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Phytopharmacological Potentials and Micropropagation of *Aegle marmelos* – A Review

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Abstract

Aegle marmelos, a popular medicinal plant and is used in traditional medicine to treat numerous ailments. *Aegle marmelos* are reported to have a wide spectrum of pharmacological properties. Its medicinal usage is well-documented in in different traditional medicine such as the Ayurveda, Unani, Siddha, and other conventional medical systems. Whole parts of the plant are rich in secondary metabolites, which impart miraculous medicinal uses to the plant. The bioactive constituents isolated from lupeol, sitosterol, amyrin, flavanoids (mainly rutin), coumarins, including aegeline, marmesin, umbelliferone etc. The extracts of different parts of plant used as antimicrobial, hepatoprotective, antidiabetes, analgesic, antipyretic, diuretic, immunomodulators, nephroprotective, antioxidant and cytotoxicity activities. Employment of techniques such as cell and tissue culture would provide means of rapid propagation and conservation of the plant species and, from the point of view of phytochemistry, give scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant. However, more advanced research is warranted to determine the activities of bioactive compounds *in vitro* and *in vivo*, establish their underlying mechanisms of action and commence the process of clinical research. This review consists all the updated information about secondary metabolites, medicinal properties and tissue culture studies on *Aegle marmelos*.

1 Introduction

Aegle marmelos (L.) is an important medicinal plant of India and are reported to have various medicinal properties in traditional medicinal systems. The medicinal properties of *Aegle marmelos* plant have been described in the Ayurveda which translates as "knowledge of life," 5000 years back to the ancient Sanskrit text, the Vedas**.** It is as fresh and useful to humans today as it was in the ancient times yet more relevant and applicable in these modern times.

2 Botanical descriptions

2.1 Vernacular names

Commonly known as Bael , Bilva, Bel, Kuvalam, Koovalam (in Malayalam), Madtoum, or Beli fruit, Bengal quince, stone apple, Maredu (in Telugu), and golden apple[1].

2.2 Taxonomy classification

Kingdom: Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Family : Rutaceae

Tribe : Clauseneae

Genus : Aegle Correa

Species : *A. mamelos*

2.3 Distribution

The tree of *A. marmelos* grows in the dry forest of hills and plains of central India and Bangladesh. In India it is throughout cultivated including moist and deciduous forests of eastern and

western Ghats. It is found in the states of Himachal Pradesh, Uttar Pradesh, West Bengal, Tripura, Maharashtra, Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu, in India, in Ceylon and northern Malaya, the drier areas of Java and also on northern Luzon in the Philippine Islands where it was fruited first in 1914.

A. marmelos tree grows throughout the dry hilly areas, reaches up to 1,300 m tall[2].

2.4 Morphological properties

A. marmelos is a medium sized, slender aromatic tree species, 6.0 to 8.0 m in height, with a fluted bole of 3.0 to 4.5 meter having spines on branches. The bark is corky soft, light grey and exfoliating in irregular flakes. Leaves are alternate, digitated have five foliates. It bears large greenish white, scented flowers in short axillary panicles.

Fruits are globas, grey or yellowish, rind, woody, seeds numerous, oblong, compressed, and embedded in sacs covered with thick orange, coloured sweet pulp. It flowers during April-May and fruits ripen during March-April[3].

3 Chemical constituents

In India, *A. marmelos* is also grown as a temple garden plant and the leaves are used to pray Lord Shiva. It is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. Recently, the plant is screened for its medicinal properties by scientific techniques and reported for various medicinal properties.

It was first described as a steroid essential oils from leaves of *A. marmelos* were analysed by GC-MS. Among the sixteen compounds in *A. marmelos* oil the major were alpha-Phellandrene (35.7%) d-limonene (29%), subinene (16.7%) and alphapinene (6.9%). Among the twenty six compounds of feronia limonia oil major compounds are methyl chavicol (74.6%) and anethole (20%)[4]. By earlier works, but a neutral alkaloid, with one methyl or diethyl groups with degradative studies the structure of Aegelin has been established[5]. The non saponifiable fraction of the ether extract of the leaves on chromatography yielded a sterol having melting point 144-145^{o}C and identified as aegelin from the leaves of *Aegle marmelos* has also been described[5].

