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# ANTIBACTERIAL SCREENING OF ETHANOLIC *AEGLE MARMELOS* LEAF EXTRACT AGAINST PATHOGENIC BACTERIA

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**ABSTRACT**

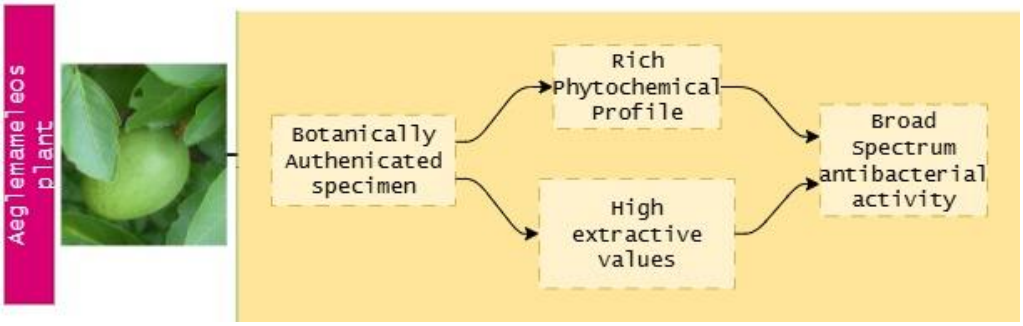
Aeglemarmelos (L.) Correa, named as Bael is one of the medicinally important plants and popular in all systems of medicine for its therapeutic properties. Present investigation focuses on collection and authentication of A. marmelos leaves in the extract preparation, phytochemical analysis, physico-chemical characterization and antimicrobial studies. An accredited botanist confirmed the plant specimen to be Herbarium collections based on its form. The plant material was collected from Botanical Garden of Chaudhary Charan Singh (CCS) University, Meerut. To assist in plant identification, the leaves macroscopic features - shape, size, venation and texture were noted. The shade-dried leaves were powdered and subsequently extracted with methanol in soxhlet apparatus. Phytochemical analysis revealed the presence of bioactive agents like alkaloids, tannins terpenoids, flavonoids phenolics, glycosides and saponin in varying concentration. Physicochemical examination confirmed the purity of the plant and its compatibility with use in medicine. The extractive value was high (84.1 + 1.54%) and the ash as well as moisture content were within allowable limits. The alcoholic leaf extract showed considerable antibacterial activity against both Gram-positive (Staphylococcus aureus, Streptococcus mutans) and Gram-negative (Escherichia coli, Klebsiellapneumoniae) bacteria, as well as antifungal against Candida albicans and Aspergillus niger. These findings suggest that the leaves of A. marmelos has significant phytotherapeutic value in support of its use in herbal formulations against microbial diseases.

**KEYWORDS:** Antibacterial, Aeglemarmelos, Pathogenic Bacteria, ethanolic leaf extract, herbal.

**Highlights**

1. Aeglemarmelos leaves were authenticated using herbarium and microscopic traits.
2. Methanolic extraction yielded key phytochemicals: alkaloids, flavonoids, tannins etc. 3. Physiochemical analysis confirmed purity; extractive values was 84.1±1.54%.
4. Reducing sugar were present; carbonyl groups absent in phytochemical profile.
5. Ethanolic extract showed broad-spectrum antibacterial and antifungal activity.

GRAPHICAL ABSTRACT




## 1. INTRODUCTION

### 1.1 Herbal Medicine

Herbal medicine, also known as botanical medicine or phytotherapy, is the oldest healing tradition in the world, and its method of practice has been spread throughout countless cultures. It relies on plants and plant extracts to prevent and treat disease, improve health, make people feel better. Beyond even modern pharmacology and synthetic medications, people have lived with plants as medicine. The practice is ancient. Even in this day herbal medicine has become common all over the world after modern western technology, even though it is still used by many people as a side treatment.[1] Herbal medicine treatment has been practiced for a long time and it is widely used by people. Archaeologically, traces of use of plants for healing have been found in a burial site and cave paintings. On different continents, tribes devised their very own ways of using herbs for medicine based upon the available plants in their region. For example Traditional Chinese Medicine (TCM) is based heavily on herbal medicine, and Ayurveda (which in Hindi means 'science of life') uses herbs and other natural substances to heal.[2] Herbal medicine is predicated on the belief that plant-based chemicals have properties which are favorable to the body. These chemicals help regulate the body's processes to reduce symptoms, fight infections and keep organs functioning properly. Many medications in use today are plant-based, including aspirin, which was originally derived from willow bark. At that time, herbal medicine utilizes whole plants, or extracts which contain tens or hundreds of different compounds, to give a more balanced and effective treatment with lower chance of adverse effects.[3]

Herbalists take in the patient's whole health and seek to restore balance to the body, not just treat symptoms. Herbal therapy has many advantages: It reduces side effects compared to synthetic drugs, supports overall health and boosts the body's natural resistance. Herbal remedies are also more accessible and less expensive, particularly in rural or low-income areas. to heal in the first place. Overall, it is an effective way to heal and although hundreds of years old, it remains a key part of healthcare for many people around the world.[4]

### 1.2 Benefits of Herbal Medicine

Herbal treatment has its benefits, but also its problems and limitations. A big problem is there is no standardization of herbal products. The potency of active constituents may vary considerably between plant species, growing conditions, time of harvest and processing. This discrepancy can result in unpredictability treatment effect. It can also be unhealthy when contaminated with heavy metals, pesticides or poisons.[5] There is also the possibility that herbal remedies could contraindicate with other medication. Some herbs may make your medication less effective, while others could have potentially harmful interactions with other medications you take. He cited St. John's Wort, which can interact with blood thinners and birth control pills. Patients should always tell their doctors if they are on any supplements, particularly herbal ones, to avoid possible complications.[6]

### 1.3 History of herbal medicine

In recent years, several scientists have conducted research on the safety and efficacy of TCMs. Some plants have obvious potential as medicines and others are lacking the clinical proof. This new research offers objective ways to validate ancestral knowledge and understand how herbal ingredients work. This laboratory investigation also leads to new drug discovery using plants.[7] And finally as we have begun to pay more attention to the strength of natural medicine, acceptance of this even in mainstream health is now beginning. In order to ensure that herbal medicine is used safely, accurate information concerning identification, dosage, preparation and risks is essential. Integration of classical knowledge and scientific evidence is essential in successful and safe herbal treatment.[8]

