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ETHNOBOTANICAL POTENTIAL OF *Carica papaya, Psidium guajava* AND *Azadirachta indica* LEAF, BARK EXTRACTS AND THEIR COMBINATION IN INHIBITING THE GROWTH OF *Salmonella typhi*

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ABSTRACT

The rise of antibiotic resistance among bacterial pathogens, including *Salmonella typhi*, represents a significant public health challenge worldwide. *Salmonella typhi*, the causative agent of typhoid fever, continues to cause high morbidity and mortality among people especially in developing countries. An Isolates of *Salmonella typhi* was obtained from Aminu Kano Teaching Hospital, Kano and treated with three different concentrations (50, 75 and 100 mg/ml) of *Azadirachta indica, Psidium Guajava* and *Carica papaya* using disc diffusion method. The results revealed that all the treatments were highly significantly effective against the test organism. The leaf extracts were more effective against the growth of *Salmonella typhi* than the bark and the combined. Similarly, *Carica papaya* performed better against the test organism than both *A. indica* and *P. guajava*. However, *Azadirachta* performed better (17.71 mm) than the other treatments (8.86 mm for *P. guajava* and 11.89 mm for *C. papaya*.) when combined. The effects of the concentration revealed that the concentration of 100 mg/ml was most effective across all the test materials. Based on the results obtained from this study, it can be concluded that the test materials highly inhibit the growth of *Salmonella typhi*. Similarly, the leaves of the test materials are more effective than the barks. Also, the leaf of *Carica papaya* inhibited more Salmonella than either *P. guajava* or *Azadirachta indica*.

Keywords: Ethno botanical, Potential, Leaf, Bark, Extracts, and Salmonella

INTRODUCTION

The genus *Salmonella* belongs to the Enterobacteriaceae family and comprises Gram-negative, facultatively anaerobic, rod-shaped bacteria. *Salmonella bongori* and *Salmonella enterica* are the two species of the *Salmonella* genus. The species *Salmonella enterica* consists of a large amount of serovars and the subspecies I (*Salmonella enterica* subsp. *enterica*) includes mostly those responsible for disease in humans and animals (Jolley *et al*., 2018). In humans, infection with *Salmonella* causes different clinical manifestations depending on the infecting serovar. While infection with *Salmonella enterica* serovar typhimurium (*S. typhimurium*) causes mild localized gastroenteritis, infection with *Salmonella enterica* serovar typhi (*S. typhi*) or *Salmonella enterica* serovar Paratyphi (*S. Paratyphi*) causes an acute systemic disease called typhoid or paratyphoid fever respectively, collectively referred to as enteric fever (Wong *et al*. (2015). Mogasale *et al*. (2014) reported that, around 14.3 million cases of enteric fever were recorded worldwide with an estimated 135.9 thousand deaths. 76.3% of cases (10.9 million) and 85.9% of deaths (116.8 thousand) were attributable to *S.* typhi.

The rise of antibiotic resistance among bacterial pathogens, including *Salmonella typhi*, represents a significant public health challenge worldwide. *Salmonella typhi*, the causative agent of typhoid fever, continues to cause high morbidity and mortality, especially in low- and middle-income countries (LMICs), where sanitation and healthcare resources are not easily available. According to the World Health Organization (WHO, 2021) approximately 11–21 million cases of typhoid fever occur annually, resulting in an estimated 128,000–161,000 deaths globally. This alarming burden is compounded by the increasing ineffectiveness of conventional antibiotics due to the emergence of multidrug-resistant (MDR) strains of *S. typhi* (Klemm *et al*., 2018). In response to this growing threat, researchers have turned to natural alternatives, such as medicinal plants, to explore their potential as sources of novel antimicrobial agents. Plants produce a broad range of biochemical compounds, i.e., alkaloids, flavonoids, tannins, and phenolics, which exhibit antimicrobial, antioxidant, and anti-inflammatory activities (Othman *et al*., 2019) Traditional medicine is also significantly relevant in healthcare worldwide, particularly in rural areas, especially in developing countries since it is readily accessible and much more affordable than other medications (Sah *et al*., 2020). This research was therefore aimed at determining the efficacy of the combination of the selected plants against the growth of *Salmonella typhi.*

For thousands of years, *Azadirachta indica* (neem) has been recognized for its wide array of beneficial properties, including those in agriculture for pest control and in traditional medicine for various common human ailments. This originally provoked world-wide interest due to its capacity as a non-toxic infection-control agent for use in farming (Olwenyi *et a*l., 2021). Almost every part of *A. indica* (e.g., the stem, bark, roots, leaves, gum, seeds, fruits, flowers, etc.) has been used as house-hold remedies for human illnesses (Ogidigo *et al*., 2022).

