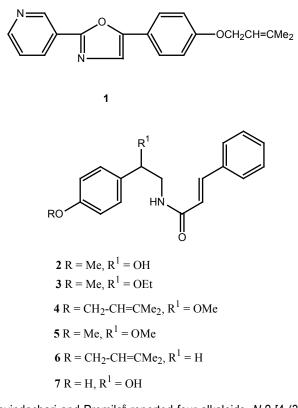
Phytoconstituents

Extensive investigations have been carried out on different parts of *Aegle marmelos* and as a consequence, varied classes of compound viz., alkaloids, coumarins, terpenoids, fatty acids and aminoacids have been isolated from its different parts. Notably, majority of reports on the isolation and compound characterizations have been reported by many Indian workers. Broadly, *Aegle marmelos* leaves contained γ -sitosterol, aegelin, lupeol, rutin, marmesinin, β -sitosterol, flavone, glycoside, *O*-isopentenyl halfordiol, marmeline and phenylethyl cinnamamides⁴. The detailed investigations on isolated compound classes are as under:

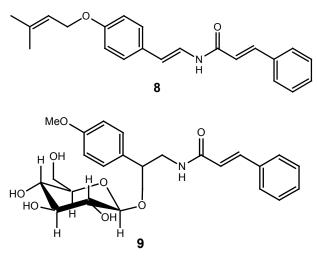
1. Alkaloids: The alkaloids comprise the largest single class of secondary plant substances. New alkaloids from the leaves of *A. marmelos* were reported viz., *O*-3,3-(dimethylallyl)halfordinol (**1**), *N*-2-ethoxy-2-(4-methoxyphenyl) ethylcinnamamide (**3**), *N*-2-methoxy-2-[4-(3',3'-dimethylallyloxy)phenyl] ethylcinnamamide (**4**), *N*-2-methoxy-2-(4-methoxyphenyl) ethylcinnamamide (**5**) and marmeline (**6**).⁵

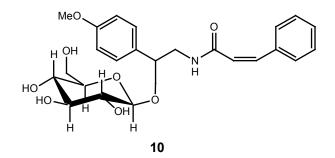
¹Animal House Facility, Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow. ²Instrumentation and Central Laboratory, Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow. *Author for correspondence E-mail: npyadav@gmail.com

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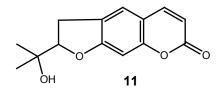


Govindachari and Premila⁶ reported four alkaloids, *N*-2-[4-(3',3'dimethylallyloxy)phenyl] ethylcinnamide, *N*-2-hydroxy-2-[4-(3',3'dimethylallyloxy)phenyl] ethylcinnamide or marmeline **(6)**, *N*-4methoxystyryl cinnamide and *N*-2-hydroxy-2-(4-hydroxyphenyl) ethylcinnamide **(7)** and aegeline **(2)** from dry leaves of *A. marmelos.* Aegeline was initially believed to be a sterol but after a critical study it was found to be a neutral nitrogenous compound. Recently, series of phenylethyl cinnamides, which included new compounds named anhydromarmeline **(8)**, aegelinosides A **(9)** and B **(10)** were isolated from *Aegle marmelos* leaves as α glucosidase inhibitors⁷.





2. Phenylpropanoids: These are naturally occurring phenolic compounds, which have an aromatic ring to which three-carbon side chain is attached. Among the phenylpropanoids are included hydroxycoumarins, phenylpropenes and lignans. The most widespread plant coumarin is the parent compound, coumarin itself, which occurs in over twenty-seven plant families. Marmesin (11) was established as a new compound from leaves, which is also a constituent of heartwood and root.^{8,9,10}



Aegelenine, a minor base of the leaves, which was initially claimed to be a new compound, was found to be in identical to halfordinol, the basic constituent of *Halfordia scleroxyla*.^{11,12}

Fresh leaves yield in distillation yellowish-green oil with a peculiar aromatic odour, marmelosin¹³ reported marmesinine, β -sitosterol- β -D-glucoside and rutin in the leaves. Ali *et al.*¹⁵ isolated Marmenol, a new 7-geranyloxycoumarin [7-(2,6-dihydroxy-7-methoxy-7-methyl-3-octaenyloxy)coumarin] from the leaves of methanolic extract of *A. marmelos*.

