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Enhanced Antibacterial Activity of Selected Medicinal Plant Extract Combinations against Food-Borne Bacterial Pathogens

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ABSTRACT

Food-borne bacterial infections remain an important concern for public health and food preservation, creating interest in plant-derived antimicrobial formulations. This study evaluated the antibacterial performance of combined aqueous and ethanolic extracts prepared from six medicinal plants: *Asparagus racemosus*, *Acacia arabica*, *Andrographis paniculata*, *Lawsonia inermis*, *Chrysanthemum odoratum*, and *Centella asiatica*. Extract combinations containing two to six plant species were screened against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Salmonella enteritis*, and *Listeria monocytogenes* using an agar disc-diffusion assay. Ciprofloxacin was included as the reference antibacterial agent. The results showed that antibacterial performance depended on the composition of the mixture and was not directly proportional to the number of plants combined. Among the evaluated formulations, a three-plant combination comprising *C. odoratum*, *L. inermis*, and *A. paniculata* displayed the most consistent broad-spectrum inhibition. The ethanolic preparation produced inhibition zones of 2.1–2.4 cm, whereas the corresponding aqueous preparation produced zones of 1.8–2.0 cm. The maximum inhibition was observed against *S. enteritis*. Larger combinations containing four to six plant species retained antibacterial activity but did not exceed the performance of the selected three-plant formulation. The findings indicate that carefully selected plant combinations may provide enhanced antibacterial effects and warrant further investigation using MIC, MBC, checkerboard interaction assays, phytochemical profiling, and food-model validation.

Introduction

Food contamination by pathogenic microorganisms continues to represent a major challenge for food safety and public health. Bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, pathogenic *Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes* are important causes of food-associated infections and outbreaks. Their presence in food products may result from inadequate processing, cross-contamination, poor storage conditions, or insufficient hygiene during production and distribution (WHO, 2024). The increasing occurrence of antimicrobial resistance has further stimulated interest in alternative approaches for controlling pathogenic microorganisms. Natural products derived from medicinal plants are increasingly investigated because they contain diverse antimicrobial constituents and may offer complementary approaches for food preservation and microbial control (Mancuso *et al.,* 2023; Li *et al.,* 2024).

Plant extracts contain a wide range of secondary metabolites, including polyphenols, flavonoids, tannins, alkaloids, saponins, and terpenoids. These compounds can interfere with bacterial growth through several mechanisms, such as membrane destabilization, disruption of cellular metabolism, enzyme inhibition, oxidative stress induction, and interference with microbial signaling systems (Cowan, 1999; Bouarab-Chibane *et al.,* 2019; Rasheed *et al.,* 2024). The antimicrobial performance of a plant preparation is influenced by several factors, including plant species, plant part, geographical origin, extraction procedure, solvent polarity, and concentration of bioactive constituents. Consequently, extracts obtained from the same plant using different solvents may exhibit substantially different antibacterial properties (Ncube *et al.,* 2008; Azwanida, 2015; Zouine *et al.,* 2024). An emerging strategy in natural-product research is the use of combined plant extracts. The combination of extracts containing chemically distinct bioactive compounds may broaden antimicrobial activity by exposing microorganisms to multiple inhibitory molecules simultaneously. Depending on their interactions, combined plant preparations may demonstrate additive, indifferent, antagonistic, or enhanced effects (Bassolé & Juliani, 2012; Hemaiswarya *et al.,* 2008).

However, combining a large number of plant extracts does not automatically result in greater antibacterial activity. The final response depends on the compatibility of the phytochemicals present and their relative concentrations. In some combinations, the inclusion of additional components may dilute active compounds or generate antagonistic interactions (Caesar & Cech, 2019; Van Vuuren & Viljoen, 2011). The present study evaluated aqueous and ethanolic combinations prepared from six medicinal plants, namely *A. racemosus*, *A. arabica*, *A. paniculata*, *L. inermis*, *C. odoratum*, and *C. asiatica*. The extracts were tested in combinations containing two, three, four, five, and six plant species against five important food-borne bacterial pathogens. The study was designed to compare the antibacterial activity of different combination sizes, determine whether increasing the number of plant components improves inhibition, and identify promising formulations for subsequent quantitative interaction studies. Particular emphasis was placed on the combination containing *C. odoratum*, *L. inermis*, and *A. paniculata*, which demonstrated the strongest broad-spectrum antibacterial response within the experimental dataset.

