

Effects of Leaf Extracts of Selected Medicinal Plants against *Salmonella typhi*

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ABSTRACT

Emerging antimicrobial resistance of *Salmonella typhi* necessitates alternative approaches to treat it, one of which is plant extracts. This paper evaluates the antimicrobial activity of the leaf extracts of five selected medicinal plants: *Azadirachta indica*, *Calotropis procera*, *Psidium guajava*, *Carica papaya*, and *Jatropha tanjorensis*, against *Salmonella typhi*. Plant materials were gathered, identified, and authenticated. They were then dehydrated by air drying, and extracted with ethanol. Disc diffusion was used as test for antimicrobial activity. The results indicated that the leaf extracts of *Carica papaya* showed maximum antimicrobial activity with an inhibition zone of 35.23 mm but, there was no statistical difference between the effect of *Calotropis procera* (22.13 mm) and *Jatropha tanjorensis* (20.00 mm). Also, the combined leaf extracts of *Azadirachta indica* with *Jatropha tanjorensis* inhibited 33.37mm zone of the organism while the least impact (12.50 mm) was observed when the leaf of *Psidium guajava* was combined with the leaf of *Azadirachta indica*, and it was not statistically different from when the leaves of *Psidium guajava* were combined with those of *Calotropis procera* (14.13 mm). This study confirms both the separate extracts of the plant and its blend possess excellent antibacterial activity against *Salmonella typhi*, indicating that such pharmacologically active plant species could be used as an alternative antimicrobial drug.

Keywords: Effects, Leaf extracts, Medicinal plants, Salmonella, Inhibition.

INTRODUCTION

Salmonella typhi has been reported (Ejikeugwu, 2024) to belong to the genus *Salmonella* and the family Enterobacteriaceae. They are facultative anaerobic, rod-shaped, gram-negative bacteria. It is a human pathogen and the etiological agent of typhoid fever. This pervasive infectious plague spreads mainly through the consumption of contaminated food and water (Crump *et al.*, 2015) and through the intake of polluted foods, meat and water (Ehuwaet *al.*, 2021; Billah & Rahman, 2024). The bacterium possesses several virulence factors, such as the Vi capsular polysaccharide, that enables it to evade the host immune system and survive in macrophages (Wang *et al.*, 2020). *S. typhi* invades the gastrointestinal tract, punctures the intestinal epithelium, and reaches the blood, causing bloodstream infection (BSI) and food poisoning (Schneider *et al.*, 2016).

Salmonella typhi infections remain a severe health problem, particularly in developing countries. Traditional antibiotics are no longer effective since resistance develops through drug-resistant strains (Akinyemi *et al.*, 2005). Herbal medicines have been discovered to contain biochemical components that would serve as good antimicrobial agents. There have been encouraging findings about the antimicrobial properties of some plants. For instance, Quartey *et al.* (2021) observed that the leaves of *Adansonia digitata* contain anti-inflammatory substances that inhibit cyclooxygenase enzymes and other inflammatory mediators. Also, Iheanacho *et al.* (2023) demonstrated that cashew (*Anacardium occidentale*) leaf extracts demonstrated vasodilation and systolic blood pressure inhibition in hypertensive models by mediation of the high antioxidant activity. However, empirical findings on the inhibitory activity of leaf extracts of *Calotropis procera*, and *Jatropha tanjorensis*, against *Salmonella typhi* has not been adequately documented.

Salmonella typhi resistance to readily available antibiotics is a public health issue of great concern, particularly where access to up-to-date medicine is not available. The pathogen's opposition to standard antibiotics like Tetracycline, third-generation Cephalosporin, Gentamycin, Quinolones, ampicillin, chloramphenicol, Quinolones, and trimethoprim-sulfamethoxazole, therefore, causes difficulty in treatment and necessitates alternative approaches to treat it (Islam *et al.*, 2015). Herbs, especially those from plants, may be very good alternatives in that they have positive biochemical compounds (Zhang *et al.*, 2023).