3. Terpenoids: The essential oil of *A. marmelos* (L.) Correa leaves were studied very much extensively in India by various workers since 1950. α -Phellandrene was found to be the common constituent of the essential oil from leaves, twigs and fruits.^{16,17} α -Phellandrene (56%) and p-cymene (17%) were reported from leaf oil.¹⁸ Later, similar report was published on leaf essential oil by many workers. p-Menth-1-en-3,5-diol was isolated and characterized from *A. marmelos* leaves.¹⁹ Limonene (**12**) (82.4%) was reported as the main constituent from *A. marmelos* leaves and it was shown that limonene is characteristic marker for identification of *A. marmelos* oil samples.²⁰

 γ -Sitosterol from the leaves was identified²¹. Investigations on stem bark, leaves, fruit and root revealed α -and β -amyrin and β -sitosterol.¹⁶

4. Miscellaneous compounds

Ali *et al.*¹⁵ reported praealtin D, *trans*-cinnamic acid, valencic acid, 4-methoxybenzoic acid, betulinic acid, *N*-p-*cis*-& *trans*-coumaroyltyramine, montanine, and rutaretin from the leaves of methanolic extract of *Aegle marmelos*. Rutin, flavan-3-ols, anthrocyanins, lecucanthrocyanins, flavone glycosides and tannins also have been reported from the leaves.^{14,22,23}

Pharmacological Activities of Leaves

The leaves of *Aegle marmelos* are made into poultice, used in the treatment of ophthalmia, and the freash juice is praised in catarrhs and feverishness. The fresh juice of leaves is given, with addition of black pepper, in anasarca with costiveness and jaundice. In external inflammations, the juice of the leaves is given internally to remove the supposed derangement of tumours². Broadly the biological activity of the plant can be categorized as follows:

1. Hypoglycemic activity

Phuwapraisirisan *et al.*⁷ reported a series of phenylethyl cinnamides, where anhydroaegeline revealed the most potent inhibitory effect against α -glucosidase with IC₅₀ value of 35.8 μ M. Sabu *et al.*²⁴ examined the action of *Aegle marmelos* against experimental diabetes as well as the antioxidant potential of the drug. *Aegle marmelos* extract effectively reduced the oxidative stress induced by alloxan and produced a reduction in blood sugar. Upadhya *et al.*²⁵ found the hypoglycemic and antioxidant activity of aqueous extract of *Aegle marmelos* leaves by analyzing the glucose, urea & GST (glutathione-S-transferase) levels in plasma and GSH (glutathione) and MDA (malondialdehyde) levels in erythrocytes of alloxan induced diabetic rats.

Sachdewa *et al.*²⁶ tested the hypoglycemic effect of *Aegle marmelos* and *Hibiscus rosa sinensis* in glucose induced hyperglycemic rats. *Aegle marmelos* leaf extract for 7 consecutive days, @ 250 mg/kg oral dose showed the significant improvements in its

Hema *et al.*²⁷ studied the effect of the aqueous, alcoholic and petroleum ether extracts of *A. marmelos* for the hypoglycaemic and other pharmacological actions and observed that the aqueous and alcoholic extracts at 500 mg/kg dose produce hypoglycaemia in normal fasted rabbits, but the petroleum ether extract did not.