The different methanolic extracts of *Aegle marmelos* plant parts like leaves, fruit, bark, pulp, flora parts were prepared and screened phytochemically by standard tests. All parts showed the presence of carbohydrates, aminoacids, proteins, anthocyanins, steroids, glucosides, etc. These extract were evaluated for antioxidant activity.

The major constituents of the leaf extract were identified to be tannins, skimmianine, essential oil (mainly caryophyllene, cineole, citral, eugenol), sterols and or triterpenoids, including lupeol, and sitosterol, and amyrin, flavanoids (mainly rutin) and coumarins, including aegeline, marmesin and umbelliferone[6].

4 Pharmacological activities

A. marmelos is one of the most important medicinal tree species used in various indigenous systems of ayurvedic medicine in India. Every part of bael plant contains specific medicinal value.

Studies have indicated the presence of phenols, alkaloids, ployphenol and flavanoids compound in the different solvent extracts of the leaves of *A. marmelos,* which correlate the therapeutic activity with the chemical marker of the plant as well as the mode of action of that compound. Various parts of the tree are used for its curative, pesticidal and nutritive properties. Fresh half ripe Bael fruit is mildly astringent and used to cure dysentery, diarrhoea, hepatitis, tuberculosis, dyspepsia and good for heart and brain. Roots have antidiarrhoetic, antidote to snake venom, anti-inflammatory and wound healing properties. The leaves and seed oil have pesticidal properties.

Generally dried fruit pulp and its powder are used for the treatment of many diseases like diarrhoea. The dried powder is also used as an important remedy for chronic dysentery conditions characterized by alternate diarrhea and constipation. It has been found that extract significantly reduces blood urea and cholesterol level[7].

5 Antimicrobial activity

A. marmelos has been traditionally used for the treatment of various infectious diseases and been extensible reported to inhibit the broad range of pathogenic microorganisms. Many in vitro studies proved the antimicrobial potential of *A. marmelos* extracts towards the pathogenic microorganisms including bacteria and fungi. The aqueous, petroleum ether and ethanol extract of the leaves of *Aegle marmelos* exhibited efficient antimicrobial activity against *Escherichia coli, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae* and *Proteus vulgaris*. The ethanolic extract shows activity against *Penicillium chrysogenum* and the petroleum ether and aqueous extract shows activity against *Fusarium oxysporum*[8]. The extract of leaves was checked against multi resistant strains of *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antimicrobial activity against gram-negative strains was higher than that of gram positive strains[9].

6 Antifungal activity

The antifungal activity of essential oil isolated from leaves evaluated, the oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500ppm. It is proposed that essential oil from leaves may interfere with the Ca^{2+}-dipicolonic acid metabolism pathway and possibly inhibit the spore

formation[10,11].. By taking into consideration present study, was designed to evaluate the phytochemical and antimicrobial traits of *Aegle marmelos* fruits from M.P. species.

7 Tissue culture studies

Most of the plants raised through seeds are highly heterozygous and show great variations in growth habit and yield and may have to be discarded because of poor quality of products for their commercial release. Moreover, many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items. In recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions rather than have indifferent populations. Tissue culture has now become a well established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions. *In vitro* propagation also called micropropagation is in fact the miniature version of conventional propagation which is carried out under aseptic conditions. Research efforts in plant cell and tissue culture have increased dramatically worldwide in recent years including efforts in developing nations. Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cells, tissues and organs under sterile and controlled environmental conditions[12].

Recently, emphasis has been on genetic transformation, especially for increased production of secondary metabolites, production of alkaloids, pharmaceutics, nematocidal compounds, and also some novel compounds not found in the whole plant, regeneration of plant resistant to herbicides, diseases, and pests, scale up of cultures in bioreactors, plants with different morphological traits, and transgenic plants for the production of vaccines etc. These developments have far reaching implications in the improvement of medicinal plants as well.

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The commercial production of these crops is restricted due to the shortage of desirable planting material. Tissue culture can play an important role in rapidly increasing new cultivars of these fruit crops.