The plants have been reported to exhibit highly antimicrobial and antibacterial activities, *Jatropha tanjorensis* has diverse therapeutic activities, with its antimicrobial activity being the focus of several ethnomedicinal studies. The various parts of the plant, especially leaves and bark, have been reported to have antibacterial and antifungal efficacy. Ewa-Udu *et al.* (2022) have indicated that *J. tanjorensis* showed antimicrobial activity against variety of fungi and bacteria pathogens including *Serratia marcescens*. Similarly, Sankarganesh *et al.* (2018) reported antibacterial activity of *C. papaya* extracts in in-vitro tests against various bacterial strains. Also, Ugbogu *et al.* (2022) showed the traditional application of guava leaf extract in gastrointestinal, oral, and cutaneous infection medications. Furthermore, Almaghrabiet *al.* (2022) documented the use of *C. procera* for the development of natural antimicrobial agents against multidrug-resistant microorganisms. Moreso, *Azadirachta indica* bark and leaves exhibit antifungal, antibacterial, and antiviral activity as reported by Ismail *et al.* (2024). Considering the importance of the plants highlighted above, this research aims to be a step towards developing newer modalities that are more affordable and accessible to treat typhoid fever in developing nations, as well as resource-poor communities.

MATERIALS AND METHODS

Study area

The experiment was conducted at the laboratory, Department of Biological Sciences, Nigeria Police Academy, Wudil, Kano State, Nigeria. The Nigerian police academy is located within the latitudes 11.8229°N and longitudes 8.8035°E.

Collection and Preparation of Research Materials

Research Plants

Plant materials, which are the leaves of *Azadirachta indica*, *Calotropis procera*, *Psidium guajava*, *Carica papaya*, and *Jatropha tanjorensis*, were randomly collected from natural vegetation at Kano State, Nigeria (Dennis *et al.*, 2023). *Azadirachta indica* was collected near the Nigeria Police Academy Stadium (11.822720°N, 8.811105°E), *Carica papaya* was collected opposite a filling station, Nigeria police Academy (11.830984°N, 8.800713°E), and *Calotropis procera* was collected behind the faculty of sciences, Nigeria Police Academy (11.819181°N, 8.805693°E), while *Psidium guajava* was collected from the botanical garden along Zoo road, Kano State (11.847221°N, 8.550921°E), and *Jatropha tanjorensis* was collected from Giwa Street, Hausawa area, Kano State (11.965143°N, 8.530203°E). The plant materials were identified and authenticated by Ibrahim Shu'aibu and certified by Professor Hajara Haruna at the Bayero University Herbarium, Kano State.

The plant materials were washed in distilled water and dried at room temperature for 14 days. The leaves were then ground into powder using a mechanical grinder as described by Ali *et al.* (2017), and the grounded plant materials were sieved to separate the fine powder from the coarse particles. Twenty-five grams of fine powdered sample were soaked in 150 mL of ethanol and kept for 72 hours at room temperature with mild shaking (Hemeg *et al.*, 2020). The solution was filtered using Whatman No.1 filter paper; the filtrate was evaporated with a rotary evaporator (RE 52A) at 45°C under vacuum pressure. The concentrated extracts were stored in airtight containers at 4°C until use (Hossain, 2024).

Research Organism and Preparation of McFarland Standard

The test microorganism used was a *Salmonella typhi* clinical isolate collected from Aminu Kano Teaching Hospital (AKTH) in Kano State. It underwent standard biochemical identification, which included the triple sugar iron (TSI) agar test, catalyst test, citrate utilization test, and oxidase test (Cheesbrough, 2006) at the Nigeria Police Academy Biology Laboratory. A fresh culture of *Salmonella typhi* was inoculated into sterile nutrient broth after sterile nutrient agar had been poured into an inoculum tube and allowed to solidify. It was then incubated at 37°C for 18–24 hours to prepare the inoculum. The turbidity was adjusted to approximately 0.5 McFarland standards, corresponding to about 1.5×10^8 CFU/mL (McFarland, 1907).

