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Available online at: http://www.iajps.com *Review Article*

A COMPREHENSIVE REVIEW ON EXTRACTION OF AEGLE MARMELOS FOR ANTIMICROBIAL ACTIVITY

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Abstract:

Beal (Aegle marmelos), commonly known for its medicinal properties, has gained significant attention due to its potential antimicrobial activity. This study aims to evaluate the antimicrobial effects of Beal leaf extracts against a range of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria. The leaves were collected, dried, and extracted using solvents such as ethanol. The antimicrobial potential was assessed using the agar well diffusion method, measuring zones of inhibition around the wells. The results indicated that Beal leaf extracts exhibited notable inhibitory effects against several bacterial strains, with the methanol extract demonstrating superior activity compared to ethanol.

These findings suggest that Beal leaves possess bioactive compounds that may be useful in the development of natural antimicrobial agents. Further studies are recommended to isolate and identify the specific compounds responsible for this antimicrobial activity, as well as to evaluate their safety and efficacy in clinical applications. The goal of this study was to assess the antibacterial properties and phytochemical components of Aegle marmelos (Linn.) Correa fruit pulps. Reducing sugars, saponins, tannis, flavonoids, and phenols were found in the crude extract after phytochemical screening.

Additionally an estimate of the total phenolic and flavonoid content was made. Additionally, the crude extract was evaluated for antimicrobial activity against two gram-positive strains of E. Colli at several concentration over a three-hour period. Ethanolic extract were found to be efficient in preventing the growth of the bacterial strain E. colli.

Key words: *Aegle marmelos, Medicinal plants, Phytochemical Screening, Antibacterial activity, Antimicrobial activity.*

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INTRODUCTION:

The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Today, as much as 80% of the world's population depends on the traditional medicine as primary health care needs.[1] Ancient literature such as Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita have been extensively studied by advanced scientific techniques and are reported for various medicinal activity.[2]

Aegle marmelos is a medicinal herb belonging to family Rutaceae. The different part of plants like leaves, fruits, bark and root shows antimicrobial activity and has the therapeutics significance in Ayurveda. Aegle marmelos commonly known as Bael Tree is a tropical plant that has been traditionally used in various cultures for its medicinal properties. A marmelos is grown as a temple garden plant and the leaves are used to pray Lord Shiva. A marmelos is an important medicinal plant with several ethanomedicinal applications in traditional and folk medicinal system. A marmelos is used in the treatment of diarrhea and dysentry. Leaves of these plants are used to cause infertility/abortion in women.[2] Modern research have successfully supported the pharmacological action of Bael by discovering the presence of valuable bioactive compounds. The antimicrobial activity of medicinal plants has gained significant attention in recent years due to the increasing resistance of pathogens to synthetic drugs. The leaves of Aegle marmelos are rich in bioactive compounds, including flavonoids, alkaloids, tannins and essential oils, which are believed to contribute to its antimicrobial effects.[5]

Anti oxidant, antiulcer, anti diabetic, anticancer, anti hyperlipidemic, anti inflammatory and anti spermatogenetic effects have also been reported on various animal model by the crude extract of this plant.[1] A poultice made from bael leaves is used to treat ophthalmic conditions and is also applied to inflamed area, often in combination with black paper to reduce edima. The juice of Aegle marmelos leaves, when mixed with honey, is commonly used to treat fever and can also help in management of tuberculosis. Beal (Aegle marmelos), commonly known as Beal, is a tropical fruit tree native to the Indian subcontinent. The leaves of the Beal tree have been used in traditional medicine for centuries, particularly in Ayurvedic practices, due to their numerous therapeutic properties.[4]

The biological screening of the compounds present in these medicinal plants proved their activity in treating diseases like dysentery, diarrhoea, stomach pain, dyspepsia. In the past few years several plant species have been assessed and evaluated for their antimicrobial activity.[6]

PLANT PROFILE:

Synonym: Bel, Bengal Quince, Golden Apple and Wood Apple

Biological Source: Bael consists of the entire unripe or its slices of Aegle marmelos.