### 1.4 Herbal Medicine as Antibacterial: An In-Depth Study

The herbal medicine is an important origin of the new antibacterial medicines, especially in view of the increasing bacteria resistance. Herbs have been used in Ayurveda and Chinese Medicine for millennia to treat infections. To some use them as in wound covering only and no others rather than alone.Some scientific publications have confirmed this uses which isolating

bioactive compounds that exhibited remarkable antibacterial activity and put prospects for future studies forward.[9]

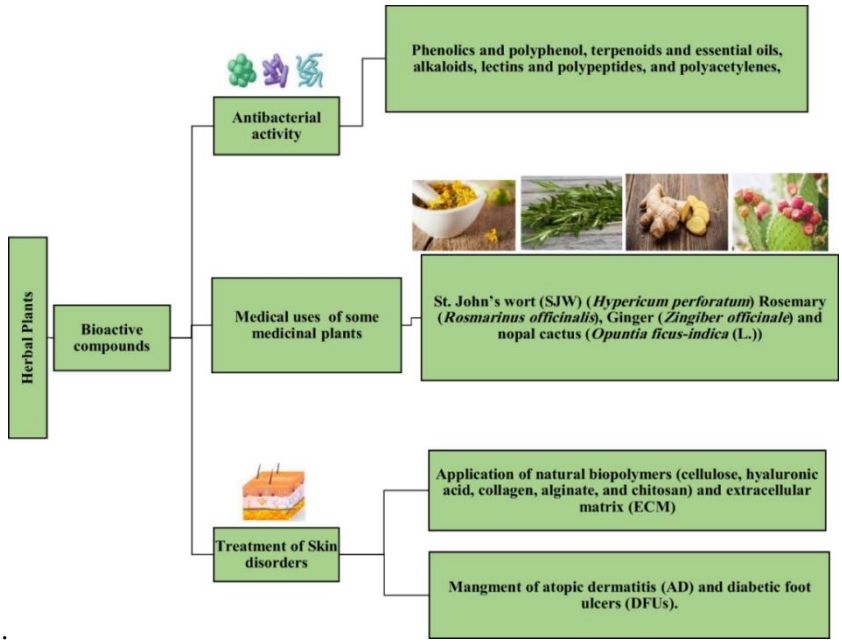



**Figure 1: Antibacterial activity of medicinal plants.**

### 1.4.1 Background on Herbal Antibacterials

The knowledge of using plants in healthcare is ancient, goes back to the times before recorded history and was transmitted through generations verbally. Historical texts as old as the Charaka Samhita and Shennong Bencao Jing described herbs with specific good effects.[10] Herbal remedies, such as garlic, neem and turmeric were traditionally used to treat infections before the advent of modern antibiotics. And after the 2d world war hum-synthesised antibiotics were largely consumed worldwide but herbal antibacterials continued to be a focus of attention especially in places were modern medicine is inaccessible.[11] The emergence and increasing problem of antimicrobial resistance has raised new scientific interest in the use of herbal remedies as alternative or complementary treatments for drug resistant bacteria.[12]

### 1.4.2 Mechanism of action of herbal antibacterial

Herbal antimicrobials exhibit a number of mechanisms by which they prevent or kill organisms. Plant chemicals tend to have multiple effects on bacteria, something that might make it harder for them to develop resistance. That sets it apart from many antibiotics whose manufacturers single out specific bacterial enzymes or processes.[13]

1. **Cell Wall Disruption**: Some of those plant chemicals interfere with how the bacterial cell wall is made, or with its structure, so that the contents of the cell ooze out and the cell itself dies. Tannins, for example, can bind to proteins in the cell wall and thus diminish the strength of bacterial envelope.[14]
2. **Membrane Permeability**: Essential oils and phenolic compounds are capable of degrading bacterial membranes causing cell lysis and the collapse of membrane potential. This is a well-known property of terpenoids and flavonoids.[15]
3. **Stopping Protein Synthesis:** Some phytochemicals stop ribosomes from working or stop bacteria from making important proteins.[16]
4. **Interference with DNA/RNA Synthesis:** Alkaloids and other substances can then interfere with bacterial DNA, or inhibit enzymes essential to nucleic acid synthesis.[17]
5. **Quorum Sensing Inhibition:** Some plant extracts prevent bacteria from communicating with each other (quorum sensing), which prevents biofilm growth and reduces their virulence.[18]
6. **Efflux Pump Inhibition:** Bacteria use efflux pumps to drive out harmful substances, such as antibiotics. Some herbs may block these pumps, thus enhancing the effect of antibiotics.[19]

### 1.4.3 Medicinal Plants with antibacterial activity

- A diverse array of plants has been recognized for their antibacterial properties, with several species subjected to substantial traditional and scientific investigation.
- **Garlic (Allium sativum):** Bacteria use efflux pumps to throw out things that are dangerous to them, like antibiotics. Some medicinal herbs block these pumps and therefore increase the activity of antibiotics.[20]
- **Neem (Azadirachta indica):** Neem contains high concentrations of azadirachtin and nimbin thus it is effective against Gram positive and Gram negative bacteria including Staphylococcus aureus and Escherichia coli.[21]

- **Turmeric (Curcuma longa):** The active component of turmeric, curcumin has antibacterial, antiinflammatory and anti-oxidative effects. It has been reported to have activity against Helicobacter pylori and other microbes.[22]
- **Tea Tree (Melaleucaalternifolia):** Its essential oil contains terpinen-4-ol, which is very effective to disintegrate the bacterial membranes and prevents biofilms.[23]
- **Echinacea:** Originally used for infections, echinacea extracts alter the workings of immune system and are bactericidal against the bacteria which cause respiratory infections.[24]
- **Oregano (Origanumvulgare):** Carvacrol and thymol, two phenolic compounds contained in oregano, are very effective at not only killing foodborne germs but also in acting as a therapeutic way to treat foodborne illness parties.[25]
- **Ginger (Zingiberofficinale):** Contains gingerol and shogaol, which fight drug resistant organisms.[26]
- **Clove (Syzygiumaromaticum):** Eugenol, the major chemical in clove oil, has been shown to kill bacteria and work well with antibiotics.[27]
- **Cinnamon (Cinnamomumverum):** Cinnamaldehyde and other parts stop germs from growing and forming biofilms.[28] The antibacterial activity of some plants are given in Table :1