Antimicrobial Sensitivity Testing

The disk diffusion method by the Clinical and Laboratory Standards Institute (CLSI, 2020) was used. Mueller-Hinton agar was melted and poured into sterile Petri plates, where it was allowed to cool for use. A sterile swab was used to evenly spread the inoculum across the surface of the solid agar to create a uniform bacterial lawn (Hossain, 2024).

Non-Combined Plant Extracts

The sterilized 6 mm discs were treated with 10µL (0.01 mL) of the different leaf extracts

using a micropipette (P20), and placed on the inoculated agar plates using a pair of sterile forceps at suitable distances apart (Bhandari *et al.*, 2024).

Combined Plant Extracts

A solution of *Azadirachta indica*, *Calotropis procera*, *Psidium guajava*, *Carica papaya*, and *Jatropha tanjorensis* combination was prepared separately in a concentration of 100 mg/ml (leaf + leaf extracts of different plants). 500µL / 0.5 mL from different plant extracts were thoroughly mixed to give a homogeneous solution of 100mg/ml (1:1). Sterile 6 mm filter paper discs were infiltrated with 10 µL (0.01 ml) of each combined extract mixture using a micropipette (P20) and placed on the inoculated agar plates using a sterile forceps at suitable distances apart according to Obuekwe *et al* (2020).

Positive and Negative Controls

As controls, ciprofloxacin was the positive control, while distilled water was used as the negative control to ensure solvent neutrality (Hemeg *et al.*, 2020).

Incubation and Measurements

The plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters with an electronic digital caliper and recorded (Hossain, 2024).

Data Collection

Data were obtained from the biochemical characteristics on triple sugar iron (TSI) agar test, catalyst test, citrate utilization test, oxidase test, of *Salmonella typhi*, morphological characteristics on shape, pigment, texture, elevation, size and margin, observations and measurements of minimum inhibitory zone.

Data analysis

Analysis of Variance (ANOVA) was used to determine the significant difference of the treatment means, and the means were separated using Duncan's multiple range test (DMRT). Analysis was done using SAS version 9.0 statistical tool.

RESULTS

Effect of the Leaves of the Research Materials on the Test Organism.

Table 1 presents the mean square estimates of leaf extracts of the selected individual plants on *Salmonella typhi*

The results show that there was a highly significant difference ($P \leq 0.01$) when the leaf extracts of the individual selected plants (*Azadirachta indica*, *Calotropis procera*, *Psidium guajava*, *Carica papaya*, and *Jatropha tanjorensis*) were used to treat the organism (*Salmonella typhi*) (Table 1).

Table 1: Mean square estimates of leaf extracts of the selected individual plants on *Salmonella typhi*

Source	Df	Leaf
Model	4	202.53**
Error	10	2.57

** - Highly significant

Effect of the selected leaf extracts on the Test Organism

Table 2 shows the mean performance of individual plants' leaf extracts against *Salmonella typhi*.

The results revealed that *Carica papaya* was more effective with a zone of inhibition of 35.23mm compared to all other plant leaf extracts used in this research. On the other hand, *Psidium guajava* had the least effect with a zone of inhibition of 13.00 mm. However, there was no statistical difference between the effect of *Calotropis procera* and *Jatropha tanjorensis* which have zones of inhibition of 22.13 mm and 20.00 mm respectively (Table 2).

Table 2: Mean performance of individual plants, leaf extracts against *Salmonella typhi*

SN	Treatment	Zone of inhibition (mm)
1	<i>Azadirachta indica</i>	18.83 ^c
2	<i>Calotropis procera</i>	22.13 ^b
3	<i>Psidium guajava</i>	13.00 ^d
4	<i>Carica papaya</i>	35.23 ^a
5	<i>Jatropha tanjorensis</i>	20.00 ^{bc}

Means with the same letters are not statistically different

Effect of the Combination of Different Leaf extracts on the Test Organism

Table 3 presents the mean square estimates of the combination of different leaves' extracts of the selected plants on *Salmonella typhi*. The results showed a highly significant difference ($P \leq 0.01$) when the different leaves of the selected plants were combined. (Table 3).

