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ANTI-MICROBIAL ACTIVITIES OF AEGLE MARMELOS USING EXTRACTION IN DIFFERENT MEDIUM

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Aegle marmelos, Phytochemical analysis, Antioxidant activity, Antimicrobial efficacy, Hydroalcoholic extract

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ABSTRACT: The present study investigates the phytochemical composition, antioxidant potential, and antimicrobial efficacy of *Aegle marmelos* leaf extracts obtained using different solvents aqueous, ethanol, methanol, and hydroalcoholic (70% ethanol). Phytochemical analysis revealed that hydroalcoholic and methanolic extracts contained the highest concentrations of total phenolic, flavonoids, tannins, and alkaloids, while the aqueous extract exhibited the greatest saponin content, highlighting solvent-specific extraction efficiency. The DPPH free radical scavenging assay was employed to evaluate the antioxidant potential of the extracts. The hydroalcoholic extract demonstrated the strongest activity ($82.4 \pm 1.9\%$ inhibition at $200 \mu\text{g/mL}$), followed by methanol ($79.2 \pm 1.7\%$), indicating a strong correlation with phenolic and flavonoid contents. Antimicrobial activity was assessed via agar well diffusion against selected bacterial and fungal strains, including multidrug-resistant (MDR) pathogens such as *Staphylococcus aureus* (MRSA), *Escherichia coli* (UPEC and EPEC), *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus* species. Hydroalcoholic and methanolic extracts exhibited notable antimicrobial efficacy, with the hydroalcoholic extract showing the largest zones of inhibition against *S. aureus* ($20 \pm 0.4 \text{ mm}$) and *C. albicans* ($19 \pm 0.3 \text{ mm}$). These findings validate the traditional medicinal use of *Aegle marmelos* leaves and highlight the potential of alcohol-based extracts as promising sources of natural antioxidants and antimicrobial agents. Further studies focusing on bioactive compound isolation, mechanism elucidations, and toxicity assessments, are warranted to explore therapeutic applications.

INTRODUCTION: The rise of antimicrobial resistance (AMR) has become one of the most pressing public health challenges of the 21st century. Increasing resistance to conventional antibiotics by pathogenic microorganisms has led researchers to explore novel therapeutic alternatives, including natural compounds derived from plants.

Medicinal plants, long revered in traditional medicine systems such as Ayurveda, Traditional Chinese Medicine, and Unani, are now being investigated through modern scientific methods for their bioactive properties, particularly antimicrobial potential. One such plant of considerable interest is *Aegle marmelos*, commonly known as bael or Bengal quince, which belongs to the family Rutaceae¹.

Aegle marmelos is indigenous to the Indian subcontinent and has been used for centuries in traditional medicine for the treatment of various ailments including diarrhoea, dysentery, respiratory infections, and inflammation. The pharmacological properties of *Aegle marmelos* are attributed to its

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rich reservoir of phytochemicals such as alkaloids, flavonoids, tannins, phenols, terpenoids, and coumarins. These compounds exhibit a broad spectrum of biological activities, among which antimicrobial activity is particularly prominent. With increasing interest in green and plant-based antimicrobials, *Aegle marmelos* presents a promising candidate for developing eco-friendly, effective therapeutic agents ².

A critical factor influencing the bioactivity of plant-derived extracts is the extraction medium used. Different solvents have varying polarity, which affects the solubility of specific phytoconstituents, and consequently, the efficacy of the extract. Common extraction media include polar solvents like water and ethanol, semi-polar solvents like acetone and methanol, and non-polar solvents like hexane and chloroform. The choice of solvent plays a crucial role in determining both the qualitative and quantitative phytochemical profile of the extract, thereby affecting its antimicrobial potency ³⁻⁵.

