Comparative Phytochemical Characterization and Total Phenolic Content of Aqueous Extracts of Azadirachta indica and Murraya koenigii with Isolation and Identification of Foodborne Microorganisms from Fresh Produce

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Abstract

Background: Medicinal plants are an important source of bioactive phytochemicals with potential applications in food safety and antimicrobial therapy. Fresh fruits and vegetables are frequently contaminated with foodborne microorganisms, highlighting the need for natural antimicrobial agents. Comparative evaluation of phytochemical composition and antimicrobial potential of medicinal plants may provide valuable insights for food preservation and public health.

Aim: The present study aimed to comparatively evaluate the phytochemical constituents and total phenolic content of aqueous extracts of Azadirachta indica (Neem) and Murraya koenigii (Curry leaves), and to investigate their antibacterial activity against foodborne bacterial isolates recovered from fresh produce.

Methods: Aqueous extracts of neem and curry leaves were prepared using 10 g dried plant powder in 100 mL of distilled water. Qualitative phytochemical screening was performed to detect major secondary metabolites, while total phenolic content (TPC) was determined using the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g extract. Fresh watermelon and tomato samples were microbiologically analyzed by total plate count and selective culture techniques. Bacterial isolates were identified using cultural characteristics, Gram staining, IMViC, Triple Sugar Iron (TSI), catalase, and oxidase tests. Antibacterial activity was evaluated by the agar well diffusion method on Mueller-Hinton agar using extract concentrations of 100%, 75%, 50%, 25%, with streptomycin as the positive control.

Results: Both plants extracts contained alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, and glycosides. Neem extract exhibited a substantially higher total phenolic content (81.8 mg GAE/g extract) than curry leaf extract (46.2 mg GAE/g extract). The total bacterial counts were 49.5 × 104 CFU/g in watermelon and 48.6 × 104 CFU/g in tomato samples. Escherichia coli was isolated from watermelon, whereas Salmonella spp. and fungal isolates were recovered from tomato samples. Both extracts demonstrated concentration-dependent antibacterial activity against E. coli, with neem extract exhibiting comparatively greater inhibitory activity, particularly at lower concentrations.

Conclusions: The findings demonstrate that aqueous extracts of Azadirachta indica and Murraya koenigii are rich in bioactive phytochemicals and possess promising antibacterial activity against foodborne bacteria. The higher phenolic content and superior antibacterial efficacy of neem extract indicate its potential as a natural antimicrobial agent for food safety applications. These results provide a foundation for future investigations involving quantitative phytochemical analysis, antioxidant efficacy against a broader range of foodborne pathogens.

Keywords: Azadirchta indica, Murraya koenigii, phytochemicals; total phenolic content; foodborne pathogens; Escherichia coli; Salmonella spp; antibacterial activity; food safetY.

1. Introduction

Wireless communication systems are fundamentally constrained by the impairments of multipath propagation, Doppler shifts, and time dispersion, which introduce severe fading and inter-symbol interference. Accurate channel estimation is critical to mitigate these effects and enable reliable signal detection. However, existing estimation techniques face significant trade-offs between computational complexity, pilot overhead, and robustness in dynamic environments.

Traditional pilot-based methods, such as Least Squares (LS) and Minimum Mean Square Error (MMSE), offer simplicity but suffer from excessive training overhead, reducing spectral efficiency. Compressive Sensing (CS)-based approaches exploit channel sparsity to reduce pilot requirements, yet they often produce coarse estimates that lack refinement in noisy conditions. Meanwhile, deep learning (DL)-based solutions, Especially Convolutional Neural Networks (CNNs), have demonstrated promise in learning local channel features but

