Plant-part specific in vitro antibacterial activity of crude ethanolic extracts of Mangifera indica against Salmonella typhi: an exploratory laboratory study

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Research Article

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Abstract

Background

Medicinal plants remain widely used in the management of infectious diseases in low- and middle-income countries, yet experimental evidence supporting the antibacterial activity of specific plant parts remains inconsistent. This study evaluated the plant-part–specific antibacterial activity of crude ethanolic extracts of *Mangifera indica* against *Salmonella typhi* under controlled laboratory conditions.

Methods

An exploratory in vitro study was conducted using crude ethanolic extracts prepared from the seed kernel, stem bark, and root of *Mangifera indica*, as well as a combined extract. Antibacterial activity was assessed using the agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using broth microdilution and sub-culture techniques. A standardized bacterial inoculum (0.5 McFarland) of *Salmonella typhi* was used. Experiments were conducted in triplicate (n = 3). Tetracycline (6.667 µg/mL) served as the positive control, while dimethyl sulfoxide (DMSO) was used as the negative control. All assays were incubated at 37°C for 24 hours.

Results

The seed kernel extract demonstrated measurable antibacterial activity, producing a mean zone of inhibition of 18.0 ± 0.7 mm. The combined extract showed lower activity (11.5 ± 0.7 mm), while stem bark and root extracts showed no detectable activity. One-way ANOVA demonstrated a statistically significant difference among treatments, $p < 0.0001$. Both active extracts exhibited an MIC of 16.67 mg/mL and an MBC of 33.34 mg/mL. The MBC/MIC ratio was 2 for both extracts, which is consistent with bactericidal activity under the in vitro conditions employed.

Conclusions

Crude ethanolic extract of *Mangifera indica* seed kernel exhibited in vitro antibacterial activity against *Salmonella typhi*, with evidence of plant-part specificity and reduced activity in combined extracts. These findings are limited to preliminary laboratory observations and do not imply clinical effectiveness.

Introduction

Infectious diseases remain a major cause of morbidity and mortality worldwide, with a disproportionate burden in low- and middle-income countries where access to diagnostic services and effective antimicrobial therapy is often limited. In such contexts, plant-based remedies continue to play an

important role in primary health care due to their accessibility, affordability, and cultural acceptance (1, 2).

The global increase in antimicrobial resistance among bacterial pathogens has renewed interest in plant-derived substances as potential sources of novel antimicrobial agents (3). While this interest is often linked to drug discovery efforts, a considerable number of studies remain at the level of preliminary in vitro screening. Reported antibacterial activity within the same plant species can vary depending on factors such as plant part, extraction solvent, extract concentration, and experimental design (4–6).

Mangifera indica L. (*M. indica*), family Anacardiaceae, commonly known as mango, is widely cultivated in tropical and subtropical regions and has a long history of ethnomedicinal use (7, 8). Different parts of the plant, including the seed kernel, stem bark, leaves, and roots, have been used traditionally in the management of gastrointestinal disorders and febrile illnesses, including conditions locally associated with typhoid fever (9, 10). Despite this broad ethnomedicinal application, experimental evaluations of *M. indica* have produced variable findings, particularly with respect to antibacterial activity across different plant parts (11, 12).

Typhoid fever, caused by *Salmonella enterica serovar* Typhi (*S. typhi*), remains a public health concern in many developing regions, including sub-Saharan Africa. In Uganda, recurrent outbreaks have been associated with inadequate water supply, poor sanitation, and high population density (13). Although antimicrobial therapy remains central to disease management, challenges related to antimicrobial resistance and access to treatment continue to motivate exploratory investigations into locally available plant-based alternatives (14, 15).

Within this context, this study evaluated the in vitro antibacterial activity of crude ethanolic extracts of selected parts of *Mangifera indica* against *Salmonella typhi*, with emphasis on plant-part specificity. This was done to generate preliminary evidence on which parts of *M. indica* demonstrate measurable antibacterial activity against *S. typhi* under controlled laboratory conditions, given the continued ethnomedicinal use of the plant and the variability in prior reports.

Materials and methods

Study design

This study was designed as an exploratory in vitro laboratory investigation aimed at evaluating the antibacterial activity of crude ethanolic extracts prepared from selected parts of *Mangifera indica* against *Salmonella typhi*.

Study area and laboratory setting

All laboratory procedures were conducted at the Biosciences Laboratory, Department of Biological Sciences, Kyambogo University, Kampala, Uganda.

Bacterial strain and culture conditions

A laboratory strain of *Salmonella typhi* previously characterized by polymerase chain reaction (PCR) was obtained from the Biosciences Laboratory, Kyambogo University (16). The strain was maintained in glycerol stocks at − 80°C, revived on tryptone soya agar, and working cultures maintained at 4°C.