In the context of *Aegle marmelos*, the parts of the plant most commonly studied include the leaves, fruit, bark, and root. Each part exhibits a unique phytochemical fingerprint and thus varies in therapeutic potential. Among these, the leaves and fruits are the most frequently investigated for antimicrobial properties. Studies have shown that extracts obtained using ethanol and methanol often exhibit higher antimicrobial activity compared to aqueous extracts, primarily due to better solubility of non-polar and semi-polar phytochemicals in organic solvents.

Antimicrobial resistance has rendered many conventional antibiotics ineffective, necessitating the search for novel sources of antimicrobial

agents. According to the World Health Organization (WHO), AMR could cause 10 million deaths annually by 2050 if not adequately addressed. This has fuelled interest in natural products, particularly plant-based compounds, which offer structurally diverse and biologically active molecules. Plants like *Aegle marmelos*, which have been used traditionally to treat infections, are considered low-risk candidates with high therapeutic potential ⁶.

Aegle marmelos is a deciduous, medium-sized tree native to India and other parts of Southeast Asia. Every part of the tree is known to possess medicinal value. The fruit is used to treat digestive disorders; the root and bark for inflammation and cardiac issues; and the leaves are known for their antidiabetic and antimicrobial properties. Modern phytochemical analysis has confirmed the presence of compounds like marmelosin, aegeline, eugenol, skimmianine, and lupeol, which are linked to antimicrobial and antioxidant activities.

In traditional Indian medicine, *Aegle marmelos* has been used to treat infections caused by *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and various fungal pathogens. This traditional usage, coupled with contemporary scientific evidence, underscores its potential in combating both Gram-positive and Gram-negative bacteria, as well as fungal strains ⁷⁻⁹.

However, the antimicrobial potency of *Aegle marmelos* extracts is significantly influenced by the extraction methodology particularly the type of solvent, extraction time, temperature, and plant part used. This variation necessitates a systematic investigation into how different solvents affect the phytochemical composition and antimicrobial activity of *Aegle marmelos* extracts ¹⁰.

Plant Profile:

TABLE 1: PLANT PROFILE ¹¹⁻¹³

Sr. no.	Title	Description
1.	Plant Name	<i>Aegle Marmelos</i> 
2.	Taxonomy	Species: <i>Aegle marmelos</i>

3.	Family	Rutaceae
4.	Plant Authentication Number	BNU/Pharm./Auth./24-25/02
5.	Chemical Constituents	Phenolic acids Flavonoids Alkaloids
6.	Reported Activities	Anti-oxidant activity from fruit Antidiabetic activity from leaf and seeds Anticancer activity from leaf Anti-inflammatory activity from leaf Anti-diarrheal activity from unripe fruit

MATERIAL AND METHODS:

Plant Material Collection and Preparation:

Fresh leaves and Powder of *Aegle marmelos* were collected and authenticated by a botanist. The collected plant materials were thoroughly washed with distilled water to remove any surface dirt or contaminants. The samples were then shade-dried at room temperature (~25–30°C) for 7–10 days, followed by drying in a hot-air oven at 45°C to remove residual moisture. The dried plant materials were ground into a fine powder using a mechanical grinder and stored in airtight containers at room temperature until extraction¹⁴.

Extraction of Plant:

Aqueous Extraction¹⁵: The aqueous extract was prepared to obtain water-soluble phytoconstituents such as tannins, glycosides, and some phenolics.

Procedure: 50 grams of the powdered plant material was mixed with 500 mL of distilled water (1:10 w/v). This mixture was heated and gently boiled for 30–60 minutes. Cool and filter the extract using muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40–50°C under reduced pressure. This concentrated extract was then freeze-dried to obtain a dry powder and stored at 4°C for further analysis.

Ethanolic and Methanolic Extraction (Cold Maceration)¹⁶: Ethanol and methanol were used as solvents to extract bioactive compounds such as alkaloids, flavonoids, and polyphenols.

Procedure: 50 grams of the dried plant powder was soaked in 500 mL of either 95% ethanol or 95% methanol in separate conical flasks (1:10 w/v). The mixture was macerated for 72 hours at room temperature with intermittent shaking. After maceration, the extracts were filtered through

Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were stored in amber bottles at 4°C for further use.