**Table 1: Medicinal Plants with Antibacterial Activity.**

| Plant Name | Active Compound(s) | Mechanism / Target | Target Bacteria / Effect |
|---|---|---|---|
| **Garlic (Allium sativum)** | Allicin | Inhibits lipid synthesis; disrupts bacterial membranes | Broad-spectrum |
| **Neem (Azadirachta indica)** | Azadirachtin, Nimbin | Damages bacterial structures | *S. aureus*, *E. coli* |
| **Turmeric (Curcuma longa)** | Curcumin | Antibacterial, anti-inflammatory, antioxidant | *Helicobacter pylori*, others |
| **Tea Tree (Melaleuca alternifolia)** | Terpinen-4-ol | Disrupts membranes; inhibits biofilms | Broad-spectrum |
| **Echinacea** | Multiple alkylamides & polysaccharides | Immunomodulatory; targets respiratory pathogens | Bacteria causing respiratory infections |
| **Oregano (Origanum vulgare)** | Carvacrol, Thymol | Phenolics that rupture cell membranes | Foodborne pathogens |
| **Ginger (Zingiber officinale)** | Gingerol, Shogaol | Active against drug-resistant bacteria | Resistant microbial strains |
| **Clove (Syzygium aromaticum)** | Eugenol | Bactericidal; synergizes with antibiotics | Broad-spectrum |
| **Cinnamon (Cinnamomum verum)** | Cinnamaldehyde | Inhibits growth and biofilm formation | Broad-spectrum |

These plants, along with many others, are stores of bioactive chemicals that might be used to combat bacterial diseases.

### 1.5 Aeglemameleos

Aegle marmelos (Kalmegh or King of Bitters) is an herbal medicine of Indian traditional systems like Ayurveda and Traditional Chinese Medicine. Biochemically, it is the bio-active elements such as andrographolide having medicinal properties against various pathogenic bacteria such as two types of gram-positive and gram-negative organism.[29] Studies show that the extracts of Aegle marmelos is capable of inhibiting bacteria such as Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The modes of action are cell-membrane disruption, nucleic acid production inhibition and protein synthesis interference in which the plant takes act; thus it is unlikely for bacteria to response with resistance. Aegle marmelos also stimulates the immune system, rendering it a hopefully natural alternative or adjuvant to antibiotics. Its antibacterial and immunomodulatory properties qualify it as an antibiotic adjuvant, particularly in view of increasing levels of bacterial resistance to antibiotics. It is included in herbal formulas and as an adjunct therapy.[30]

#### 1.5.1 Plant profile

**Name: Aeglemarmelos**

Aeglemarmelos, known as bael, Bengal quince and wood apple, is a tree that people have used in traditional medicine for centuries in India. It is a deciduous plant that belongs to the Rutaceae family and indigenous to India and Southeast Asia.[31] Valued in Ayurveda for its salubrious properties, the bael tree is invested with religious and medicinal sanctity. Every part of the tree,

such as leaves, fruit, bark, sap etc have been used for various medicinal purposes to treat a huge array of ailments and conditions; this is making it a very cherished plant in traditional and contemporary herbal medicine.[32]

**Figure 2: Leaves of Aeglemarmelos.**

Scientific Classification

- **Kingdom**: Plantae
- **Clade**: Angiosperms
- **Clade**: Eudicots
- **Clade**: Rosids
- **Order**: Sapindales
- **Family**: Rutaceae
- **Genus**: Aegle
- **Species**: Aeglemarmelos (L.) Corrêa

### 1.5.2 Chemical Parts

Aegle marmelos, or bael fruit, has several bioactive constituents including: Alkaloids (e.g., aegeline), Flavonoids (e.g., rutin and quercetin), Tannins, Volatile oils (e.g. limonene and cineole),Marmelosin, Phenolic acids, Terpenes.[33]

These substances are responsible for its biological activities including antimicrobial, anti-inflammatory and antioxidant effects.

### 1.5.3 Aeglemarmelos uses in medicine

Aeglemarmelos is used in medicine, including Ayurveda and traditional Chinese medicine. Some of its uses include:

1. Digestive issues: Bael fruit is used to treat diarrhea, dysentery, and constipation.[34]
2. Antimicrobial properties: It has been shown to have antimicrobial properties, making it effective against certain bacteria and viruses.[35]
3. Anti-inflammatory properties: It has anti-inflammatory compounds, which can help lower inflammation and symptoms.

Antioxidant benefits: Being rich in antioxidants, bael also helps protect from oxidative stress and cell damage

## 2. MATERIAL AND METHODS

### 2.1 Collection and authentication of *Aeglemarmelos* plant

The plant material of Aeglemarmelos employed in this work was obtained from Botanical Garden of Chaudhary Charan Singh (CCS) University, Meerut. After collecting, the plant was thoroughly inspected and identified by the distinguished botanist Professor Vijay Malik of CCS University. The authentication process was morphological Identification in which the plant was identified and compared with herbarium specimens were confirmed. Prof. Malik expertise confirmed that the specimen used in this study was identified as *Aeglemarmelos*, a member of the Rutaceae family.

### 2.2 Preparation of course powder

The leaves of *Aeglemarmelos* were dried in the shade carefully for preservation of bioactive compounds. Leaves will be grind into fine powder using a mechanical grinder after complete drying. After passing through 40 mesh sieve, powder will be stored in an airtight container to protect it from moisture and contamination to ensure its suitability for further experimental use.[37]

### 2.3 Preparation of extract

The dried leaves of plant have been pulverized to form a rough powder. This powder was extracted with methanol using a Soxhlet apparatus. After successive extraction the obtained extract was filtered with muslin cloth to remove solid materials. The filtrate was evaporated with a rotary evaporator under reduced pressure and vacuum dried to obtain the crude ethanolic extract as solid for further studies.[38]

### 2.4 Phytochemical Analysis of *Aeglemarmelos* Leaf Extract

The complete tests used in Phytochemical analysis of *Aeglemarmelos* Leaf Extract has been shown in table: 2

