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ADVANCED APPROACHES: AEGLE MARMELOS CORREA AS HERBAL MEDICINAL PLANT

Shital Devkar[1]*, Samradhnyee Sawant[2], Vaishnavi Mali[3], Vaishnavi Patil[4], Prachi Khochage[5] and Dr. Nilesh Chougule[6]

[1,2,3,4]Student of Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur 416112, Maharashtra, India.
[5]Assistant Professor of Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur 416112, Maharashtra, India.
[6]Principal of Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur 416112, Maharashtra, India.

***Corresponding Author: Shital Devkar**
Student of Ashokrao Mane Institute of Pharmacy, Ambap, Kolhapur 416112, Maharashtra, India.

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ABSTRACT
The sophisticated methods of beal plant are studied in this review liturature. These methods include the following: green synthesis from silver nanoparticles, pharmacological activity, chemical constituents, botanical profile, botanical description, and genetic polymorphism. Additionally, RP-HPLC was developed for standardization. Bael also known as aegle marmelos is a naturally occurring herb which containing numerous parts, such as roots, fruits, leaves and bark which are used in traditional indian medicine throughout history. A wealth of clinical and extensive experimental data provides strong backing for the utilization of marmelosin, a prominent phytoconstituent in this plant, in addressing a wide spectrum of health conditions. Its actions include mending ulcers, angiogenotoxic, diuretic, antidiabetic, antibacterial, antiinflammatory, anticancer, antipyretic, antidiarrheal, antimicrobial, antiviral, radioprotective, chemopreventive and antifertility. In-vivo studies conducted on animal models have substantiated the plant's antidiabetic potential, suggesting its utility in managing conditions like diabetes, cancer, and cardiovascular diseases. Advanced multifactorial approaches of this plant which are nanotechnology, biotechnology, modern standardization methods and modern method of identification. In recent times this advanced approaches are usefull in future research purpose. This review article which includes recent advanced technologies which are very beneficial for research experiments.

KEYWORDS: Multifactorial, Chemopreventive, Ailments, Approaches, Complementary.

INTRODUCTION
This plant is known as "Bael" in Marathi. The "Aegle Marmelos" was the scientific name of it and Rutaceae is its family. Aegle marmelos having several parts that are useful for treating different illnesses and have uses in cutting-edge technologies. Extracts from its fruits or leaves can be used to create silver nanoparticles in a green way, and genetic engineering techniques can be used to genetically modify bael. These are a few applications for the various bael plant parts. Because of the variety of phytochemicals found in Aegle marmelos, such as tannins, gums, resins, coumarin, polysaccharides, gums, and essential oils, it can effectively treat a broad range of illnesses. When it comes to the nutritional value, it is far more important than other fruits. It also releases a higher percentage of oxygen into the atmosphere than other trees, which helps to create a cleaner climate, which makes it an essential component of environmental conservation efforts. The plant possesses a number of significant therapeutic qualities, including antiinflammatory, analgesic, insecticidal, antipyretic, antilipidemic, immunomodulatory and antiproliferative actions. Post-harvest technologies are applied to the fruits to produce an array of products, which encompass candies, toffees, jams and juices. Which increases their shelf life and lowers post-harvest losses. This plant is a valuable source of revenue for farmers.[1] Owing towards their unique biological and physical properties, nanoparticles was gained importance in medicine, electronics and biology fields. The strong bactericidal and inhibitory effects of silver, along with its extensive antimicrobial properties at low concentrations, have been well-established for a long time.[2] AgNPs have garnered substantial attention within the realm of metallic nanoparticles due to their multifarious applications. These encompass but are not limited to, their utilization in water purification systems, food packaging and as coatings safeguarding biomedical apparatus.[3] In higher animals it is one of the least hazardous and safest antibacterial agents.[4] In skin infection condition the

silver nanoparticals produces their anitimicrobial activity.[5] For the purposes of studying species polymorphism and crisis identification, molecular markers are an essential component. There are several medicinal uses for the Aegle marmelos plant. It has religious significance in addition to containing a greater nutritional value. India's national medicinal plants board has been trying to increase the country's medicinal plant resource base through cultivation and preservation. A great deal of wildlife has been successfully domesticated and farmed for use in farming. Indian Council of Forestry Research and Education (ICFRE) was greatly aided development of medicinal plants. For a number of medicinal plant species which established domestication and cultivation protocol that are of conservation concern.[6] Worldwide, there is a growing trend in the utilization of complementary and alternative medicine (CAM) for diabetes management. CAM includes medical procedures that have been used in the past or that have non-traditional medical roots.[7] Supplementary foods, herbal remedies, and complementary therapies like yoga and meditation are also included. Owing to their millennia-long history of use, herbal antidiabetic remedies are currently used either in conjunction with other treatments or independently in the early stages of diabetes.[8]

PLANT PROFILE

Scientific Name: Aegle marmelos
Sanskrit Term: Bilva
Commonly Known As: Bael Tree
Family Classification: Rutaceae
Utilized Parts of the Plant: Root, bark, Fruit, leaf.

SCIENTIFIC CLASSIFICATION

Kingdom: Plantae Order: Sapindales
Division: Angiosperms Family: Rutaceae
Class: Eudicots Subfamily: Aurantioideae
Subclass: Rosids Genus: Aegle Correa
Tribe: Clauseneae Species: A. marmelos.[9]

Table 1: Vernacular designations of aegle marmelos.[10]

Name	Language
Billi	Gujarati
Bele	Orissa
Bel	Urdu
Kaveenth	Marathi
Bel, Shreefal	Bengali
Vilva Pazham, Vilva Maram	Tamil
Maredu	Telugu
Toum	Lao
Bnau	Khmer
Modjo	Javanese
Oranger du Malabar	French
Ohshit, Opesheet	Burmrse
Mojo tree	Indonesian
Pokok Maja Batu	Malay
Mapin, Matum, Tum	Thai
Shreephal, Bilva, Bilwa	Sanskrit
Sir Phal	Old Hindi
Aegle Marmelos	Latin
Bengal Quince, Wood/ Stone apple	English
Trai mam, Mabau nau	Vietnamese
Gudu, Bel	Nepali

BOTANICAL DISCRIPTION

India's deciduous forests are home to the medium-sized, slender, aromatic bael fruit tree, also known as aegle marmelos. It is growing, with a 3.0-4.5 metre somewhat grooved bole, and reaches a height of 1200 metres in the andaman islands and the western himalayas. Hindus generally revere this tree because, during worship, its leaves are offered to Lord Shiva.[11] This tree represents lord kailashnath in a different way, according to hindu mythology. Traditional chinese medicine uses this tree stem, root, fruit and leaves for treatment of a extensive ranges of illness at all phases of enlargement.[12] Aegle marmelos is belongs to monotropic genus. The height of aegle marmelos plant was 25-30 feet. This is medium sized herb and height was 25-30 feet. It has spreading, soft, thick, short and peeling bark containing stem. They have thorny branches. The branches of the lower stem droop. This tree has sharp axillary spines. The leaflets have an oval or lanceolate shape and measure 2.5-10 cm in width and 4–10 cm in length. Each leaflet has three to five. The lateral leaflets do not have petioles; only the terminal leaflet does. The petiole has a diameter of one to two inches. A strange smell comes from crushed mature leaves. India experiences flowering in April and May. The fruits are ripened in 10-11 months.[13] This plant produces flowers that are usually grouped in groups of four to seven along the young branches. The flowers have four succulent, recurved petals. These greenish-

white flowers have an unusual and distinctive aroma. Fruits range in size from 2 to 4 inches in diameter, with spherical or oval shapes possible. The fruit has a firm, thin shell that is inherently woody. It has a greenish hue when unripe; as it ripens, this colour changes to a yellowish one. Fruit pulp has a yellow, thick, sweet, resinous, and fragrant texture. It is divided into 8 to 15 segments. Each of the almost one-centimeter-long seeds is embedded in the pulp. Encased in a sticky sac, these hard, flattened oblong seeds have fuzzy hairs on them.[14] Rich, well-drained soil is ideal for aegle marmelos to grow, but it can also bear fruit in southern Florida's oolitic limestone. Additionally, it may grow in soils that range in pH from 5 to 8 and are stony, alkaline, or swampy. It has a reputation for doing well in india in environments that other fruit trees find difficult.[15]

a) Bael Plant Leaves.

b) Bael Stem.

c) Bael Plant Bark.

d) Bael Fruit

Figure 1: Aegle marmelos plants parts.

SOURCE AND GEOGRAPHICAL DISTRIBUTION

The bael tree was first domesticated in central india and the eastern ghats. It is found mostly in subtropical and tropical areas which is innate to indian subcontinent. In the lower himalayan hills this plant is seen and which is up to an elevation of 500 metres. The plant known as bael grows along the east coast, in madhya pradesh, deccan plateau, jharkhand, chattisgarh, uttar pradesh, bihar, and uttarakhand.[16] Chinese buddhist traveler hiuen tsiang, who arrived in India in 1629 A.D., recorded this tree and other trees in the area.[17] In addition, a few egyptian gardens in trinidad and surinam are growing it. The citrus collection in florida is home to specimens of bael that have been acquired and are being maintained.[18] Due to the tree's hypoglycemic qualities, it has been used in bangladesh for antiproliferative and fertility-control purposes as well as in sri lanka.[19] Europe first saw the introduction of the bael fruit in 1959.[20] The tree is allegedly grown in ceylon, java, philippines islands and the northern malaya, where it was initial reported to have produced fruit in 1914.[21]

ETHNOMEDICINAL SINGNIFICANCE OF BAEL PLANT

In conventional medicine, this plant is used to treat ichthyosarcotoxism, postnatal care, gastrointestinal problems and recurrent fevers.[22] Due to its potency against diarrhoea and dysentery, aegle marmelos fruit has been included to the british pharmacopoeia.[23] Furthermore, chopra (1982) correctly noted that "No drug has been longer and better known, nor has it been more appreciated by the inhabitants of India than the Bael fruit".

