PHYTOCHEMICAL CHARACTERIZATION, HPTLC FINGERPRINT PROFILING, AND ANTIMICROBIAL EVALUATION OF AEGLE MARMELOS SEED EXTRACTS

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Abstract

Medicinal plants represent an important source of naturally occurring bioactive compounds with potential therapeutic applications. Aegle marmelos (L.) Correa, commonly known as Bael, has a long history of use in traditional medicine. The present study aimed to investigate the phytochemical constituents of Aegle marmelos seed extracts, establish their High-Performance Thin-Layer Chromatography (HPTLC) fingerprint profiles, and evaluate their antimicrobial activity. The seeds were collected, authenticated, dried, and powdered before extraction using selected solvents, including methanol, ethanol, and water. Preliminary phytochemical screening was performed to identify the major classes of secondary metabolites present in the extracts. HPTLC analysis was conducted using an appropriate chromatographic system to obtain characteristic fingerprint profiles based on the observed spots and their corresponding retention factor (Rf) values. The antimicrobial activity of the extracts was evaluated using the selected experimental method. The combined findings of phytochemical screening, chromatographic profiling, and antimicrobial testing were intended to provide information on the chemical composition and biological potential of the seed extracts. HPTLC fingerprinting may also serve as a useful analytical approach for the identification, comparison, and quality assessment of the investigated extracts. The study provides a basis for further investigation of Aegle marmelos seeds as a potential source of biologically active constituents.

Keywords: *Aegle marmelos, Phytochemical screening, HPTLC fingerprinting, Antimicrobial activity, Seed extract, Disc diffusion method, Bioactive compounds.*

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1. INTRODUCTION

Medicinal plants are important sources of naturally occurring compounds with potential applications in pharmaceutical and biomedical research. Their biological properties are associated with diverse phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, coumarins, and other secondary metabolites. Systematic phytochemical investigation helps characterize these constituents and provides a scientific basis for examining the biological properties of medicinal plants. Padghan et al. (2026) reviewed the phytochemical composition and pharmacological properties of *Aegle marmelos*, highlighting its importance as a medicinal species. Similarly, Jindal (2026) discussed contemporary approaches to herbal drug standardization, emphasizing the role of analytical techniques and biological evaluation in improving the quality and consistency of herbal materials.

Aegle marmelos (L.) Corrêa, commonly known as Bael, belongs to the family Rutaceae and has a long history of use in traditional medicine. Different parts of the plant, including the leaves, fruits, roots, bark, and seeds, have been investigated for their chemical constituents and biological properties. However, the phytochemical composition of an extract may vary according to the plant part, geographical origin, extraction procedure, and solvent employed. Therefore, findings obtained from one plant part cannot automatically be extrapolated to another. Mishra et al. (2010) isolated antifungal constituents from *A. marmelos* seeds, providing direct evidence of the chemical diversity of this plant part and supporting further investigation of its potential biological activities.

Solvent extraction is an important step in phytochemical research because the recovery of plant constituents depends on their solubility and the physicochemical properties of the extraction medium. Solvents such as methanol, ethanol, and water may extract different proportions of phytochemicals because of differences in polarity. Preliminary phytochemical screening provides an initial indication of the major classes of secondary metabolites present in individual extracts. Comparing extracts prepared with different solvents can therefore help characterize their phytochemical composition and guide subsequent chromatographic and biological investigations. Nevertheless, preliminary screening provides presumptive evidence of phytochemical classes and does not independently establish the identity or concentration of individual compounds.

High-Performance Thin-Layer Chromatography (HPTLC) is a useful analytical technique for separating and comparing the chemical constituents of complex plant extracts.

Chromatographic fingerprinting provides characteristic profiles based on the number, distribution, and appearance of separated bands under specified analytical conditions. The retention factor ((Rf)) values and band characteristics can be used to compare extracts and assess their chromatographic similarities and differences. Shukla and Yadav (2026) discussed modern techniques for herbal standardization, including HPTLC, HPLC, spectroscopic methods, and chromatographic fingerprinting. The World Health Organization (2011) also described quality-control approaches for evaluating medicinal plant materials. Accordingly, HPTLC fingerprinting can contribute to the comparative assessment and quality standardization of herbal extracts. However, the identification of individual compounds requires suitable reference standards or additional analytical evidence because matching (Rf) values alone does not establish chemical identity.

