

“COMPARATIVE PHYTOCHEMICAL PROFILING AND IN VITRO ANTIBACTERIAL ACTIVITY OF ALCOHOLIC LEAF EXTRACTS OF *MORINGA OLEIFERA* AND *AEGLE MARMELOS* AGAINST *BREVUNDIMONAS DIMUTA*”

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ABSTRACT:

Background: The increasing prevalence of foodborne bacterial contamination and growing concerns regarding antimicrobial resistance have intensified the search for natural antimicrobial agents from medicinal plants. Fresh fruits and vegetables may serve as vehicles for bacterial contamination, highlighting the need for effective and neutral approaches to microbial control. Medicinal plants contain diverse bioactive phytochemicals with potential antibacterial properties.

Aim : The present study aimed to investigate the phytochemical composition and antimicrobial activity of alcoholic leaf extracts of *Moringa oleifera* and *Aegle marmelos* against *Brevundimonas* spp. isolated from fresh fruit samples.

Methods : Alcoholic leaf extracts of *Moringa oleifera* and *Aegle marmelos* were prepared using standard extraction procedures. Qualitative phytochemical screening was performed, including alkaloids, terpenoids, and glycosides. The antibacterial activity of the extracts against *Brevundimonas* spp. was evaluated using the agar well diffusion method at 25%, 50%, 75%, 100% concentrations. Zone of inhibitions were measured in millimetres using an antimicrobial zone measuring scale. Streptomycin was used as the standard antibiotic.

Results : Phytochemical screening revealed the presence of several bioactive constituents in both plant extracts, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides. Both extracts demonstrated antibacterial activity against *Brevundimonas* spp. The *Moringa oleifera* extract produced zones of inhibition of 22, 21, 20, and 19 mm at 100%, 75%, concentrations, while both extracts showed comparable activity at 50% and 25 %.

Conclusions : The findings demonstrate that alcoholic leaf extracts of *Moringa oleifera* and *Aegle marmelos* possess promising antibacterial activity against *Brevundimonas* spp. isolated from fresh fruits. The observed activity may be associated with the presence and combined effects of bioactive phytochemical constituents. These findings suggest that both medicinal plants may represent potential sources of natural antibacterial agents for controlling bacterial contamination in fresh produce. Further studies involving minimum inhibitory concentration determination, quantitative phytochemical analysis, and characterization of individual bioactive compounds are warranted.

KEYWORDS : *Moringa oleifera*, *Aegle marmelos*; alcoholic leaf extracts; *Brevundimonas* spp ; phytochemical screening; antibacterial activity ; mango; muskmelon ; foodborne pathogens.

1. INTRODUCTION

Fresh fruits constitute an important component of a healthy diet because they provide essential vitamins, minerals, dietary fibre, antioxidants, and other biologically active compounds. However, their consumption may also present microbiological safety concerns because fresh produce is frequently handled through multiple stages from cultivation to consumption. Microbiological safety concerns because fresh produce is frequently handled through multiple stages from cultivation may occur during cultivation, harvesting, transportation, storage, processing, retail handling, or preparation. Unlike foods subjected to cooking or other effective microbial inactivation processes, many fresh fruits are consumed with minimal processing, allowing microorganisms introduced during production and handling to remain associated with the product. Consequently, maintaining the microbiological quality of infections and represents a major global health concern.

The World Health Organization has emphasized that antimicrobial resistance is driven in part by the misuse and overuse of antimicrobial agents and that resistant bacteria can enter the food chain through agricultural and environmental pathways. Fresh fruits and vegetables may also become contaminated with antimicrobial – resistant bacteria through agricultural production and subsequent handling.

In this context, medicinal plants have attracted considerable scientific interest as potential sources of naturally occurring antimicrobial compounds. Plants synthesize a wide range of secondary metabolites, including phenolic compounds, flavonoids, alkaloids, tannins, saponins, terpenoids, and glycosides, many of which have been associated with antimicrobial and antioxidant activities. The use of plant-derived extracts may therefore provide an alternative approach for investigating naturally occurring antibacterial agents, particularly against microorganisms associated with food and food processing environments. However, antimicrobial activity can vary considerably according to plant species, plant part, extraction solvent, concentration, and the susceptibility of the target microorganisms.

Moringa oleifera ., commonly called known as the drumstick tree, is an important medicinal and nutritional plant belonging to the family Moringaceae . Its leaves are particularly rich in biologically active constituents, including phenolic compounds, flavonoids, alkaloids, tannins, saponins, and other secondary metabolites. Previous investigations have demonstrated antimicrobial activity of *Moringa oleifera* leaf extracts against a range of bacterial microorganisms, suggesting that its phytochemical constituents may contribute to its inhibitory effects. Reviews of *Moringa oleifera* have documented antimicrobial activity associated with different plant parts and extraction systems, supporting continued investigation of its leaves as a potential source of natural antimicrobial compounds.