Table 2: Ethnomedicinal importance of bael plant parts.

Plant Part	Ethnomedicinal Importance
Leaves	Leaf extracts are effective treating ulcers, abscesses, backaches, and vomiting. cuts, heart disease, bronchitis, blood sugars, dropsy, diarrhoea, beriberi, and animal-related injuries.[24] Leaf juice has laxative properties, treat ocular infectionand asthmatic symptoms. In the combination with cumin seeds used as hair tonic, coughs and other respiratory illnesses. Additionally, leaves are employed in veterinary medicine for the treatment of wounds, as animal feed, and for stimulating the denervated nictitating membrane in anaesthetized cats.[25]
Root bark	Fish toxicity, heart palpitations, melancholia, and intermittent fevers can all be treated with the root bark. Bark Juice and cumin in milk increase the volume of the seminal fluid. Extracts from alcoholic roots can treat hypoglycemia.[26] Additionally, it is used for rheumatism, anti-amoebic, cardiac issues, stomach problems, and dog bites.[27]
Flower	Distilled floral extracts are used as a diuretic, anti-diabetic, anti-dysentery, local anaesthetic and stomach and intestine tonic. Used for treatment of epilepsy.[28]
Fruit	The usage of dry powder and mustard oil to handle burn cases. Fruits are utilised for ulcer, intestinal parasites, tonic, digestive, brain and heart issues, gonorrhoea, dysentery, stomach issues, epilepsy and constipation. Extracts derived from fresh fruit have demonstrated the ability to lower blood pressure effectively.[29] Furthermore, a finely powdered form of unripe fruit presents an alternative treatment for intestinal parasites like ascaris lumbricoides and entamoeba histolytica.[30]

CHEMICAL CONSTITUENTS

The Aegle marmelos plant has a number of chemical constituents that are important for managing a number of illnesses. Different plant components have yielded chemical compounds that have been identified and isolated, including steroids, coumarins and alkaloids which is used in multiple disorder.

Table 3: Chemical constituents in bael.

Coumarins	It includes 7-geranyloxycoumarin [7-(2, 6-dihydroxy-7-methoxy-7-methyl-3-octaenyoxy) coumarin], marmelid, marmenol, scoparone, methyl ether, imperatorin, xanthotoxol, marmesin, marmelosin, alloimperatorin, psoralen, umbelliferone and scopoletin.[31]
Alkaloids	The array of compounds found within aegle marmelos leaves is diverse, encompassing aeglin, aegelenine, dictamine, fragrine (C13H11O3N)32, and a myriad of other complex molecules such as O-methylhalfordinine, isopentenylhalfordinol, N-2-[4-(3', 3'-dimethylallyloxy) phenyl] ethyl cinnamide, N-2-hydroxy-2-[4-(3', 3'-dimethylallyloxy) phenyl] ethyl cinnamide, N-2-hydroxy-(4-hydroxyphenyl) ethyl cinnamide and many more.[32,33] Notably, the fresh leaves harbor a substantial amount of shahidine, an uncommon alkaloid characterized by an unstable oxazoline core. Shahidine is identified as the parent compound of aegeline and several other amides, exhibiting sensitivity to moisture but displaying stability when in a dimethyl sulfoxide environment. Its structural determination relied on a comprehensive spectroscopic investigation. Conceptually, oxazolines serve as precursors to hydroxyl amides and oxazoles prevalent in various plant species. Intriguingly, certain gram-positive bacteria demonstrate resistance to shahidine.[34] Initially classified as a sterol, aegeline was later elucidated as a neutral nitrogenous molecule following meticulous analysis. Recent revelations introduced anhydromarmeline, a novel chemical category discovered within a

	series of phenylethyl cinnamides. Additionally, aegelinosides A and B were identified as potent alpha-glucosidase inhibitors sourced from Aegle marmelos leaves.[35] These discoveries further reinforce the traditional medicinal use of Aegle marmelos in treating diabetes.[36]
Polysaccharides	Upon hydrolysis, a distinctive blend of carbohydrates surfaces, including L-rhimanose, galactose, arabinose and uronic acid. Notably, the seed oil comprises a composition rich in linolenic acid, oleic, palmitic and stearic.[37] These compounds, recognized for their organic nature, boast an aromatic ring coupled with a three-carbon side chain, defining them as phenolic compounds. Among the array of phenylpropenes, phenylpropanoids, lignans stand prominent and hydroxycoumarins. An intriguing addition to the chemical inventory is marmesin, a novel compound derived from leaves and found as a constituent within the heartwood and roots. This unique chemical signature presents a fascinating glimpse into the diverse chemical constituents harbored within this natural source.[38]
Terpenoids	In the Indian research landscape of the 1950s, a wave of significant investigations delved into exploring the essential oil extracted from Aegle marmelos (L.) Correa leaves. Among the consistent discoveries across the fruits, leaves and twigs, phellandrene emerged as a prevalent component within the essential oils.[39] Further analysis revealed alpha-phellandrene (at 56%) and p-cymene (at 17%) as the primary constituents in the leaf oil.[40] Subsequent reports from various researchers echoed these findings, reinforcing the dominance of these elements in the essential oil extracted from A. marmelos leaves. Additionally, the isolation and characterization of p-Menth-1-en-3,5-diol stemmed from A. marmelos leaves.[41] Limonene, identified as a distinctive marker, surfaced as the predominant compound within A. marmelos leaves, constituting a staggering 82.4% of the oil's composition.[42] Moreover, the presence of Gamma-Sitosterol was discerned within the leaves, adding to the diverse chemical profile revealed through these investigations. These collective findings delineate the nuanced composition of essential oils derived from aegle marmelos, shedding light on its varied chemical constituents and their concentrations.[43]
Tannnins	During the month of January, bael fruit reaches its zenith in tannin content, boasting the highest concentration among its seasonal phases. Wild variants exhibit a remarkable 9% tannin content within the pulp, whereas cultivated varieties tend to display comparatively lower levels. Remarkably, skimmianine tannins are prevalent in the leaves, known scientifically as 4, 7, 8-Trimethoxyfuroquinoline. This compound stands as a distinctive identifier within the chemical makeup of bael leaves, contributing to their unique properties and biological significance.
Caretenoids	The delicate hue of the bael fruit owes its existence to the presence of carotenoids. Within the bael plant, compounds such as marmelosin, skimmianine, and umbelliferone contribute significantly to the active constituents of carotenoids, rendering them invaluable in medicinal applications. Notably, a diverse array of compounds constitutes the bael's chemical makeup, albeit in minimal quantities. Sitosterol, ascorbic acid, crude fibers, tannins, -amyrin, carotenoids and crude proteins are among these constituents, albeit in trace amounts. Delving into the roots, one encounters additional components like praealtin D, trans-cinnamic acid, 4-methoxy benzoic acid, betulunic acid and montanin. Not to be overlooked are the presence of psoralen and the xanthotoxin scopoletin, further enriching the intricate chemical composition of the bael plant. This intricate blend of diverse compounds forms the foundation of the bael's biological significance and its potential in various medicinal applications.[44]
Seed oil	It includes linlenic acid, palmitic, stearic, oleic and linoleic acid. This are the phytochemical present in bael plants and have power to treat various illness in human being. This chemical constituents can produced activity like anti-diabetic, anti-bacterial, anti-microbial, anti-protozoan and many other.[45]

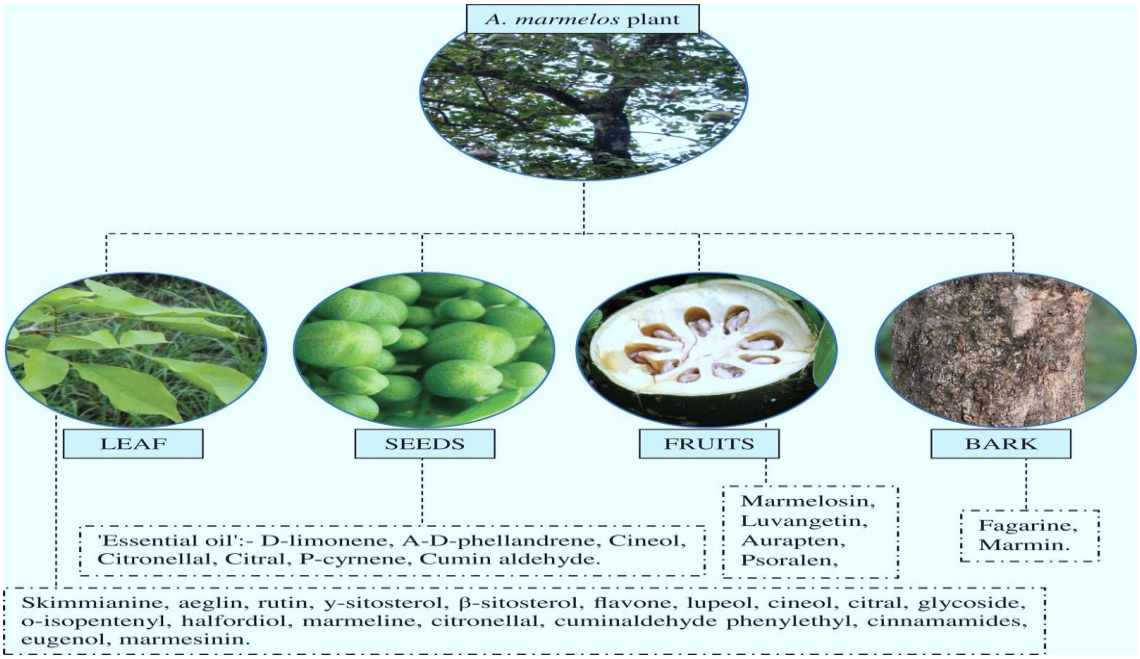
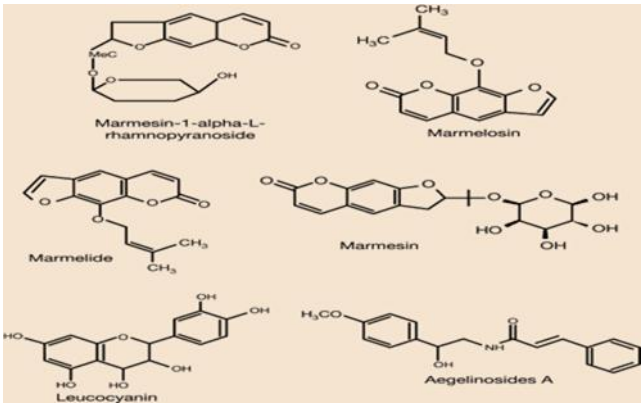
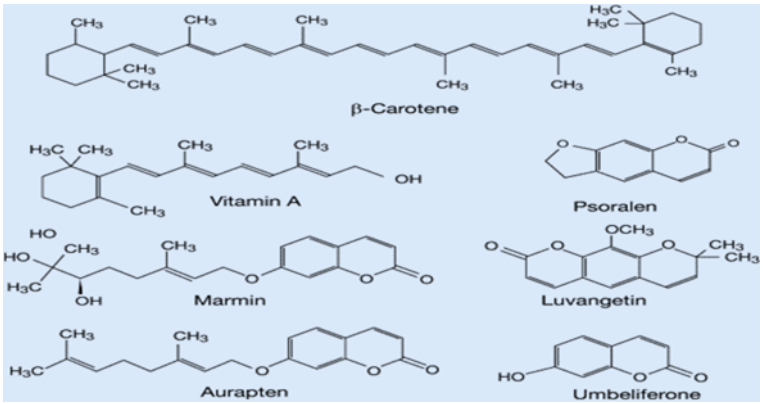