The evaluation of antimicrobial activity is another important component of medicinal plant research. Plant-derived compounds have attracted scientific interest because certain phytochemicals may inhibit the growth of selected microorganisms under appropriate experimental conditions. Kumar et al. (2026), in a review published in the *Global Journal of Pharmaceutical and Scientific Research* (GJPSR), discussed plant-derived therapeutics and their potential relevance to antimicrobial-resistance research. Mujeeb et al. (2014) also investigated the phytochemical composition and antimicrobial activity of *A. marmelos* leaves. Although these studies provide useful background for investigating plant-derived antimicrobial candidates, their findings do not establish the antimicrobial activity of seed extracts. Consequently, the biological activity of *A. marmelos* seeds must be evaluated experimentally using defined microbial strains, appropriate controls, and a suitable antimicrobial assay.

Herbal standardization is essential for improving the consistency, reliability, and quality assessment of plant-derived materials. The integration of phytochemical screening, chromatographic fingerprinting, and biological evaluation can provide complementary information about the chemical characteristics and potential biological properties of plant extracts. These approaches are particularly relevant when comparing extracts prepared using different solvents because chemical profiles and antimicrobial responses may differ among extraction conditions. However, chromatographic profiles and preliminary phytochemical findings should be interpreted alongside experimental antimicrobial results rather than treated as direct evidence of biological efficacy.

Considering these aspects, the present study was undertaken to investigate the phytochemical constituents of *Aegle marmelos* seed extracts prepared using selected solvents, develop their

HPTLC fingerprint profiles, and evaluate their antimicrobial activity against selected test microorganisms. The study further aimed to compare the phytochemical and chromatographic characteristics of the extracts and examine their relationship with the observed antimicrobial responses, where supported by the experimental findings. The results may contribute to the preliminary characterization of *A. marmelos* seed extracts and provide a basis for further investigation of their potentially bioactive constituents.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

Seeds of *Aegle marmelos* (L.) Corrêa, belonging to the family Rutaceae, were selected for phytochemical characterization, chromatographic fingerprinting, and antimicrobial evaluation. The seeds were collected from [insert collection location] during [insert collection period]. Botanical authentication was performed by [insert botanist or institution], and the voucher specimen number was recorded as [insert number, if available].

The collected material was inspected to remove foreign matter, visibly damaged seeds, and other unsuitable materials. Authentication and documentation of the plant material are important components of botanical quality control and reproducibility, as described by the World Health Organization (WHO, 2011).

2.2. Processing of Plant Material

The collected seeds were cleaned, sorted, and dried under the conditions adopted in the laboratory. After drying, the seeds were ground into a coarse powder using a suitable grinding apparatus. The powdered material was transferred to a clean, dry, airtight container and stored until further analysis.

The drying temperature, duration, and storage conditions should be specified according to the laboratory records because these parameters may influence the stability and composition of plant constituents.

2.3. Preparation of Seed Extracts

The powdered seeds were extracted using the solvents employed in the study, including methanol, ethanol, and water, as applicable to the experimental procedure. The powdered material was mixed with the respective solvent and processed according to the extraction method used in the laboratory. The resulting mixtures were filtered, and the filtrates were concentrated using a method appropriate for the solvent employed. The prepared extracts were collected in labelled containers and stored under suitable conditions until further analysis.

For reproducibility, the quantity of powdered material, solvent-to-material ratio, extraction technique, extraction duration, temperature, number of extraction cycles, and concentration procedure should be reported from the original experimental records.

Where the extraction yield was determined, it was calculated using the following equation:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered plant material}} \times 100$$

2.4. Preliminary Phytochemical Screening

Qualitative phytochemical screening was performed to investigate the major classes of phytoconstituents present in the seed extracts. The screening included tests for alkaloids, flavonoids, tannins, saponins, glycosides, cardiac glycosides, terpenoids, steroids, carbohydrates, proteins, and fixed oils, where the corresponding procedures were performed.

The results were assessed based on characteristic colour changes, precipitate formation, or persistent foam formation, depending on the test. Qualitative phytochemical screening is commonly used as an initial approach to characterizing medicinal plant extracts, including extracts of *Aegle marmelos* (Mujeeb et al., 2014). The observations were interpreted as preliminary indications of the presence or absence of particular phytochemical groups rather than definitive identification of individual compounds.

2.4.1. Tests for Alkaloids

Mayer's test: Approximately 0.5 g of extract was treated with 5 mL of 1% hydrochloric acid and heated on a water bath. The mixture was filtered, and a few drops of Mayer's reagent were added to 1 mL of the filtrate. The formation of a white or cream-coloured precipitate was considered a positive reaction for alkaloids.

Wagner's test: The acidified extract was prepared and filtered using the procedure described above. A few drops of Wagner's reagent were added to 1 mL of the filtrate. The formation of a reddish-brown or dark precipitate was recorded as a positive reaction.

Hager's test: The acidified extract was filtered, and a few drops of Hager's reagent were added to 1 mL of the filtrate. The development of a yellow precipitate was interpreted as a positive reaction for alkaloids.