Materials and Methods

Plant materials

Six medicinal plants were selected for antibacterial screening: *Asparagus racemosus*, *Acacia arabica*, *Andrographis paniculata*, *Lawsonia inermis*, *Chrysanthemum odoratum*, and *Centella asiatica*. Plant leaves were used for preparing crude extracts. The selected species were evaluated both individually and as multi-plant formulations.Medicinal plants are known to contain variable concentrations of antimicrobial secondary metabolites, and differences in phytochemical composition may influence the activity of combined preparations (Bouarab-Chibane *et al.,* 2019; Rasheed *et al.,* 2024).

Preparation of aqueous and ethanolic extracts

Plant materials were processed for the preparation of crude aqueous and ethanolic extracts. The use of water and ethanol enabled comparison between two extraction systems with different solvent characteristics. Ethanol is generally capable of recovering a broader range of moderately polar phytochemicals than water, although extraction efficiency depends on the chemical characteristics of individual compounds (Azwanida, 2015; Ncube *et al.,* 2008). The resulting extracts were subsequently used to prepare different combinations for antibacterial screening.

Preparation of plant combinations

The extracts were combined to produce formulations containing different numbers of plant species. The combinations included two-, three-, four-, five-, and six-plant formulations. Both aqueous and ethanolic combinations were tested. This design allowed the comparative evaluation of whether antibacterial activity increased with formulation complexity or whether specific combinations provided greater activity than mixtures containing a larger number of plants.

Test microorganisms

Antibacterial activity was evaluated against five food-borne bacterial pathogens: *Staphylococcus aureus, Bacillus cereus, Escherichia coli* O157:H7, *Salmonella enteritis* and *Listeria monocytogenes.*

These bacteria represent important food-associated pathogens with different biological characteristics and mechanisms of pathogenicity (WHO, 2024).

Antibacterial assay

The antibacterial activity of the combined extracts was determined using the agar disc-diffusion method. Sterile discs containing the plant extract preparations were placed on inoculated agar media, followed by incubation under appropriate conditions. The diameter of the clear area surrounding each disc was measured and expressed as the zone of inhibition in centimetres.

Disc-diffusion testing remains a useful preliminary method for comparing antimicrobial activity among crude extracts, although inhibition-zone size may also be influenced by diffusion characteristics and physicochemical properties of the test compounds (Balouiri *et al.,* 2016).

Ciprofloxacin was used as the reference antibacterial agent at 20 µg/disc. The activity of the plant combinations was subsequently compared with the inhibition zones obtained for the standard antibiotic.

Interpretation of combination effects

The antibacterial responses of the different combinations were compared using their respective inhibition-zone diameters. Since the available dataset is based primarily on disc-diffusion measurements, the results were interpreted as evidence of enhanced antibacterial performance rather than definitive pharmacological synergy.

Formal confirmation of synergy requires quantitative methods such as checkerboard assays, fractional inhibitory concentration indices, and time-kill experiments (Doern, 2014; Odds, 2003). Therefore, the present study represents a preliminary screening approach for identifying promising plant combinations for more detailed antimicrobial investigation.

Results and Discussion

Comparative antibacterial response of plant combinations

The combined medicinal plant extracts showed measurable antibacterial activity against all five tested food-borne pathogens. However, the magnitude of inhibition varied according to the plant species included in the formulation, the extraction solvent, and the number of extracts combined. The overall pattern obtained from the experimental dataset is summarized in Table 1.

As shown in Table 1, the most notable observation was that antibacterial activity was related to the composition of the formulation rather than simply to the number of plants included. The selected three-plant combination produced a stronger overall response than several larger mixtures. This finding agrees with previous evidence indicating that plant-derived antimicrobial combinations can produce different interaction patterns depending on the chemical compatibility of their constituents (Bassolé & Juliani, 2012; Caesar & Cech, 2019). An increase in the number of ingredients may therefore result in additive or antagonistic interactions rather than enhanced antibacterial performance. The results also support the concept that rational formulation is more important than formulation complexity. The incorporation of additional extracts may alter the concentration of active compounds or modify interactions among phytochemicals, thereby influencing the overall antibacterial response (Hemaiswarya *et al.,* 2008; Van Vuuren & Viljoen, 2011).