**Table 2: Phytochemical Analysis of *Aeglemarmelos* Leaf Extract.**

| Phytochemical | Test Name | Reagent/Procedure | Positive Indication |
|---|---|---|---|
| **Alkaloids** | Mayer's Test | Potassium mercuric iodide solution | Creamy/white precipitate |
| | Dragendorff's Test | Dragendorff's reagent | Orange/reddish-brown precipitate |
| **Flavonoids** | Shinoda Test | Magnesium turnings + concentrated HCl | Pink/red coloration |
| **Tannins** | Ferric Chloride Test | 5% ferric chloride solution | Blue-black or greenish-black precipitate |
| **Saponins** | Frothing Test | Shake vigorously with water | Persistent foam lasting several minutes |
| **Terpenoids** | Salkowski Test | Chloroform + concentrated $H_2SO_4$ | Reddish-brown coloration at interface |
| **Phenolic Compounds** | Ferric Chloride Test | 5% ferric chloride solution | Deep blue or black coloration |
| **Glycosides** | Keller-Kiliani Test | Glacial acetic acid + ferric chloride + $H_2SO_4$ along tube wall | Brown ring at the interface |
| **Steroids** | Liebermann–Burchard | Acetic anhydride + concentrated $H_2SO_4$ | Violet → blue/green color change |

### 2.5 Physicochemical content

The shade-dried and powdered leaves of Aeglemarmelos were used to find out different physicochemical properties, using the World Health Organization's (WHO) standard procedures for checking the quality of medicinal plant materials.[39]

#### 2.5.1 Ash values

Ash values are significant analytical tools for figuring out the quality, identification, and purity of powdered crude medicines. The ash content is the non-organic substance that is left over after burning the plant material. This residue could include mineral salts that are naturally present in the plant tissues, dirt or dust particles that have stuck to the plant, or contaminants that were added as adulterants which helps to find any contamination or purposeful adulteration.[40]

#### 2.5.2 Total ash

Total ash is the total quantity of inorganic material that is left over after burning the plant material all the way through. There are two types of ash: physiological ash, which comes from the plant's intrinsic mineral content, and non-physiological ash, which comes from things like dirt, sand, or other foreign matter that sticks to the plant's surface. The air dried powdered samples (2 g) were weighed in a clean and dry crucible made of silica crucible, heated to red hot for 30 min, cooled in desiccator. The ash was free of carbon and was incinerated at below 600°C and crucibles were left to cool in a desiccator for 30 min, then weighed. The percent ash weight was calculated by dividing the weight of the ash by the weight of the plant material.[41]

#### 2.5.3 Water soluble ash content

The ash was treated with 25 cc distilled water for 5 min. and filtered. The resultant insoluble residue was washed with hot water, dried, and finally incinerated in a muffle furnace (≤450 °C) for 15 min. Residue was cooled in desiccator and weights were noted.[42]

#### 2.5.4 Acid insoluble Ash content

When you boil the whole ash with weak hydrochloric acid and then light the rest of the insoluble particles, you get acid-insoluble ash. This test is usually performed to find out how much silica is in plant materials. Silica may be found in sand or siliceous soil. An oven dried ash made on the test plate was treated with 25 ml of dilute HCl, evaporated to acid fume, and filtered in ashless paper. The solid was hot water-washed to neutral, suspended on a crucible, ignited, cooled in desiccator for 30 mins and weighed. The acid insoluble ash was subsequently calculated as a % of the air dry sample.[43]

#### 2.5.5 Sulphate ash

A silica crucible was heated red hot, cooled in a desiccator and weighed out with 2g of the sample for ash content. The material was carefully ignited, moistened with 1mL of concentrated sulfuric acid then heated to fumes. It was heat-treated at 800±25°C to get rid of black particles. The mixture was boiled after cooling and sulphuric acid was added. This was repeated until two consecutive measurements were within 0.5g of each other.[44]

#### 2.5.6 Determination of extracting value

Extractive values are an essential way to measure the phytoconstituents in plant materials, especially when other methods can't readily do this. These numbers give you an idea of the chemical makeup and amount of the

active components in a crude medication. By figuring out the extractive values, you may learn more about how well different parts of the plant dissolve in different solvents. Different solvents may pull out different types of chemicals. Extractive values are a good way to tell how good and strong the plant material is. They may also help you find bioactive components that are important for therapeutic uses.[45]

**2.5.7 Determination of soluble extractive value**

Powder leaf (5 g) samples were macerated in 100 mL chloroform water (95 mL dist. water + 5 mL chloroform) in a closed jar for 24 h, stirring at intervals in 6 h; on filtration of the maceration volume, 25 mL of the filtrate was evaporated on a weighed dish. The residue was weighed and the amount of dissolve was calculated as a % of the air-dried sample.[46] To calculate the water soluble extractive value:-

**Water-soluble extractive (%) = (Weight of dried extract / Weight of sample) × 100**

**2.5.8 Alcohol-soluble extract**

Five grams of powdered plant material were macerated in 100 ml of 90% ethanol for 24 h with occasional shaking for the initial 6 h. 25 mL of the filtrate were evaporated to dryness in a pre-weighed dish and dried at 105 °C for 1 hr and cooled in desiccator and weighed. The drying process was further continued until a constant weight has been achieved. The extract yield was presented as the percentage of air dried sample.[47] To find the alcohol-soluble extractive value, use the following formula:

**Alcohol-soluble extractive (%) = (Weight of dried extract / Weight of sample) × 100**

**2.5.9 Estimation of fixed oil**

Powder plant material was extracted with petroleum ether (boiling range, 40-60°C) in Soxhlet extraction thimble for 6 hr. The alcohol was transferred to a pre-weighed evaporating dish and evaporated. Dried at 105°C in hot air oven to reach a constant weight.[48] The fixed oil content was calculated:

**Ether-soluble extractive (%) = (Weight of dried residue / Weight of sample taken) × 100**

**2.5.10 Estimation of volatile ether-soluble extract**

Two grams of powdered plant material was extracted continuously for 20 h in anhydrous diethyl ether with a continuous extraction apparatus. The extracted ether was then transferred to a pre-weighed porcelain dish and left to evaporate at room temperature. The residue was subsequently dried over phosphorus pentoxide for 18 h to dry off residual moisture and ether. The extract was weighed and the weight value was then dried in hot air oven at 105°C to constant weight. The weight of dried sample after was the content of the volatile matter.[49]

**2.5.11 Loss on drying**

A known amount of sample was introduced into a pre-heated and cooled silica crucible, dispersed, covered, and weighed with its own lid to measure the initial weight. The crucible was dried at 105°C in a hot air oven until a constant weight was obtained, indicating total removal of moisture. It was then cooled under desiccator and its crucible was weighed again. The loss on drying (LOD) was calculated by the difference of initial and final weights, the amount of the weight loss divided by the weight of the air-dried sample.[50]

**2.5.12 Determination of the foaming index**

One gram of coarsely powdered plant material was boiled in 100mL of boiling water (80-90°C) for 30 min, allowed to cool, filtered, and made up to 100mL with distilled water. The decoction was serially diluted and 10mL volumes (1–10mL) of the decoction were transferred to ten stoppered test tubes (16cm × 16mm), made to 10mL mark with distilled water. Tubes were shaked for 15 s and then left at room temperature for 15 min.[51] Foam were measured to determine the saponin content. The foaming index was calculated using the formula:

**Foaming Index = 1000 / a**,

where *a* is the volume (in mL) of decoction producing 1 cm of foam. If all tubes showed 1000 and further dilution was required.