Table 3: Mean square estimates of the combination of different leaves extracts of the selected plants on *Salmonella typhi*

Source	Df	Different Leaves
Model	9	178.77**
Error	20	7.09

** - Highly significant

Effect of the Combination of Different Leaves extracts on the Test Organism

Table 4 shows the mean performance of the combination of different leaves extracts of the selected plants against *Salmonella typhi*. The results show that the combination of *Azadirachta indica* leaves with *Jatropha tanjorensis* has the highest effect (33.37). The least impact (12.50) was observed when the leaf of *Psidium guajava* was combined with the leaf of *Azadirachta indica*, and it was not statistically different from when the leaves of *Psidium guajava* were combined with those of *Calotropis procera* (14.13). However, there was no statistical difference from the leaf combination effect of *Jatropha tanjorensis* with the bark of either *Carica papaya* (33.17) or *Calotropis procera* (31.80) (Table 4).

Table 4: Mean performance of the combination of different leaf extracts of the selected plants against *Salmonella typhi*.

SN	Treatment	Mean inhibition (cm)
1	<i>Azadirachta indica</i> / <i>Calotropis procera</i>	25.20 ^b
2	<i>Azadirachta indica</i> / <i>Psidium guajava</i>	12.50 ^c
3	<i>Azadirachta indica</i> / <i>Carica papaya</i>	21.63 ^b
4	<i>Azadirachta indica</i> / <i>Jatrophanjorensis</i>	33.37 ^a
5	<i>Calotropis procera</i> / <i>Psidium guajava</i>	14.13 ^c
6	<i>Calotropis procera</i> / <i>Carica papaya</i>	33.23 ^a
7	<i>Calotropis procera</i> / <i>Jatrophanjorensis</i>	31.80 ^a
8	<i>Psidium guajava</i> / <i>Carica papaya</i>	26.17 ^b
9	<i>Psidium guajava</i> / <i>Jatrophanjorensis</i>	23.93 ^b
10	<i>Carica papaya</i> / <i>Jatrophanjorensis</i>	33.17 ^a

Effect of the positive and Negative Controls on the Research Organism

The results of ciprofloxacin which was the positive control indicated a very high

inhibitory effect (33.80) on *Salmonella typhi*, confirming that the isolate is sensitive to the standard antibiotic. On the other hand, distilled water as the negative control revealed no inhibitory effect (0.00) verifying solvent neutrality (Table 5).

Table 5: Shows the zone of inhibitions of ciprofloxacin (positive) and distilled water (negative) as the controls used against *Salmonella typhi*

Treatment	Zone of Inhibition
Ciprofloxacin	33.80
Distilled water	0.00

DISCUSSION

Leaf of extracts of individual plants

The leaf extracts of the plants were found to exert a very strong influence ($P \leq 0.01$) on the growth of *Salmonella typhi*, even though the different plant leaves varied in their inhibition against the organism. Among the five plants tested, the greatest inhibition activity was observed in *Carica papaya* with a mean inhibition zone diameter of 35.23 mm. This was followed by *Calotropis procera* with 22.13 mm, then *Jatropha tanjorensis* with 20.00 mm, *Azadirachta indica* with 18.83 mm, and the lowest for *Psidium guajava* at 13.00 mm (Table 2). *Carica papaya* leaves are active owing to high alkaloid, flavonoid, and saponin contents. Destabilization of bacterial cell membranes has been achieved in metabolites, which inhibit enzyme activity. Also, the extraction procedure and solvent can facilitate the bioavailability of bioactive molecules, according to Alhodieb *et al.* (2025). Likewise, Habeeb *et al.* (2024) & Babayemi *et al.* (2021) identified that phenolic compounds, tannins, and cardiac glycosides of *Calotropis procera* and *Jatropha tanjorensis* were found to be more effective in antibacterial activities under laboratory conditions.