Antibacterial activity of the selected three-plant formulation

The combination containing *Chrysanthemum odoratum*, *Lawsonia inermis*, and *Andrographis paniculata* showed the most consistent antibacterial performance among the tested formulations. The detailed inhibition-zone values for the aqueous and ethanolic preparations are presented in Table 2.

The data in Table 2 demonstrate that the ethanolic formulation consistently produced larger inhibition zones than the corresponding aqueous combination. The strongest response was recorded against *S. enteritis*, with an inhibition zone of 2.4 cm, followed by *L. monocytogenes* at 2.3 cm. The improved activity of the ethanolic formulation may be associated with differences in the range and concentration of phytochemicals extracted by ethanol. Solvent selection can substantially affect the recovery of phenolic compounds, flavonoids, alkaloids, and other antibacterial metabolites (Azwanida, 2015; Ncube *et al.,* 2008).

The broad activity of the three-plant formulation may also reflect the simultaneous presence of different antimicrobial constituents. Plant phenolics and other secondary metabolites can affect bacterial cells through multiple targets, including membrane integrity, enzymatic pathways, and cellular metabolism (Cowan, 1999; Rasheed *et al.,* 2024). Nevertheless, the enhanced response observed in Table 2 should not be interpreted as confirmed synergy. Disc-diffusion results provide useful comparative information but cannot independently distinguish between synergistic and additive interactions. Checkerboard-based MIC analysis would be necessary to quantify the interaction among the three extracts (Doern, 2014; Odds, 2003).

Effect of extraction solvent

The superiority of the ethanolic preparation was one of the consistent findings of the present investigation. As illustrated in Figure 1, the ethanolic three-plant combination produced larger inhibition zones against each of the five bacterial pathogens than the corresponding aqueous formulation.

The results shown in Figure 1 indicate that ethanol was more effective than water for recovering antibacterial constituents from the selected plant mixture. This observation is consistent with reports showing that solvent polarity strongly influences phytochemical extraction and subsequent biological activity (Azwanida, 2015; Ncube *et al.,* 2008). However, ethanol should not be regarded as universally superior for all medicinal plants or all antimicrobial compounds. Extraction efficiency depends on the polarity, molecular structure, and stability of the compounds responsible for biological activity (Zouine *et al.,* 2024). Therefore, optimization of extraction conditions remains important for developing reproducible plant-based antibacterial formulations.

Influence of the number of plants in the formulation

The experimental findings demonstrated that antibacterial activity did not increase progressively from two-plant to six-plant formulations. Instead, selected smaller combinations, particularly the three-plant preparation, produced the most favorable responses. This pattern is represented schematically in Figure 2.

The results suggest that the antibacterial performance of a combination depends on the interaction among its individual components. When compatible bioactive compounds are combined, they may contribute to complementary antibacterial effects. Conversely, the addition of further extracts may reduce the relative concentration of important compounds or introduce interactions that do not contribute positively to bacterial inhibition (Caesar & Cech, 2019; Van Vuuren & Viljoen, 2011). Similar concerns have been highlighted in studies of botanical combination therapy, where both synergistic and antagonistic outcomes have been reported depending on the composition and ratio of individual constituents (Bassolé & Juliani, 2012; Hemaiswarya *et al.,* 2008). Therefore, the present findings suggest that the identification of optimal plant combinations should be based on systematic screening rather than the assumption that formulations containing more plant species will necessarily produce stronger antibacterial effects.

Comparison with the reference antibiotic

Ciprofloxacin produced larger inhibition zones than all of the evaluated plant combinations. The standard antibiotic showed inhibition zones between 2.6 and 2.8 cm, whereas the ethanolic three-plant formulation produced zones ranging from 2.1 to 2.4 cm, as shown in Table 2. The lower activity of the crude plant formulation compared with ciprofloxacin is expected because ciprofloxacin is a purified compound with a defined concentration and established antibacterial mechanism. In contrast, crude plant extracts contain numerous compounds, and the concentration of individual antibacterial constituents may vary considerably. Despite this difference, the relatively high inhibition zones obtained for the ethanolic three-plant formulation indicate promising antibacterial potential. Natural antimicrobial preparations are increasingly considered as complementary tools for food safety and preservation, particularly when their active components and safety profiles are properly characterized (Li *et al.,* 2024; Zouine *et al.,* 2024). Further studies should determine MIC and MBC values for the selected formulation and compare its activity with that of individual extracts. Such experiments would clarify whether the improved performance is additive or truly synergistic.