**2.5.13 swelling index**

Plant material powders (1.0 g) were added to 25 mL glass-stoppered graduated cylinders (125mm × 16mm, graduated in 0.2mL intervals). The mixture was mixed with 25 mL of distilled water and shaken every 10 min for 1 h. It was subsequently left at room temperature for 3 hr for complete swelling. The volume of the hydrated material in the sample, with the inclusion of any mucilage layer, was measured. The S index was expressed as the average volume of ml/g of sample.[52]

**2.6 Test organisms and inocula**

This study utilized Gram-positive bacterial strains, including Staphylococcus aureus and Streptococcus mutans, as well as Gram-negative strains, such as Escherichia coli and Klebsiellapneumoniae. There were other fungus strains including Candida albicans and Aspergillus niger in the mix. The Department of Microbiology at Chaudhary Charan Singh (CCS) University in Meerut, Uttar Pradesh, provided all of the microbial strains.

**2.7 Standard Drugs**

Standard antibacterial drugs, amoxicillin (30 µg/disc) and the antifungal agent ketoconazole (30 µg/disc), were procured as complimentary samples from Akums Drugs and Pharmaceuticals, Haridwar, Uttarakhand. These standard discs used as positive controls in the assessment of antibacterial activity.

**2.8 Preparation of Nutrients Agar media**

This study used dehydrated nutrient agar media as the medium for bacterial growth. To make the medium, we

dissolved the commercially available dehydrated powder in distilled water, following conventional lab protocols to make sure it was clean and consistent. Peptone, beef extract (or yeast extract), sodium chloride, and agar were some of the important parts of the nutritional agar. Peptone is a source of organic nitrogen, amino acids, and peptides that microbes need to thrive. Extract of beef is rich in vitamins, salts, & other nitrogenous compounds, which partially satisfies the nutritional requirements of many bacteria. Sodium chloride maintains the osmotic equilibrium in the medium and agar solidifies surface, making it possible to isolate and observe bacterial colonies. For the preparation of nutritional agar medium: 28 gm of dried powder was accurately weighed and suspended in 1000 ml distilled water in a clean conical flask. Subsequently, the combination was heated in a water bath until all components of the medium were dissolved. Depolymerisation of the medium will prevent as it would hinder the efficiency of the support material for microbial growth, by avoiding direct heating. After the medium was completely dissolved, it was sterilized by autoclaving at 15 psi and 121°C for 15 min. This ensured everything was as sterile as it possibly could be, so you could use the medium to inoculate and grow bacterial strains in a lab setting.[53]

### 2.8.1 Cleaning of Media

To prevent contamination of the prepared nutritional agar medium from dust or any airborne particles, a cotton bung that did not absorb was used to close up firmly the conical flask. To prevent contamination of the medium during sterilisation, aluminium foil was tightly folded over the neck. The flask was then placed at autoclave and sterilized for 20 minutes where the pressure of the autoclave was set to 15psi, and temperature up to 121°C. The application of this method assured that all microbial flora were destroyed resulting in sterility of nutrient agar medium which is suitable as a growth culture medium in bacterial studies.[54]

### 2.8.2 Getting Ready for Test Organisms

Test organisms were maintained on nutrient agar slants initially and transferred to fresh slants weekly for verification of continued viability and purity. To promote optimal growth, these slants were incubated at 37°C for 24 h. The inoculum was prepared by thorough washing of the bacterial cells aseptically from agar slants to large nutritional agar surface (15-20 cm in diameter) by adding around 3 ml of sterile saline solution. This surface was then incubated again at 37°C for 24 hours to get fresh, active cultures. After the bacteria had grown, 50 ml of distilled water was used to gently wash the growth off the nutrient agar surface. This made a bacterial suspension. We used a spectrophotometer to measure the optical density of this solution and then changed the concentration until it let 25% of the light through at 520 nm. Preliminary studies utilizing test plates or broth cultures were used to figure out the right amount of bacterial suspension to add to each 100 ml of nutritional agar or broth based on this standardization. Prepared bacterial cultures were kept in the refrigerator until they were needed for studies to keep them alive.[55]

### 2.8.3 Method of experimentation

Cup and Plate Method is used to test the antibacterial activity of the ethanolic extract of Aeglemarmelos leaves. We put sterilized nutritional agar medium, which had been liquefied and sterilized, into sterile 100 mm Petri plates. Ten plates were made and let harden. Using a stainless steel borer, five wells with a diameter of 6 mm were made in each plate. To make the ethanolic extract, 1% Dimethyl Sulfoxide (DMSO) was used as the solvent and the concentration was 10 mg/ml. Amoxicillin (30 µg/disc) and ketoconazole (30 µg/disc) functioned as standard controls for antibacterial and antifungal activity, respectively. To keep things even, 0.1 ml of the extract solution was carefully put into each well with a micropipette. In addition, strips of amoxicillin and ketoconazole were inserted in the middle well of each plate in a sterile manner to serve as standards. The plates were then left alone for an hour at room temperature so that the test solutions could spread out evenly throughout the agar substrate. After the diffusion, the plates were kept at 37°C for 24 hours. After the incubation period, the plates were checked for zones of inhibition. The widths of these zones were then properly measured to see how well the extract worked as an antibiotic.[56]

## 3. RESULTS AND DISCUSSION

### 3.1 Collection and authentication of *Aeglemarmelos* plant

The plant material of *Aeglemarmelos* used in this study was collected from the Botanical Garden of Chaudhary Charan Singh (CCS) University, Meerut. After collection, the plant was carefully examined and authenticated by Professor Vijay Malik, a renowned botanist associated with CCS University. The authentication process involved morphological identification and comparison with herbarium specimens to confirm the taxonomic identity of the plant. Professor Malik's expertise ensured that the specimen used in this study was correctly identified as *Aeglemarmelos*, a member of the Rutaceaefamily.