2.4.2. Test for Flavonoids

Shinoda test: Approximately 0.5 mL of the alcoholic extract was transferred to a test tube. A small piece of magnesium or zinc was added, followed by a few drops of hydrochloric acid. The development of a pink, red, or orange-red colour was recorded as a preliminary indication of flavonoids.

2.4.3. Test for Tannins

Approximately 0.5 g of powdered material was boiled with 20 mL of distilled water and filtered. A few drops of 0.1% ferric chloride solution were added to the filtrate. The development of a blue-black or greenish colour was interpreted as a positive reaction consistent with the presence of tannins or other phenolic compounds.

2.4.4. Test for Phlobatannins

Approximately 0.1 g of extract was heated with 1% aqueous hydrochloric acid. The formation of a red precipitate was recorded as a positive indication of phlobatannins.

2.4.5. Test for Terpenoids

Approximately 0.1 g of extract was dissolved in 1 mL of chloroform. Concentrated sulfuric acid was carefully added along the side of the test tube to form a separate layer. The development of a reddish-brown colour at the interface was recorded as a preliminary indication of terpenoids.

2.4.6. Test for Steroids

Approximately 0.1 g of extract was dissolved in 1 mL of chloroform. Acetic anhydride was added, followed by the careful addition of concentrated sulfuric acid. The development of a characteristic greenish colour was interpreted as a preliminary indication of steroidal constituents.

2.4.7. Test for Saponins

Approximately 0.5 g of extract was mixed with 5 mL of distilled water and shaken vigorously. The formation of persistent foam was recorded as a positive indication of saponins.

2.4.8. Tests for Glycosides and Cardiac Glycosides

The extracts were subjected to the glycoside tests performed in the laboratory. General glycoside screening was distinguished from specific tests for cardiac glycosides. Legal's and Baljet's tests were included where performed. The reagents, concentrations, reaction conditions, and criteria used to interpret the results should be documented according to the actual laboratory procedures.

2.4.9. Tests for Carbohydrates, Proteins, and Fixed Oils

Carbohydrates, proteins, and fixed oils were examined only where the corresponding tests were performed. The specific reagents, experimental procedures, and criteria for interpreting positive reactions should be reported according to the laboratory protocol.

2.5. Thin-Layer Chromatography (TLC)

Thin-layer chromatography was performed for the preliminary chromatographic examination of the seed extract. Silica gel-G plates with a reported layer thickness of 0.25 mm were used.

The plates were dried at room temperature and activated in an oven at 100°C for 2 hours, according to the supplied experimental procedure.

The hydroalcoholic seed extract was applied to the plate using a capillary tube, with the application position marked approximately 1 cm from the lower edge. The reported mobile phase consisted of toluene, ethyl acetate, formic acid, and methanol in a ratio of 3:3:0.3:0.2 (v/v/v/v).

The plate was developed in a chromatographic chamber containing the mobile phase. After development, the solvent front was marked, and the plate was dried. The separated bands were examined under the detection conditions used in the experiment.

The observed (Rf) values were used to describe the preliminary chromatographic profile of the extract. These values were treated as comparative parameters rather than definitive evidence of the identity of individual compounds.

2.6. High-Performance Thin-Layer Chromatography (HPTLC) Fingerprinting

HPTLC fingerprinting was performed to establish the chromatographic profile of the *Aegle marmelos* seed extract. This technique allows separated chromatographic zones to be examined and compared under defined analytical conditions. Chromatographic profiling is useful in the identification and quality assessment of herbal materials when supported by appropriate method documentation and reference standards (WHO, 2011).

The seed extract was prepared and applied to an HPTLC plate using the procedure and equipment employed in the laboratory. The plate was developed using the mobile phase selected for the analysis. After development, the plate was dried and examined under the detection conditions used in the study. The chromatographic bands were recorded, and their corresponding (Rf) values and densitometric parameters were obtained from the available analytical output.

For adequate reproducibility, the plate type, sample concentration, application volume, band dimensions, mobile-phase composition, chamber saturation time, development distance, detection wavelengths, derivatization reagent, if applicable, and scanning conditions should be reported from the original records. Reference standards should also be specified if they were used.

The resulting chromatographic fingerprint was interpreted as a comparative profile of the extract. Individual peaks were not assigned to specific chemical compounds without supporting reference-standard or analytical evidence.

2.7. Evaluation of Antimicrobial Activity

The antimicrobial activity of the seed extracts was evaluated against the selected test microorganisms using the assay procedure adopted in the laboratory. The microorganisms included *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, as indicated in the experimental results.