Similarly, *Aegle marmelos* commonly known as bael, is medicinal plant of the family Rutaceae that has a long history of use in traditional medicine, particularly in the Indian subcontinent. Different parts of the plant contain diverse phytochemicals, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, glycosides. Several studies have reported biological activities of *Aegle marmelos* , including antimicrobial, antioxidant, anti-inflammatory, and gastrointestinal – related effects. In particular, investigations of its leaves have demonstrated the presence of biologically active phytochemicals and antibacterial activity against several bacterial species, supporting its potential as a source of plant – derived antimicrobial compounds.

Phytochemical screening represents an important preliminary approach for evaluating medicinal plants because it provides qualitative information about the major classes of secondary metabolites present in an extract. The identification of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and glycosides can provide preliminary basis for relating the chemical composition of plant extracts to their observed biological activities.

Although food-safety investigations traditionally focus on well – established foodborne pathogens, the presence of other environmental bacteria in fresh-produce environments also deserves attention . Members of the genus *Brevundimonas* are gram negative bacteria that occur in environmental habitats and have been recovered from food – associated environments. Importantly , *Brevundimonas* has been isolated from a commercial fresh-cut-produce processing facility, where the strain was reported to exhibit strong biofilm – forming ability. This finding demonstrates that *Brevundimonas* can occur within fresh – produce processing environments and supports the need for further investigation of this bacterial group in relation to produce – associated microbiology.

The occurrence of *Brevundimonas* in food – associated environments, together with the increasing interest in plant – derived antimicrobial compounds, provide a relevant basis for evaluating is available concerning the antibacterial activity of *Moringa oleifera* and *Aegle marmelos* leaf extracts specifically against *Brevundimonas* spp. isolated from fresh fruits. Comparative evaluation of these two medicinal plants may therefore provide against a less extensively investigated food-associated bacterial group.

Therefore, the present study was undertaken to comparatively evaluate the phytochemical composition and antibacterial activity of alcoholic leaf extracts of *Moringa oleifera* and *Aegle marmelos* against *Brevundimonas* spp. isolated from fresh fruit samples. The antibacterial activity of the extracts was assessed at different method, with streptomycin used as the standard antibiotic. The study was designed to determine the inhibitory potential of the two medicinal plant extracts and to explore their possible significance as natural sources of antibacterial compounds for applications related to fresh – produce safety and antimicrobial research .

MATERIALS AND METHODS

1.1 Collection of Fruit Samples

Fresh mango (*Mangifera indica*) and Muskmelon (*Cucumis melo*) samples were collected from local retail markets in sterile polyethylene bags and transported to the Microbiology Laboratory for analysis. All samples were processed immediately after collection under aseptic conditions.

1.2 Isolation and Identification of Bacterial Isolates

Ten grams of each fruit samples was aseptically transferred into 100 mL sterile buffered peptone water and homogenized. Serial dilutions were prepared, and appropriate dilutions were inoculated onto selective and differential media. Presumptive *Escherichia coli* and *Salmonella* spp. colonies were purified and identified based on colony morphology, Gram staining, and standard biochemical tests.

1.3 Collection and authentication of Plant materials

Fresh leaves of *Moringa oleifera* and *Aegle marmelos* were collected from Gwalior, Madhya Pradesh. The plant materials were washed thoroughly with distilled water, shade-dried at room temperature, and ground into a fine powder. The botanical identity of both plant species was **authenticated by the CSIR-National Botanical Research Institute (NBRI), Lucknow, India**. After authentication, the powdered plant materials were stored in airtight containers until extraction.

1.4 Preparation of Methanolic Extract of *Moringa oleifera* & *Aegle marmelos*

Shade dried leaves of *Moringa oleifera* were ground into a fine powder. 10 gm of the powdered leaf material was extracted with **methanol** using a **Soxhlet apparatus**. The extraction was continued until the solvent in the siphon tube became colorless. The extract was filtered and concentrated under Heat mantle. The concentrated extract was dried and stored in airtight containers at 4°C until further phytochemical screening and antibacterial analysis.

1.5 Qualitative Phytochemical Screening

Qualitative phytochemical screening of the methanolic extracts was performed using standard phytochemical methods to detect the presence of major secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, glycosides. Alkaloids were identified using Dragendorff's test, Flavonoids by the alkaline reagent test, phenolics and tannins by the ferric chloride test, saponins by the froth test, terpenoids by the Salkowski test, glycosides by the Keller- Killiani test. The presence of each phytochemical based on the development of characteristic colour changes or precipitate formation according to established standard procedures.