struggle to capture long-range temporal dependencies due to their limited receptive fields.
Recent advances in Vision Transformers (ViTs) present a compelling alternative, as their self-attention mechanisms model global relationships across the entire input sequence. Unlike CNNs, ViTs excel at identifying both sparse and non-sparse channel components while maintaining robustness against far-reaching signal correlations—a critical requirement in large delay-spread environments (e.g., massive MIMO and high-mobility scenarios). However, standalone ViT architectures may lead to increased computational costs when processing raw channel observations without prior sparsity exploitation.
Fresh fruits and vegetables are essential components of a healthy diet because they provide vitamins, minerals, dietary fiber, and numerous bioactive compounds that contribute to human health. The increasing consumption of fresh produce has also raised concerns regarding microbial contamination, as these foods are often consumed raw or with minimal processing. During cultivation, harvesting, fruits and vegetables may become contaminated with pathogenic microorganisms, posing a significant public health risk. Foodborne pathogens such as Escherichia coli and Salmonella species are among the most common bacterial contaminants associated with fresh produce and are responsible for outbreaks of foodborne illnesses worldwide. (5, 16)
Conventional methods for controlling microbial contamination in food largely depend on chemical preservatives and synthetic antimicrobial agents. Although effective, their prolonged use has raised concerns regarding antimicrobial resistance, environmental impact, and consumer health. Consequently, there is growing interest in identifying naturally occurring bioactive compounds from medicinal plants that may serve as alternative sources of antimicrobial attention because they are biodegradable, relatively safe, and contain a diverse range of secondary metabolites with biological activities.
Among medicinal plants, Azadirachta indica (Neem) has been extensively recognized for its therapeutic importance in traditional medicine. Various parts of plant, particularly the leaves, contain biologically active constituents including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and glycosides. These phytochemicals are associated with antioxidant, antimicrobial, anti-inflammatory, and other beneficial biological activities. The abundance of phenolic compounds in neem leaves has made the plant an important subject of research in natural product chemistry and food preservation. (2,6)
Murraya koenigii (L.) spreng., commonly known as curry leaf, is another medicinal plant widely used as a culinary herb and in traditional medicine. Curry leaves contain several biologically active compounds such as carbazole alkaloids, flavonoids, phenolic compounds, tannins, and essential nutrients. These phytochemicals have been reported to exhibit antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties (1,16). Because of these characteristics, curry leaves have attracted increasing scientific interest as a potential natural source of functional ingredients for food and pharmaceutical applications.
Phytochemical screening provides preliminary information regarding the presence of biologically active secondary metabolites in medicinal plants. Qualitative identification of alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, and glycosides serves as an important first step in evaluating the therapeutic potential of plant extracts. Furthermore, determination of total phenolic content offers as estimate of phenolic constituents present in plant extracts, which are frequently associated with antioxidant capacity and may contribute to other biological activities. Comparative evaluation of different medicinal plants based on their phytochemical composition and phenolic content provides useful information for selecting suitable plant materials for further biological investigations.
Assessment of the microbiological quality of fresh produce is equally important for ensuring food safety. Enumeration of total bacterial count provides an indication of the overall microbial load present in food samples, while isolation and identification of microorganisms assist in determining the occurrence of potential foodborne pathogens. Selective and Differential culture media, together with biochemical characterization, remain reliable approaches for identifying bacterial contaminants in routine microbiological investigations.
In the present study, aqueous extracts of Azadirachta indica and Murraya koenigii were prepared and evaluated for their phytochemical composition and total phenolic content. Fresh watermelon and tomato samples obtained from a local market were analyzed to determine total bacterial count, and microorganisms were isolated and identified using selective culture media and biochemical tests.
The study aimed to compare the phytochemical characteristics of the selected medicinal plants while simultaneously assessing the microbiological quality of fresh produce. The findings provide baseline information that may support future investigations into the application of medicinal plant- derived bioactive compounds for food safety and preservation.

Materials And Methods

Collection Of Plant Material

Fresh leaves of Azadirachta indica (Neem) and Murraya Koenigii (Curry leaves) were collected from local area of Gwalior, Madhya Pradesh, India. The plants materials were authenticated by the National Botanical Research Institute (NBRI), Lucknow, India. The leaves were washed thoroughly with distilled water to remove dust and other impurities, shade-dried at room temperature, and pulverized into a fine powder using a laboratory grinder. The powdered samples were stored in airtight containers until further anlaysis.

Preparation Of Aqueous Extracts

Aqueous extracts were prepared by mixing 10 g of dried plant powder with 100 mL of distilled water (1:10 w/v). The mixture was allowed to extract under laboratory conditions and subsequently filtered through Whatman No.1 filter paper. The filtrates were collected and stored at 40C until further analysis.

Qualitative Phytochemical Screening

Preliminary phytochemical analysis of the aqueous extracts was carried out using standard qualitative methods to detect the presence of major secondary metabolites, including alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, and glycosides.

Determination Of Total Phenol Count

The total phenolic content (TPC) of the plant extracts was determined using the Folin-Ciocalteu method. Briefly, an aliquot of each extract was mixed with Folin-Ciocalteu reagent and sodium carbonate solution. After incubation under dark conditions, the absorbance was measured spectrophotometrically at the appropriate wavelength. Gallic acid was used as the standard reference compound, and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract.