Micropropagation of mature trees has been a difficult task due to various factors like exogenous and endogenous infection, presence of Phenolic compounds, hard tissues long complex life cycles, great genetic variation, etc. as reported by Zimmerman (1985)[16].

It is well known that tissue culture propagation of plants is influenced by various factors, like genotype, age and source of initial plant tissues[17].

Aegle marmelos is conventially propagated through seeds. seeds have short viability and are prone to insect attack. Vegetatively it is propagated through root suckers which is slow, difficult and cumbersome*.* Indiscriminate collection resulted in the disappearance of this plant from the wild source and the species is reported to be vulnerable. Due to the use of root for medicinal purposes destructive harvesting poses a serious threat to the sustenance of the tree. propagation through tissue culture ia a viable alternative method for this species. Several workers have reported in vitro propagation by using axillary bud multiplication[18, 19], nuclear calli[20] and from leaf explants[21]*.*

kumar and Seeni (1998)***[22]*** has been reported rapid clonal multiplication by enhanced axillary bud proliferation by using single node segment of 25 years old tree on MS medium on 1.0 mg/l indole -3-acetic acid (IAA). The 12.1 number of shoots was initiated upto 5.2 cm length in 7 weeks culture. All sterile plant parts like node, leaves and internodes were subcultured on the medium having BAP (0.05mg/l- 2.5mg/l) were equally shows regeneration potential. Most respondive plant part for shoot multiplication is nodal region which produces uniform (3.8-5.3 cm) shoots were harvested for rooting. Shoots were best rooted in the medium containg 0.5 mg/l IAA (70%) or 10.0 mg/l IBA (90%)

Hossain *et al., (1994b)*[20] has reported regeneration of plantlets from in vitro cultured cotyledons from from seedlings of various ages of *Aegle marmelos* were cultured on Murashige and Skoog medium supplemented with different combinations of growth regulators. The ten days old seedlings was shows best shoot induction response on BA (benzyladenine) in the concentration of 2.0 mg/l. The additional of indole-3- acitic acid (IAA) in the concentratio of 0.2 mg/l improved shoot induction and multiplication efficiency. The studies shows that the proximal part of cotyledon had the highest regeneration potential. Multiple shoots were elongated on MS medium containing 0.5mg/l kinetin and 0.1 mg/l gibberllic acid. About 25% of regenerated shoots were rooted on half strength MS with 0.5 mg/l indole-3-butyric acid.

Types of explants, explants collection season is also one of the most important factors for the establishment and growth of *in vitro* cultures.

An improved micropropagation protocol for Bael tree has been reported by Raghu et al., (2007)[23] by enhanced axillary shoot proliferation method from mature node explants. This report shows the seasonal variation response of explants under in vitro conditions. The explants collected and used for shoot induction in the month of October and November shows maximum bud break response (72.8% -78.66%). For the multiplication protocol Murashige and Skoog medium with 0.5 mg/l BA (6-benzyl adenine) was used which produces an average of 6.2 shoots per explants, increases an avrage of 16.3 shoots after third subculturing. invitro rooting was achieved in the medium with different auxins at varing concentration and combinations. In the experiment in vitro raised shoots wre rooted ex vitro by giving pulse treatment with naphthoxy acetic acid (NOA) and IBA and then in chlorogenic acid followed by planting in moist sand. By this method 83.9% of plantlets get survived and the standardised mehtod was used for large scale production and conservation of this endangered medicinal plant. Chandra and Padaria (1999)[24] has also initiated shoots buds from axillary meristem of *Lichi* cv. seedless on MS medium supplimented with 0.2 mg/l BAP with 0.1 IAA mg/l and 0.5 mg/l GA_3 to achieved adventitious shoot development.

Micropropagation of *A. marmelos* by *in vitro* techniques has been reported from different explants, i.e. cotyledonary node**,** root segments**,** nucellus**,** and single-node segments***[25].***

First report of tissue culture studies through somatic embryogenesis in *A. marmelos* is by using zygotic embryos showed the somatic embryo (18 percent) and the number of somatic embryo per explants (12) was observed in the presence of 2,4-D and BA as per the observation after 42 days**.**. High frequency regeneration from zygotic embryos, maximum number of shoots per explants was obtained from 10-150 days old cotyledons of the seeds has reported by Islam *et al.,* (1995)[26].