Collection and identification of plant materials

Fresh, disease-free seed kernels, stem bark, and roots of *Mangifera indica* L. (family Anacardiaceae) were collected from a single mature cultivated mango tree located in Omaa Village, Lira City East Division, Lira City, Uganda (2°10'26.6"N, 32°54'49.1"E; 1101 m above sea level). Permission to collect the plant materials was obtained from the landowner.

The plant material was identified by the first author, Ivan Ronald Omara, based on standard morphological characteristics of *Mangifera indica* L. A voucher specimen was subsequently deposited at the Makerere University Herbarium, Makerere University, Kampala, Uganda, under voucher number MUH51645.

Samples were washed thoroughly with sterile distilled water, shade-dried at room temperature for 14 days, and ground into fine powder using a sterilized electric grinder. The powdered samples were stored in sealed containers until extraction.

Preparation of crude ethanolic extracts

Crude extracts were prepared using ethanolic maceration (10). Five grams (5 g) of each powdered plant material were soaked in 50 mL of ethanol (99.9%) for 72 hours at room temperature with intermittent agitation. The mixtures were filtered using sterile filter paper (Whatman No. 1) and concentrated at 40°C using a rotary evaporator to obtain dried crude extracts. Each extract was reconstituted in DMSO to obtain a stock concentration of 66.67 mg/mL. A combined extract was prepared by mixing equal volumes (1:1:1) of the seed kernel, stem bark, and root extracts.

Preparation of bacterial inoculum

Bacterial inoculum was prepared by suspending fresh colonies of *Salmonella typhi* in Mueller–Hinton broth. The turbidity of the suspension was adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL), verified spectrophotometrically at 600 nm to ensure consistency across assays (17). For each independent replicate, a fresh inoculum was prepared.

Agar well diffusion assay

Antibacterial activity was assessed using the agar well diffusion method (18). Mueller–Hinton agar plates were uniformly inoculated with the standardized bacterial suspension using sterile cotton swabs. Wells of 6 mm diameter were aseptically punched into the agar and filled with 100 µL of each plant extract at a concentration of 66.67 mg/mL. Tetracycline (6.667 µg/mL) served as the positive control. The tetracycline concentration was selected based on laboratory standard operating procedures for comparative susceptibility screening. DMSO was used as the negative control.

Plates were incubated at 37°C for 24 hours. Zones of inhibition (including the well diameter) were measured in millimeters using a digital caliper. All assays were performed in triplicate using three independent bacterial inoculum preparations on three separate days.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined using the broth microdilution method (19). Two-fold serial dilutions of each extract were prepared in Mueller–Hinton broth to obtain final concentrations ranging from 33.34 mg/mL to 2.08 mg/mL in 96-well microtiter plates. Each well was inoculated with 100 µL of standardized bacterial suspension (approximately 5×10^5 CFU/mL final concentration). Plates were incubated at 37°C for 24 hours. MIC was defined as the lowest concentration of extract that showed no visible turbidity. Each MIC determination was performed in triplicate with independent inoculum preparations.

Determination of minimum bactericidal concentration (MBC)

Minimum bactericidal concentration was determined by sub-culturing 10 µL aliquots from wells that showed no visible growth during MIC determination onto fresh Mueller–Hinton agar plates (20). Plates were incubated at 37°C for 24 hours. The lowest concentration of extract that showed no bacterial growth was recorded as the MBC. Each MBC determination was performed in triplicate.

Statistical analysis

All experiments were performed in triplicate, meaning three independent replicate experiments each conducted on separate days with fresh bacterial inoculum preparations. Data are presented as mean ± standard deviation (SD) calculated from these three replicates (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using GraphPad Prism version 8. A p-value < 0.05 was considered statistically significant.

Biosafety and ethical considerations

All procedures involving bacterial handling were conducted in accordance with standard biosafety practices for non-clinical laboratory strains. No human participants or animal subjects were involved in this study; therefore, formal ethical approval was not required. Plant material collection complied with applicable local regulations and institutional guidelines for cultivated plant species. No protected, endangered, or regulated plant species were involved in the study.

Results

Antibacterial activity

The seed kernel extract produced a mean zone of inhibition of 18.0 ± 0.7 mm (Table 1). The combined extract produced 11.5 ± 0.7 mm. Stem bark and root extracts showed no detectable activity (0.0 ± 0.0 mm). Tetracycline (positive control, 6.667 µg/mL) produced 14.0 ± 0.7 mm. The negative control (DMSO) showed no inhibition.

Table 1
Zones of inhibition (mm, mean ± SD) from three independent replicate experiments (n = 3)

Extract	Zone of inhibition (mm)
Seed kernel	18.0 ± 0.7
Stem bark	0.0 ± 0.0
Root	0.0 ± 0.0
Combined extract	11.5 ± 0.7
Tetracycline (6.667 µg/mL)	14.0 ± 0.7
DMSO	0.0 ± 0.0

Note: The combined extract consisted of equal volumes (1:1:1) of seed kernel, stem bark, and root extracts.