Taxonomical Classification:

1. Kingdom: Plantae
2. Phylum: Angiosperm
3. Class: Eudicots
4. Order: Sapindales
5. Family: Rutaceae
6. Genus: Aegle
7. Species: Aegle marmelos
8. Common name: Bael patra
9. Sub-family: Aurantioideae



Fig. 1: Leaves of Bael Plant

Chemical Constituents:

Fruit contains marmelosins (0.4%), carbohydrates (11-15%) including reducing sugars (3-4%), allo-imperatorin, ß-sitosterol, tannins (20%), proteins (1%), vitamins A and C and volatile oil. Fruits are also the source of alkaloids namely O-methylhalfordinol and isopentylhalfordinol. It also contains marmelide, psoralen, gum, etc. The fruit yields 2% water-soluble gum which on hydrolysis gives galactose, arabinose, Ogalacturonic acid, rhamnose.

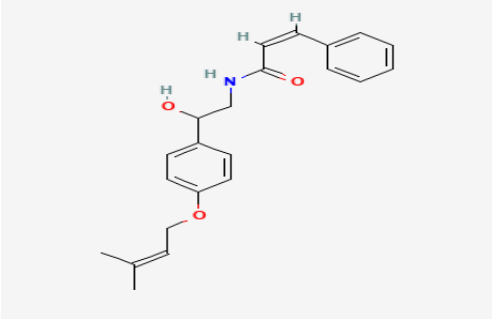


Fig. 2: Chemical Formula – Marmeline (C22H25NO3)

Uses:

- It is used as an Antioxidant.
- It should be used as an Antibacterial drug.
- It play a crucial role in the Antifungal treatment.
- It is used in the treatment of diarrhea.
- It is also helpful for the diabetic patients.
- It is used as Antiploriferative.
- It is used as Cytoprotective & Hepatoprotective.
- It works as a antifertility drug.
- It is an Analgesic drug.
- It treats the arthritis.
- It is used as Antihyperlipidemic.



Fig. 3: Bael Tree

➢ **BACTERIAL PROFILE:-**

Name - Escherichia coli (E. Coli)
Staining - Gram-negative, facultative anaerobic bacteria
Life span - 2 to 4 month
PH range - 7.2 to 7.5
Shape - Rod-Shaped
(2.0 um long and 0.25-1.0 um diameter)

Clinical significance -

1. Intestinal infection
2. Extraintestinal infection
3. Urinary tract infection
4. Food borne illness

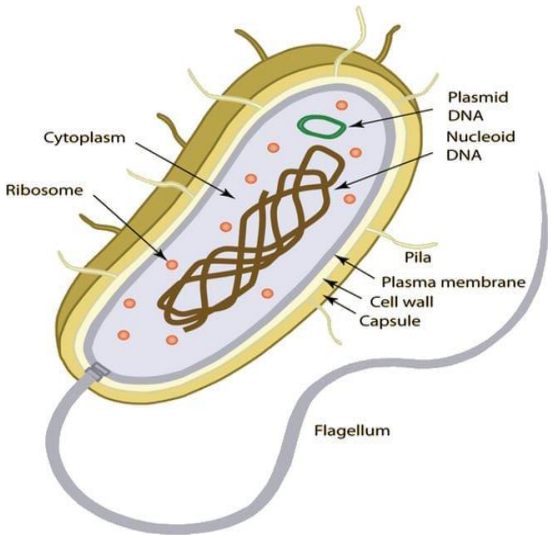


Fig.4: Parts of Escheria coli

➢ **BACTERIAL HISTORY :-**

Escherichia coli (E. coli) was first discovered in 1885 by Theodor Escherich, a German pediatrician, who isolated it from the feces of healthy individuals. He originally named it Bacterium coli commune, later renamed Escherichia coli in 1919, and officially recognized in 1958.

Here's a more detailed look at its history:

- 1885: Theodor Escherich isolates a bacterium from infant stool samples, later named Bacterium coli commune.
- 1895: Migula reclassifies the bacterium as Bacillus coli.
- 1919: Castellani and Chalmers propose the name Escherichia coli.
- 1958: The name Escherichia coli is officially recognized.
- 1982: E. coli O157:H7 is recognized as a pathogen during an outbreak investigation, linked to undercooked ground beef.
- 1997: The first complete E. coli genome sequence (K-12 MG1655 strain) is published.