The assay findings were recorded as zones of inhibition in millimetres under the reported test conditions. Where agar well diffusion or disc diffusion was used, the diameter of the inhibition zone was measured and recorded for each extract and test organism. The results were compared across the different solvent extracts and reported test conditions.

For reproducibility, the exact assay method, culture medium, microbial strain identifiers, inoculum preparation, extract concentrations, incubation temperature and duration, and positive and negative controls should be specified according to the experimental records. The meaning of the reported 1:1, 1:2, and 1:3 conditions should also be clarified. If the minimum inhibitory concentration (MIC) was determined, the dilution method and endpoint criteria should be described.

Plant-extract studies have previously investigated the relationship between phytochemical composition and antimicrobial activity in *Aegle marmelos*, although findings from leaf extracts cannot be directly assumed to apply to seed extracts (Mujeeb et al., 2014). Therefore, the antimicrobial results in the present study were interpreted in relation to the extracts and microorganisms actually investigated.

2.8. Data Recording and Analysis

The qualitative phytochemical observations, TLC retention factors, HPTLC parameters, and antimicrobial inhibition-zone measurements were recorded according to the available experimental results. The findings were summarized using descriptive tables and chromatographic or graphical presentations where appropriate.

Statistical analysis should be reported only if replicate measurements and the corresponding statistical calculations were performed. Where replicate data are available, the number of replicates, summary statistics, statistical tests, and significance threshold should be specified. No statistical significance should be claimed solely from differences between individual reported inhibition-zone measurements.

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out to identify the major classes of phytoconstituents present in the seed extract of *Aegle marmelos* Linn. The analysis involved

qualitative chemical tests based on characteristic colour changes, precipitate formation, and other observable reactions.

Positive reactions were reported for alkaloids, glycosides, cardiac glycosides, terpenoids, phlobatannins, flavonoids, tannins, and saponins. The steroid test produced a negative result under the conditions employed. These findings suggest that the investigated seed extract contains several classes of secondary metabolites that warrant further chemical characterization.

Table 3.1. Qualitative phytochemical screening of *Aegle marmelos* seed extract

Phytochemical group	Test performed	Result
Alkaloids	Mayer's test	Positive
Alkaloids	Wagner's test	Positive
Alkaloids	Hager's test	Positive
Glycosides	General glycoside test	Positive
Cardiac glycosides	Legal's test	Positive
Cardiac glycosides	Baljet's test	Positive
Terpenoids	Salkowski test	Positive
Phlobatannins	Phlobatannin test	Positive
Flavonoids	Flavonoid test	Positive
Tannins	Tannin test	Positive
Saponins	Foam test	Positive
Steroids	Steroid test	Negative

3.2 Thin-Layer Chromatography (TLC) Analysis

Thin-layer chromatography was performed to examine the chromatographic profile of the hydroalcoholic seed extract of *Aegle marmelos* Linn. The reported mobile phase consisted of toluene, ethyl acetate, formic acid, and methanol in the ratio of 3:3:0.3:0.2, respectively.

Three chromatographic spots were reported, with retention factor ((Rf)) values of 0.03, 0.28, and 0.40. The differences in their migration indicate variations in the chromatographic behaviour of the detected components under the selected conditions. These observations provide a preliminary chromatographic profile for the extract and form a basis for comparison with the subsequent HPTLC findings.

Table 3.2. TLC profile of the hydroalcoholic seed extract of *Aegle marmelos* Linn.

Plant material	Extract analyzed	Mobile-phase composition	Reported (Rf) values
Aegle marmelos Linn. seeds	Hydroalcoholic extract	Toluene acetate acid (3:3:0.3:0.2)	0.03, 0.28, 0.40

3.3 High-Performance Thin-Layer Chromatography (HPTLC) Fingerprinting

HPTLC fingerprinting was used to characterize the chromatographic profile of the seed extract in greater detail. Five peaks were reported, each with corresponding retention factor values, peak heights, integrated areas, and percentage values. Differences in these parameters demonstrate the distribution of detector responses across the chromatogram.

Peak 3 exhibited the largest reported integrated area of 244,924.5, followed by Peak 4 with an area of 138,440.5. Peak 2 had an area of 11,202.2, whereas Peaks 1 and 5 showed areas of 380.9 and 724.3, respectively. The reported maximum heights were 715.2 for Peak 2 and 710.9 for Peak 3, representing the two largest maximum-height values in the supplied dataset.