Table.1 Comparative antibacterial performance of different categories of medicinal plant combinations**.**

Combination type	General antibacterial response	Interpretation
Two-plant combinations	Variable activity depending on the selected plants	Some combinations showed promising inhibition
Three-plant combinations	Strongest and most consistent antibacterial response	Most effective combination category
Four-plant combinations	Moderate antibacterial activity	Additional plants did not consistently improve inhibition
Five-plant combinations	Detectable antibacterial effect	No proportional enhancement observed
Six-plant combination	Broad but comparatively lower enhancement	Greater formulation complexity did not improve performance
Selected three-plant formulation	Strong inhibition against all five pathogens	Most promising formulation

Table.2 Antibacterial activity of the selected three-plant combination.

Test bacterium	Aqueous extract (cm)	Ethanolic extract (cm)	Ciprofloxacin (cm)
Staphylococcus aureus	1.9	2.1	2.7
Bacillus cereus	2.0	2.2	2.8
***Escherichia coli* O157:H7**	1.8	2.2	2.6
Salmonella enteritis	1.9	2.4	2.7
Listeria monocytogenes	1.8	2.3	2.6

Figure.1 Comparative antibacterial activity of aqueous and ethanolic extracts of the selected three-plant combination against food-borne bacterial pathogens.

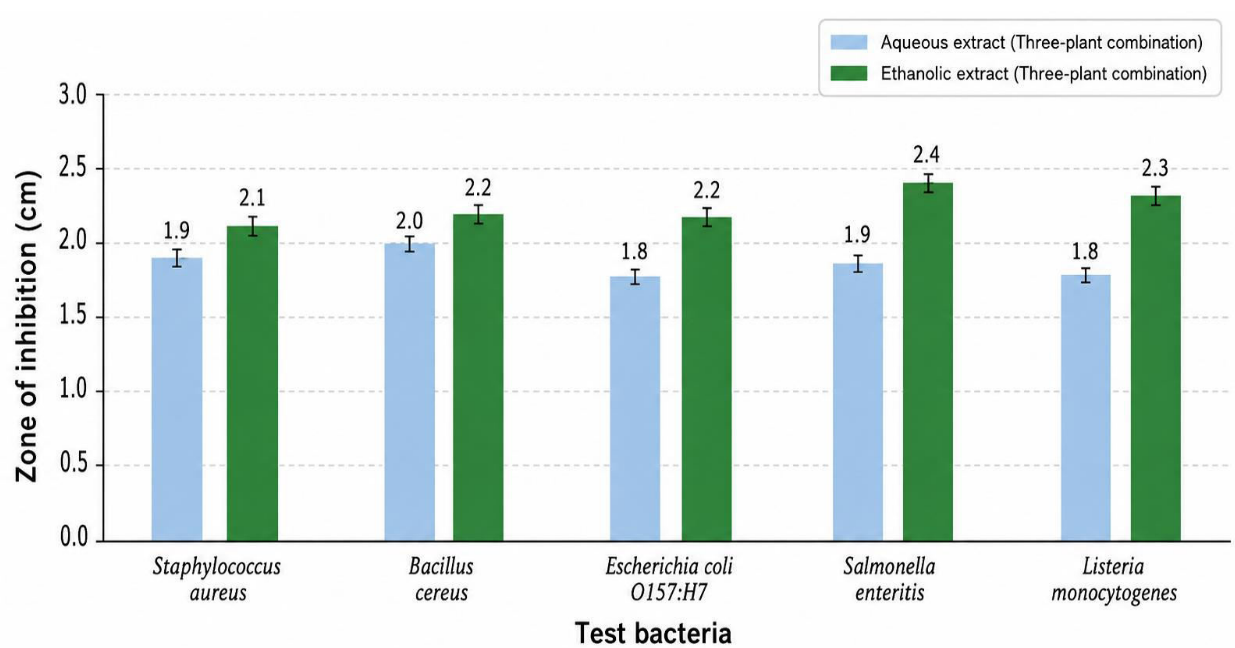
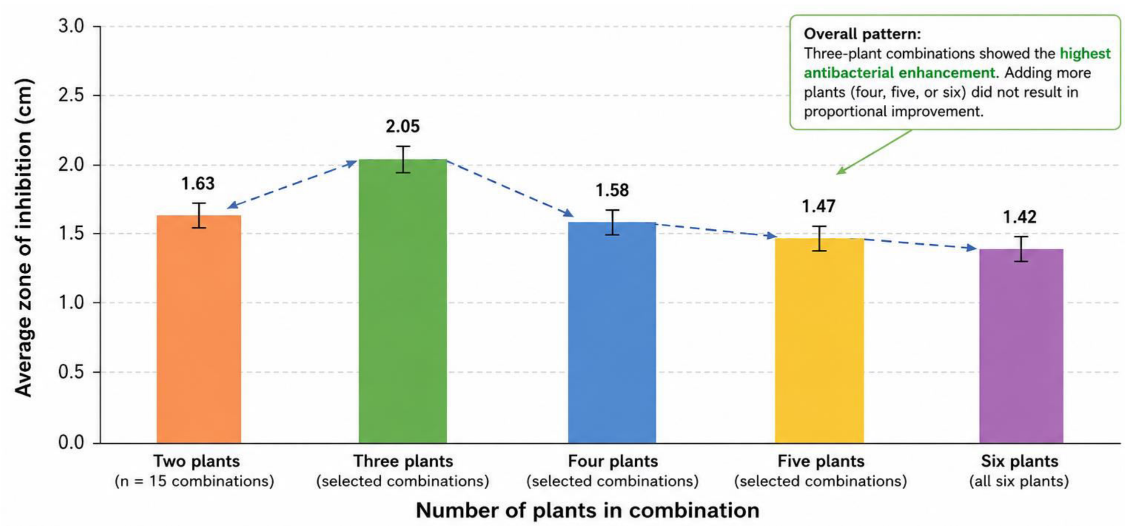