Gupta *et al.,* (2008)[27] had reported a protocol for micro propagation of Bael (*Aegle marmelos* (L.) Corr.) by using the nodal explants of 30 year old tree to initiate cultures. Two cytokinins, viz., 6-benzylaminopurine (BAP) and kinetin (KN) were used in varied concentration (0.1-2 mg/l) for shoot multiplication. BAP (2 mg/l) was found better than KN, where a 3- fold increase in the number of shoots was recorded in 4 weeks. A synergistic influence of cytokinin and auxin was also observed in the present study. A combination of 0.5 mg/l BAP and 0.1 mg/l IAA induced the formation of maximum number (4.5) of shoots (2.5 cm). For rooting of in vitro raised shoots, different auxins like NAA, IAA and IBA (0.1–2.0 mg/l) were tested. IAA (0.01 mg/l) was found better than NAA and IBA. It was concluded that elite cultivars of Bael can be micro propagated, without undergoing callus phase, using BAP (0.5 mg/l) with IAA (0.1 mg/l) for shoot multiplication and IAA (0.1 mg/l) for rooting, to produce true-to-type in vitro plants. The in vitro raised plantlets were acclimatized with 30% success.

Pati *et al.,* (2008)[28] studied that the season of explants collection is one of the most important factor in establishment and growth of *in vitro* cultures to evaluated *in vitro* clonal propagation of *Aegle marmelos* CV. CISH-B1 through enhanced axillary multiplication. It was reported that the position of node on explants also plays role in determining the growth and differentiation of cultures. Three centimeter long shoots having one axillary bud excised from 10-15th nodal region of shoots during September gave quick *in vitro* bud burst (5.33 days) and maximum number of proliferated shoots (9.0 per explant) when cultured on Murashige & Skoog medium supplemented with BAP (8.84 µM) and IAA (5.7 µM). The micro shoots were rooted (100%) on ½ strength Murashige & Skoog (MS) medium supplemented with (IBA) indole butric acid (49.0) and (IAA) indole acitic acid (5.7 µM). *in vitro* rooted plants were acclimatized on autoclaved coconut husk containing ½ strength MS plant salt mixture and under shade net house (50 % shade 70-80 % RH). The micropropagated plants were tested for its genetic fidelity using 13 RAPD, 3 ISSR and 2 DAMD primers. Profile obtained by all the three single primer amplification reaction (SPAR) technique from mother tree and micropropagated plants revealed genetic integrity of micropropagated plants with that of mother tree.

Callus induction and plantlet regeneration of *A. marmelos* by using cotyledon explants has achieved by Hazeena and Sulekha (2008)[29] on Murashige Skoog medium supplemented with BAP (2.2 µM) and 2, 4-D (2,4-dichlorophenoxy acetic acid) in the concentration of 2.26 µM. This medium was recorded the best combination for highest growth of callus formation and proliferation. Callus regenerated from shoots was best recorded on MS medium having 8.8 µM BA with 2.8µM IAA. Callus derived shoots shows best rooting in the medium contains 12.3µM IBA (indole-3- butric acid) and the plantlets were acclimatized in highest percentage in sand before transfer to soil.

Warrier *et al., (2010)*[30] has studied *invitro* propagation of *A. marmelos* by using mature nodal explants. Rapid clonal multiplication protocol was developed by using different media combinations. For initiation the medium treated with benzyl amino purine (BAP) in the concentration of 0.10 to 1.0 ppm for five weeks, followed by transfer the culture to the higher BAP (2.5mg/l) in woody plants medium. This medium combination proved the most beneficial for the induction and multiplication of shoots (6.0 per explants). Roots were induced from the micro shoots in the medium treated with 3000ppm Indole butric acid (IBA).