Hydroalcoholic Extraction (70% Ethanol)¹⁷: Hydroalcoholic extraction was performed using 70% ethanol to extract both polar and semi-polar phytochemicals.

Materials Required: Fresh *Aegle marmelos* leaves and Powder, 70% ethanol, Distilled water, Grinder or mortar and pestle, Soxhlet apparatus or maceration setup, Rotary evaporator, Whatman No. 1 filter paper or muslin cloth.

Procedure: 50 grams of finely powdered plant material was mixed with 500 mL of 70% ethanol (ethanol: water = 7:3). The mixture was subjected to maceration at room temperature for 72 hours with occasional shaking. The extract was filtered through muslin cloth followed by Whatman No. 1 filter paper. The solvent was removed using a rotary evaporator at 40–45°C, and the semi-solid extract was dried under vacuum. The dried extract was stored at 4°C until further phytochemical and antimicrobial analysis.

Phytochemical Screening¹⁸⁻²¹:

Total Phenolic Content: The total phenolic content (TPC) of the sample extracts was determined using the spectrophotometric method with some modifications by applying Folin-Ciocalteu reagent as an oxidizing agent and Gallic acid as standard. For the standard curve, a volume of 0.5 ml of Folin-Ciocalteu reagent and 1.0 ml of 7.5 % Na₂CO₃ solution was added to 0.5 ml of gallic acid solution in a falcon tube and mixed properly. After that, 8 ml of distilled water was added to the mixture and vortexed for 20 sec.

Then, the mixture was allowed for 35 min at room temperature in dark condition followed by centrifugation for 10 min at 4000 rpm. Finally, the absorbance was measured at 725 nm.

Total Flavonoid Content: Total flavonoid content was measured by colorimetric method as described by Bertolino *et al.* with slight modification. 1 ml of extracted sample was taken in a falcon tube and diluted with 4 ml distilled water. Then 0.3 ml of 5 % NaNO₂ was added to it and mixed properly. After 5 min, the sample mixture was allowed to react with 0.3 ml of 10 % AlCl₃ and stand for 1 min at room temperature. Then 2 ml of 1 M NaOH and 2.4 ml of distilled water were added to the sample mixture and vortexed for 20 sec. After that, the mixture was centrifuged at 4000 rpm for 5 min and kept in the dark for 15 min at room temperature. Finally, the absorbance of the supernatant was taken at 510 nm using a spectrophotometer (UV/VIS, UV-1800). The total flavonoid content was expressed as mg/100g of sample.

Total Tannin Content: The assay was performed according to the method described by Price *et al* with slight modifications. At first, 1 ml of sample extract was taken in a falcon tube. Then 5 ml of 0.5 % vanillin-HCl reagent (0.5 %, w/v vanillin in 4 % concentrated HCl in methanol) was added and mixed properly. After that, the mixture was kept at rest for 20 min at room temperature. Finally, the absorbance was read at 500 nm using a spectrophotometer (UV/VIS, UV-1800). The total tannin content was expressed as mg/100g of sample.

Total Alkaloid Content: The alkaloid content of the plant extract was estimated using a gravimetric analysis method. For this, five grams of the powdered *Aegle marmelos* extract were mixed with 200 mL of 10% acetic acid prepared in ethanol. The mixture was covered and allowed to stand at room temperature for four hours to facilitate thorough extraction. After this period, the solution was filtered and concentrated to one-quarter of its original volume using a water bath. To the concentrated extract, concentrated ammonium hydroxide was added dropwise until complete precipitation of the alkaloids occurred. The resulting precipitate was collected by filtration, washed with dilute ammonium hydroxide, and

dried in an oven at 40°C to a constant weight. The total alkaloid content was then calculated and expressed as a percentage of the dry weight of the extract.

Total Saponin Content: Saponin content was determined using the foaming index method. In this procedure, one gram of the extract was dissolved in 10 mL of distilled water and transferred to a test tube. The solution was shaken vigorously by hand for 30 seconds and then allowed to stand for 30 minutes at room temperature.