1.6 Antibacterial Activity Assay

The antibacterial activity of the methanolic extracts of *Moringa oleifera* and *Aegle marmelos* leaves was evaluated using the agar well diffusion method. Fresh bacterial cultures of *Escherichia coli* and *Salmonella spp.* were prepared and uniformly spread onto Muller – Hinton agar (MHA) plates using sterile cotton swabs. Wells of approximately 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Plant extracts at concentrations of 25, 50, 75, 100 mg/mL were dispensed into the respective wells, while Streptomycin standard antibiotic was included as the positive control, where applicable. The plates were incubated at 37°C for 18-24 hr, after which the antibacterial activity was assessed by measuring the diameter of the clear inhibition zones (mm). All experiments were performed in duplicate, and the mean inhibition zone was recorded.

1.7 Data Analysis

The diameters of the inhibition zones were measured in millimetres (mm). All experiments were performed in duplicate, and the mean inhibition zone was calculated and presented for each extract concentration against the test microorganisms.

2. RESULTS

2.1 Qualitative Phytochemical Screening



Fig 1. Alcoholic extraction of *Moringa oleifera*

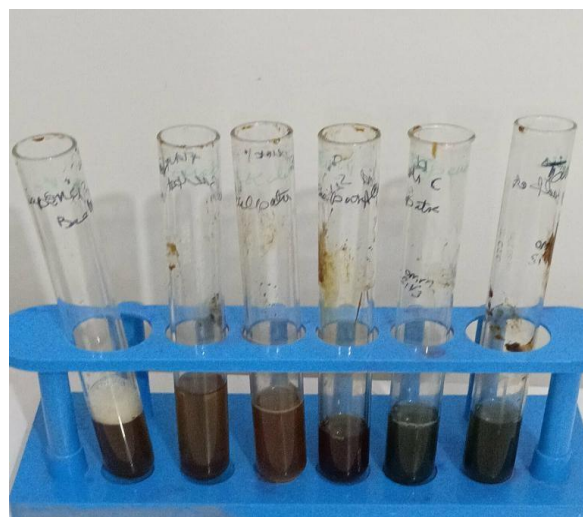


Fig 2. Alcoholic extraction of Bael patra

2.2 Antimicrobial Activity



Fig 3. Alcoholic *Moringa* extract against *Brevundimonas* spp.



Fig 4. Alcoholic *Aegle marmelos* against *Brevundimonas* spp.

The *Aegle marmelos* extract produced inhibition zones of 23 mm, 22 mm, 20 mm, 19 mm at concentrations of 100 %, 75 %, 50 %, 25 % respectively. The results demonstrated a gradual reduction in the inhibition zone with decreasing extract concentration. The 100% concentration exhibited the greatest antibacterial activity, whereas the 25% concentration produced the lowest inhibition zone. Streptomycin was used as the standard antibiotic for comparison.

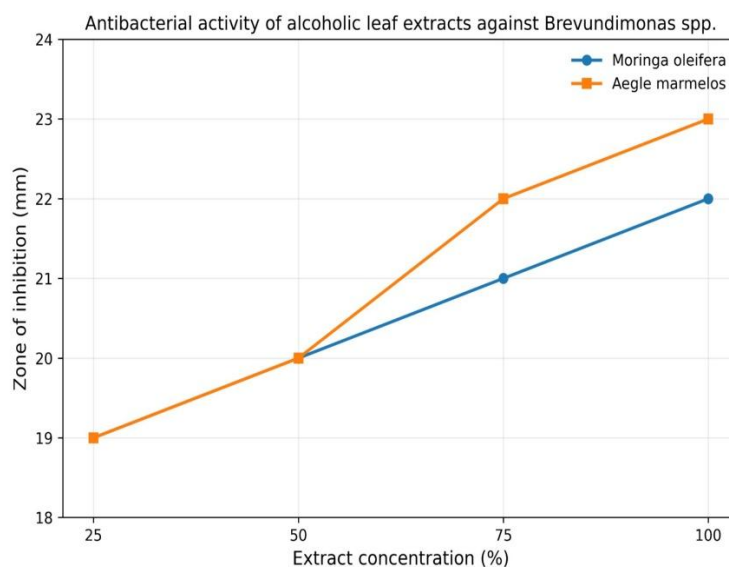


Fig 5. Antibacterial activity of Alcoholic leaf extracts against *Brevundimonas* spp.