The aqueous extract of Azadirachta indica exhibited a total phenolic content of 81.8 mg GAE/g extract, whereas the extract of Murraya koenigii showed 46.2 mg GAE/g extract.

Microbiological Analysis of Food Samples

Fresh watermelon and tomato samples were collected from a local market and analyzed in duplicate. Total bacterial count was determined using standard plate count procedures, and the results were expressed as colony-forming units per gram (CFU/g).

Watermelon samples yielded 49.5 × 104 CFU/g

While tomato samples yielded 48.6 × 104 CFU/g

Isolation And Identification Of Microorganisms

Serially diluted food samples were inoculated onto selective and differential media. Escherichia coli was isolated from watermelon samples using EMB agar and MacConkey agar. Tomato samples yielded Salmonella spp. and fungal isolates.

Presumptive bacterial isolates were characterized using standard biochemical tests, including IMViC, Triple Sugar Iron (TSI), catalase, and oxidase tests.

Biochemical Characterization of Bacterial Isolates

The bacterial isolates obtained from the food samples were subjected to conventional biochemical characterization. The tests included the IMViC test series, Triple Sugar Iron (TSI) agar reaction, catalase test, and oxidase test. These biochemical assays were selected to provide phenotypic information useful for the preliminary characterization and differentiation of the bacterial isolates.

For the IMViC test, isolates were evaluated for indole production, methyl red reaction, Voges-Proskauer reaction, and citrate utilization. TSI agar was used to assess carbohydrate fermentation patterns and related biochemical reactions. Catalase activity was determined by observing the production of visible oxygen bubbles following exposure of the bacterial culture to hydrogen peroxide. Oxidase activity was determined using an appropriate oxidase reagent according to standard microbiological procedures.

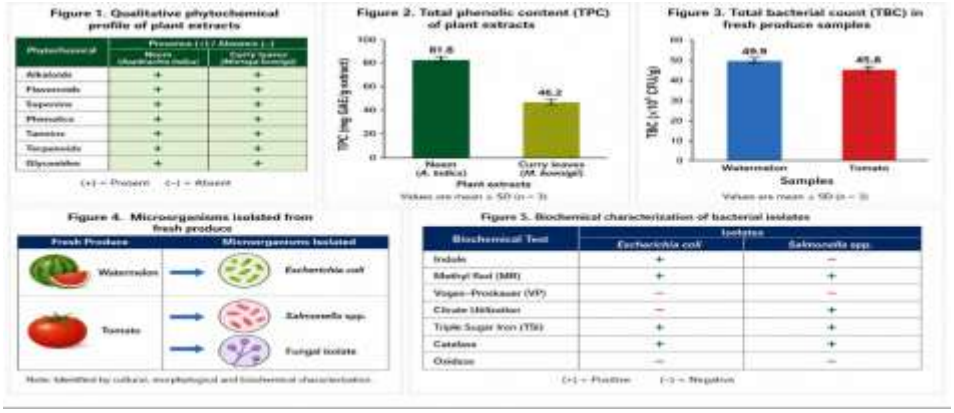
The results obtained from the biochemical tests were interpreted together with the colony characteristics and growth with the colony characteristics and growth patterns observed on selective and differential media. The identification of isolates was reported at the level supported by the experimental tests performed.

Antibacterial Activity

Determination Of the Zone of Inhibition

The antibacterial activity of the prepared plant extracts was evaluated against the identified bacterial isolates using the agar well diffusion method on Mueller – Hinton agar (MHA). The bacterial cultures were uniformly inoculated onto the surface of MHA plates using a sterile swab. Aqueous plant extracts were tested at concentrations 100%, 75%, 50% and 25% were added to the respective wells and Streptomycin was used as the standard antibiotic (positive control) for comparison. The inoculated plates were incubated at 340C for 24 h. After incubation, the antibacterial activity was determined by measuring the diameter of the clear zone surrounding each well. The diameter of the zone of inhibition (ZOI) was measured in millimeters (mm) using a transparent ruler or Vernier caliper. The recorded ZOI. The experiment was performed in two independent experiments.