In the same vein, several studies have shown that *Carica papaya* (pawpaw) has antiparasitic and antibacterial properties. Ezike *et al*. (2010) examined the antimalarial potential of *Carica papaya* seeds in the treatment of malaria fever and reported significant antiplasmodial activity, with a marked reduction in parasite growth observed at higher extract concentrations. Doughari and Elmahmood (2008) also carried out a research using different concentrations of the leaf extracts of carica papaya against some intestinal bacteria and concluded that *Carica papaya* leaf extracts hold potential as alternative antimicrobial agents for treating gastrointestinal infections caused by enteric bacteria.

Similarly, *Psidium guajava* (guava) has been reported as a multipurpose medicinal plant that finds application all over the world. Current research has proved that guava leaves possess outstanding hepato-protective activity since they contain phenolic compounds that can suppress liver injury caused by oxidative stress (Chechani *et al*., 2024).

Antimicrobial activity of guava has also been adequately documented, where leaf extracts exhibit strong antibacterial and antifungal activity against the bacterial pathogens like *Escherichia coli* and *Staphylococcus aureus* (Ruksiriwanich *et al*., 2022). However, the synergy between the leaf and bark extracts of these plants have not been exhaustively examined, Hence this research intends to bridge that gap.

MATERIALS AND METHODS

Study Area

This research was carried out in the Department of Biological Sciences, Nigeria Police Academy (POLAC) in Wudil Local Government Area, Kano state. The area is located on Longitude 11.794244 and latitude 8.839021(Wikipedia, 2026)

Sample Collection

Test Organism

An isolate of Salmonella typhi was obtained from Aminu Kano Teaching Hospital, Kano. It was transported to Nigeria Police Academy, Wudil and stored in the refrigerator until required for use.

Reconfirmation of the Test Isolate

In order to ascertain that it was *Salmonella typhi*, the following reconfirmatory tests were carried out. They included Citrate Test, Triple Sugar Iron Test (TSI), Sulfur, Indole, Motility Test (SIM) as well as PCR.

Collection, Identification and Handling of Plants Materials

The plants materials which included *Psidium guajava, Carica papaya* and *Azadirachta indica* leaves and barks were collected from Danziyal Agricultural and Poultry Services LTD, at Yalwan Danziyal town of Rimin Gado LGA, Kano State. They were then authenticated at the herbarium unit of the Department of Plant Biology, Bayero University, Kano, Nigeria, and transported to the Laboratory, Nigeria Police Academy Wudil, Kano. The leaves and the stem barks were air-dried at room temperature for 7 days and pulverized to coarse powder using clean pestle and mortar. Sieve with tiny mesh was used to remove the coarse particles. The fine powder was then stored in a clean, sterile container as described by Ali *et al*. (2017).

Preparation of Plant Extracts

Five (5g) each of the powder of leaf and bark of *Azadirachta indica, Carica papaya* and *Psidium guajava* were dissolved in 100ml of ethanol in a stoppered reagent bottle respectively. The mixtures were then kept under room temperature for 7 days. Within those 7 days, the mixtures were shaken vigorously every 24hours. After 7 days, the resulting mixtures were filtered using a cheesecloth and then with Whatman filter paper-1. Sterile filter paper discs (Whatman No.1 (6mm) was then labelled accordingly (name of plant and part of the plant extracted). Using a micropipette, the discs were impregnated with a drop of extract according to the label on each of the disc. This was replicated three times.