### 3.2 Macroscopy of *Aeglemarmelos* leaves

Examine the leaves and stem carefully with the naked eye and note the following characteristics:

**Leaves**

- Shape: Observe the overall shape (e.g., lanceolate, ovate).
- Size: Measure the length and width using a ruler or caliper.
- Arrangement: Note if leaves are opposite or alternate on the stem.
- Margin: Observe the leaf edges (entire, serrated, etc.).
- Surface texture: Note if the leaf surface is smooth, hairy, or rough.

- Color: Record the color on both upper and lower surfaces.
- Venation: Observe the pattern of veins (e.g., reticulate, parallel).
- Odor and taste: Crush a leaf gently and note any characteristic odor or taste (e.g., bitter).

**3.3 Preparation of course powder**

The leaves of *Aeglemarmelos* were carefully dried in the shade to preserve their bioactive compounds. Once completely dried, the leaves were ground into a fine powder using a mechanical grinder. The resulting powder was then sieved through a 40-mesh sieve to obtain uniform particle size and subsequently stored in an airtight container to protect it from moisture and contamination, ensuring its suitability for further experimental use.

**3.4 Preparation of extract**

The air-dried leaves of *Aeglemarmelos* were ground into a coarse powder. This powdered material was then subjected to extraction using methanol as the solvent in a Soxhlet apparatus. After continuous extraction, the resulting extract was filtered through muslin cloth to remove solid residues. The filtrate obtained was concentrated by evaporating the solvent under reduced pressure using a rotary evaporator, followed by vacuum drying to obtain the crude ethanolic extract in a solid form for further analysis.

**3.5 Phytochemical examination**

**Table 3: Results of phytochemical examination of Aeglemarmelos.**

| S. No. | Name of phytochemicals | Observation |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Saponin | + |
| 3 | Tannins | + |
| 4 | Flavonoids | + |
| 5 | Terpenoids | + |
| 6 | Phenolic Compound | + |
| 7 | Steroids | + |
| 8 | Glycoside | + |
| 9 | Carbonyl | - |
| 10 | Reducing Sugar | + |

**3.6 Physiochemical constant examination**

**Table 4: Results of Physicochemical contents examination of *Aeglemarmelos.***

| S. no. | Parameters | Percentage w/w |
|---|---|---|
| **Ash value** | | |
| 1 | Total ash | 6.25±0.21 |
| 2 | Water soluble ash | 3.45±1.13 |
| 3 | Acid soluble ash | 1.12±0.19 |
| 4 | Sulphate ash | 6.20±0.23 |
| **Extractive value** | | |
| 5 | Ethanol solubility extractive | 84.1±1.54 |
| 6 | Water soluble extractive | 9.80±0.42 |
| 7 | Ether insoluble non-volatile | 22.30±0.23 |
| 8 | Ether insoluble volatile | 12.12±0.92 |
| 9 | Loss on drying | 6.42±0.72 |
| 10 | Crude fiber content | 5.76±0.01 |
| 11 | Foaming index | Less than 100 |

**3.7 Test organisms and inoculums**

Gram-positive bacterial strains including *Staphylococcus aureus* and *Streptococcus mutans* (AHCC 28653), along with Gram-negative strains such as *Escherichia coli* and *Klebsiellapneumoniae* were used in this study. Additionally, fungal strains including *Candida albicans* and *Aspergillus niger* were also included. All microbial strains were procured from the Department of Microbiology, Chaudhary Charan Singh (CCS) University, Meerut, Uttar Pradesh.

### 3.8 Antibacterial activity

**Table 5: Antibacterial activity gram positive of ethanolic extract of *Aeglemarmelos.***

| S. No. | Drug | Concentration | Zone of inhibition value (mm) ± SD | |
|---|---|---|---|---|
| | | | ***Staphylococcus aureus*** | ***Streptococcus mutans*** |
| 1 | Ethanolic extract of *Aeglemarmelos* | 10 (mg/ml) | 17.08±0.01 | 14.15±0.47 |
| 2 | Ethanolic extract of *Aeglemarmelos* | 15 (mg/ml) | 22.03±0.23 | 23.15±0.38 |
| 3 | Amoxicillin | 30 (µg/disc) | 24.81±0.32 | 24.99±0.23 |

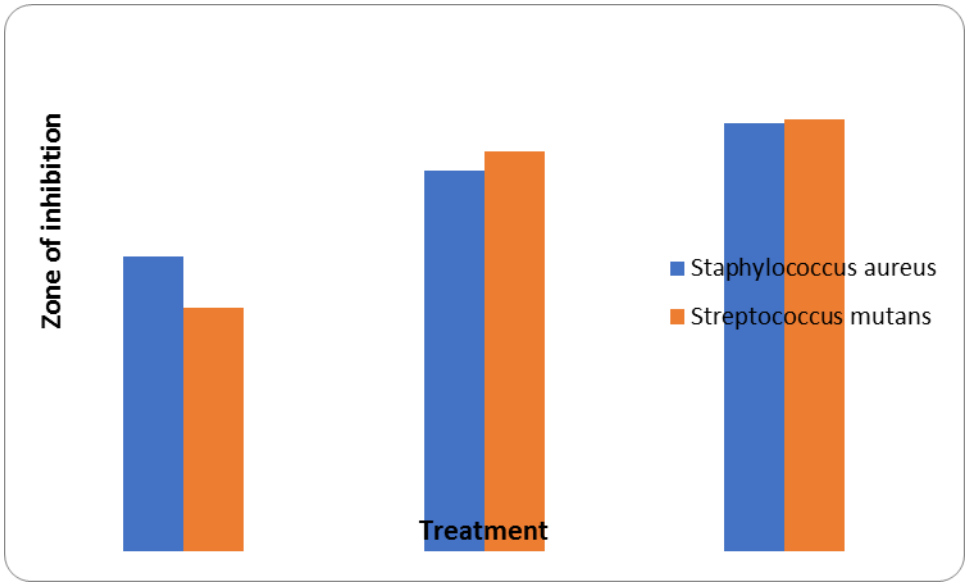



**Figure 3: Antibacterial activity gram positive of ethanolic extract of *Aeglemarmelos.***

**Table 6: Antibacterial activity gram negative of ethanolic extract of *Aeglemarmelos.***

| S. No. | Drug | Concentration | Zone of inhibition value (mm) ± SD | |
|---|---|---|---|---|
| | | | ***Escherichia coli*** | ***Klebsiellapneumoniae*** |
| 1 | Ethanolic extract of *Aeglemarmelos* | 10 (mg/ml) | 14.01±0.06 | 15.21±0.29 |
| 2 | Ethanolic extract of *Aeglemarmelos* | 15 (mg/ml) | 19.02±0.13 | 18.32±0.11 |
| 3 | Amoxicillin | 30 (µg/disc) | 21.27±0.33 | 21.08±0.39 |