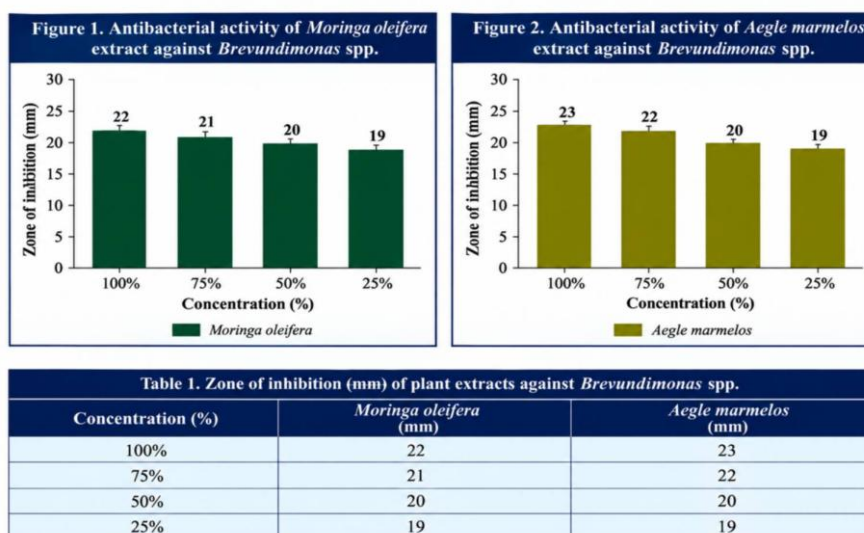


Table 1. Zone of inhibition of Antibacterial activity of *Moringa oleifera* and *Aegle marmelos*

3. DISCUSSION:

The present study demonstrated that alcoholic leaf extracts of *Moringa Oleifera* and *Aegle marmelos* exhibited measurable antibacterial activity against *Brevundimonas* spp., as evidenced by the formation of distinct zones of inhibition in the agar well diffusion assay. Both extracts showed a concentration – dependent pattern, with inhibition increasing. For *Moringa Oleifera*, the inhibition zone increased from 19 mm at 25 % concentration to 22 mm at 100 %, whereas *Aegle marmelos* produced zones ranging from 19 to 23 mm over the same concentration range. This progressive increase suggests that the concentration of bioactive constituents available in the extract may influence the extent of bacterial growth inhibition.

The slightly greater inhibition with *Aegle Marmelos* at the highest concentration indicates comparatively stronger activity under the conditions employed in the present experiment. At 100 % concentration, *Aegle marmelos* produced a zone of 23 mm for *Moringa oleifera*. However, the difference was relatively small, and because the present measurements represent zone diameters rather than replicated mean values, the apparent difference should not be interpreted involving biological replicates and statistical analysis would be required to establish whether the two extracts differ significantly in antibacterial potency.

The antibacterial activity observed for *Moringa oleifera* is consistent with previous investigations demonstrating that its leaves contain diverse secondary metabolites, including phenolics compounds, flavonoids, tannins, alkaloids, Terpenoids, and other bioactive constituents. These compounds have been associated with antimicrobial effects against both Gram – positive and Gram – negative bacteria. Previous studies have also demonstrated that the antimicrobial activity of *Moringa oleifera* depends considerably on extraction solvent and extraction procedure because these factors influence the quantity and composition of recovered phytochemicals.

The activity of *Aegle marmelos* observed in the present investigation is likewise supported by earlier phytochemical and antimicrobial studies. Kothari et al. reported broad – spectrum antimicrobial activity of *Aegle marmelos* leaf extracts and identified phenols, tannins, flavonoids, coumarins, saponins and triterpenoids among

the phytochemical constituents. Other investigations have reported antibacterial activity of *Aegle marmelos* leaf extracts against several Gram – negative and Gram – positive bacteria and have suggested that phenolic, flavonoid, terpenoid and related compounds may contribute to this activity.

The concentration – dependent response observed in the present study may therefore reflect an increased availability of antibacterial phytochemicals at higher extract concentrations. Plant – derived phenolics and flavonoids can interfere with bacterial cells through several mechanisms, including alteration of membrane integrity, interaction with cellular proteins and enzymes, and disruption of essential metabolic processes. Experimental studies with *Moringa oleifera* extracts have also demonstrated effects on bacterial membrane permeability and biofilm – associated processes, supporting the possibility that multiple mechanisms, may contribute to the observed inhibition.