Yadav and singh (2011)[31] have reported in vitro propagation and biochemical analysis of field established wood apple (*Aegle marmelos*) and describes an improved protocol for rapid multiplication and shoot regeneration from nodal segments on Murashige and Skoog (1962) medium with various concentration of auxins and cytokinins alone and in combinations.

BAP was found to be more effective than kinetin for shoot multiplication. Nodal explants responded most favorably at BAP (2.0 mg/l) producing maximum number of shoots (8.0) and uniform shoots facilitating their simultaneous harvest for rooting. The medium supplemented with 2.0 mg/l BAP with 1.0 mg/l IAA was found to be most prolific combination of the treatments with regard to number and length of shoots. Creamish friable compact callus accompanying multiple shoots (8.0) was achieved from nodal segment on MS medium fortified with 2.0 mg/l BAP with 0.5 mg/l 2, 4-D (2, 4-Dichloroacetic acid) within 8 days of culture. Besides that the biochemical parameters, like chlorophyll, total sugars, reducing sugars and proteins were estimated in leaf tissue from both *in vivo* and *in vitro* raised plants in order to establish the sustainability of plants.

Micropropagation of Guava (Psidium guajava L.) has been done by Mishra *et al.,* (2007)[32] by using shoot bud culture, proliferation. The explants were pretreated in solution containing 0.1% Carbendazime and 100 mg/L PVP for 1 hour and then washed with Tween-20 wetting agent. The pretreated explants were further treated with HgCl2 0.1% for 5 minutes aseptically followed by six washing in autoclaved distilled water. The sterilized explants were cultured on MS medium supplemented with BAP (3.0 mg/L) for shoot bud induction and proliferation. The proliferated shoots were then subcultured on MS medium containing 10 mg/L IBA for rhizogenesis. The rooted plants were finally shifted to autoclaved coconut husk fortified with ½ MS salt mixture for acclimatization.

Puhan and Rath (2012)[33] has established an efficient protocol for rapid in vitro propagation by using meristem explants of *A. marmelos* through axillary bud multiplication. High frequency bud break were induced on Murashige and Skoog's medium supplemented with 0.5 mg/l benzyladenine (BA). After ten days the nodal initiated cultures started multiplying with the formation of callusing. The culture shows good growth when transferred to the medium having BA (0.5 mg/l) with either Kinetin or gibberellic acid (GA_3). Excised multiple shoots of 2 to 3 cm long were transferred on MS half concentration having 2.5 mg/l IBA (Indole butric acid) and 0.5% AC (Activated charcoal) for root formation.

Baher *et al.,* (2013)[34] has reported adventitious plantlet regeneration from different explants of *Aegle marmelos* such as epicotyls, cotyledon, hypocotyls and root explants obtained from four week old axenic seedlings. For shoot induction and multiplication the cytokinin BAP shows more effective cytokinin. MS medium supplemented with 2.2 µM BAP with 1.425 µM indole-3-acetic acid (IAA) produced maximum number of shoots. Addition of an auxin along with cytokinin improved shoot multiplication capacity of all explants tested, it was observed that epicotyls shows highest average number of shoots. The regenerated and elongated shoots were transferred for root induction on the medium containing different concentration of auxins and was best in the medium augmented with 2.85 µM IAA (Indole acetic acid) and about 90% of the rooted plants survived hardened and transfer to the field.

While *in vitro* micropropagation through nodal explants was reported by Bindu (2013)[35] on two cytokinins, viz., 6- benzyl amino purine (BAP) and Kinetin (KN) in varied concentration (0.1 to 2 mg/l) for shoot induction and multiplication. In that investigation 2.0 mg/l BAP was found better than other treatment where a threefold increase in shoot numbers within four weeks. A synergistic effect of cytokinins with auxins was also observed in that study where a combination of BAP (0.5 mg/l) and IAA (0.1 mg/l) initiate the maximum number of shoots. Here IAA (0.1mg/l) was found better than NAA and IBA for root formation to produce true to type *in vitro* plantlets.