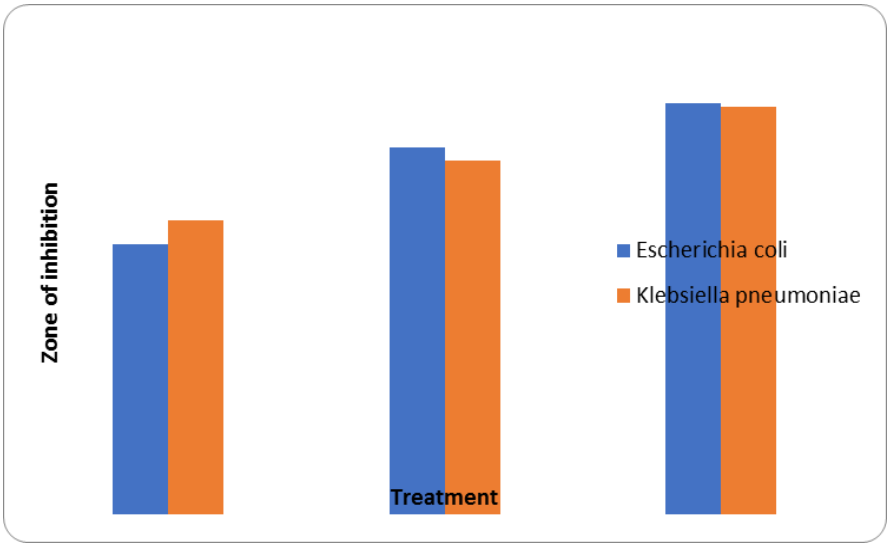



**Figure 4: Antibacterial activity gram negative of ethanolic extract of *Aeglemarmelos.***

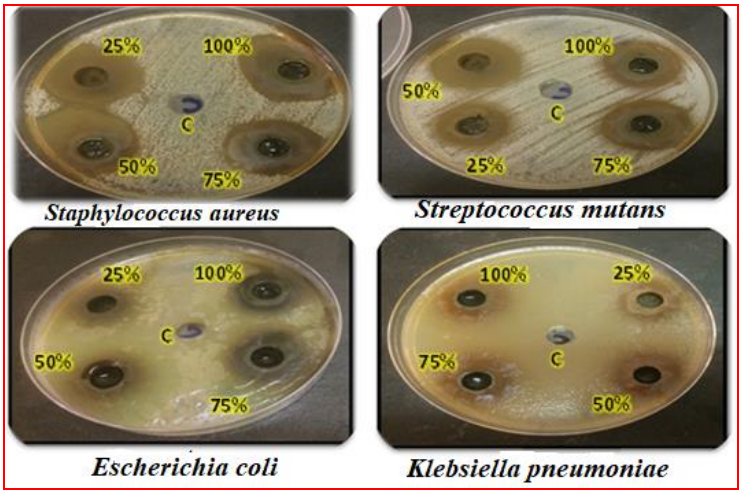



**Figure 5: Antibacterial activity of ethanolic extract of *Aeglemarmelos on Staphylococcus aureus, Streptococcus mutans, Escherichia coli and Klebsiellapneumoniae.***

**Table 7: Antifungal activity of ethanolic extract of *Aeglemarmelos.***

| S. No. | Drug | Concentration | Zone of inhibition value (mm) ± SD | |
|---|---|---|---|---|
| | | | *Candida albicans* | *Aspergillus niger* |
| 1 | Ethanolic extract of *Aeglemarmelos* | 10 (mg/ml) | 17.01±0.07 | 11.03±0.04 |
| 2 | Ethanolic extract of *Aeglemarmelos* | 15 (mg/ml) | 21.04±0.16 | 17.12±0.19 |
| 3 | Ketoconazole | 30 (µg/disc) | 23.27±0.13 | 25.01±0.43 |

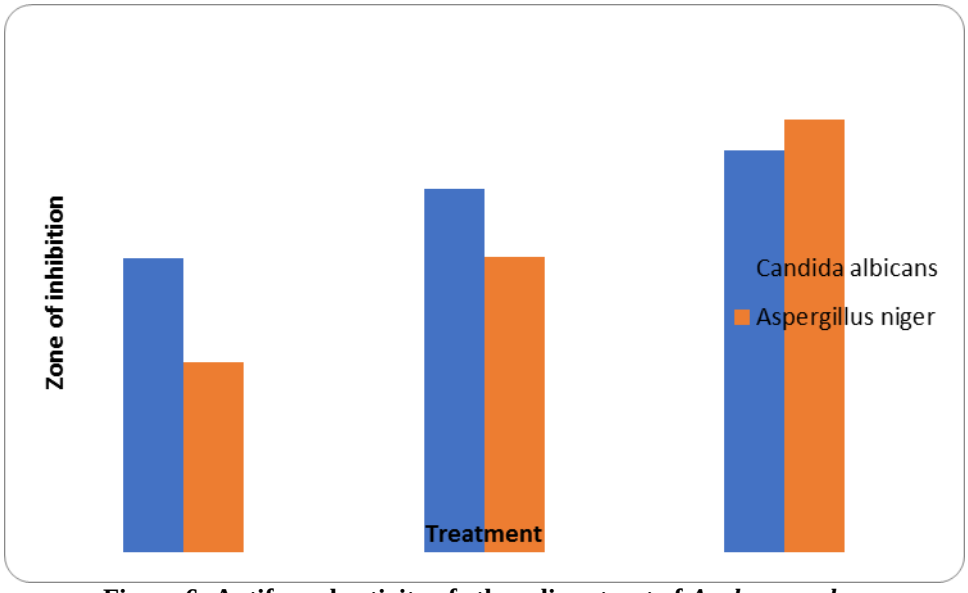



**Figure 6: Antifungal activity of ethanolic extract of *Aeglemarmelos.***

## 4. DISCUSSION

The current study concentrated on the pharmacognostic and antibacterial evaluation of Aeglemarmelos (often referred to as Bael), a plant of considerable ethnomedicinal significance. The complete investigation started with the methodical collecting and scientific validation of the plant material, guaranteeing the dependability and precision of ensuing studies.