Table 3.3. HPTLC peak characteristics of *Aegle marmelos* seed extract

Peak	Start (Rf)	Start height	Maximum (Rf)	Maximum height	Max (%)	End (Rf)	End height	Area	Reported area (%)
1	0.05	2.2	0.04	51.1	2.39	0.02	1.7	380.9	0.74
2	0.01	3.0	0.03	715.2	33.40	0.09	14.9	11,202.2	21.82
3	0.19	15.3	0.29	710.9	33.20	0.33	20.4	244,924.5	48.55
4	0.35	22.3	0.42	612.44	28.60	0.46	20.9	138,440.5	26.96
5	0.46	20.9	0.49	28.5	1.33	0.52	6.8	724.3	1.41

Note: Values are reproduced from the supplied HPTLC table. The reported area percentages should be checked against the original instrument report before publication.

The HPTLC profile showed that Peaks 3 and 4 accounted for the largest reported integrated peak areas. Peak 3 had the greatest area, while Peak 4 had the second-largest area. By comparison, Peaks 1 and 5 had substantially smaller areas. These differences indicate that the detector response was not distributed uniformly across the five reported peaks.

Peak area, maximum peak height, and their respective percentages describe different aspects of the chromatographic signal and should not be treated as interchangeable measurements. Moreover, detector response is influenced by the analytical conditions and the properties of the detected compounds. Therefore, the relative peak area cannot automatically be interpreted as the mass percentage of an individual phytochemical.

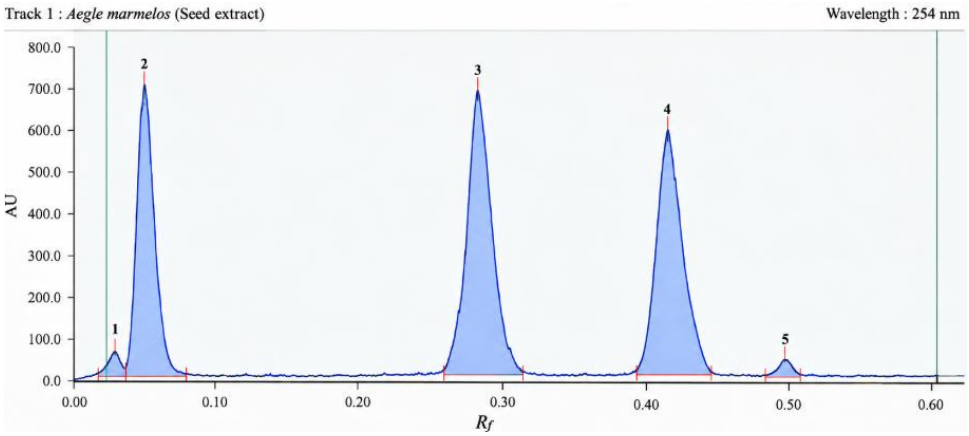


Figure 3.1. HPTLC densitometric fingerprint of *Aegle marmelos* seed extract.

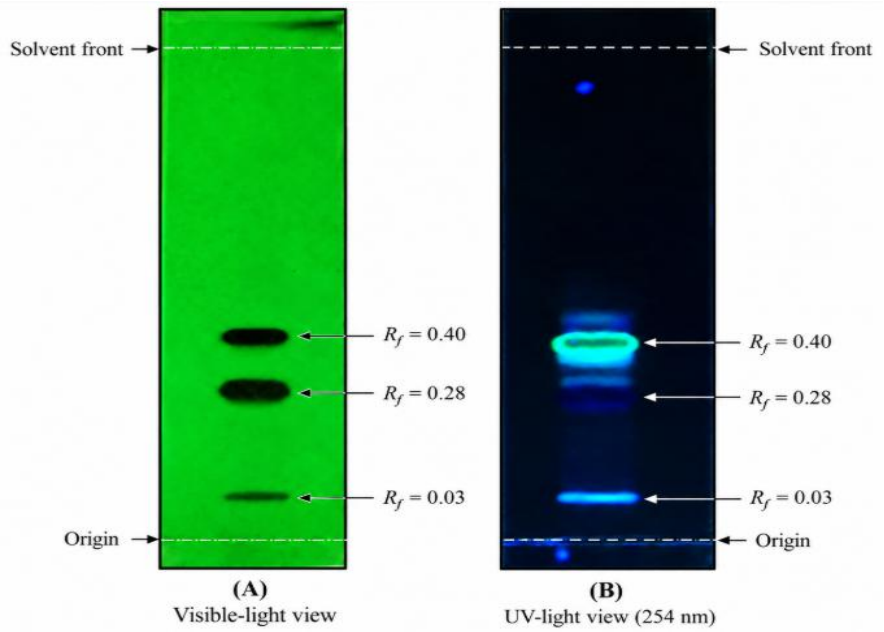


Figure 3.2. TLC chromatographic profile of the hydroalcoholic seed extract of Aegle marmelos under (A) visible light and (B) UV light (254 nm)

3.4 Evaluation of Antibacterial Activity

The antibacterial activity of aqueous, methanol, chloroform, and petroleum ether seed extracts was assessed against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The results were expressed as zones of inhibition in millimetres for the reported 1:1, 1:2, and 1:3 test conditions.