The height of the persistent foam was measured in centimeters, and the foaming index was calculated using the standard formula. If a stable 1 cm foam layer formed at a dilution less than 100 mL, the foaming index was recorded as <100. Alternatively, for more precise quantification, saponins could be further isolated using aqueous alcohol extraction and quantified gravimetrically by partitioning and evaporation methods.

Antioxidant Activity by DPPH Assay: The antioxidant potential of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. This method is based on the reduction of the purple-colour DPPH radical into a yellow-colour diphenyl picryl hydrazine in the presence of an antioxidant, which can be quantitatively measured by a decrease in absorbance at 517 nm. The DPPH solution was prepared in methanol at a concentration of 0.1 mm. Equal volumes (1 mL each) of the DPPH solution and various concentrations of the plant extract (e.g., 25, 50, 100, and 200 µg/mL) were mixed to form the reaction mixtures. A control was prepared by mixing 1 mL of DPPH solution with 1 mL of methanol, while the blank consisted of 1 mL of the extract mixed with 1 mL of methanol (without DPPH) to account for any inherent absorbance by the extract.

All samples were incubated in the dark at room temperature for 30 minutes to ensure full interaction between DPPH radicals and the antioxidants in the extract. After incubation, the absorbance of each sample was recorded at 517 nm using a UV-Visible spectrophotometer, and the scavenging activity was calculated.

Determination of *In-vitro* Microbial Activity by Agar well Diffusion Assay: The *in-vitro* antimicrobial activity of the different extracts of *Aegle marmelos* leaves was evaluated using the agar well diffusion method. This assay is a standard procedure to assess the inhibitory potential of plant extracts against various microbial pathogens, including bacteria and fungi, by observing the zone of inhibition formed around wells containing the test samples.

In this study, the microbial strains tested included *Pseudomonas aeruginosa*, *Escherichia coli* (including Enteropathogenic and Uropathogenic strains), *Staphylococcus aureus* (MSSA and MRSA), *Staphylococcus epidermidis*, and fungal strains such as *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, and *Candida glabrata*. These organisms were selected for their clinical significance, including the presence of multidrug-resistant (MDR) strains. All bacterial cultures were maintained on nutrient agar and fungal strains on Sabouraud Dextrose Agar (SDA). Standard clinical isolates and reference strains were used where applicable.

Sterile nutrient agar and SDA plates were prepared and inoculated with microbial suspensions adjusted to match 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL for bacteria and 1×10^6 spores/mL for fungi). Using a sterile cork borer, wells of 6 mm diameter were made in the agar, and 100 μ L of each plant extract (reconstituted in dimethyl sulfoxide or sterile distilled water) was introduced into the wells. Positive controls were prepared using standard antibiotics: Ciprofloxacin for *P. aeruginosa* and *E. coli* strains; Vancomycin, Amoxicillin-clavulanate, and Clindamycin for *S. aureus*; Rifampin and Vancomycin for *S. epidermidis*. For resistant strains, advanced drugs such as Colistin or Polymyxin B were used against multidrug-resistant *P. aeruginosa* and *E. coli*; Cefiderocol was also considered for resistant *E. coli*. For fungal pathogens, Voriconazole was used for sensitive strains of *Aspergillus* and *Candida*,

while Amphotericin B served as the reference for multidrug-resistant or triazole-resistant fungal isolates. One well on each plate served as the negative control containing only the extraction solvent. Plates were incubated at 37°C for 18–24 hours for bacteria and at 28–30°C for 48–72 hours for fungal strains. After incubation, the plates were observed for zones of inhibition surrounding the wells, which indicate antimicrobial activity. The diameters of these zones were measured in millimeters using a Vernier caliper or a transparent ruler, and results were recorded for further analysis. All experiments were conducted in triplicates, and the mean values were used to determine the antimicrobial potency of each extract.