Results



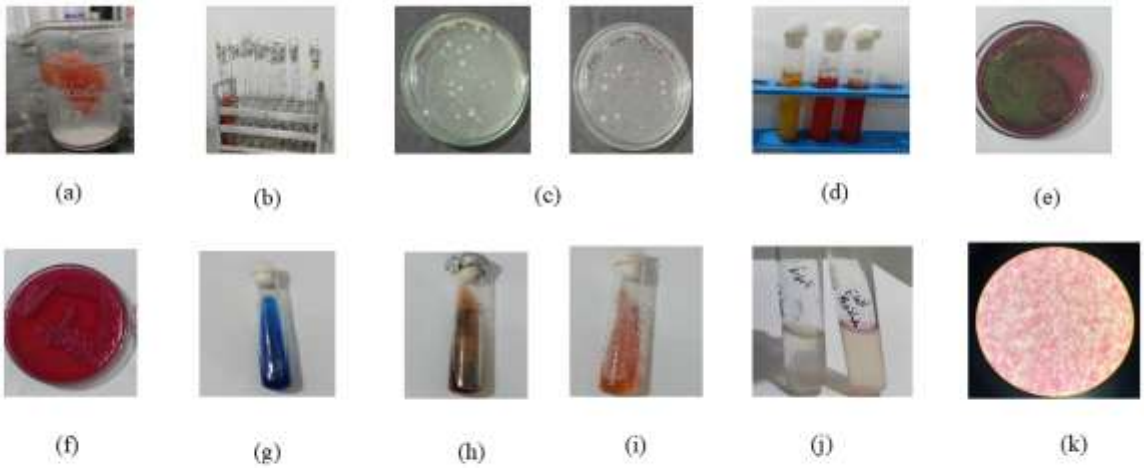


Figure 6. Isolation and identification of microorganisms from watermelon samples: (a) Watermelon sample,(b) serial dilution (c) Total plate count (TPC), (d) pre- enrichment culture, (e) isolation of Escherichia coli on eosin methylene blue (EMB) agar, (f) Isolation of Escherichia coli on MacConkey agar ,(g) (h) (i) (j)Biochemical characterization ,(k) Gram staining showing Gram – negative bacteria.

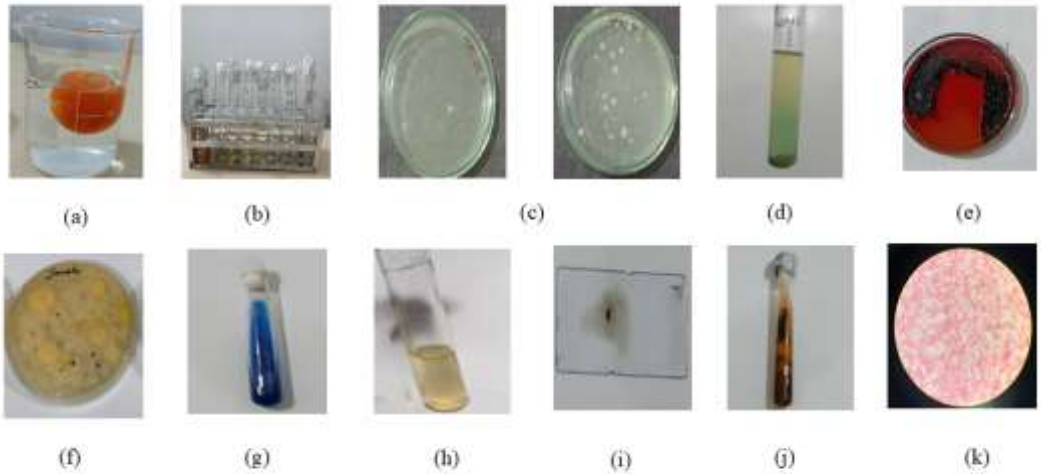


Figure 7. Isolation and identification of microorganisms from Tomato sample : (a) Tomato (b) serial dilution (c) Total plate count (TPC), (d) pre- enrichment culture, (e) isolation of *Salmonella* on Xylose Lysine Deoxycholate Agar (XLDA), (f) isolation of Fungal on Czapek Yeast Extract Agar (CYZA) (g) (h) (i) (j) Biochemical test (k) Gram staining showing Gram – negative bacteria