Figure 2: Phytoconstituents existent in numerous plants parts of Aegle marmelos.



(A)



(B)

Figure 3: Phytoconstituents in Aegle marmelos.

PHARMACOLOGICAL ACTIVITY

One of the most commonly utilised medicinal plant is aegle marmelos which is in the rutaceae family. Recent reports suggest this plant has a number of therapeutic benefits. The bael plant has major therapeutic benefits in all parts of it. Numerous illnesses, including respiratory disorders, chronic diarrhoea, dysentery, peptic ulcers, and constipation, are treated with bael herbal medicinal formulations.[46] The bael has traditionally used for treatment of multiple disorders, including sores, swelling, thirst, upper repiratory tract infection, thyroid disorders, ulcers, nausea, eye disorders, ulcers and

mental illnesses. It is also used to treat dysentery, stomachache, stomachic, jaundice, constipation, chronic diarrhoea, febrile delirium, acute bronchitis, snakebite, fever, asthma and inflammations. This plant containing various plant parts which produces different types of activity that are antipyretic, wound healing, ulcer healing potential, analgesic, antidiarrhoeal, antiarthitis, contractile, diuretic, immunomodulatory, antithyroid, antimicrobial, antifungal, antioxidant and radioprotective activity.[47] Bael is a sacred plant, and all of its parts are very beneficial. Typically, if one component of a plant has a pharmacological action, the remaining sections of the plant will also have the same effects. There is a good chance that the other component will provide the same or a related activity. In this case, the bael tree illustrates the same idea.[48]

Table 4: Promoted Invention of Aegle Marmelos Plant.[49]

Company Name	Marketed Formulation
Maharishi Ayurveda	Glucomap
Bliss Ayurveda	Ulco Bliss Tablet
Oushadhi	Vilwadi gulika
Ayurvedic sanjivani	Capsule Bilv Giri
Tates Remedies	Ojamin
Oushadhi	Manasamithra vatakram
Himalaya	Chayawanprash
La-Medicca (India) Pvt. Limited	Aegle Marmelos Capsule
Ambika Medico	Kof-Rid Syrup
Sydler Remedies Pvt. Ltd	Pregeight
Shrey Nutraceuticals and herbals	Leucare Capsules
Ambika Medico	Entrostat Syrup
Oushadhi	Pushyanugam gulika

ADVANCED APPROACHES OF AEGLE MARMELOS PLANT

1. STANDARDIZATION OF AEGLE MARMELOS BY USING RP-HPLC METHOD

Indian herbs like bael (aegle marmelos) have long been used for treatment of a assortment of disorders. The present project aims to standardise aegle marmelos by employing marmelosin, a product of research utilising the RP-HPLC method. The devised approach has demonstrated exceptional precision, simplicity and accuracy in achieving the specified objective. Its reliability makes it an ideal method for consistently standardizing herbal remedies incorporating raw fruit extract. This innovative technique offers a unique and reliable means to ensure the quality and consistency of herbal preparations containing such extracts, providing a robust framework for their production and utilization. The Aegle marmelos (bael) fruit pulp is an essential chemical ingredient used in many traditional herbal remedies. RP-HPLC was developed as a way to ensure a consistent quality for this fruit pulp.[50]

MATERIAL AND METHOD

Plant material

Aegle marmelos fruits were bought in the neighbourhood area. Following a 15-day incubation period at a controlled 35°C, the fruit pulp underwent a meticulous drying process for 30 days under ambient conditions in a shaded environment. Subsequently, the meticulously dried fruit pulp underwent a sequence of sieving and grinding procedures, culminating in its preservation within an airtight container, ensuring its integrity and readiness for future application as required.

Chemical

The UICT in Mumbai provided the marmelosin reference standard (Maharashtra, India). Only compounds of analytical grade were used in this investigation.

HPLC method devlopment and optimization

Physical and chemical characteristics of marmelosin, including partition coefficeint, pKa and absorption maxima were studied. The following technique is suggested.

Table 5: Chromatographical condition.

Agilent-1220 LC Infinity	Instrument
1 ml/min	Flow Rate
20µl	Injection Volume
20 mins	Run Time
Agilent TC-C18, 4.6x5 µm	Column
UV-VIS Detector at 247 nm	Dectection
Acetonitrile: Water (70:30)	Mobile Phase

Preparation of standard solution: Validation method: By dissolving 10 mg of marmelosin in 10 millilitres of methanol at a concentration of one milligramme per millilitre, a solution was created. The devised technique

involved creating a range of standard solutions with concentrations of 30, 25, 20, 15, 10 and 5 µg/ml using methanol from the stock solution. Validation of the technique was conducted according to ICH criteria, encompassing assessments of linearity, limit of quantification, range, accuracy, precision and limit of detection.

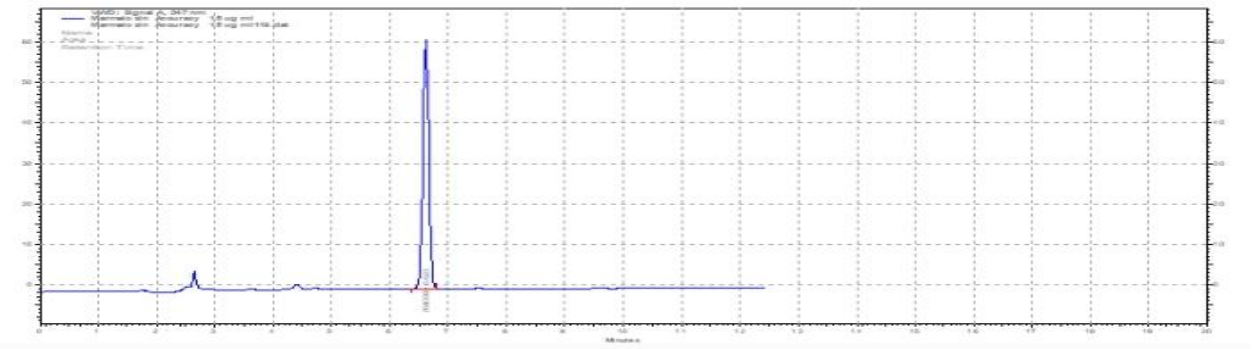


Figure 4: Demonstrative chromatogram of standard marmelosin.

Crude extract standardization

The treated fruit pulp underwent extraction via the soxhlet method to isolate its components. The ethanolic solution obtained from this process facilitated the quantification of marmelosin within the fruit solution. Dissolving one milligram of the fruit solution in ethanol initiated the preparation stage. Employing whatman filter paper, the mixture underwent filtration post 30 minutes of sonication, ensuring purity. The subsequent step involved the meticulous preparation of test solutions at a concentration of 10 µg/ml, which were then precisely injected into the High-Performance Liquid Chromatography (HPLC) system for analysis.

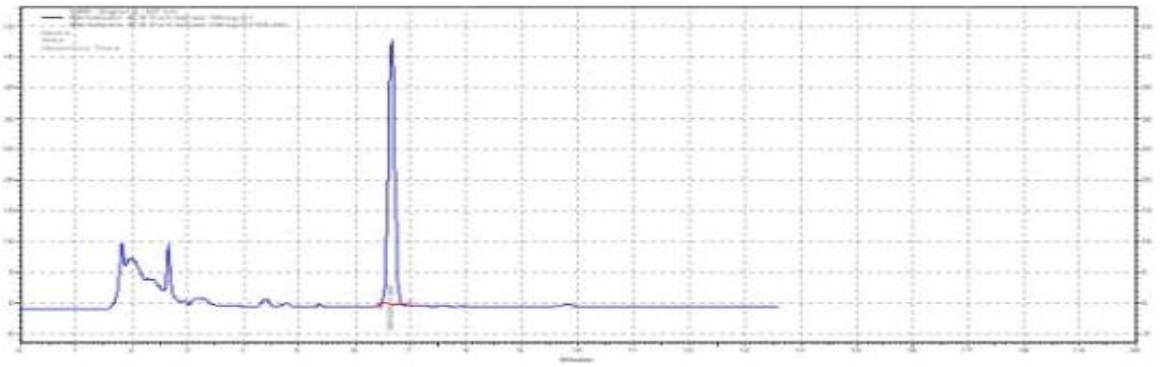


Figure 5: Demonstrative chromatogram of marmelosin from crude extract.