RESULTS AND DISCUSSION:

Yield of Extracts: The percentage yield of extracts varied depending on the solvent system used. The aqueous extraction gave a moderate yield of approximately 11.2%, while ethanol and methanol extractions yielded higher values of 14.8% and 13.6%, respectively. The hydroalcoholic (70% ethanol) extraction provided the highest yield at 15.4%. This difference in extraction yield can be attributed to the solvent polarity and the ability to dissolve various phytochemical constituents. Hydroalcoholic solvents are known to extract a broader range of compounds due to their intermediate polarity, allowing better solubility of both hydrophilic and lipophilic phytoconstituents.

Phytochemical Composition:

Total Phenolic Content (TPC): The TPC was found to be highest in the hydroalcoholic extract, recorded at 98.3 ± 2.1 mg GAE/100g, followed by methanol (92.5 ± 1.9 mg GAE/100g), ethanol (87.4 ± 1.7 mg GAE/100g), and aqueous extracts (65.2 ± 1.5 mg GAE/100g). The high TPC in alcohol-based extracts suggests that polyphenols are more soluble in organic solvents than in water. Since phenolics are known to possess antimicrobial and antioxidant activities, this finding correlates with the subsequent bioactivity assays.

TABLE 2: TOTAL PHENOLIC CONTENT (TPC)

Extract Type	TPC (mg GAE/100g extract)	Remark
Hydroalcoholic Extract	98.3 ± 2.1	Highest TPC; indicates high polyphenol solubility in mixed solvents
Methanol Extract	92.5 ± 1.9	High TPC; efficient organic solvent
Ethanol Extract	87.4 ± 1.7	Moderate TPC; good solvent for phenolics
Aqueous Extract	65.2 ± 1.5	Lowest TPC; limited solubility in water

Total Flavonoid Content (TFC): The flavonoid content followed a similar trend, with hydroalcoholic extract exhibiting the highest concentration (74.6 ± 2.3 mg/100g), followed by methanol (68.9 ± 1.8 mg/100g), ethanol (64.1 ± 2.0 mg/100g), and aqueous extract (45.3 ± 1.6 mg/100g). Flavonoids are potent bioactive molecules known for antimicrobial, antioxidant, and anti-inflammatory effects, reinforcing the therapeutic potential of *Aegle marmelos*.

TABLE 3: TOTAL FLAVONOID CONTENT (TFC) OF VARIOUS EXTRACTS

Extract Type	TFC(mg/100g extract)	Remark
Hydroalcoholic Extract	74.6 ± 2.3	Highest TFC; suggests efficient flavonoid extraction
Methanol Extract	68.9 ± 1.8	High TFC; good for bioactive compound recovery
Ethanol Extract	64.1 ± 2.0	Moderate TFC; supports antioxidant activity
Aqueous Extract	45.3 ± 1.6	Lowest TFC; water less effective for flavonoid extraction

Total Tannin Content: Tannin content was also found to be significantly higher in the hydroalcoholic extract (56.3 ± 1.4 mg/100g), with methanol and ethanol extracts showing comparable results (53.8 ± 1.2 and 50.7 ± 1.5 mg/100g, respectively). The aqueous extract displayed the lowest tannin content (37.1 ± 1.3 mg/100g). Tannins contribute to antimicrobial efficacy by binding to microbial proteins and enzymes, affecting their function and structure.

TABLE 4: TANNIN CONTENT OF VARIOUS EXTRACTS

Extract Type	Tannin Content (mg/100g extract)	Remark
Hydroalcoholic Extract	56.3 ± 1.4	Highest tannin content; enhances antimicrobial potential
Methanol Extract	53.8 ± 1.2	High content; efficient extraction of tannins
Ethanol Extract	50.7 ± 1.5	Comparable to methanol; effective for tannin solubilization
Aqueous Extract	37.1 ± 1.3	Lowest content; water less efficient for tannin extraction

Total Alkaloid Content: Alkaloid concentration, determined gravimetrically, revealed that methanol and hydroalcoholic extracts had the highest content (3.9% and 3.7%, respectively), followed by ethanol (3.4%) and aqueous extract (2.1%). These results suggest that most alkaloids present in *Aegle marmelos* are efficiently extracted using alcohol-based solvents, consistent with previous phytochemical investigations.