Plant regeneration of *Aegle marmelos* from cotyledonary explants has been reported by Pradeepa *et al.,* (2014)[36] by the formation of organogenic callus on Murashige and Skoog (1962) medium supplemented with BAP and Zeatin-6-furfurylamine (0.5 to 2.5mg/l) with 0.5 mg/l NAA (α-Napthalene acitic acid) under dark condition. Callus was transferred to hormone free medium to produce shoots under light condition which was rooted on the medium contains different concentrations of IBA (0.5 to 3.0mg.l) and BAP (1.0mg/l).

Shahina *et al.,* (2015)[37] has studied the effect of explants origin on clonal propagation of *Aegle marmelos* from nodal explants of aerial and root suckers on with various concentrations of growth regulators. Results proved that the nodal segments of root suckers have more potential to regenerate large number of shoots rapidly compare to aerial shoots on similar medium containing BAP and NAA. Cultures were maintained up to the fifth subculture stage on the regeneration medium and by the end of passage e*x vitro* rooting in micro shoots was employed to shorten the propagation span, with an added effect on acclimatization success.

In vitro seed germination and clonal propagation through epicotyls explants of *A. marmelos* has been studied by Parihar and Kumar (2015)[38] to develop an efficient and rapid clonal production of this important medicinal. The explants which were used were obtained by *in vitro* seed germination in the presence of different growth regulators on MS medium. The presence of Kinetin shows best seed germination and seedling growth. *in vitro* raised seedlings were used for shoot multiplication on cytokinins containing medium by which maximum (19.35±0.32) multiple shoots were obtained on the combination of BAP (1.5

mg/l) with the same concentration of Kinetin. The regenerated shoots were best rooted on the medium containing 1.0 mg/l IBA (Indole butric acid). This standardized method is proved the best used for large production of *A. marmelos.*

The technology of plant tissue culture played important role in the conservation of medicinal plants in the rapid multiplication and reintroduction to nature of endangered species in the assessment and monitoring of biodiversity, as a source of new tools for large production and conservation and in the search for new gene product of therapeutic use. Species of medicinal and aromatic plants at risk need to be multiplied with minimum loss of time and reintroduced for establishment in their natural habits. *In vitro* protocol for multiplication of endangered species could be very useful for those species whose propagation through conventional means was difficult.

Researchers aim to obtain increased production of secondary metabolites, increased plant production, higher nutritional value and greater plant resistance to adverse weather, pathogenic agent and pests.

8 Microbiological studies

Medicinal components from plants play an important role in conventional medicine. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory**.** Since their discovery, antimicrobial drugs have proved remarkably effective for the control of bacterial infections.

However, it was soon evident that bacterial pathogens were unlikely to surrender unconditionally, because some pathogens rapidly become resistant to many of the first discovered effective drugs. New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains**.**

Examples of some microorganisms that gained resistance to antimicrobials are *Escherichia coli*, *Proteus* sp., *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella enteritidis*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Candida albicans.* Different parts of plants like leaf, fruit (both unripe and ripe) and their extracts have been reported to have anti-bacterial, anti-fungal, anti- viral activities.

Fruits and leaves of *Aegle marmelos* were subjected to antibacterial screening but were found to be inactive[39].The essential oil obtained from the leaves has shown a broad spectrum of anti-bacterial and anti-fungal activities[40]. The aqueous decoction of the leaves has been reported to have a significant hypoglycemic effect[41]. Extract obtained from leaves and fruits of *Aegle marmelos* showed antibacterial activity, against *Staphylococcus aureus, Escheritia coli* at 0.5% concentration.

Valasaraj et al., (1997)[39] has studied antifungal activity of leaves and fruits of *A. marmelos* by using 80% ethanol extract by agar well diffusion method, against two fungi, *Candida albicans* and *Aspergillus niger*.

The antifungal activity of essential oil of leaves of *A. marmelos* has been evaluated[11] by using spore germination assay of the most resistant fungus *Fusarium udum* which inhibited 8% at 400ppm. And the aqueous extract of *Aegle marmelos* were screened against two rice fungal pathogens, *Magnaporthea grisea* and *Rhizoctonia solani*.