Herbal prepartion assay

After meticulously weighing 20 herbal tablets, an exact 10 mg quantity of marmelosin was meticulously dissolved in methanol. Subsequently, following a thorough 30-minute sonication process, filtration of this solution was executed utilizing Whatman filter paper. The ensuing step involved a careful dilution of the resulting solution before its precise introduction into the High-Performance Liquid Chromatography (HPLC) system for comprehensive analysis.[51,52]

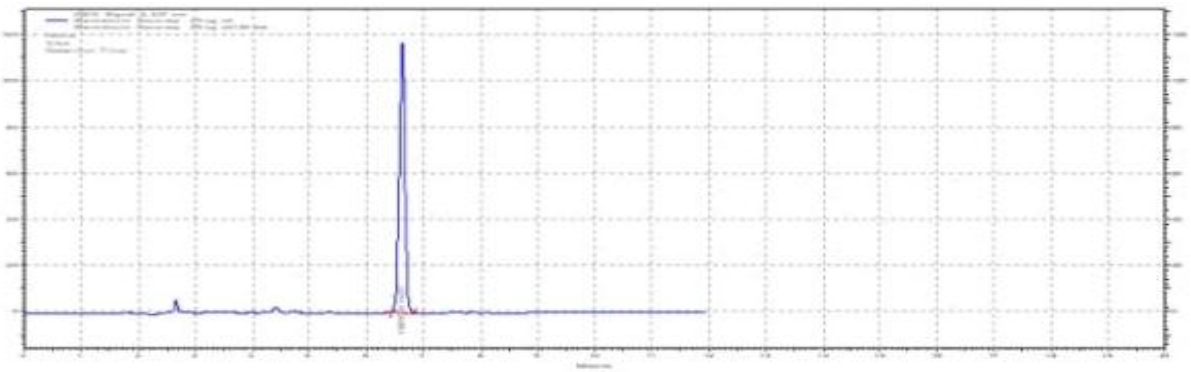


Figure 6: Representative chromatogram of marmelosin of herbal preparation.

2. UTILIZATION OF AgNPs BY GREEN SYNTHESIS FROM THE BAEL PLANT

One of the scientific fields that is developing the fastest, nanotechnology, has grown significantly in the last several years. It is an interdisciplinary field that integrates information from several fields, such as material science, biology, chemistry, physics, and engineering. One of the most well-known applications of nanotechnology is "sustainable synthesis" or "green synthesis".[53] Eliminating the usage of excessive energy, hazardous chemicals, high pressure and high temperature, this method places an emphasis on eco-friendly and efficient production.

Comparing green synthesis to traditional chemical synthesis reveals a number of benefits. Its applications in the biomedical and culinary fields are noteworthy, which makes it a desirable option for the synthesis of nanoparticles.[54] The simple and potentially more economical method of producing nanoparticles from plant extracts has garnered increased attention in recent times. The objective of this study is to fabricate silver nanoparticles through a user-friendly technique employing the aqueous extract derived from bael leaves. Plant extracts possess the capacity to serve as stabilizing and reducing agents in the production of nanoparticles.[55] Many ancient ayurvedic treatises that document the medicinal properties of bael, such as siddha, unani, sushruta and charaka samhita, attest to the plant's significant therapeutic value.[56]

This study's main goal is to determine how well synthetically produced silver nanoparticles work as antibacterial agents against aeromonas hydrophila, pseudomonas aeruginosa, streptococcus pyogenes and pseudomonas aeruginosa. These nanoparticles also have antioxidant, photocatalytic degradation and anticancer properties.

MATERIALS AND METHOD

Material

The leaves of aegle marmelos were gathered at madukkarai, coimbatore, South India, for the production of silver nanoparticles. The sample's authenticity was confirmed by coimbatore, the southern regional centre for a botanical survey of India. Merck limited, an indian company, provided the chemicals, media, and solvents.

Methods

Extract preparation

The leaves are then carefully washed under running tap water and sanitised with milli-Q water to get rid of any remaining pollutants. The leaves of nearby Aegle marmelos plants were picked. After that, they were cut and let to air dry at room temperature. In a 250 mL erlenmeyer flask, 100 mL of water and 10gm of leaves are placed. This was followed by boiling deionized water for 30 minutes at 80°C. Whatman No. 1 filter paper was used to isolate undesirable chemicals. The filter that was produced was yellow in colour and was used in further studies.

Silver Nanoparticle synthesis

Silver nitrate (AgNO3) underwent dissolution in a 0.1 mM aqueous solution to initiate the formation of silver nanoparticles. To attain the desired concentration of silver nanoparticles, a solution of 0.1 mM was synthesized by amalgamating 25 ml of plant extract with 75 ml of silver nitrate. Remarkably, within a span of fewer than two minutes, the solution underwent a rapid transformation from a transparent state to a deep, vibrant yellow hue, strongly indicating the successful generation of silver nanoparticles. After 5 minutes, the hue became a deeper shade of dark brown. Reduced silver ion levels were verified by UV-Vis spectra. To extract the synthesized nanoparticles, the mixture was centrifuged at 10,000 rpm for 20 minutes.[57] The pellet obtained from three to four repetitions of the centrifugation procedure was suspended in distilled water to eliminate the organic components of the leaf extract. Carefully, the material was extracted from the bottom of the centrifuge tube and transferred to a watch glass. Subsequently, it was subjected to drying in a hot air oven set at 600°C.

Description of AgNPs

In this investigation, the UV-Vis spectra of the reaction mixture disclosed the reduction of pure silver ions. Spectra within the 200–800 nm wavelength range were observed utilizing a shimadzu spectrophotometer. FTIR analysis was implemented to ascertain the functional groups accountable for stabilizing and reducing silver nanoparticles. An FTIR (Fourier Transform Infrared) spectrum with a resolution of 4 cm^{-1} was employed to scrutinize the nanoparticles, with the spectrum measured in the 4000-600 cm^{-1} region. The determination of the size of the silver nanoparticles involved utilizing the X'Pert Pro X-ray diffractometer. Elemental analysis of silver nanoparticles created through eco-friendly procedures was conducted using Energy Dispersive X-ray (EDX). Scanning Electron Microscopy (SEM) was deployed to scrutinize synthesized silver nanoparticles at varying magnifications. For determining the zeta potential of the silver nanoparticle suspension, the hobira technique was utilized and the stability of the silver nanoparticle aqueous solution was assessed utilizing hobira's zeta potential analyzer.

Anti-bacterial Activity

The antibacterial effects of silver nanoparticles on aeromonas hydrophila, staphylococcus aureus, streptococcus pyogenes, escherichia coli and pseudomonas aeruginosa were evaluated using the well diffusion technique.[58] The generated silver nanoparticles were added to water at 100, 50 and 25 µg/ml concentrations. The reference sample plates containing 10 µg/ml of tetracycline were cultured at 37 °C for 24 hours. Subsequent to the incubation duration, the measurement of the diameter of the inhibition zones surrounding the well was performed.[59]

Anti-oxidant Activity

The procedure was conducted with slight adjustments following the M.S. Blois protocol.[60] A volume of one milliliter of methanol and 2.5 milliliters of 0.5 mM DPPH in methanol were mixed with A. marmelos leaf extract (ranging from 25 to 500 g/mL) and Ag NPs at different concentrations. Then, for thirty minutes, this combination was kept in the dark. Using a UV-vis spectrophotometer, an absorption measurement was made at 517 nm. The ascorbic acid-DPPH-methanol reagent and a blank were utilised as controls. Every concentration trial was repeated three times or more. The following formula shows how to calculate the scavenging activity as a percentage.

DPPH scavenging activity (effect % inhibition) = [(A _(0)− A_ (1))] /A_ (0)]

Antineoplastic Activity of a AgNPs Against MDA-MB-231 Cell Line

Concurrently, a thousand human breast cancer MDA-MB-231 cells were seeded into 96-well plates using DMEM medium supplemented with a thousand times the antibiotic antimycotic solution (Himedia, India) and ten percent fetal bovine serum. Following a wash with 200 µl of 1X PBS, varying concentrations of 500, 250, 100, 50 and 25µg/mL of bael leaf extract were applied to the cells, followed by exposure to different concentrations of Ag NPs. The plate system was then placed in an incubator for the entire day. Post-incubation, MTT solution was introduced into each well, and the plate was further incubated in a CO2 incubator at 37°C for an additional four hours in the absence of light. Subsequently, the supernatant was aspirated, and 100 µL of dimethyl sulfoxide (DMSO) was added to reduce the development of the purple-blue color. The photosensitive density was utilized to assess the degree of cell cytotoxicity. Absorbance at 570 nm was measured using a microplate reader. The untreated cells were used as the negative control in determining the cytotoxicity index.[61]

% Cell viability = (OD value for test / OD value for control) × 100

% cytotoxicity = 100 − % cell viability

Basic Fuchsin Dye: Photocatalytic Degradation

Using artificially produced silver nanoparticles (Ag NPs) as a photocatalyst, basic fuchsin (BF) dye that had been subjected to environmental pollution was broken down. The dye was dissolved in a 25 mL solution with a concentration of 25 mg/L, followed by the addition of approximately 1 mL of the Ag NPs catalyst. Direct sunshine was used to illuminate the reaction mixture. Throughout the irradiation procedure, three millilitres of the reaction mixture were collected regularly in a quartz cuvette. These samples were then examined using a UV-vis spectrophotometer at various time intervals, covering the wavelength range of 200 to 800 nm.[62]