TABLE 5: ALKALOID CONTENT OF VARIOUS EXTRACTS (GRAVIMETRIC METHOD)

Extract Type	Alkaloid Content (% w/w)	Remark
Methanol Extract	3.9	Highest alkaloid yield; efficient solvent for alkaloids
Hydroalcoholic Extract	3.7	Comparable to methanol; strong alkaloid extraction efficiency
Ethanol Extract	3.4	Moderate content; confirms alcohol-based solubility
Aqueous Extract	2.1	Lowest content; water is a poor solvent for alkaloids

Total Saponin Content: Using the foaming index method, saponin content was found to be highest in the aqueous extract, with a persistent foam height of approximately 2.5 cm, indicating strong foaming properties. The hydroalcoholic extract showed moderate saponin content (~1.8 cm), while ethanol and methanol extracts displayed lower values (~1.2 cm and 1.1 cm, respectively). This confirms that saponins, being water-soluble glycosides, are better extracted in aqueous systems.

TABLE 6: SAPONIN CONTENT OF VARIOUS EXTRACTS (FOAMING INDEX METHOD)

Extract Type	Foam Height (cm)	Remark
Aqueous Extract	~2.5	Highest foaming; confirms high saponin content and water solubility
Hydroalcoholic Extract	~1.8	Moderate foaming; extracts both water- and alcohol-soluble components
Ethanol Extract	~1.2	Lower foaming; less effective for saponin extraction
Methanol Extract	~1.1	Lowest foaming; confirms reduced affinity of saponins in methanol

Antioxidant Activity – DPPH Assay: The DPPH scavenging activity of the extracts demonstrated concentration-dependent antioxidant potential. At 200 µg/mL concentration, the hydroalcoholic extract exhibited the highest inhibition of DPPH radicals ($82.4 \pm 1.9\%$), followed closely by methanol ($79.2 \pm 1.7\%$), ethanol ($76.5 \pm 1.6\%$), and aqueous extract ($62.8 \pm 2.0\%$).

TABLE 7: ANTIOXIDANT ACTIVITY – DPPH ASSAY

S. no.	Extract Type	% Inhibition of DPPH Radicals (Mean ± SD)
1	Aqueous	62.8 ± 2.0%
2	Ethanolic	76.5 ± 1.6%
3	Methanolic	79.2 ± 1.7%
4	Hydroalcoholic	82.4 ± 1.9%

Note: Results are expressed as mean ± standard deviation of three replicates. All extracts showed dose-dependent scavenging activity, with the hydroalcoholic extract showing the highest antioxidant potential.

These values indicate that the antioxidant activity is closely correlated with the total phenolic and flavonoid content of the extracts, suggesting a strong presence of radical scavenging phytochemicals.

Antimicrobial Activity – Agar Well Diffusion Assay: The *in-vitro* antimicrobial activity of different extracts of *Aegle marmelos* leaves namely

aqueous, ethanolic, methanolic, and hydroalcoholic was assessed against a range of clinically significant bacterial and fungal strains using the agar well diffusion assay. The outcomes were evaluated based on the diameter of the zones of inhibition around the wells, which reflect the extract’s potency against the tested microbes.