Taxonomical Classification: -

Domain: Bacteria

Kingdom: Pseudomonadati

Phylum: Pseudomonadota

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Tribe: Escherichieae

Genus: Escherichia

Species: E. coli

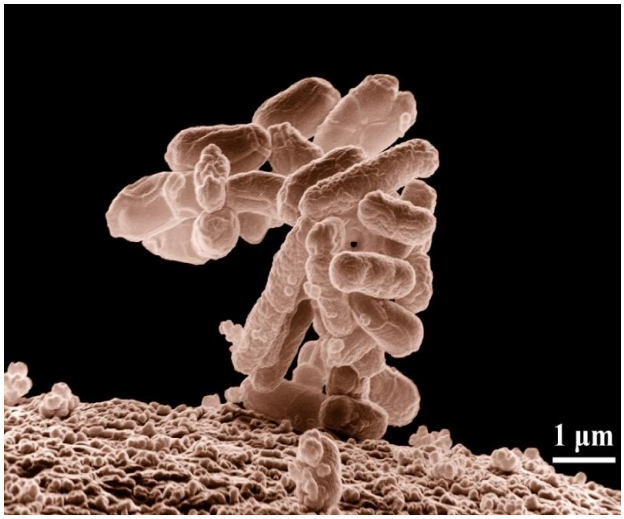


Fig. 5: Structure of E. coli

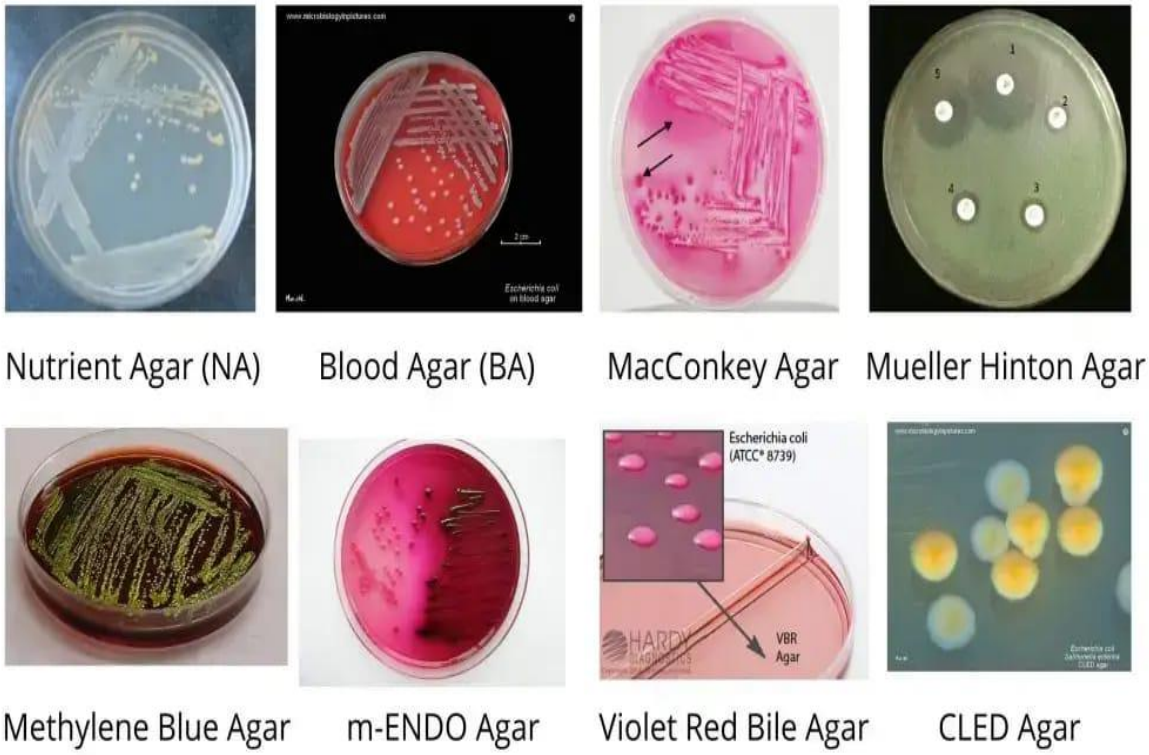


Fig.6: Growth of bacteria in different agar media

Material used:-

Drug: - Bael leaves

Solvent: - Ethanol

Bacteria: - E.Coli

Equipments :- Soxhlet apparatus, Incubator.

Apparatus :- Grinder, Test tube, Glass rod, Sieve, Petri plate, Disc, funnel and beaker.



Fig. 7: Incubator and Autoclave

- **EXPERIMENTAL METHODOLOGY*:* -**

Extraction procedure:-

Drug :- Bael leaves

Apparatus:- Soxhlet Apparatus, Round bottom flask, funnel, Beaker.