3. GENETIC TRANSFORMATION OF AEGLE MARMELOS PLANT

Genetic transformation of bael plant is one of the part of biotechnology in which we can use genetic engineering method for production of transgenic plants. This transgenic plants utilise techniques to increase its resistance to illness. Significant quantities of chemical components with medicinal potential can be produced by these plants. As a result, genetic engineering-based genetic transformation is a sophisticated and practical technique. Breeding programmes for Aegle marmelos, a woody perennial tree, are particularly difficult because of its inherent recalcitrance. To improve the bael's ability to regenerate and resist sickness, it is essential to introduce particular genes into the animal. Transgenic technologies and genetic engineering have a lot to offer this crop's enhancement. The mainstream of illnesses are produced by fungi, and the alkaloids and phenolics found in the tree have important therapeutic uses. Although it is only generated in small amounts in ripe fruits, the pulp contains the laxative and diuretic compound marmelosin ($C_{13}H_{12}O_3$). Genetic engineering has the potential to greatly expand its output. Agrobacterium rhizogenes may be a feasible avenue for genetic alteration, which would increase the plant's innate resistance by augmenting secondary metabolite synthesis. Chitin and glucan are easily broken down by the enzymes chitinases and glucanases from the fungal cell wall. These enzymes' cDNA clones are widely available for use in creating genetically altered plants that express the enzymes. Effective transformation is possible by Bael's regeneration method, which is based on somatic embryogenesis. Replanting plants in Bael can also be effectively accomplished with cotyledonary explants.[69]

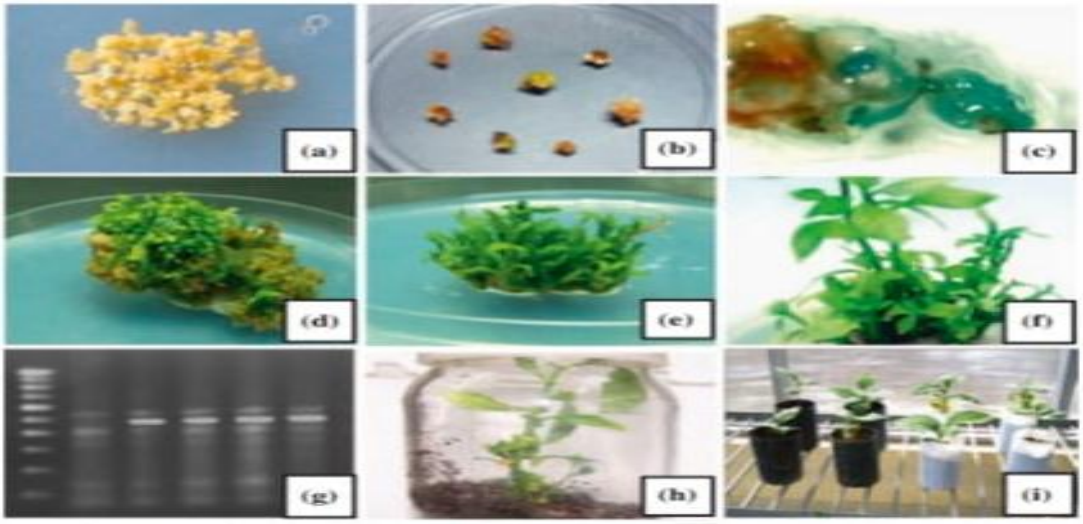


Figure 7: Steps involved in genetic transformation of aegle marmelos Plant.

a]. Transformed embryos b]. Selection of transformed embryos in kanamycin 150 mg/l c]. GUS assay d]. Regenration in transformed callus e] & f]. Proliferation in regenerated plantlets g]. PCR analysis for npt –II (480bp) h]. Acclimatization i]. Plant in transgenic glasshouse under controlled condition.

Development of Transgenic

Donor gene

Within the realm of agricultural plants, the practice of genetic engineering stands as a well-established methodology, encompassing the formulation of gene constructs featuring precise promoters tailored for specific purposes. The genes of interest can be used, depending on the situation, to isolate cDNA clones from a library or to use gene-specific PCR to produce gene constructs. Since bael's rough peel is the main cause for worry, two potential options include changing the genes responsible for fruit peel to lessen fruit shell hardness and using dwarfing genes previously discovered in agricultural plants, particularly perennials, to shorten the tree's height. Transgenic techniques in bael are essential in these domains.

Method of transformation

Early studies aimed at genetically changing medicinal plants used Agrobacterium rhizogenes, which produces hairy root cultures, and Agrobacterium tumefaciens, which produces altered cells for cultivation or the regeneration of whole plants. The transformation of the cell lines went well in the early stages, but the regeneration that followed was difficult. Two notable constraints encompass the elevated expense associated with bioreactors and the precarious stability of cell lines, frequently resulting in the gradual loss of their capacity to produce the intended substances over time. Agrobacterium-mediated and biolistics (Gene gun) are the two approaches that are most frequently utilised in the field of plants. The Agrobacterium-mediated transformation approach is often chosen because it is consistent and has a decreased risk of gene silencing.

Explant to be transferred

Generating complete transgenic plants is the main goal of the majority of plant transformation research conducted in plant biotechnology. The explants used for transformation should be able to grow into fully developed plants and have a significant number of transformable cells.

In transgenic integration vector from the tree genetic makeup was used

Ti plasmid variations are used as the transformation vector in agrobacterium-mediated plant transformation. They typically only retain the left and right border sequences for Ti-DNA transfer, eliminating most of the intrinsic characteristics of Ti plasmids. The vector's T-DNA contains a selectable marker, usually Kanamycin sulphate, to help identify transformed plants. Within the bacterium, virulence genes necessary for T-DNA transmission could be found on a different plasmid. Binary and co-integrate vector systems are the two types of systems utilised in agrobacterium-mediated transformation. Depending on certain needs, unique gene constructs are created and added to the vector system.

Agrobacterium strain to be used

There are a number of popular Agrobacterium strains that may convert plants. The selection of the strain has a significant role in whether the experiment is successful or unsuccessful for more difficult-to-train plant species. The next section discusses a general methodology for agrobacterium-mediated plant transformation.

Sterilization and explant preparation

Transformation claims that aegle marmelos is an immature zygotic embryo that makes for the greatest explants since it thrives in a variety of crops. The fruits, which were still immature, were cleaned with running water and immersed in a solution of bevestine (0.1 µl) with one drop of Tween 20. This was cleaned five times with sterile distilled water following an hour-long surface sterilisation process. The surface has previously

been sanitised for effectiveness. The explants were surface sterilised with 0.1% $HgCl_2$ for five minutes in order to check for impurities. Prior to the extraction of the immature zygotic embryo from the unripe fruits and its placement into the culture medium, the explants underwent a meticulous process of thorough cleansing, involving five successive washes with a sterile solution of distilled water.

Cocultivation

Five grammes of yeast extract, ten grammes of tryptone, and ten grammes of NaCl were added to fifty millilitres of LB broth made with agrobacterium strain LBA4404). There was a single colony with the necessary gene within this strain. The colony reached an optical density of 0.8–1.0 at 600 nm after a 24-hour cultivation period at 100 rpm, 28°C and no light. Ten millilitres of liquid MS media were used to facilitate reconstitution after the pellet was centrifuged at 10,000 rpm and 10 µM spermidine and 100 µM acetosyringone were added. Remarkably, after three hours, no infection developed. A. tumefaciens was cultivated for 30 minutes after the explants were divided into 2-3 mm-long sections. The tissues were cleaned of any remaining bacteria by rinsing them with MS solutions before drying them on sterile filter paper. Subsequently, they were co-cultivated for 72 hours without employing a selection agent in MS semi-solid medium, aiming to expedite the transfer of the T-DNA into plant cell tissues. Following co-cultivation, the explants underwent treatment with antibiotics (a solution containing Cefotaxime at 500 mg/l or carbenicillin at 250 mg/l).[70]

Selection and Regeneration

The explants were subsequently moved to the somatic embryo induction medium, formulated using a half-strength Murashige and Skoog (MS) base, complemented by 400 mg/l glutamine, MS vitamins, 10 mg/l 2,4-D, 6% sucrose and 0.8% agar. To curtail the growth of untransformed cells, a blend of 500 mg/l cefotaxime or 250 mg/l carbenicillin was incorporated with kanamycin at a concentration of 150 mg/l. The pH of the medium was adjusted to 5.8. These cultures were maintained in total darkness at 25 ± 2°C for a duration of four weeks. After this initial period, the kanamycin concentration was elevated to 150 mg/l. Following this four-week interval, an array of white to light yellow globular embryos emerged, contingent upon the plant species. Subsequently, these somatic embryo clumps were transferred to a regeneration medium to foster their development into plants. The regeneration medium consisted of half-strength MS medium supplemented with 400 mg/l glutamine, MS vitamins, 0.5 mg/l BAP, 0.1 mg/l NAA, 3% sucrose, and 0.8% agar. The cultures were then placed in a growth chamber maintaining a relative humidity of 55% and a temperature of 25 ± 2°C for 16 hours, with a light intensity set at 4 $\mu molm^2s^{-1}$.

Rooting and Accumulation

Subsequently, to promote the growth of colonies, plantlets measuring 4-5 cm in length were introduced into a rooting medium free from kanamycin, containing 3 mg/l of IBA, and supplemented with 500 mg/l of activated charcoal. Once the root systems were established, the plantlets were transplanted into culture bottles filled with coconut husk, sterilized vermiculite, and a 1/2 ms salt solution. These cultures were maintained for a span of two to three weeks at 25±2°C under a 16-hour photoperiod with light intensity set at 40 $\mu molm^2s^{-1}$ and a relative humidity of 55%. Once the plants attained four or six leaves, they were relocated to poly houses equipped with 50% shade and an integrated misting system. To prepare the poly houses, a mixture comprising a 1:1:1 ratio of soil, sand and field yard manure should be utilized.