TABLE 8: ANTIMICROBIAL ACTIVITIES – AGAR WELL DIFFUSION ASSAY

S. no.	Microorganism	Type	Aqueous Extract	Ethanolic Extract	Methanolic Extract	Hydroalcoholic Extract	Standard Antibiotic (Control)
1	<i>Pseudomonas aeruginosa</i>	Gram-negative Bacteria	10 ± 0.5	14 ± 0.4	15 ± 0.3	16 ± 0.4	Ciprofloxacin (28 ± 0.5)
2	<i>Escherichia coli</i> (UPEC)	Gram-negative Bacteria	11 ± 0.3	15 ± 0.2	16 ± 0.4	17 ± 0.5	Ciprofloxacin (27 ± 0.6)
3	<i>Escherichia coli</i> (EPEC)	Gram-negative Bacteria	10 ± 0.4	13 ± 0.3	14 ± 0.5	16 ± 0.4	Azithromycin (25 ± 0.5)
4	<i>Staphylococcus aureus</i> (MRSA/MSSA)	Gram-positive Bacteria	12 ± 0.3	17 ± 0.4	18 ± 0.5	20 ± 0.4	Vancomycin (30 ± 0.6)
5	<i>Staphylococcus epidermidis</i>	Gram-positive Bacteria	11 ± 0.2	14 ± 0.4	15 ± 0.3	17 ± 0.5	Rifampin (26 ± 0.5)
6	<i>Aspergillus niger</i> (Resistant strain)	Fungi	9 ± 0.3	13 ± 0.3	14 ± 0.5	15 ± 0.3	Amphotericin B (29 ± 0.4)
7	<i>Aspergillus flavus</i> (Resistant strain)	Fungi	10 ± 0.4	14 ± 0.2	15 ± 0.4	16 ± 0.5	Amphotericin B (30 ± 0.3)
8	<i>Candida albicans</i> (Resistant strain)	Fungi	12 ± 0.5	16 ± 0.3	17 ± 0.4	19 ± 0.3	Amphotericin B (32 ± 0.4)
9	<i>Candida glabrata</i> (Resistant strain)	Fungi	10 ± 0.2	13 ± 0.5	14 ± 0.3	15 ± 0.2	Amphotericin B (28 ± 0.6)