In a clinical trial, a branded formulation having leaves of *Aegle marmelos* as one of the constituents was found effective in mild to moderate diabetic patients, which included even the insulin dependent ones. The treatment, reportedly, tends to increase insulin secretion from pancreas.²⁸ Das *et al.*²⁹ studied the effect of leaf of *Aegle marmelos* on histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. The treatment of leaf extract on diabetic pancreas showed improved functional state of pancreatic beta cells. This study indicates the hypoglycemic nature of the leaf extract, helping in regeneration of damaged pancreas.

Seema *et al.*³⁰ investigated the potential of the leaf extract of *Aegle marmelos* as an anti-diabetic agent on the liver of streptozotocindiabetic rats. The leaf extract of *Aegle marmelos* was found to be as effective as insulin in restoring of blood glucose and body weight to normal levels. Rao *et al.*³¹ reported that aqueous extract of leaves given in the dose equivalent to 1 gm powder/kg/day produced significant (p<0.01) anti-hyperglycemic effect within three days in alloxan induced diabetic rabbits while similar treatment in normal rabbits produced decrease upto 35.3% in blood glucose level after 4 hours of administration. Moderate hypoglyceamic effect was recorded even after 12 hours.

Ponnachan *et al.*³² studied the potential antidiabetic effect of *Aegle marmelos* leaf extract in diabetic rats. The diabetic animals were given insulin injection and another group *Aegle marmelos* leaf extract orally. This study indicated that the active principle in *Aegle marmelos* leaf extract has similar hypoglycemic activity to insulin treatment.

2. Antimicrobial activity

Rani *et al.*³³ studied the 54 plant extracts (methanol and aqueous) for their activity against muti-drug resistant *Salmonella typhi*. The methanol extracts of *Aegle marmelos, Salmalia malabarica, Punica granatum, Myristica fragrans, Holarrhena antidysenterica, Terminelia arjuna* and Triphala showed strong antimicrobial activity. The antifungal activity of essential oil isolated from the leaves

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of *Aegle marmelos* was studied using the spore germination assay.³⁴ The oil exhibit variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi, the most resistant fungus, *Fusarium udum* was inhibited 80% at 400 ppm. Kinetic studies showed concentration as well as time dependent complex inhibition of spore germination by the essential oil. Pattnaik *et al*³⁵ studied the essential oils of *Aegle marmelos* and some other plants for antibacterial activity against 22 bacteria (including Gram positive cocci and Gram negative rods) and 12 fungi (3 yeast like and 9 filamentous) by the disc diffusion method. *Aegle marmelos* essential oil inhibited the 21 bacteria and all 12 fungi.

3. Anti-inflammatory activity

Arul *et al.*³⁶ studied that the leaves of *Aegle marmelos* possess the anti-inflammatory, antipyretic and analgesic properties. The extract of leaves of *Aegle marmelos* caused a significant inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats. Jagtap *et al.*³⁷ showed the effect of

polyherbal formulation (of *Aegle marmelos* & some other plants) on experimental models of inflammatory bowel diseases (IBD).

4. Radioprotective activity

The radioprotective activity of a leaf extract of *Aegle marmelos* (AM) in mice exposed to different doses of gamma-radiation was investigated.³⁸ AM treatment reduced the symptoms of radiation-induced sickness and increased survival. The radioprotective action might be due to free-radical scavenging and arrest of lipid peroxidation accompanied by an elevation in glutathione.

5. Anti-oxidative activity

Rajadurai *et al.*³⁹ reported that pretreatment with *Aegle marmelos* leaf extract at doses of 100mg/kg and 200mg/kg body weight for 35 days showed a significant effect on the activities of marker enzymes, lipid peroxides, lipids, lipoproteins and antioxidant enzymes in isoproterenol treated rats. The effect of extract 200mg/kg was found to be equal to the effect of alpha-tocopherol 60mg/kg.

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6. Anticancer activity

Costa-Lotufo *et al.*⁴⁰ studied the anticancer potential of 11 plants used in Bangladeshi folk medicine and found among all tested extracts, only the extracts of *Oroxylum indicum*, *Moringa oleifera* and *Aegle marmelos* showed potential anticancer cactivity.