Determination of the Antibacterial Properties of the Leaf and Bark Extracts of the Selected Plants (*A. indica, C. papaya* and *P. guajava*) against *Salmonella typhi*

Mueller Hinton Agar plates were labelled accordingly (name of plant and the part extracted). McFarland 0.5 standard was used to regulate turbidity and then a streak was made on the Mueller Hinton plate. The disc impregnated with plant extracts at different concentrations (50mg/ml, 75 mg/ml and 100 mg/ml) respectively were placed using a pair of forceps on the plate according to the label on the plate. Ciprofloxacin disc was used as control. The plate was then incubated for 24hours after which a transparent ruler was then used to measure the zone of inhibition. This experiment was replicated in three places

Data Analysis

Data was analysed for summary statistics and ANOVA was also carried out to determine significance of the effects of the extracts. Where such extracts are observed, the means were separated using New Duncan's Multiple Range Test (NDMRT).Correlation analysis was also carried out to determine relationship between the organism and the different parts of the plant materials.

RESULTS AND DISCUSSION

Effects of the leaf extracts of *A. indica, C. papaya* and *P. guajava* on the test organism were examined and the results revealed that there was a highly significant effect of all the tested plants' leaf and bark extracts on the test organism (Tables 1, 2 and 3).

Table 1: Mean Square Estimates of the Effects of Neem's Leaf, Bark and their Combination on the Growth of Salmonella Typhi

Source of Variation	DF	Leaf	Bark	Combined
Plant extract	2	38.88**	34.19**	93.44**
Error	6	1.64	0.72	4.06
F-value		28.78	47.34	28.07
Pr>F		0.0014	0.0002	0.0015

Table 2: Mean Square Estimates of the Effects of Guava's Leaf, Bark and their Combination on the Growth of Salmonella Typhi

Source of Variation	DF	Leaf	Bark	Combined
Plant extract	2	37.90**	21.15**	40.08**
Error	6	0.26	0.41	0.13
F-value		147	51.03	308.32
Pr>F		0.0001	0.0002	<0.0001

** = highly significant

Table 3: Mean Square Estimates of the Effects of Pawpaw's Leaf, Bark and their Combination on the Growth of Salmonella Typhi

Source of Variation	DF	Leaf	Bark	Combined
Plant extract	2	315.52**	44.12**	41.35**
Error	6	2.29	0.81	0.49
F-value		137.38	54.77	85.17
Pr>F		<0.0001	0.0001	<0.0001

** = highly significant

Mean Performance of the Different Parts of the Plants on Salmonella Typhi

Azadirachta indica

Table 4 presents the effects of the leaf, bark and combined extracts on the growth of Salmonella typhi. The results revealed that there was a progressive increase in the inhibition of the test organism as the concentration of the plant extract increased. Hence, it was observed that, the highest inhibition (19.01cm) occurred when 100mg/l of the leaf extracts was administered while, the lowest inhibition (12.00cm) was noticed when 50mg/l was used. A similar trend was noticed when the bark and the combined extracts were administered (Table 4).

In the same vein, when the extracts of *Psidium guajava* was used, the results indicated that there was a proportional effect of the different plant parts. The highest inhibition of 12.97 (leaf), 11.40cm (bark) and 12.17cm (combined) were observed when 100 mg/l of the extracts were used. The lowest effects of 5.90, 6.60 and 4.93 cm respectively were observed when 50 mg/l were administered. Furthermore, when the leaf extract of pawpaw was used to treat the test organism, the results showed that, the highest inhibition was observed at 100 mg/l and the lowest of 14.83 cm was observed when 50 mg/l of the extract was administered.

Furthermore, when the effects of the plant parts were compared, it was observed that the leaves of the three plants performed better against the organism compared to the barks and the combination of the parts in all the plant species except in *Azadirachta indica* where the combined treatment performed better at 100 and 75 mg/l (21.70 and 20.10) respectively. Comparing the effects of the different plant materials, the results revealed that the effects of C. papaya leaf and bark had more effect than those of *A. indica and P. guajava* but, when the leaves and barks were combined, *Azadirachta indica* performed better at 100 and 75 mg/l than the rest plants (Table 4).