Figure.2 Relationship between the number of medicinal plants combined and the observed antibacterial response. Selected three-plant formulations demonstrated greater overall enhancement than larger combinations.



Implications for the development of natural antibacterial formulations

The present findings support further development of selected medicinal plant combinations as potential natural antibacterial preparations. The strongest formulation contained *C. odoratum*, *L. inermis*, and *A. paniculata*, suggesting that these plants may possess complementary phytochemical properties. efore practical application, several important issues must be addressed. These include chemical standardization, identification of active constituents, stability during storage, toxicity, sensory effects, and antibacterial performance in complex food matrices. The effectiveness of plant extracts observed in laboratory media may change substantially when proteins, lipids, carbohydrates, pH, and other food components are present (Li *et al.,* 2024; Rasheed *et al.,* 2024). Accordingly, the present results should be considered an initial step toward identifying promising antibacterial plant combinations rather than evidence for immediate food application. The selected three-plant formulation represents a suitable candidate for detailed quantitative and phytochemical investigation.

In conclusion, the present study demonstrated that combined medicinal plant extracts can inhibit important food-borne bacterial pathogens, although antibacterial activity depended more strongly on the composition of the formulation than on the total number of plants included. Among the combinations evaluated, the formulation containing *Chrysanthemum odoratum*, *Lawsonia inermis*, and *Andrographis paniculata* showed the most consistent broad-spectrum activity. The ethanolic preparation produced inhibition zones ranging from 2.1 to 2.4 cm and consistently outperformed the corresponding aqueous combination. Increasing the number of plant components from three to four, five, or six did not result in proportional improvement in antibacterial activity. These findings suggest that rational selection of compatible plant extracts may be more effective than increasing formulation complexity. Because the present results are based on disc-diffusion screening, the observed enhanced effects require confirmation using MIC and MBC assays, checkerboard analysis, fractional inhibitory concentration indices, time-kill studies, and phytochemical characterization before definitive synergy or practical food applications can be established.

Author Contributions

K. Arivazhagan: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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