The measured inhibition zones varied among the extracts and bacterial species. The complete set of reported values is presented in Table 3.4.

Table 3.4. Antibacterial activity of seed extracts of *Aegle marmelos*

Test organism	Aqueous 1:1	Aqueous 1:2	Aqueous 1:3	Methanol 1:1	Methanol 1:2	Methanol 1:3	Chloroform 1:1	Chloroform 1:2	Chloroform 1:3	Petroleum ether 1:1	Petroleum ether 1:2	Petroleum ether 1:3
E. coli	18	12	9	22	16	14	16	14	8	27	24	18
S. aureus	18	13	11	28	22	20	18	13	10	27	22	17
S. typhi	16	14	10	25	16	15	16	15	13	23	19	15
P. aeruginosa	11	-	-	-	-							

3.4.1 Antibacterial Activity Against *Escherichia coli*

At the reported 1:1 condition, the petroleum ether extract produced the largest inhibition zone against *E. coli* (27 mm). The methanol extract produced a zone of 22 mm, followed by the aqueous extract at 18 mm and the chloroform extract at 16 mm.

At the 1:2 and 1:3 conditions, petroleum ether also showed the largest reported inhibition zones among the four extracts, with values of 24 and 18 mm, respectively. The recorded inhibition zones decreased across the successive conditions for all extracts. If these labels represent progressively lower extract concentrations, this pattern may indicate a concentration-related reduction in the observed antibacterial response.

3.4.2 Antibacterial Activity Against *Staphylococcus aureus*

Against *S. aureus*, the methanol extract showed the largest inhibition zone at the reported 1:1 condition, measuring 28 mm. The petroleum ether extract produced a zone of 27 mm, while the aqueous and chloroform extracts each produced zones of 18 mm.

At the 1:2 condition, the methanol extract produced an inhibition zone of 22 mm, equal to that of the petroleum ether extract. At the 1:3 condition, the methanol extract showed the largest reported zone at 20 mm, followed by petroleum ether at 17 mm, aqueous extract at 11 mm, and chloroform extract at 10 mm.

These results indicate that the methanol extract produced the largest recorded response against this organism at the 1:1 and 1:3 conditions, while the methanol and petroleum ether extracts showed equal values at 1:2.

3.4.3 Antibacterial Activity Against *Salmonella typhi*

For *S. typhi*, the methanol extract produced the largest inhibition zone at the reported 1:1 condition (25 mm), followed by petroleum ether (23 mm). The aqueous and chloroform extracts each produced zones of 16 mm.

At the 1:2 condition, petroleum ether produced a zone of 19 mm, compared with 16 mm for methanol, 15 mm for chloroform, and 14 mm for the aqueous extract. At the 1:3 condition, the chloroform extract produced a zone of 13 mm, while the methanol and petroleum ether extracts each produced zones of 15 mm and the aqueous extract produced a zone of 10 mm.

The comparative pattern varied across the test conditions, indicating that the relative performance of the extracts was not identical at every condition.

3.4.4 Antibacterial Activity Against *Pseudomonas aeruginosa*

Against *P. aeruginosa*, the methanol extract produced the largest reported inhibition zone at the 1:1 condition (25 mm). Petroleum ether produced a zone of 18 mm, followed by chloroform at 15 mm and aqueous extract at 11 mm.

At the 1:2 condition, the methanol extract again produced the largest zone, measuring 22 mm. The petroleum ether, aqueous, and chloroform extracts produced zones of 15, 9, and 10 mm, respectively. At the 1:3 condition, the methanol and petroleum ether extracts produced reported inhibition zones of 18 and 13 mm. Results were not supplied for the aqueous and chloroform extracts at this condition.

The available data indicate that the methanol extract produced the largest reported inhibition zones against *P. aeruginosa* at the first two test conditions. However, the missing observations prevent a complete comparison across all extracts at 1:3.

3.4.5 Comparative Analysis of Antibacterial Activity

Comparison of the results at the reported 1:1 condition showed that the extract producing the largest inhibition zone differed according to the test organism. Petroleum ether produced the largest recorded zone against *E. coli* (27 mm), whereas methanol produced the largest recorded zones against *S. aureus* (28 mm), *S. typhi* (25 mm), and *P. aeruginosa* (25 mm).