Sivaraj *et al.,* (2011)[42] The antimicrobial activity of the leaves of *A. marmelos* was performed by agar well diffusion method. The aqueous, petroleum ether and ethanol extract of the leaves of *Aegle marmelos* exhibited efficient antimicrobial activity against *Escherichia coli*, *Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae* and *Proteus vulgaris.* The ethanolic extract shows activity against *Penicillium chrysogenum* and the petroleum ether and aqueous extract shows activity against *Fusarium oxysporum*.

Antibacterial activity of leaves extracts of *Aegle marmelos* has been investigate by Yadav *et al.,* (2014)[43] by using crude methanolic and chloroform extracts through agar disc diffusion method. Two gram positive (*Bacillus subtilis*, *Staphylococcus aureus* and three gram negative bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia, Escherichia coli)* were used for the study. The zone of iinhibition obtained from the method showed that methanolic and chloroform leaf extracts exhibited maximum (15mm) activity against *P. aeruginosa* and 18mm against *S. aureus* respectively.

The antibacterial activity was maximum at higher concentration (100 mg/ml) and decreases gradually with the decrease in the concentration of extracts against all pathogens. The studies may be attributed to different phytocontituents.

Mujeeb *et al.,* (2014)[44] has evaluated phytochemicals, antimicrobial activity, and determination of bioactive components from leaves of *Aegle marmelos* through aqueous and methanolic extracts of 18 varieties/accessions. The crude extracts of *A. marmelos* revealed the presence of several biologically active phytochemicals with the highest quantity of alkaloids, flavonoids, and phenols in Pant Aparna variety.

The antibacterial efficacy was investigated against pathogenic bacterial strains and the highest inhibitory activity of aqueous extract was obtained against *S. epidermidis*, whereas methanolic extract was found to be most potent against *S. aureus* at 40 mg/ml concentration.

However, in aqueous: ethanol, the best results were observed against *E. aerogenes* followed by *K. pneumonia* and *S.*

epidermidis. The MIC of aqueous and methanol extract of *Aegle marmelos* ranged from 10 mg/ml to 40 mg/ml whereas in aqueous and ethanol it ranged between 40 mg/ml and 160 mg/ml. The GC-MS analysis revealed the presence of many bioactive compounds such as flavonoids, alcohols, aldehydes, aromatic compounds, fatty acid methyl esters, terpenoids, phenolics, and steroids that can be postulated for antibacterial activity.

Phytochemical and antimicrobial activity of fruit pulp of Aegle marmelos has studied by Behera *et al.,* (.2014)[45]. The investigation was carried out to evaluate the phytochemical constituents and antibacterial activities of fruit pulps of Aegle marmelos (Linn.) Correa. The crude extract revealed the presence of Reducing Sugars, Saponins, Tannis, Flavonoids and Phenols.

Further the total phenolic and flavonoid content was also estimated. Besides, the crude extract was tested for antimicrobial activity against two gram positive strains of *Staphylococcus aureus* (ATCC 29213, ATCC 700699) at different concentrations of 10, 50, 100, 250 and 500ug/ml at different time span of 3hrs. It was found that a concentration range 50-100ug/ml of the ethanolic extracts was effective in inhibiting the growth of bacterial strain *Staphylococcus aureus* ATCC 29213. 250 ug/ml was effective for aqueous extract and 500ug/ml concentration was effective for petroleum ether extracts in inhibiting the growth of the above strain.

When the similar study was carried out using other strain, *Staphylococcus aureus* ATCC 700699, it was found that 100ug/ml of ethanolic extract, 250ug/ml and 500ug/ml of petroleum ether was effective in inhibiting the growth of bacteria, whereas the concentration of aqueous extracts taken were ineffective against the growth of the bacteria.

9 Conclusion

The review given here can be used for multiplication of the above said medicinal and economical plants commercially. *Aegle marmelos* offers many promising prospects for both traditional and modern medicine. *Aegle marmelos* is apparently a potential herbal therapy for many ailments. This review summarized the existing ethnobotanical uses, phytochemistry, pharmacological activities, safety evaluation, and conservation status on *Aegle marmelos*.

10 Conflict of interests

None

11 Authors contributions

AG and TT carried out literature review of work and SK drafted the manuscript.

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