An important observation from the present study is that both plant extracts produced relatively substantial inhibition zones even at the lower concentrations tested. Nevertheless, agar diffusion results are influenced not only by antibacterial potency but also by factors such as extract concentration, diffusion characteristics, molecular size of active compounds, solvent composition, agar properties and bacterial inoculum. Consequently, inhibition-zone diameter alone cannot establish minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), or the precise antibacterial mechanism. Previous work on both *Moringa oleifera* and *Aegle marmelos* has demonstrated that antimicrobial activity can vary substantially according to extraction solvent and bacterial species.

The present findings are particularly relevant because *Brevundimonas* spp. were investigated in association with fresh produce. However, the antibacterial activity demonstrated in vitro should not be directly equated with practical food- preservation efficacy. The effectiveness of plant extracts on fresh produce may be affected by surface attachment, organic matter, plant tissue characteristics, extract stability and the physiochemical properties of the food matrix. Therefore, further work should evaluate the extracts under food-model or actual fresh – produce conditions before any food – safety application is proposed.

Overall, the findings indicate that both *Moringa oleifera* and *Aegle marmelos* possess promising in vitro antibacterial activity against the tested *Brevundimonas* isolate, with *Aegle marmelos* showing a slightly higher inhibition zone at the highest concentration tested. The results support further investigations of these medicinal plants as potential sources of plant derived antimicrobial compounds. Future studies should include replicate experiments with statistical analysis, determination of MIC and MBC values, quantitative phenolic and flavonoid estimation, chromatographic identification of active compounds, and evaluation of the extracts against multiple *Brevundimonas* isolates and other relevant food associated bacteria.

4. CONCLUSION :

The present study demonstrated that alcoholic leaf extracts of *Moringa oleifera* and *Aegle marmelos* appreciable **in vitro antibacterial activity against *Brevundimonas* spp.** evaluated by the agar well diffusion method. Both extracts exhibited inhibition at all tested concentrations, with the antibacterial effect generally increasing with increasing extract concentration. *Moringa oleifera* produced inhibition zones of 19 – 22mm, while *Aegle marmelos* produced inhibition zones of 19 – 23 mm at concentrations ranging from 25 % to 100 %.

Among the two plant extracts, *Aegle marmelos* exhibited slightly higher concentrations, whereas comparable activity was observed antibacterial activity may be associated with the presence of biologically active phytochemicals such as phenolics, flavonoids, tannins, alkaloids, saponins and terpenoids compounds present in the leaves of these plants. The findings therefore support the potential of *Moringa oleifera* and *Aegle marmelos* as sources of plant – derived antibacterial compounds against *Brevundimonas* spp.

However, the present findings are based on agar diffusion measurements and should not be interpreted as evidence of clinical efficacy or direct food – preservation effectiveness. Further investigations involving MIC, MBC, replicated experiments with statistical analysis, characterization of active phytochemicals, biofilm assays, and validation on fresh – produce matrices are required to establish the antibacterial potential and practical applicability of these extracts. Overall. The study provides a useful preliminary basis for further investigation of *Moringa oleifera* and *Aegle marmelos* as natural sources of antibacterial agents against *Brevundimonas* spp.

5. Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this study.

6. Limitations of the study :

The present study has certain limitations that should be considered when interpreting the findings. First, the antibacterial activity was evaluated using the **agar well diffusion method**, which provides inhibition – zone measurements but does not directly determine the minimum inhibitory concentration (MIC) or minimum bactericidal concentrations (MBC) of the extracts. Therefore, the observed inhibition zones should be interpreted as preliminary evidence of antibacterial activity rather than a direct measure of bactericidal potency.

Second, the study evaluated the activity of the extracts against the tested ***Brevundimonas* spp. isolate**, and the findings may not necessarily represent the response of other strains or species of *Brevundimonas*. Testing multiple independent isolates would provide a stronger assessment of the consistency and spectrum of antibacterial activity. Third, the present measurements were based on inhibition – zone diameters, and further studies involving biological replicates and appropriate statistical analysis are required to determine the reproducibility and

statistical significance of differences between *Moringa oleifera* and *Aegle marmelos* extracts and among different concentrations.

Another limitation is that although the antibacterial activity may be associated with the phytochemicals present in the plant extracts, the individual compounds responsible for the observed activity were not identified or quantified in the present investigation. Advanced analytical approaches such as HPLC, LC-MS or GC- MS could be employed in future studies to characterize the active constituents.

Finally, the antibacterial activity was evaluated in vitro and under controlled laboratory conditions. The effectiveness, stability and practical applicability of these extracts on fresh produce were not assessed. Further investigations using food – matrix models, biofilm assays, MIC/MBC determination and safety / toxicity evaluation are therefore necessary before considering these extracts for practical food – safety considering these extracts for practical food – safety or antimicrobial applications.

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