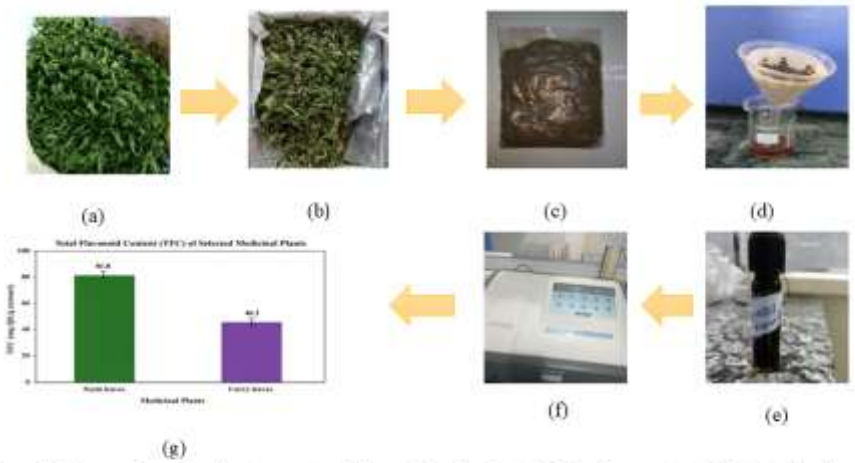
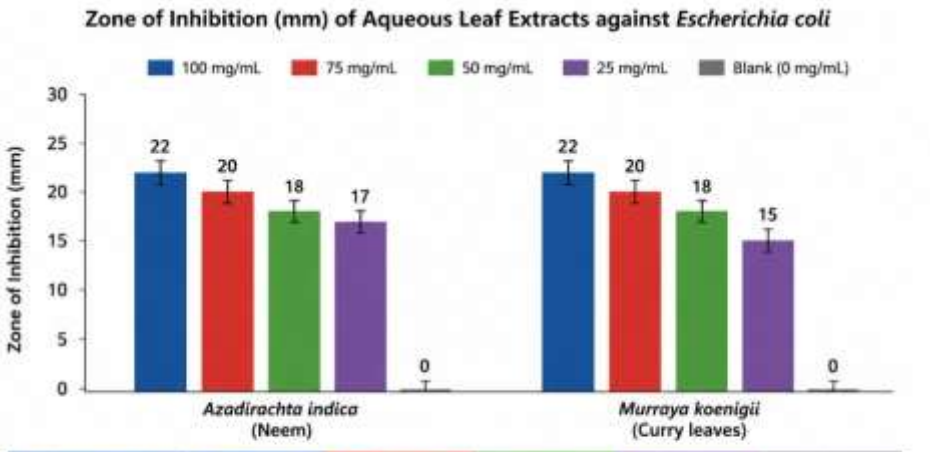


Figure 8. Preparation of plant extract and determination of total phenolic content (TPC). (a) Fresh plant leaves ,(i) Neem Leaves,(b) Curry leaves, (c) shade dried leaves powder, (d) Filtration of the extract, (e) concentrated plant extract, (f) UV Visible spectrophotometric analysis, and (g) Total Phenolic content (TPC) of the plant extract.



Plant extract	100 mg/mL	75 mg/mL	50 mg/mL	25 mg/mL	Blank
Azadirachta indica (Neem)	22	20	18	17	0
Murraya koenigii (Curry leaves)	22	20	18	15	0

Values are mean ± SD (n = 3)

Figure 9. Zone of Inhibition (mm) of aqueous leaf extracts against Escherichia coli

Discussion

The present study combined the phytochemical characterization of two medicinal plants with the microbiological examination of fresh produce. This integrated approach provides two complementary perspectives: first, the identification of bioactive chemical constituents in medicinal plants; and second, the detection of microorganisms associated with commonly consumed fresh foods

The phytochemical screening revealed alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, and glycosides in the investigated plant extracts. The detection of multiple classes of secondary metabolites demonstrates the chemical diversity of both Azadirachta indica and Murraya koenigii. These compounds are of considerable interest because plant secondary metabolites may contribute to various biological activities. However, the presence of a particular phytochemical group alone does not establish its biological effectiveness. Further quantitative and bioactivity – based investigations are required to determine the contribution of individual compounds or compound groups to biological activity.

A notable finding of the present investigation was the difference in total phenolic content between the two aqueous extracts. The neem extract showed a total phenolic content of 81.8 mg GAE/g extract, whereas the curry leaf extract showed 46.2 mg GAE/g extract. The higher value observed for neem suggests that the extraction conditions used in this study resulted in a recovery of Folin-Ciocalteu- reactive phenolic constitutes from neem leaves. Differences in plant species, geographic origin, maturity, environmental conditions, drying procedures, extraction ratio, and solvent polarity may all influence the measured phenolic content.