Table 6: Confirmation of Transformation.

1	Examined were the transformed tissues capacity to proliferate in the presence of specific antibiotics (Kanamycin 150 mg/l).
2	Additionally, altered calli, somatic embryos, fruit, roots and leaf sections sections from plants were subjected to a histochemical GUS assay to assess the expression of GUS.[71]
3	DNA extracted from transgenic and control plants was substantially processed as described and PCR analysis was conducted employing npt II specific primers, resulting in the detection of a 480 bp band.[72]
4	The genomic DNA is isolated from the plant for DNA hybridization. By using specific restriction enzyme 50 microorganism was restricted and separated in 0.8% of agarose gel. Then specific cDNA probe is combined with DNA and transferred onto a nylon memebrane. The probe used to estimate T-DNA copies.[73]
5	In order to identify target proteins, such as those produced by the CP gene and PRV npt II, in allegedly transgenic leaves, the Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) method uses both polyclonal and monoclonal antibodies.[74]

Stage	Lab/Field	Analysis	Remark
T0	Lab testing or Green house/Phytotron	• Molecular analysis • Target trail data	Select a few promising plants
T1	Green house/Phytotron	• Molecular analysis of selected single plant progenies • Trial segregation analysis	Identify individual positive plants
T2	Green house/Phytotron	• Molecular analysis of single plant progenies • Target trial analysis	Identify non segregating progenies
T3	Limited field trial	• Agronomic performance including DUS(distinctiveness, Uniformity and Stability) features • Target trial expression • Toxicity data • Allergenicity data • Environmental impact analysis • Pollen flow study • Soil data	• Integration of transgenic into IARI breading programme • Identify promising progenies with desired level of target trait expression and advance to T4 generation
T4 and T5	Multilocation field evaluation	• Agronomic data • Target trait expression • Pollen flow data	Dialogue with project Directors/Coordinators of respective crops for VCU testing
T6	Value for cultivation and use (VCU) testing in all India coordinated trials	• Seed multiplication	Variety identification and release

Figure 8: Transgenic Crop Development and Evaluation.

4. ISSR MARKER USED FOR ASSESSMENT OF GENETIC POLYMORPHISM OF BAEL PLANT

The genomic DNA of the bael plant was extracted using the CTAB (Cetyl Trimethyl Ammonium Bromide) method in order to look into genetic polymorphism. With the use of this sophisticated technique, it is easier to separate DNA from leaves or seeds and purify it afterwards. The genetic polymorphism between several Aegle marmelos genotypes was investigated using the ISSR (Inter Simple Sequence Repeats) technique. DNA purification was also accomplished using the CTAB technique. The ISSR approach is a fast, precise, and highly informative tool for DNA fingerprinting that is used in many studies, including molecular ecology, identification, genetic mapping, cultivar analysis, gene tagging, and gene diversity assessment.

The initial step towards linking genetic resource application and conservation in various breeding programmes is the characterization of germplasm. Aegle marmelos (L.) Correa is a highly prized shrub because of its high nutritional content and many medicinal applications. It is important in the community's therapeutic and religious contexts. In the current study, five A. marmelos genotypes that were gathered from various Upper Assam districts were examined for genetic polymorphisms using the ISSR-DNA marker approach.

MATERIAL AND METHOD

Genetic material collection

The natural habit and homestead gardens in the five administered districts of upper assam were used to gather five genotypes of aegle marmelos. Latitude and longitude were entered using a 60 CSx, garmin system.

Standardization of genomic DNA isolation and qualification

A little tweak to the CTAB methodology allowed for the standardisation of the DNA isolation process.[76] Basically, each of the five genotypes of Aegle marmelos, which consisted of young, healthy leaves (0.5 gm/plant), were separately ground in liquid nitrogen. Then, DNA was quantified by comparing the findings of a DNA ladder and using spectrophotometric analysis.

Using the following formula, determine the concentration of isolated genomic DNA

Genomic DNA (µg/mL) = 50 × OD obtained at 260 nm × dilution factor[77]

Table 7: Collection of genotype and their location.

Genotype (accessioons code)	District	Location	Latitute (^{o}N)	Longitude (^{o}E)
AC1	Jorhat	Komolabari hatra	26^{o}48’37”	94^{o}14’21”
AC2	Dibrugarh	Dibrugarh University	27^{o}27’23”	94^{o}52’59”
AC3	Sivasagar	Bet-bari	27^{o}04’07”	94^{o}34’02”
AC4	Jorhat	Majuli	26^{o}48’33”	94^{o}14’21”
AC5	Dhemaji	Gogamukh	27^{o}21’25”	94^{o}33’04”

ISSR profiling

Once the DNA was isolated, it was diluted to 40–50 ng/mL.[78] UBC primer was used for ISSR profiling in the thermocycler.

T_m= Melting temperature of primer, T_A= Annealing temperature in PCR.

Primer Code	T_A°C	T_m °C	Sequence
UBC834	52.6	52	AGAGAGAGAGAGAGAGYT
UBC836	52.6	52	AGAGAGAGAGAGAGAGYA
UBC849	52.6	52	GTGTGTGTGTGTGTGTYA
UBC855	52.6	52	ACACACACACACACACYT
UBC819	50.4	50	GTGTGTGTGTGTGTGTA
UBC809	52.8	53	AGAGAGAGAGAGAGAGG

Figure 9: Primer code for ISSR profiling.

The amplification mixture was assembled as follows: within a 20 µL PCR tube, 2 µL PCR buffer (lacking $MgCl^{2+}$), 2.5 µL $MgCl^{2+,}$ 1 µL dNTP, 1 µL Taq-polymerase, 2 µL template, 1 µL primer, and 10.5 µL nuclease-free water were combined. The annealing temperature, set at 52–53 ℃, was determined by the primer's Tm. The 36-cycle thermal profile comprised intervals of 30 seconds at 94 °C, 45 seconds at 52–53°C, 1 minute at 72 °C, and a final extension of 10 minutes at 72°C. The initial step in genomic DNA denaturation involved heat treatment at 95°C for 4 minutes. Subsequently, the amplified products were subjected to electrophoresis in a 1.5% agarose gel utilizing 1X TBA buffer for 90 minutes at 70 volts, following staining with ethidium bromide (10 mg/mL). Assessment of the amplified DNA fragments involved comparison to a 100 bp molecular DNA ladder (Biolit, ProxiO 100 bp DNA marker, SRL). Visualization of the fragments was carried out under an ultraviolet light source post-gel electrophoresis.

Data Analysis

The single primer used in the polymorphism study provided scoreable band patterns in genotypes.[79,80]

1. STANDARDIZATION OF AEGLE MARMELOS PLANT BY USING RP-HPLC METHOD

The fruit known as aegle marmelos, or bael, has been used traditionally because of its diverse pharmacological properties, which call for more study. Memelosin is a valuable biomarker and a significant chemical constituent of bael fruit. Because HPLC has a higher sensitivity and accuracy, it was used for this. Previous methods were noticeably less sensitive and required longer elution times for marmelosin. The protocol was updated and enhanced as a result. The mobile phase consisted of acetonitrile:water (70:30), with a peak absorbance at 247 nm and a flow rate of 1 ml/min. The LOD, LOQ, linearity, repeatability accuracy and precision of this procedure were all validated. LOD and LOQ were found to be 0.1 mg/ml and 1 mg/ml, respectively. With a r^2 value of 0.998, the method demonstrated good linearity in the 1–30 µg/ml range. A thorough examination revealed that the procedure's accuracy was demonstrated by the coefficient of variation, which was less than 20%. Using a percentage recovery technique, the accuracy research produced a 95% recovery result. 8.1% w/w of marmelosin was found in the marmelos fruit pulp's crude ethanolic extract. It was found that the Aegle herbal formulation had a 95% purity percentage for marmelosin content.[51,52]

2. UTILIZATION OF AgNPs BY GREEN SYNTHESIS FROM AEGLE MARMELOS PLANT

UV Visible Spectroscopy Analysis

To create environmentally friendly silver nanoparticles (Ag NPs), 15 mL of a 1 mM silver nitrate solution and leaf extract from Aegle marmelos were used. The reaction mixture began to turn a yellowish-brown colour, which was a clear indicator that Ag NPs were forming (Fig. 10). The identification of a surface Plasmon resonance (SPR) absorption peak at 450 nm in the UV-visible spectroscopy analysis (Fig. 10) verified the synthesis of Ag NPs. Using UV-Vis spectra, the evolution of Ag NPs conversion over different time periods was regularly observed. The absorbance intensity barely changed after 48 hours, indicating that most of the silver ions had been converted to Ag NPs. Notably, stabilising agents, surface-bound particles, and the medium's dielectric constant all have a major impact on the Ag NPs SPR properties and attributes.[63]

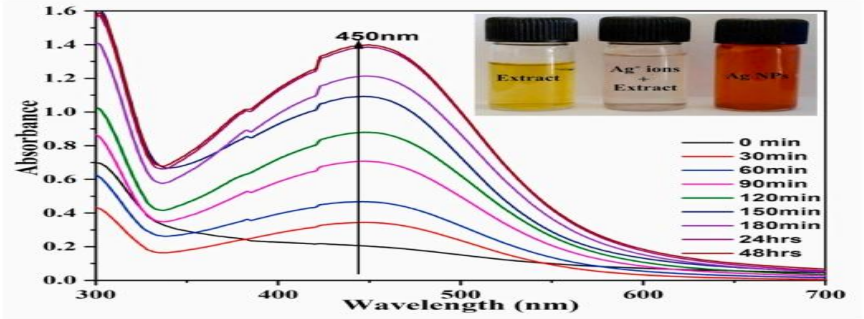


Figure 10: UV-visible analysis silver nanoparticles of aegle marmelos.