These findings suggest that the observed antibacterial response was associated with both the extraction solvent and the bacterial species tested. Differences in the compounds extracted by the various solvents may contribute to the observed patterns, but the present results do not establish the identity of the substances responsible for the inhibition.

The measurements should be interpreted as reported inhibition-zone observations rather than definitive evidence of antibacterial potency. The numerical results for the DMSO control, replicate measurements, and statistical variability were not supplied. Consequently, the data do not support claims of statistical significance. The precise meaning of the 1:1, 1:2, and 1:3 conditions should also be clarified in the Methods section.

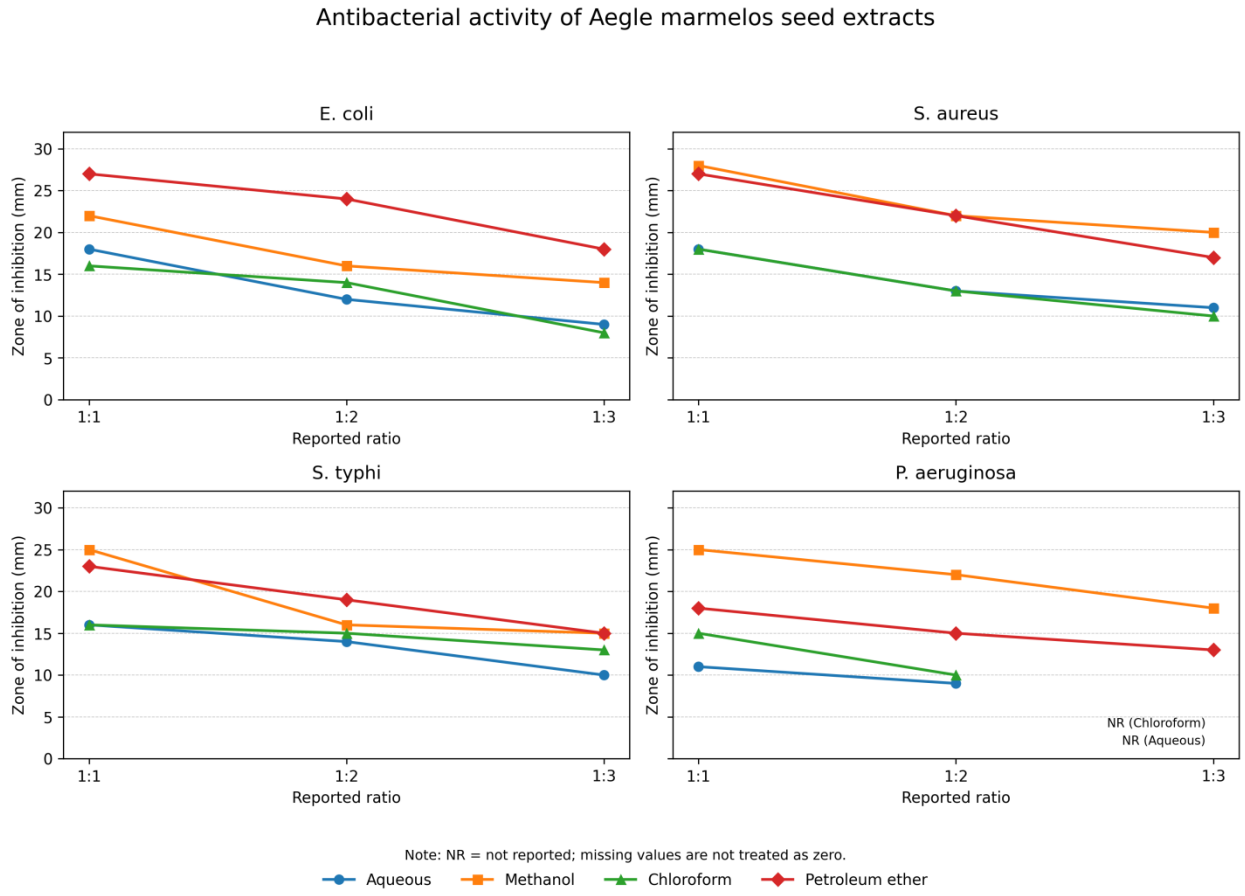


Figure 3.3: Comparative antibacterial activity of aqueous, methanol, chloroform, and petroleum ether extracts of Aegle marmelos seeds against Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa, expressed as zones of inhibition (mm) at the reported ratios