Decreased *Azadirachta indica* leaf activity can be due to phytochemical concentration variation or seasonality in the production of secondary metabolites, since neem leaves contain limonoids, nimbin, and azadirachtin, though bioactive, which under controlled conditions are susceptible to medium inhibition (Ali *et al.*, 2021). *Psidium guajava* leaf, even though earlier reported to be highly antibacterial by its possession of quercetin and guajaverin, was the least active, and this may be either due to the susceptibilities of the strains or the inefficient extraction of its bioactive molecules (Gutierrez-Montiel *et al.*, 2025). When compared with the control, the 33.83 ± 0.15 mm zone diameter of ciprofloxacin (5 µg/disc) *Carica papaya* leaf extract was comparable with the standard drug and was concluded as a strong natural antimicrobial agent against *S. typhi*. A similar finding by Ogunleye *et al.* (2021) reported that the leaf of *Carica papaya* exhibited a zone of inhibition of 26.00 mm. This was however lower than the present study.

The results showed that the different leaf extract combinations were highly significant ($P \leq 0.01$) against *Salmonella typhi*. The different Leaf extract combinations of the selected plants showed varied inhibition against *Salmonella typhi*, with a range of mean

inhibition zones of 12.50 mm to 33.37 mm. The most potent among them were *A. indica* with *J. tanjorensis* (33.37 mm) and *C. papaya* with *C. procera* (33.23 mm), followed by *C. papaya* with *J. tanjorensis* (33.17 mm), and *J. tanjorensis* with *C. procera* (31.80 mm). These actively retaining blends are an example of a good synergistic effect due to the convergence of disparate phytochemical types such as alkaloids, flavonoids, saponins, and phenolic acids with differential antimicrobial action as supported by Donkor *et al.* (2023). Medium inhibitory zones were observed for *P. guajava* + *C. papaya* (26.17 mm) and *P. guajava* + *J. tanjorensis* (23.93 mm), and were shown with partial synergism according to Mauya and Mwakabona (2025), *P. guajava* was seen to exhibit medium activity upon supplementation with phenol-containing extracts.

Mixtures of *A. indica* and *C. procera* (25.20 mm) as well as *A. indica* and *C. papaya* (21.63 mm) showed that the organism was significantly inhibited as well. This is probably due to the synergistic action of neem limonoids, coupled with the bioactive cardenolides and saponins in *C. procera* and papaya alkaloids, as pointed out by Nurudeen and Falana (2021). Minimum inhibition was, however, obtained with *A. indica* + *P. guajava* (12.50 mm) and *C. procera* + *P. guajava* (14.13 mm), the antagonist interaction pattern whereby bioactive compounds with similar activities also inhibit the activity of the other (Caesar & Cech, 2019). In Comparison with the control (ciprofloxacin 33.83 ± 0.15 mm), *A. indica*+ *J. tanjorensis*(33.37 mm), *C. procera*+ *C. papaya* (33.23 mm), and *C. papaya*+ *J. tanjorensis*(33.17 mm), which are the most active combinations, attained the same range.

CONCLUSION

This current investigation revealed that the leaves of *Azadirachta indica*, *Calotropis procera*, *Psidium guajava*, *Carica papaya*, and *Jatropha tanjorensis* exhibited highly potent antibacterial activity against *Salmonella typhi*. Inhibition zones indicate that bioactive potential is immensely varied across plant parts and species. Significantly, the leaf of *Carica papaya* showed the greatest inhibition (35.23 mm). Also, the leaf of *Azadirachta indica* and *Jatropha tanjorensis* (33.37mm) combination showed the highest effect. The findings demonstrate the pharmacological potential of plant molecules and enhance maximum antimicrobial activity. The results obtained from this study revealed that the effect of the leaf extract of *Carica papaya* and the combination of the leaf extracts of *Azadirachta indica* and *Jatropha tanjorensis* are comparable to the effects of the positive control, Ciprofloxacin (33.80 mm). Based on the results obtained from this study, it can be concluded that the test materials were highly effective against the growth of *Salmonella typhi*.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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