Fourior Transport Infrared (FT-IR)

The FT-IR study in Figure 11 a) demonstrated the diversity of phytochemicals present in the aegle marmelos leaf extract. Strong hydrogen bonding in alcohols and phenolic compounds was linked to the stretching band at 3360 cm^{-1}, whereas the -NH stretching band of amino groups was suggested by the band at 3439 cm^{-1}. It is possible to link the absorption band at 2900 cm^{-1} to -CH groups. Stretching vibrations observed at 1626 cm^{-1} and 1653 cm^{-1} in flavonoid and tannin derivatives indicated the presence of a carboxyl group (C=O) derived from aldehyde or ketone functional groups. The band at 1384 cm^{-1} might be connected to the alkane/alkene -CH bending vibration. The FT-IR spectra showed additional bands at 1203 cm^{-1}, 896 cm^{-1} and 660 cm^{-1}. The C-O groups in aliphatic esters and the N-H groups from primary and secondary amines, respectively, may be related to these bands. The leaf extracts contain phytochemical components like flavonoids, tannin and coumarins, which are polyphenolic chemicals that may help to slow down and stabilise the formation of nanoparticles, according to the whole spectrum results. This indicates that the synthesis of stable, spherical, silver nanoparticles with an average particle size of 15–30 nm involves these compounds. In comparison to aeromonas hydrophila and staphylococcus aureus, these nanoparticles showed a stronger inhibitory effect at concentrations of 25, 50, and 100 g/ml against escherichia coli, streptococcus pyogenes and pseudomonas aeruginosa. The antibacterial activity of these nanoparticles was assessed using the well diffusion method. These nanoparticles also have antioxidant, photocatalytic degradation, and anticancer properties.[64]

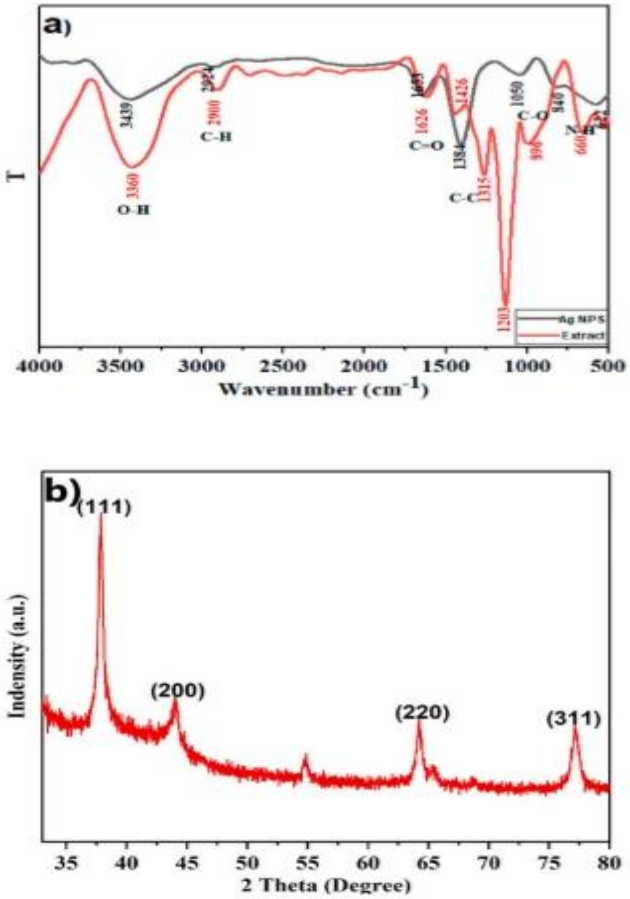


Figure 11: FT-IR anaylsis of silver nanoparticles of aegl marmelos plant.

SEM analysis (Scanning Electron microscopy)

The shape and structure of the synthesised nanoparticles were ascertained through SEM analysis. The nanoparticles were analysed at magnifications of x15000, x30000, x45000 and x55000 relative to their initial size. SEM pictures of the generated silver nanoparticles, most of which are spherical, are shown in Figure 12. Aggregation of two or more reducing agents adhered to the surface of the preexisting particle nuclei may have resulted in the development of long, large and spherical nanoparticles.[65]

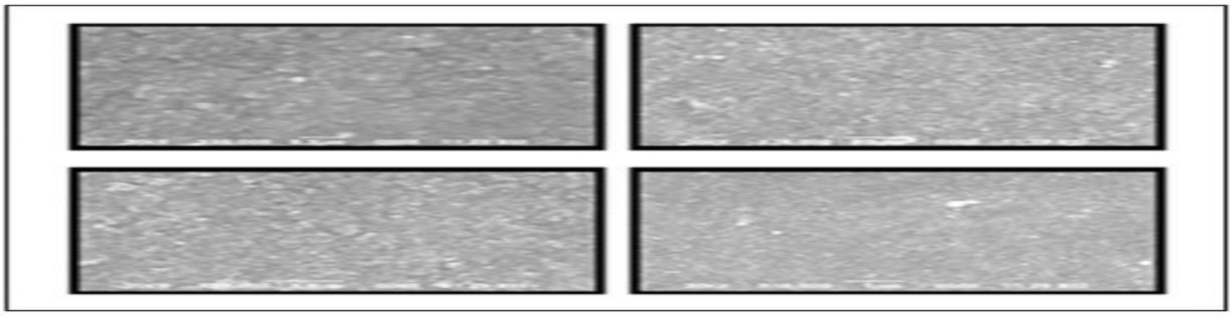


Figure 12: SEM anaysis of silver nanoparticles of aegle marmelos plant.

Energy Dispersive X-rays (EDX) analysis

This work employed Energy Dispersive X-ray Spectroscopy (EDX) to analyse the elements of silver present in the nanoparticles. An identifiable silver peak was observed by EDX analysis, indicating the presence of silver in the nanoparticle solution. An EDX spectrum is shown in Figure 13. The presence of chemical groups from the leaf extract on the surface of the nanoparticles may be the cause of the additional peaks that were seen.

X- rays Diffraction (XRD) analysis

Silver nanoparticles were synthesised using leaf extract from aegle marmelos, and structural information is provided by the X-ray diffraction (XRD) pattern, which amply demonstrates the material's crystalline nature. In Figure 14, two discrete diffraction peaks were identified at 32.12°, 46.14°, 38.01° and 43.9° in the XRD spectrum. The JCPDS data was compared to the diffraction lines, which were identified as face-centered cubic (fcc) silver planes 98, 101, 111 and 200. Using silver nanoparticles made with trdax procumbens, ondari and nalini reported comparable outcomes.[66] The Debye-Scherrer equation was used to determine the average size of the nanoparticles.[67]

$D = K \lambda/\beta \cos\theta$

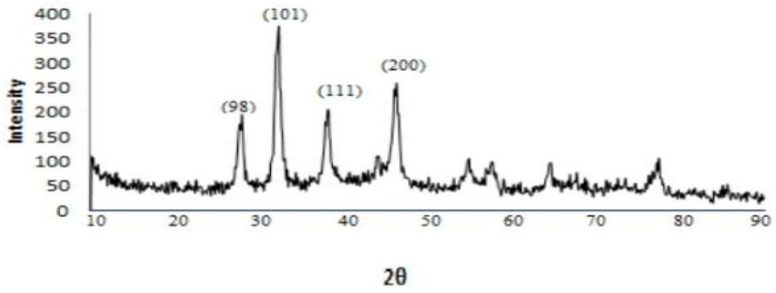


Figure 13: EDX analysis of silver nanoparticles of aegle marmelos plant.

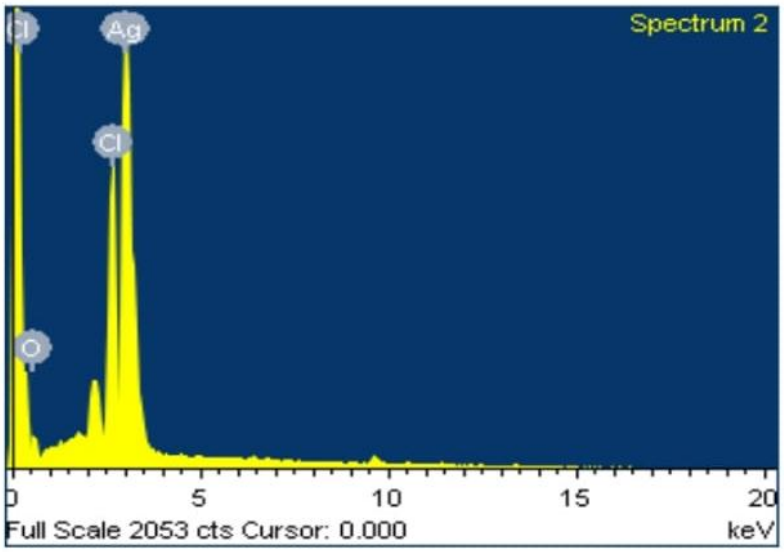


Figure 14: XRD analysis of silver nanoparticles of aegle marmelos plant.

Zeta potential analysis

As demonstrated by their zeta potential of -35.4 mV in Figure 15, the silver nanoparticles produced by the green method are stable. Zeta potential measurements are based on how nanoparticles move in response to an applied electric field; the movement is affected by the surface charge and the particle's immediate surroundings.[68]

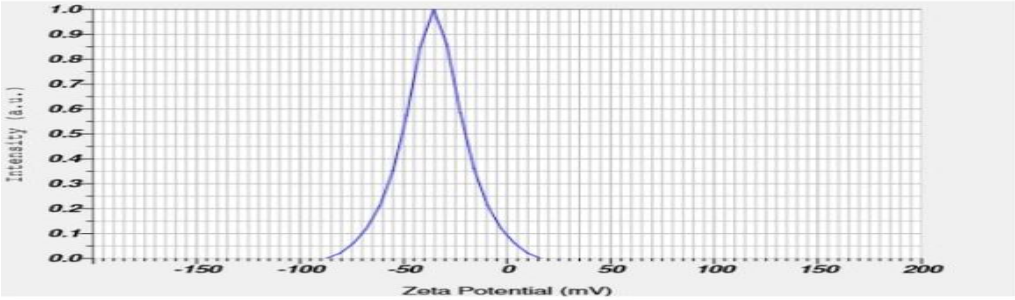


Figure 15: Zeta potential analysis of silver nanoparticles of Aegle marmelos plant.