It was also reported that *Aegle marmelos* (L.) inhibits the proliferation of transplanted Ehrlich ascites carcinoma in mice. The anticancer effect of hydroalcoholic extract of *Aegle marmelos* (AM) was studied in the Ehrlich ascites carcinoma bearing Swiss albino mice. The spatial effect of various AM administration schedules showed that six-day administration increased the survival of tumor bearing mice. The best antineoplastic action of AM was obtained when AM administered through intraperitoneal route than the oral route at equimolar dose. Dose of 400 mg/kg was considered as the best dose⁴¹.

Lambertini *et al.*⁴² studied the effects of extracts from Bangladeshi medicinal plants on *in-vitro* proliferation of human breast cancer cell lines and expression of estrogen receptor alpha gene, according to this study extract from *Aegle marmelos* is antiproliferative on both cell line MCF7 and MDA-MB-231cells, but at a higher concentration.

7. Chemopreventive potential

The effect of hydroalcoholic (80% ethanol, 20% water) extract of leaves of *Aegle marmelos* on carcinogen-metabolizing phase-I and phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation was studied. The changes in the levels of drug-metabolizing enzymes and antioxidative profiles are strongly indicative of the chemopreventive potential of this plant, especially against chemical carcinogenesis.⁴³

8. Role in heart diseases

Prince et al.⁴⁴ evaluated the preventive effects of an aqueous *Aegle marmelos* leaf extract (AMLEt) in isoprenaline (isoproterenol)induced myocardial infarction in rats. Pretreatment with AMLEt decreased the activity of creatine kinase (CK) and lactate dehydrogenase (LDH) in serum and increased them in the heart, also AMLEt pretreatment increased the activity of Na+K+ ATPase and decreased the activity of Ca2+ATPase in the heart and aorta simultaneously and the levels of cholesterol and triglycerides decreased whereas phospholipids increased in heart and aorta of AMLEt-pretreated rats. All the deranged biochemical parameters were restored with 200 mg kg-1 AMLEt. Hema *et al.*²⁷ studied the effect of the aqueous, alcoholic and petroleum ether extracts of *A. marmelos* for the hypoglycaemic and other pharmacological actions and observed that the aqueous extract acts as a cardiac stimulant, smooth-muscle relaxant and uterine stimulant while the alcoholic extract revealed cardiac depressant, smooth muscle relaxant and uterine relaxant properties.

9. Effect on Testicular Activities

The aqueous extract of leaves of *Aegle marmelos* (Bael) at the dose 50 mg/100 g body weight in rats resulted a significant diminution in the activities of key testicular steroidogenic enzymes along with low levels of plasma testosterone and relative wet weights of sex organs in respect to control without any significant alteration in general body growth. Germ cells numbers in different generation at stage VII of seminiferous epithelial cell cycle were diminished significantly after the treatment of the above extract. The above mentioned dose did not exhibit any toxicity in liver and kidney. Therefore, it may be predicted that the aqueous extract of leaf of *Aegle marmelos* has a potent antitesticular effect at a specific dose.⁴⁵

10. Acute and subacute toxicity studies

Total alcoholic, total aqueous, whole aqueous and methanolic extracts of leaves of *A. marmelos* were used for the toxicity studies. Acute, subacute and LD50 values were determined in experimental rats. There were no remarkable changes noticed in the histopathological studies after 50mg/kg body wt of the extracts of *A. marmelos* when administered intraperitoneally for 14 days successively. Pathologically, neither gross abnormalities nor histopathological changes were observed. After calculation of LD50 values using graphical methods, researcher found a broad therapeutic window and a high therapeutic index value for *A. marmelos* extracts.⁴⁶