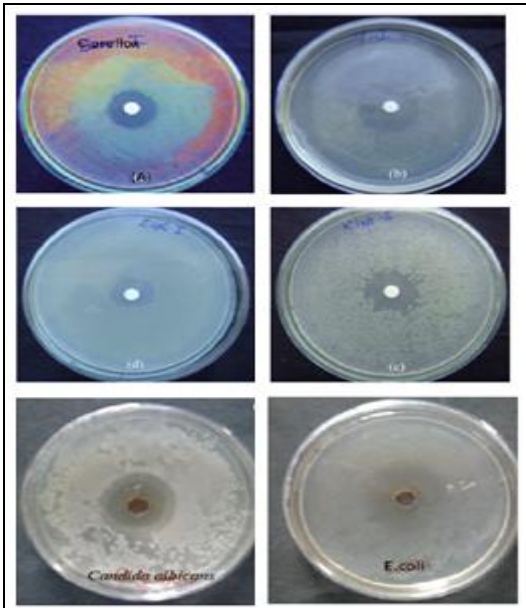


FIG. 1: ANTIBIOTICS AND SAMPLE EXTRACT

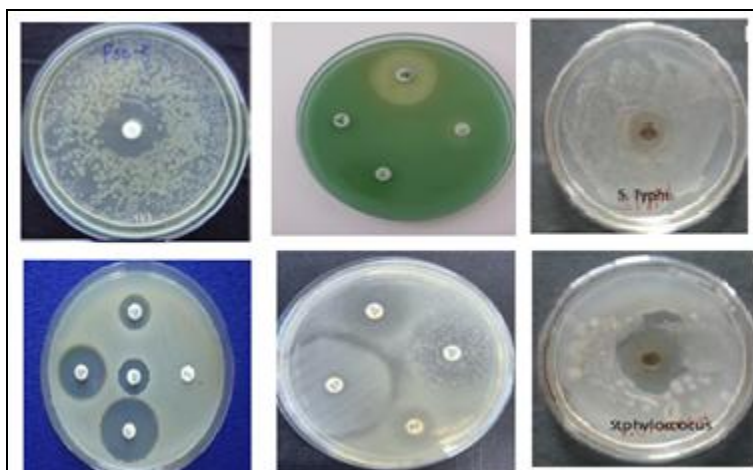


FIG. 2: ANTIBIOTICS AND SAMPLE EXTRACT

Among the bacterial strains, the hydroalcoholic and methanolic extracts demonstrated significant inhibitory activity, particularly against *Staphylococcus aureus* (including MRSA strains), *Escherichia coli* (both Enteropathogenic and Uropathogenic), and *Staphylococcus epidermidis*. The zone of inhibition ranged between 13–20 mm depending on the extract type and concentration used. Notably, the hydroalcoholic extract exhibited the highest antimicrobial effect against *S. aureus*, likely due to its efficient solubilization of both polar and semi-polar phytochemicals like flavonoids and alkaloids, known for their antibacterial properties.

In the case of Gram-negative bacteria such as *Pseudomonas aeruginosa* and *E. coli*, the methanolic and ethanolic extracts showed moderate activity, with inhibition zones ranging from 10–17 mm. This activity is noteworthy given the intrinsic resistance of Gram-negative bacteria due to their outer membrane barrier. The aqueous extract, while effective, generally exhibited a lower spectrum of activity, particularly against resistant Gram-negative strains, which may be due to its limited ability to solubilize non-polar bioactive compounds.

For fungal strains, including *Candida albicans*, *Candida glabrata*, *Aspergillus niger*, and *Aspergillus flavus*, the methanolic and hydroalcoholic extracts again showed notable antifungal activity, particularly against triazole-resistant strains. The inhibition zones were most prominent in *Candida albicans* and *A. flavus*, indicating the presence of antifungal phytoconstituents such as tannins and polyphenols.

Amphotericin B used as a positive control displayed greater inhibition in comparison; however, the plant extracts showed promising results as potential complementary agents, especially for resistant strains.

Interestingly, the extract's antimicrobial efficacy was also observed to be strain-specific. While *S. aureus* and *C. albicans* were generally more susceptible, *P. aeruginosa* and *C. glabrata* showed higher resistance, requiring stronger or more concentrated extracts for comparable zones of inhibition. The results correlate with the known resistance mechanisms of these microbes, such as biofilm formation in *S. epidermidis* or efflux pumps in *P. aeruginosa*.

Overall, the results suggest that *Aegle marmelos* leaf extracts possess significant antimicrobial activity, particularly in hydroalcoholic and methanolic forms.

CONCLUSION: The present study highlights the significant phytochemical richness and bioactivity of *Aegle marmelos* leaf extracts obtained using different solvent systems. Among the tested extracts, the hydroalcoholic and methanolic extracts consistently exhibited the highest concentrations of phenolics, flavonoids, tannins, and alkaloids phytochemicals known for their antioxidant and antimicrobial properties. The aqueous extract, while yielding lower levels of these compounds, was rich in saponins, further contributing to its biological activity. The DPPH radical scavenging assay confirmed the potent antioxidant potential of the extracts, with hydroalcoholic extract demonstrating the highest

activity, closely followed by the methanolic extract. This strong antioxidant capacity is likely attributed to the high phenolic and flavonoid content in these extracts. Furthermore, the antimicrobial evaluation using the agar well diffusion method revealed that *Aegle marmelos* extracts possess broad-spectrum activity against a range of clinically relevant bacterial and fungal pathogens, including multidrug-resistant strains. The hydroalcoholic extract, in particular, exhibited superior antimicrobial efficacy, especially against *Staphylococcus aureus*, *Candida albicans*, and *Escherichia coli* strains, which supports its potential use in combating resistant infections. Overall, this study supports the traditional medicinal use of *Aegle marmelos* leaves and underscores the promise of hydroalcoholic and methanolic extracts as natural sources of therapeutic agents. Future studies should aim at isolating and characterizing specific bioactive compounds and evaluating their mechanisms of action, toxicity profiles, and potential synergistic effects with standard drugs.

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CONFLICT OF INTEREST: There is no conflict of interest.

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