Table 4: Effects of Different Concentrations of *Psidium guajava* on the Growth of Salmonella Typhi

Plant	Treatment	Mean inhibition (cm)		
	Mg/ml	Leaf	Bark	Combined
A.Indica	100	19.01a	11.83a	21.70a
	75	16.98a	8.10b	20.10a
	50	12.00b	5.10c	11.33b
Mean		15.98	8.34	17.71
S.E		1.28	0.85	2.01
P.Guajava	100	12.97a	11.40a	12.17a
	75	10.00b	7.03b	9.49b
	50	5.90c	6.60b	4.93c
Mean		19.65	8.34	8.86
S.E		0.61	0.64	0.36
C.Papaya	100	35.23a	19.93a	19.29a
	75	23.83b	18.10a	16.10b
	50	14.83c	12.57b	11.89c
Mean		24.61	16.87	16.74
S.E		1.51	0.89	0.69

Note: Means with the same letter within a column are not significantly different ($P \leq 0.05$)

Discussion

Medicinal plants have been employed a number of times to treat different ailments in the last several years. Attempt was made to use a few of them to inhibit the growth of *Salmonella typhi* in this research. The results showed a significant ($P \leq 0.01$) effect on the growth of the test organism. However, such effects were different among the different plant materials. The results showed that the leaf extracts were highly inhibitory to the growth *Salmonella* species. The results revealed a proportional relationship between the leaf extracts and the test organism. As the concentration of extract increased, a greater inhibitory zone was observed. Nevertheless, all treatments were noted to be very effective. However, the highest concentration of 100mg/ml was observed to be most effective in all the plant extracts used in this research. In comparison, leaves of *Carica papaya* out-performed the others producing 35.25 mm inhibition. This may be as a result of the high content of alkaloid, flavonoids and saponin which have the propensity for destabilizing the cell membranes of most bacteria. This can cause the inhibition of enzyme activities. This assertion had also been alluded to by Alhodieb *et al*. (2025). Decreased *Azadirachta indica* leaf activity may be as a result of variation in phytochemical concentration or seasonality in the production of secondary metabolites. Although neem leaves contain limonoids, nimbin, and azadirachtin which are bioactive, however, under controlled conditions they are susceptible to medium inhibition (Ali *et al*., 2021). *Psidium guajava* leaf has been reported to contain quercetin and guajaverin which makes them to be highly antibacterial, it performed the least in this study (13.21 mm).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 the results obtained from this study was compared with the standard drug (Ciprofloxacin) which served as the positive control, it was observed that there was no significant difference in their effects suggesting that the test materials was highly effective in inhibiting the growth of Salmonella typhi compared with the control (33.83 ± 0.15 mm). 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 but equally highly effective.

Furthermore, the bark of *Carica papaya* had the greatest effect on the test organism when compared with the other plants. This effect is however moderate when compared with the control with 33.80mm activity. This result suggests that there is lower alkaloid and phenolic extractable concentrations in the bark, as compared to the leaves. This result was similarly reported by Sharma *et al*. (2025). *Azadirachta indica* bark was less inhibitory due to the variation of bioactive compounds like nimbin and limonoids, and having lower bark extract activity compared to the leaves (Wylie & Merrell, 2022). *Psidium guajava* bark was lowest as reported by Defino (2024), indicating that its crude bark extracts have poor activity towards Gram-negative bacteria.

When the leaf and bark of the plants were combined, the results indicated that, the inhibitory effect of Azadirachta was significantly improved, out-performing the combinational effects of *P. guajava* and *C. papaya* respectively. This may be due to the complimentarity of bioactive elements of the two parts of the plant. Although the results do not match the effect of the control, it gives an indication that the combination of the leaf and bark of *Azadirachta indica* may be more useful in inhibiting the growth of *Salmonella typhi* than the separate parts of this plant.

CONCLUSION

Based on the results obtained from this study, it can be concluded that the plant extracts highly inhibited the growth of *Salmonella typhi.* Similarly, the leaf extracts of the plants were more effective than their barks. Also, the leaf of *Carica papaya* inhibited more *Salmonella* than either *P. guajava* or *Azadirachta indica.* Furthermore, the concentration of extracts at 100 mg/ml was more nhibitory to the growth of the organism than 50 or 75 mg/ml.

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