Conclusion

A number of chemical constituents and various therapeutic effects of leaves of *A. marmelos* have been reported by different workers. The most important pharmacological activity of the leaves of *A. marmelos* has been found to be its antidiabetic activity but the mechanism of hypoglycemic action of leaves is not clear and may be the result of improvement in the functional status of beta cells, and by reversing the histologic and ultrastructural changes in the pancreas and liver of rats with streptozotocin-induced diabetes²⁹. The leaf extract of this plant also exhibits the effect on metabolic

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enzymes involved in glucose metabolism. The kinetic parameters such as Michelle's constant (Km) and V_{max} value of liver enzyme malate dehydrogenase (MDH) and its purified cystolic isoenzyme (S-MDH) were increased significantly in the diabetic state compared to respective controls³⁰. Therefore there is the dire need of correlating the therapeutic activity with the chemical marker of the plant as well as studying the mode of action of that marker compound. Anhydroaegeline can be used as marker to standardize the plant material with respective to its potential anti diabetic activity.

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HERBAL NEWS

Government to be ready with nutraceuticals policy draft by December 2009

Mumbai: Nutraceuticals are dietary supplements, which are generally used to fill nutritional deficiencies in food and to prevent diseases. The initial draft regulations for the nutraceuticals industry will be ready by the year end, according to Food Safety and Standards Authority of India (FSSAI) Chairman, Dr. P. I. Suvrathan. He was speaking after inaugurating the 2nd International Conference on Nutraceuticals, Functional Foods and Dietary Supplements organized by FICCI, in association with HADSA in Mumbai recently.

As a concept, nutraceuticals is in its stage of infancy with several developed countries having defined it only in last 15 years. While some countries define nutraceuticals based on the segments it constitutes, other define it based on the benefits it provides to the consumers. There is also no clear consensus on inclusion or exclusion of traditional medicines.

FSSA will be aided by several scientific panels and a central advisory committee to lay down standards for food safety. These standards will include specifications for ingredients, contaminants, pesticide residues, biological hazards and labels. All entities in the food sector would be required to get a licence or registration, which would be issued by local authorities.

All India Ayurvedic Center

New Delhi: President Pratibha Devisingh Patil expressed concern over climate change that was threatening the existence of several Indian herbs which are key ingredients in the traditional Ayurvedic system of medicine. "The Ayurvedic medicines make intelligent use of herbs. Climate change is disturbing the ecological balance which is making herbs, used in Ayurvedic medicines, extinct. It is a big challenge for us," she said while inaugurating the centenary celebrations of the All-India Ayurvedic Congress here. She said herbs and plants which are getting extinct should be properly categorized and efforts made to protect them. Ms. Patil sough the help of the National Medicinal Plants Board and the Central Institute of Medicinal and Aromatic Plants in this endeavour. Ms. Patil also sought strict action against "quacks" who, she said, were bringing disrepute to Ayurveda. She said there was an urgent need for patenting Ayurvedic drugs. Only then, it would be accepted as Indian knowledge. She advocated proper registration of "vaidyas" – practitioners of Ayurveda – along with their proper education and training. Some Ayurvedic medicine manufacturers were using modern methods to increase their production which was affecting the efficacy of the drugs.

Certificate for Ayush Products

New Delhi: The Department of Ayush has launched a voluntary certification scheme in collaboration with the Quality Council of India (QCI) to issue quality asserting certificate for common Ayurveda, Unani, Siddha and Homoeopathy (AYUSH) products both for Indian and overseas markets. The scheme has been designed and will be managed by QCI, India's apex quality facilitation organization. The scheme has been designed and will be managed by QCI, India's apex quality facilitation organization. The scheme envisages two levels of certifications – an AYUSH Standard Mark for meeting the domestic regulatory requirements and an AYUSH Premium Mark for meeting international norms such as those set by WHO or regulations of European Commission, USA and other countries, according to the QCI officials.

Having designed as a voluntary scheme, the certification will be issued to the willing manufacturers who volunteer to undergo the rigour of an independent third party assessment system.