The Folin- Ciocalteu assay is widely used for estimating total phenolic content; however, the response is not exclusively specific to phenolic compounds. Consequently, the values should be interpreted as total Folin-Ciocalteu reactive to the gallic acid standard. Nevertheless, the assay provides a useful comparative estimate of phenolic-rich extracts and allows comparison between the two plant materials investigated in this study.

The microbiological analysis of the fresh produce revealed total bacterial counts of 49.5 × 104 CFU/g in watermelon and 48.6 × 104 CFU/g in tomato. The relatively similar bacterial loads observed in two samples indicate that both products carried substantial microbial populations at the time of analysis. The microbial load of fresh produce may be influenced by agricultural practices, irrigation water, soil contact, harvesting, transportation, handling, storage, and market conditions.

The isolation of E. coli from watermelon is particularly relevant from a food microbiology perspective because its presence may indicate contamination associated with inadequate hygiene or environmental exposure. Similarly, the recovery of Salmonella spp. from tomato is important because members of this genus are recognized foodborne pathogens. The isolation of a fungal microorganism from tomato additionally indicates that microbial contamination of fresh produce may involve both bacterial and fungal nd fungal organisms.

The use of selective and differential media, including EMB and MacConkey agar, supported the recovery and preliminary differentiation of bacterial isolates. Further biochemical using IMViC, TSI, Catalase, and oxidase tests provided additional phenotypic information for bacterial identification. However, biochemical identification should be interpreted within the limitations of the methods used. Molecular confirmation, such and 16S rRNA gene sequencing, could provide stronger taxonomic confirmation in future work.

The concentration dependent antibacterial activity observed in the present study suggests that increasing the extract concentration enhances its inhibitory effect against Escherichia coli. Neem extract demonstrated slightly greater antibacterial activity than curry leaf extract at the lowest concentration tested, indicating a comparatively higher antimicrobial potency. The observed antibacterial activity may be attributed to the presence of phenolic compounds, flavonoids, tannins, and other bioactive phytochemicals known to disrupt bacterial cell structure and function. These findings support previous reports demonstrating the antibacterial potential of neem and curry

leaves against Gram – negative bacteria.

Conclusion

The present study demonstrated that aqueous extracts of Azadirachta indica and Murraya koenigii contain a broad range of phytochemical constituents, including alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, and glycosides. The neem extract exhibited a higher total phenolic content (81.8 mg GAE/g extract) than the curry leaf extract (46.2 mg GAE/g extract) under the conditions used in this investigation.

Microbiological analysis of fresh produce revealed bacterial loads of 49.5 × 104 CFU/g in watermelon and 48.6 × 104 CFU/g in tomato. Escherichia coli was isolated from watermelon, while Salmonella spp. and a fungal isolate were recovered from tomato. These findings demonstrate the importance of microbiological monitoring of fresh produce and provide baseline information regarding the phytochemical characteristics of two widely available medicinal plants.

The present study demonstrated that aqueous extracts of Azadirachta indica and Murraya koenigii possess significant antibacterial activity against Escherichia coli. Both extracts showed maximum activity at 100 mg/mL, while antibacterial efficacy decreased with decreasing concentration. Neem extract exhibited slightly higher antibacterial activity than curry leaf extract at the lowest concentration tested. These findings indicate that both medicinal plants are promising natural source of antibacterial agents.

The present work establishes a foundation for future studies investigating the antimicrobial and antioxidant potential of these aqueous plant extracts against food-associated microorganisms. Further research involving standardized antimicrobial assays, quantitative flavonoid analysis, antioxidant evaluation, molecular confirmation of microbial isolates, and chemical profiling would strengthen the biological and applied significance of these findings.

Conflict Of Interest

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

Limitations of the Study

This study has certain limitations that should be considered while interpreting the findings. Although phytochemical screening and total phenolic content (TPC) analysis confirmed the presence of bioactive constituents, the individual phytochemicals responsible for the observed biological activities were not identified or quantified using advanced analytical techniques such as High-performance (HPLC), or other advanced technique. Furthermore, bacterial identification was based on conventional microbiological methods, including pre-enrichment, selective culture media, colony morphology, and biochemical characterization, whereas molecular identification techniques were not employed. The antimicrobial activity was evaluated using the agar well diffusion assay only; in addition, the findings are based on in vitro experiments and require further validation through toxicity assessment and in vivo studies before potential therapeutic applications can be established.

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