Solvent :- Ethanol

- **LEAF EXTRACT :-**

Method of preparation:-



1. The leaves of Aegle marmelos collected from in and around area of Nanded, Maharashtra.
2. The collected leaves were washed with running tap water and shade dried.
3. After drying they were powdered using a grinder.
4. Then measure the 50 gm of Aegle marmelos leaf powder. The with measured powder was dissolved with 100 ml Ethanol as a 1:2 ratio using Soxhlet apparatus separately.



Fig. 8; Weighing Drug Powder

5. After setting the assembly, start the flow of water from inlet to outlet with continuous heating**.**



Fig.9: Soxhlet assembly

6. After 5 to 6 hours, the crude extract was collected in the round bottom flask and after freezing store it in umber coloured container.

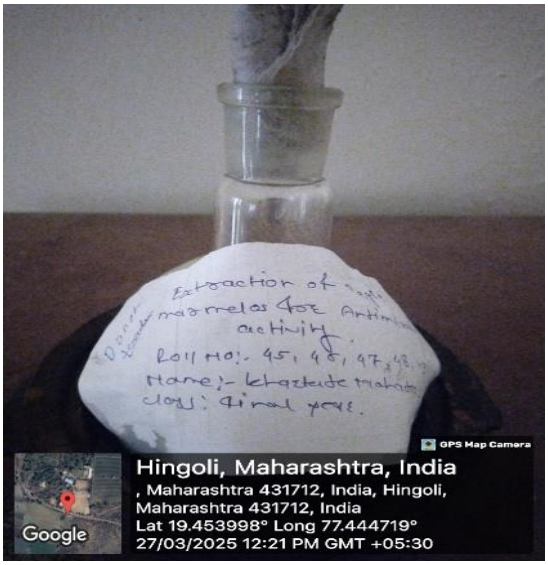


Fig. 10: Freshly prepared extraction.



Fig. 11: Stored extraction

- **EXPERIMENTAL WORK :-**

Experimental Procedure:-

- The bacteria Escheria colli was collected from the Digambarrao Bindu College, Bhokar, Nanded to check the antimicrobial activity.
- The bacterial strain was isolated by Slant tube method.
- The bacteria was then isolated in Saraswati Institude of Pharmacy, Kurtadi, Hingoli.
- E. colli was kept in the incubator at 37°c having condition 72 hours with suitable environmental**.**

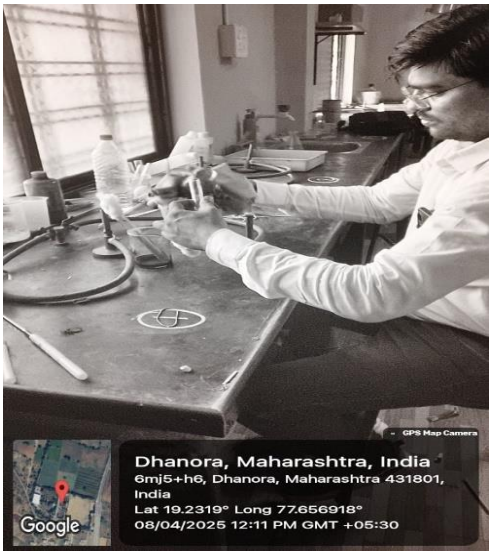


Fig:12: Collection of E.colli



Fig. 13: Slant tube method

Media Preparation Steps:-

1. **Weigh the nutrient agar powder:** Measure the desired amount of nutrient agar powder as per requirement (1.5gm).
2. **Mix with distilled water:** Combine the nutrient agar powder with the required amount of distilled water in a flask or beaker.
3. **Heat and stir:** Heat the mixture gently, stirring occasionally, until the powder is fully dissolved.
4. **Autoclave:** Sterilize the solution by autoclaving it at 121°C (250°F) for 15-20 minutes.
5. **Cool:** Allow the agar solution to cool to around 45-50°C (113-122°F) before pouring it into sterile Petri dishes.
6. **Solidify:** Let the agar solidify at room temperature.