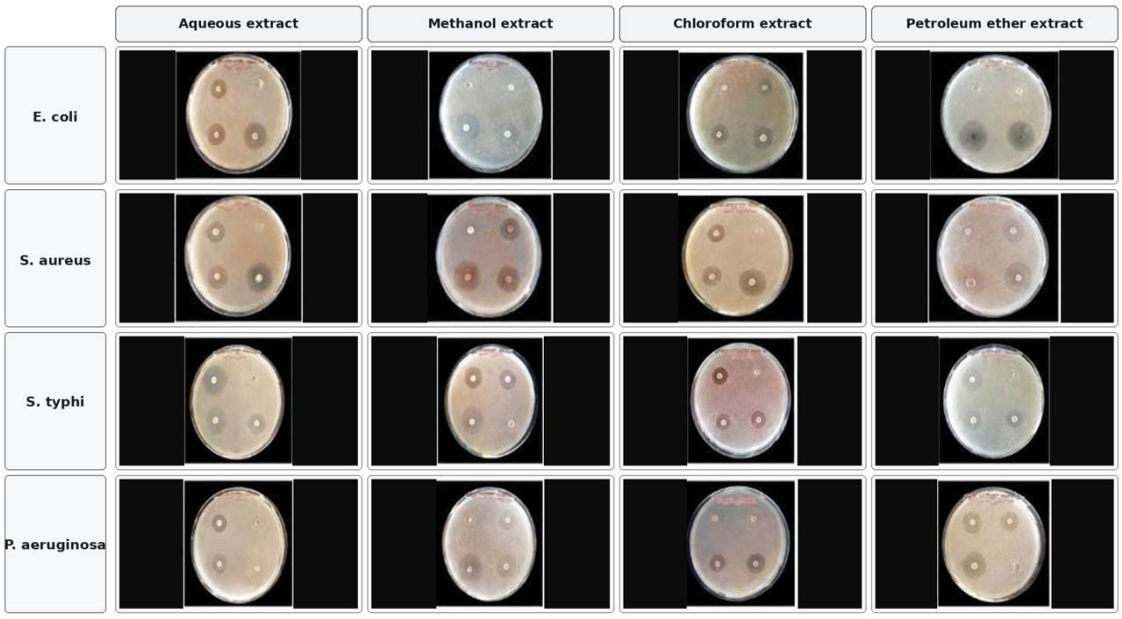


Figure 3.4: Antibacterial activity of four Aegle marmelos seed extracts against four bacterial species.

3.5 Overall Discussion

The findings provide an initial assessment of the phytochemical composition, chromatographic characteristics, and antibacterial activity of *Aegle marmelos* seed extracts. Qualitative phytochemical screening indicated positive reactions for several classes of secondary metabolites. TLC analysis provided three reported (Rf) values for the hydroalcoholic seed extract, while HPTLC analysis produced a five-peak chromatographic profile with differences in retention characteristics and integrated peak areas.

The antibacterial results demonstrated variation among the extracts and test organisms. Petroleum ether produced the largest reported inhibition zone against *E. coli*, whereas methanol produced the largest reported zones against *S. aureus*, *S. typhi*, and *P. aeruginosa* at the 1:1 condition. These observations indicate that the recorded antibacterial response was not uniform across the tested solvent extracts.

Several limitations should be considered when interpreting these findings. Qualitative phytochemical reactions do not confirm the identity of individual constituents, and the reported chromatographic peaks have not been identified using reference standards. The discrepancy between the supplied HPTLC peak areas and area percentages also requires verification. In addition, the absence of numerical DMSO control results, replicate variability, and statistical analysis limits the strength of conclusions that can be drawn from the antibacterial findings.

Further work involving validated chromatographic identification, replicated antibacterial testing, appropriate controls, and statistical analysis would improve the reliability of the findings. Overall, the present results provide a preliminary characterization of *Aegle marmelos* seed extracts and a basis for further investigation of their phytochemical composition and in-vitro antibacterial properties.

4. Conclusion

The present study demonstrated that the hydroalcoholic extract of Aegle marmelos contains a number of phytochemicals that could be the precursors for its therapeutic qualities and hence justify its use as a remedy for a wide range of illnesses. Important phyto-compounds with pharmacological characteristics should be isolated and identified to form novel medicine formulations. By comparing the Rf values of the compounds with the reference standards the HPTLC analysis carried out for the hydroalcoholic extract of Aegle marmelos chemical profiling will prove to be useful in identifying bioactive chemicals and markers.

The present study which evaluated Aegle marmelos leaves and seeds for in vitro antibacterial activity revealed that there were several plant material extracts with significant concentrations of antimicrobial chemicals. Nearly all the extracts show significant inhibition at three times the concentration indicating a low IC 50 value for the crude preparation (data not shown) allowing these purified compounds to be used as lead candidates in the process of finding new drugs. Various solvent extracts can be used to purify specific active ingredients, determine their IC50 values as well as explore them to create formulations that may be used as medicinal agents.

5. Acknowledgement

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6. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review.

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