One-way ANOVA revealed a statistically significant difference among the extracts $p < 0.0001$. Tukey's post-hoc analysis indicated that the seed kernel extract produced significantly larger zones of inhibition compared to the combined extract (mean difference = 6.50 mm, $p < 0.0001$), stem bark (mean difference = 18.00 mm, $p < 0.0001$), and root extract (mean difference = 18.00 mm, $p < 0.0001$). No significant inhibition was observed for stem bark and root extracts compared to the negative control.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The seed kernel extract exhibited an MIC of 16.67 mg/mL and an MBC of 33.34 mg/mL (Table 2). The combined extract showed identical values. The MBC/MIC ratio was 2 for both extracts, which is consistent with bactericidal activity under the in vitro conditions employed.

Table 2
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active extracts from three independent replicate experiments (n = 3)

Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
Seed kernel	16.67	33.34	2
Combined extract	16.67	33.34	2

Discussion

In this study, antibacterial activity against *Salmonella typhi* was observed primarily in the seed kernel extract of *Mangifera indica* and, to a lesser extent, in the combined extract. Stem bark and root extracts did not exhibit measurable activity under the experimental conditions used. This clear differentiation among plant parts indicates that antibacterial effects of *M. indica* against *S. typhi* are plant-part specific.

The observed inhibitory activity of *M. indica* seed kernel extract is consistent with previous laboratory studies reporting antibacterial effects of mango seed kernel extracts against enteric bacteria (10, 21).

The absence of detectable antibacterial activity in the stem bark and root extracts contrasts with some reports in the literature that have described antimicrobial effects from these plant parts under different experimental conditions (6). This discrepancy likely reflects differences in extraction solvent, extract concentration, bacterial strain susceptibility, or assay conditions.

Although the combined extract demonstrated inhibitory activity against *S. typhi*, its effect was significantly lower than that of the seed kernel extract alone (mean difference = 6.50 mm, $p < 0.0001$). This may reflect dilution of active constituents when extracts from different plant parts are combined. Antagonistic interactions among extract components also cannot be excluded, though no formal synergy testing was conducted (22, 23).

The MIC and MBC values obtained for the seed kernel and combined extracts (16.67 mg/mL and 33.34 mg/mL, respectively) are relatively high compared to purified antibiotics, which is expected for crude plant extracts. The MBC/MIC ratio of 2 for both extracts is consistent with bactericidal activity under the in vitro conditions employed.

Limitations

This study has several limitations inherent to preliminary in vitro screening investigations. Only one laboratory strain of *S. typhi* was tested, and the MIC of tetracycline was not determined. Plant material was collected from a single tree, which may limit generalizability across trees, growing conditions, and seasons. Crude extracts were used without phytochemical characterization or toxicity assessment. These limitations constrain generalizability but do not invalidate the primary finding that the *M. indica* seed kernel extract exhibited antibacterial activity under the tested conditions.

Conclusions

This study demonstrated that crude ethanolic extract of *Mangifera indica* seed kernel exhibited measurable in vitro antibacterial activity against the tested laboratory strain of *Salmonella typhi* under the experimental conditions used (triplicate independent experiments, 37°C incubation, 24 hours). Stem bark and root extracts showed no detectable activity. The combined extract showed lower inhibitory activity than the seed kernel extract alone. These findings provide laboratory evidence that antibacterial activity in *M. indica* against *S. typhi* is plant-part specific. The results are preliminary and do not imply clinical effectiveness or therapeutic applicability.

Future studies should include testing against multiple clinical isolates and ATCC reference strains of *S. typhi*, phytochemical characterization of the active seed kernel extract, and cytotoxicity assessment to evaluate safety.

Abbreviations

ANOVA
Analysis of variance
CFU
Colony-forming units
DMSO
Dimethyl sulfoxide
MBC
Minimum bactericidal concentration
MIC
Minimum inhibitory concentration
PCR
Polymerase chain reaction
SD
Standard deviation
M. indica
Mangifera indica
S. typhi
Salmonella typhi

Declarations

Ethics approval and consent to participate

This study did not involve human participants or animal subjects; therefore, ethical approval and consent to participate were not required.

Consent for publication

The authors declare that this manuscript has not been published or accepted for publication or under editorial review for publication elsewhere and thus consent to publish this manuscript with the *BMC Complementary Medicine and Therapies*.

Availability of supporting data

The datasets used in this study are available from the corresponding author upon reasonable requests.

Competing interests

The authors declare that they have no conflict of interest regarding the publication of this paper.

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Authors' contributions

Ivan Ronald Omara oversaw concept development, methodology; investigation; data curation; formal analysis; writing-original draft; writing-review and editing.

Juliet Kyayesimira: Supervision; methodological guidance; critical review of the manuscript.
Both authors read and approved the final manuscript.

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