Ingredients :-

Sr. no.	Chemical	Quantity
1.	Peptone	0.5 gm
2.	Yeast extract	0.2 gm
3.	NaCl	0.5 gm
4.	Agar	1.5 gm
5.	Starch	1.0 gm
6.	Distilled water	97.3 gm

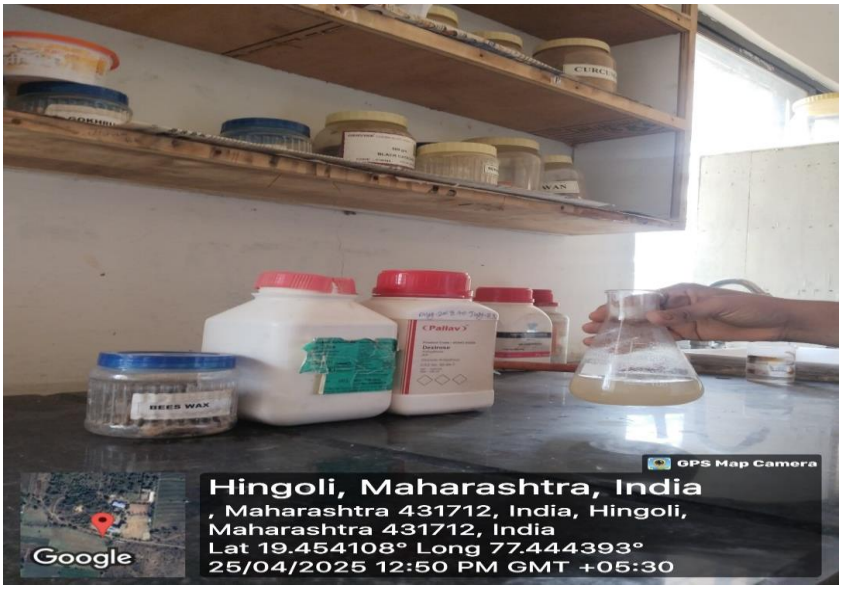


Fig.14: Ingredients collection

➢ **METHODOLOGY:-**

1. Weigh accurately all the ingredients as per the given quantity. Mix all the ingredients together and add in the conical flask and make up the volume make ap the up to 100 ml by using measuring cylinder. The prepared media is then heated for 5 min to remove any microbial contamination if present.

2. After heating, plug the cotton to cover the mouth of conical flask the to prevent contamination entry of microbes present in air.
3. Now, put the conical flask into autoclave for 15 min to main the sterility with specific pressure. After 15 ming of autoclaving, the media is removed from autoclave and allow to cool.



4. Then kept the media in aseptic area i.e. in between the two burners. Take the two sterilized petri plates and transfer the media in petri plates in between two burners.





5. Keep this two petriplates in incubator to incubate at 37°C for 24 hours. After incubation, the petriplates are again kept at aseptic area for further procedure. Now take the inoculating loop & and heat until it turns red and allow to cool for 5 min.
6. Take a single colony of bacteria i.e. E. coli by Inoculating loop and spread on the media in petri plate by using the glass spreader in both petri plate. By using a spread plate method, you can spread the drug evenly use a sterile spreader to distribute the solution across the agar surface.
7. To check the antimicrobial activity, streak the drug solution i.e. Aegle marmelos leaves extraction solation in one petri plate.



Fig.15: - Insertion of Ethanolic extract

8. Streak the Azithromycin drug in the another petri plate as a standard drug to check the antimicrobial activity. Kept both the petri plates in incubator at 37°C for 72 hours for the growth of bacteria to check the antimicrobial activity. After 72 hours, check the bacterial growth & formation of the zone of inhibition around the streaked drug in both plates.



Fig.16: - Bacterial Growth

9. Measure the zone of inhibition by using the metric ruler & Compare the zone of inhibition in test drug and the standard drug. If zone of inhibition Aegle marmelos is similar to standard drug Azithromycin. Then the Aegle marmelos extraction show the antimicrobial activity.

- **PHYTOCHEMICAL EVALUTION TEST:-**

Table 1: Phytochemical Evaluation

Tests	Observation	Inference
1. Flavonoid test	Yellow solution turns colourless	Presence of flavonoid
2. Tannin test	White precipitate appeared	Presence of Tannin
3. Ferric chloride test	Bluish black colour is formed	Presence of phenol
4. Alkaloid tests		
a. Mayer's test	Yellow colored precipitate is formed	Presence of Alkaloid
b. Wagner's test	Brown/reddish precipitate formed	Presence of Alkaloid
c. Dragendroff's test	Red precipitate formed	Presence of Alkaloid
5.Saponin test	Formation of foam	Presence of Saponin

RESULT & DISCUSSION:

The Ethanolic extract of Aegle marmelos was successfully shows the antimicrobial activity by using the bacteria. These drug shows the antimicrobial activity againt the E. colli by using the Disc diffusion method.

CONCLUSION:

This experiment concludes that *Aegle marmelos* is highly valuable medicinal plant with significant antimicrobial activity. It shows the minimal similar antimicrobial activity as compaired to the Antibacterial drug Azithromycin

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