The leaves of Aeglemarmelos came from the Botanical Garden of Chaudhary Charan Singh (CCS) University in Meerut, which is recognized for having a lot of different plants and growing medicinal plants. The authentication and the identification process were carried out by Professor Vijay Malik, botanist from the same university. Authentication is an important process of pharmacognostic investigation in that it ensures the proper taxonomical identification of plant material, following botanical properties including morphological

and anatomical features. This step could be considered not only a control for the study material but also as an insurance of reliability and reproducibility of the experiments. In such a case, morphological validation was performed by comparing the samples collected to standard herbarium specimens and known floral databases or taxonomic keys. The sample was proven as Aeglemarmelos (L.) Correa of rutaceae family and other tests were carried out.

**4.1 Pharmacognostic Evaluation**

From outside view of Aeglemarmelos leaves, things to be considered include form, margin, venation texture and color. The leaves appeared 3-foliolate as does one of A. marmelos. They were also reticulate-veined and ovate-lanceolate to oblong-ovate in shape. Their upper half was a smooth, dark green color while the lower was a slightly lighter one. The leaves, when crushed, emited a good smell and tasted a little bitter, which are all indicators that they have essential oils and flavonoids in them.

**4.2 Making Coarse Powder and Extract**

The examined plant material was crushed after slight hand scratches, according to general pharmacognostic operations. Leaves were dried in shade to preserve thermolabile chemical constituents and preserve the activity of phytoconstituents. The leaves were dried and ground into powders which passed through a 40-mesh sieve in order to obtain particles of uniform size for extraction. Methanol was selected as the solvent for Soxhlet extraction due to its higher polarity which is useful for purposes of extracting a broad range of phytoconstituents. The extract was then concentrated under reduced pressure to obtain crude methanolic extract which was retained for phytochemical and antibacterial tests.

**4.3 Examination of Phytochemicals**

Phytochemical screening of the extract revealed the presence of bioactive secondary metabolites like alkaloids, saponins, tannins, flavonoids, terpenoids, phenolic compounds and steroids glycosides and reducing sugars. No carbonyl groups were detected. People are aware that these substances have medicinal effects, like reducing inflammation, killing germs, protecting cells from damage and easing pain. Trace high levels of flavonoids and phenolic compounds in the plant also indicate its potential as an antioxidant.

**4.4 Analysis of Physicochemical Properties**

We also examined the physicochemical parameters that are essential for determining the quality and purity of herbal drugs:

The total ash (6.25%) and sulphated ash (6.20%) were also normal that indicated not much of inorganic impurities are present. The ethanol soluble extractive value was quite high at 84.1% indicating that there are many phytoconstituents dissolvable in ethanol. The water-soluble extractive was 9.80%, indicating that hydrophilic substances were only moderately soluble. The drug was also found to be pharma copoeially acceptable based on its crude fiber content (5.76%), loss on drying (6.42%) and foaming index less than 100. These numbers validate the use of the plant in traditional medicine and indicate that it has been used as a remedy for an extended period.

**4.5 Antimicrobial Activity**

Ethanolic extract ability has been tested to kill variety of microbes i.e. gram positive, gram negative and fungal infections.

**4.6 Strains that are Gram-positive**

Staphylococcus aureus and Streptococcus mutans were found to be highly sensitive towards the extract where the diameter of inhibition zones reached up to 22.03 mm at a concentration of 15 mg/mL. The results indicate that the extract generally possesses the antibacterial activity even to a lesser extent compared with usual Amoxicillin (24.99 mm).

**4.7 Strains that are gram-negative**

Escherichia coli, and Klebsiellapneumoniae were also sensitive as indicated by the inhibitory zone of up to 19.02 mm reveals that the drug has antimicrobial action against numerous groups of bacteria.

**4.8 Strains of fungi**

Candida albicans and Aspergillus niger were inhibited at both test doses, whereas with the control Candida albicans results possessed a zone of 21.04 mm (at 15 mg/mL) similar to that of ketoconazole (23.27 mm). These findings indicate that Aeglemarmelos may be an antibacterial agent, particularly when its ethanolic leaf extract is applied. Its anti-bacterial and anti-fungal activity is a good reason why it is used in traditional medicine to cure conditions of the skin, teeth, stomach or lungs.

## 4. CONCLUSION

The present study presents the pharmacognostic, phytochemical, physico-chemical as well as antibacterial study of Aeglemarmelos (L.) Correa which is a highly valued plant in the Indian traditional system of medicine. The plant material was collected from reliable botanical source through authenticating lady veternerian, ensuring the taxanomic identity and scientific validation. The macroscopic characteristics of leaves confirmed characteristic features, such as a trifoliate arrangement and reticulate veins, which are consistent with A. marmelos identification. The leaves were dried, pulverised and defatted with methanol to retain the bioactive principles for further exploration according to the method described in a literature. Preliminary phytochemical screening of the plant indicated that it contained useful secondary metabolites such as alkaloids, tannin, flavonoid, terpenoid, phenolic compound and glycoside. All of these are known to

contribute to the plant's medicinal properties. The phytochemical profile was further enriched as it had no carbonyl and reducing sugars. The physicochemical analysis also revealed that the percentage of total ash, moisture content and extractive values were within the acceptable ranges. The relatively high (84.1 ± 1.54%) extractive content indicates the existence of a good number of active constituents in the sample. The low foaming index and median crude fiber observed also indicated that the plant was of high quality, in which it can be applied for medicinal purposes. The antimicrobial investigations further validate the medicinal value of A. marmelos by showing that its ethanolic leaf extract displayed broad spectrum activity almost against all types of pathogenic bacteria such as Staphylococcus aureus, Streptococcus mutans, Escherichia coli and Klebsiellapneumoniae. The extract was also effective against Candida albicans and Aspergillus niger, but not as powerful as drugs such as ketoconazole.

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