3. GENETIC TRANSFORMATION OF AEGLE MARMELOS PLANT

Even though it is possible to produce genetically altered annual crops for commercial use, commercially produced genetically altered fruit trees remain rare. Most woody fruit species often limit commercial cultivar regeneration and transformation to a small subset of genotypes or seedlings. The development of genotype-independent methods based on the application of genes that promote regeneration and the transformation of meristematic cells with high regeneration ability are required for the long-term use of genetic modification as a fruit tree breeding technique. It was once thought that agrobacterium tumefaciens was the vector system that could transfer genes to any species or type of plant.[75]

4. ISSR MARKER USED FOR ASSESSMENT OF GENETIC POYMORPHISM OF BAEL PLANT

Standardization and qualification of extracted DNA

It was discovered that aegle marmelos DNA could be successfully and significantly separated using the 4X CTAB buffer. When the DNA band was extracted using the 4X lysate buffer, it was more intense as seen in the following image. Nevertheless, it was noted that genomic DNA pieces larger than 5000 bp were acquired. Spectrophotometry was used to quantify the DNA recovered from the 2X and 4X lysis buffers in order to verify the method's reproducibility. Table 3 shows that in the 4X buffer, there was more extracted DNA for every genotype.

ISSR studies

In the experimental genotypes, six of the twelve UBC series primers that were used for ISSR profiling exhibited unique, reliable, and repeatable bands. 31 amplicons in all, with an average value of 5.16, were produced. Significant genetic variety is revealed by the current research, with an average of 24.09% monomorphic bands and 79.24% polymorphic bands. Six of these primers showed complete polymorphism. With 33.00% of the total monomorphic bands, UBC 849 and UBC 819 had the largest share. With seven bands ranging in size from 150 bp to 1000 bp, UBC 836 and UBC 809 produced the greatest number of bands, indicating 86.72% polymorphism.

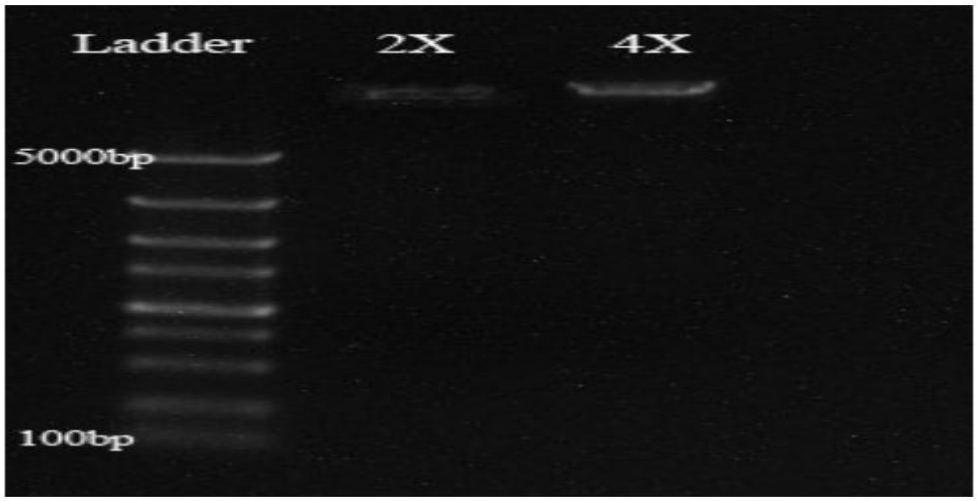


Figure 16: Qualification of extracted DNA From Aegle marmelos in Agarose gel electrophoresis.

Table 8: Qualification of extracted DNA from the five genotype of aegle marmelos.

Genotype	2XCTAB		4XCTAB	
	DNA (µg/mL)	OD Ratio at 260/280nm	DNA (µg/mL)	OD Ratio at 260/280nm
Ac1	132.40	1.59	73.60	1.61
Ac2	68.50	1.70	51.70	1.69
Ac3	89.70	1.56	56.30	1.51
Ac4	111.80	1.70	64.40	1.74
Ac5	139.50	1.68	57.09	1.79

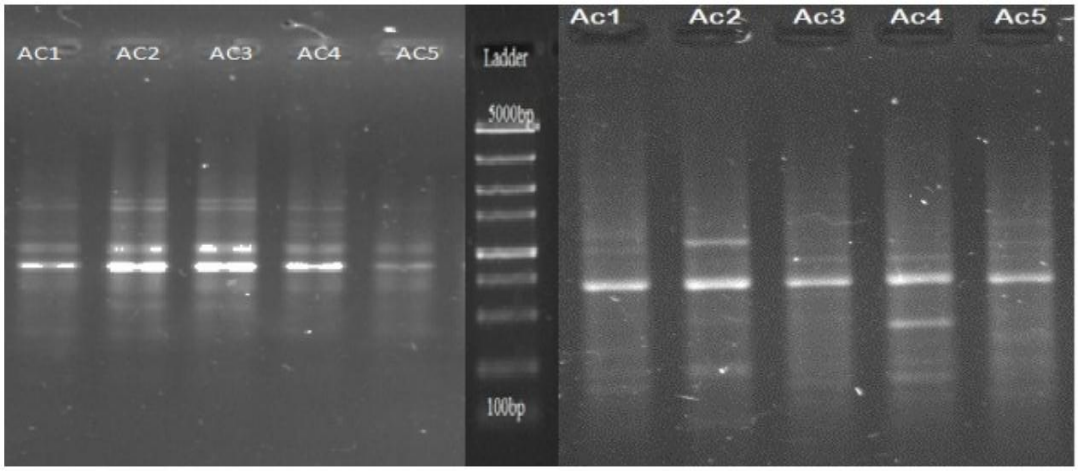


Figure 17: ISSR banding pattern of UBC 855 and UBC 819 of aegle marmelos.

Intraspecies genetic diversity information of Aegle marmelos

Primer name, MB= Monomorphic band, PB= Polymorphic band, TNB= total number of the band, PMB= percentage of monomorphic bands, PPB= percentage of polymorphic bands.

Primer code	TNM	MB	PB	PMB	PPB
UBC834	4	1	3	25	75
UBC836	7	1	6	14.28	85.72
UBC849	3	1	2	33	77
UBC855	4	1	3	25	75
UBC819	6	2	4	33	77
UBC809	7	1	6	14.28	85.72
TOTAL	**31**	**7**	**24**		
AVERAGE	**5.16**	**1.16**	**4**	**24.09**	**79.24**

Figure 18: Intraspecies genetic diversity of aegle marmelos plant.

Found in northeastern india's indo-burma megabiodiversity hotspot, aegle marmelos is a delicate and significant ethnomedicinal plant belonging to the Rutaceae family. As the DNA separation method was standardised for this investigation, it was found that the 4X CTAB buffer functioned better as a lysis buffer than the 2X CTAB buffer. This conclusion was reached by means of quantitative analysis with a spectrophotometer and qualitative evaluation utilising gel electrophoresis. However, intragenetic variety was found in Aegle marmelos according to the ISSR molecular marker used for genetic diversity study, with primers UBC836 and UBC 809 showing the most genetic diversity. On the other hand, UBC834 and UBC855 showed the least amount of genetic diversity.[81]

CONCLUSION

This review article includes advanced approaches of aegle maemelos plant which are very beneficial for future prospects. In which we will discussed botanical profile, chemical constituents, pharmacological activity, marketed formuation, Development of RP-HPLC for standardization of bael fruit, green synthesis of silver nanoparticle from bael plant, genetic transformation and assessment of genetic polymorphism. The reverse-phase high-performance liquid chromatography (RP-HPLC) technique has been devised and validated as an efficient tool for standardizing diverse formulations derived from bael plants. This technique cost-effectiveness, sensitivity, accuracy and precision make it a popular choice for standardising herbal drugs. The green synthesis has admirable traits in that it is ecofriendly, practical, simple

and economical. This review examines the process of producing silver nanoparticles from the Aegle marmelos plant's aqueous extract. This method is a new development in nanotechnology that might have positive effects on synthesis projects in the future. These nanoparticles are studied using UV-visible and FT-IR spectroscopy. Also this synthesized nanoparticle have anticancer activity, antioxidant activity, photocatalytic degradation activity and antibacterial activity was studied. It is worthwhile to produce this plant due to its numerous uses and wide range of prospects large-scale, especially on barren and unproductive ground. In the genetic transformation in which genetic engineering (biotechnological method) was deliberated. This genetic engineeried plant have high disease resistance ablity and they can produce large amount of phytoconstituents. Farmers who are impoverished and without land will benefit financially as a result of this. The full potential of this underutilised species must also be explored via methodical, scientific investigation. Bael possesses a variety of traits that highlight their importance for a variety of purpose, weather they be therapeutic and religious. This plant is used in various diseases and shows property like antifungal, analgesics, antidiabetic, antipyretic, antibacterial, antioxidant, antifertility and wound healing. Genetic polymorphism was investigated using the novel CTAB DNA extraction method, and polymorphism was explored using the ISSR methodology. Bael has a wide range of uses, thus it makes sense to cultivate it extensively, especially in isolated and varied locations. This study of the literature is at the forefront of herbalism studies. We expanded on a number of conventional and cutting-edge aegle approaches in our review paper. To completely comprehend the significance of bael plant, more investigation is required.

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