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Evaluation of Antimicrobial Susceptibility of *Salmonella* Isolated from Household Cockroaches Using *Carica Papaya* Leaf Extract

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ABSTRACT

The human pathogen, *Salmonella,* constitutes a significant source of human infections through poultry products. *Salmonella* is a bacteria that exists in both domestic and wild animals, and there are need to subdue its strength since its remains a significant source of food borne infection worldwide. The aims of these work are to investigate the antimicrobial susceptibility of *Salmonella* when exposed to natural organic substances, specifically *carica papaya* (pawpaw) leaf extract, and to bring to the understanding of the medicinal and vital importance of pawpaw leaf. Household cockroaches, known as carrier of salmonella, were used as the as source of bacterial isolation. To do this, isolation of *Salmonella* were obtained from the bodies of thirty household cockroaches samples by immersion in peptone water, followed by a serial dilution was performed in order to reduce the microbial load, which is aseptically cultured in a sterile disposable petri dish containing Xylose lysine deoxycholate agar to selectively grow *Salmonella* colonies. The distilled water and ethanol were used as solvent in producing the *carica papaya* leaf extract in varying concentration to perform an antimicrobial susceptibility tests. In conjunction, Antimicrobial susceptibility tests were conducted using the agar well diffusion method to evaluate the inhibitory effects of the extracts on the *Salmonella* isolates. The results indicated that pawpaw leaf extracts possess bioactive compound capable of inhibiting *Salmonella* growth, with ethanol extracts showing higher antimicrobial activity compared to aqueous extracts. However, limitations of this research were explored, and we considered ways to improve its accuracy. This research has implications for food safety, human well-being, and the development of natural antimicrobial agents and future studies should focus on optimizing extraction method and identifying specific active compounds to enhance its efficacy.

Keywords: Antimicrobial susceptibility, *Salmonella,* Food borne, Cockroach, Food safety.

INTRODUCTION

For a number of years, the increasing demand for herbal products has led to a quantum jump in the volume of plants (Beyene *et al*., 2016; Rahman *et al*., 2022). Therefore, the use and history of herbs date back to the time of early man (Petrovska, 2012), who had the crudest tools as his implements and used stones to start his fire. They used herbs in raw and cooked forms to keep fit. Medicinal herbs are used as medicine in traditional systems (Sofowora *et al*., 2013), which have helped eradicate infections since that time (Isola, 2013). Some of the issues encountered in the usage of medicinal plants (i.e., herbs) include little information on trading possibility and product standardization (Kunle *et al*., 2012).

Salmonella is a bacteria of the *enterobacteriaceae* family, which are gram-negative microorganisms classified as non-lactose fermenters (McDonough *et al.,* 2000). The genus *Salmonella* consists of a variety of serotypes that have a wide host range due to their versatile pathogenic capabilities (Fàbrega and Vila, 2013). The nomenclature of *Salmonella* has evolved over the past decades, and currently, a two-species system is widely used (Abatcha *et al.,* 2014). Over 99% of serotypes are grouped under the species *S. enterica,* while only a handful of serotypes belong to the species *S. bongori* (Yan *et al.*, 2004). Serotyping and phage typing, together

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with ever-improving molecular subtyping techniques. The two known species of *Salmonella* are *Salmonella* enterica and *Salmonella bongari* (Saporito *et al*., 2017). However, *Salmonella* is a deadly bacteria causing a lot of danger to human health (WHO, 2018). Especially in rural areas where pit toilets have been adopted and the rate of household pests such as cockroaches is at its peak (Fathpour and Emtiazi, 2003; MMPC, 2022). *Salmonella* has been a global concern, affecting individuals in both developed and developing countries. Its ubiquity in the environment, coupled with its ability to infect a variety of hosts, including humans, animals, and even plants, makes it a formidable public health challenge (Wiedemann *et al.*, 2015). Each year, millions of cases of salmonellosis are reported worldwide, making *Salmonella* a leading cause of food-borne illnesses (He *et al*., 2023). *Salmonella* infections primarily occur through the consumption of contaminated food, especially poultry, eggs, and other animal-derived products (WHO, 2018; Giannella, 1996). However, it can also be transmitted through direct contact with infected animals, their environments, or through person-to-person transmission in certain instances (NCEZID, 2022). This versatility in transmission routes underscores the importance of comprehensive public health measures to mitigate its impact.

Household pest, particularly cockroach, according to Britannica (2023), a cockroach is a black or brown straight-winged insect of the order *blattodae.* It's a scavenging insect that resembles a beetle, having long antennae and legs and a typically flattened body. Several tropical kinds have become established worldwide as household pests. Cockroaches are insects of significant medical importance because of their tendencies to transmit diseases mechanically (Ikeh et al., 2023). The perception on the role of cockroaches in disease transmission revealed that cockroaches are potential mechanical transmitters of disease pathogens (Wahedi *et al.,* 2020). The biology of cockroaches facilitates the adhesion of bacteria to their exoskeletons, especially on their legs and antennae, and they can swallow bacteria that persist in their gut. When they navigate food-preparation areas, their bodies and excretions may contaminate surfaces, food, and utensils, disseminating pathogens such as *Salmonella* to people.

To address *salmonella* infection, natural plant derived antimicrobial agents have gained attention. *Carica papaya* (pawpaw) belongs to the family *Caricaceae* (Adeneye, 2014). It has the following common names: Pawpaw Tree, Papaya, Papayer, Tinti, Fan Kua, Wan Shou Kuo, Kavunagaci, Kepaya, etc. (Anibijuwon and Udeze, 2009). The parts of pawpaw used include the leaves, fruit, seed, latex, and root (Ganaie, 2021). Pawpaw plants are characterized as an erect, unbranched tree or shrub, fast-growing, 7-8 m tall with copious latex, and trunks of about 20 cm in diameter (Islam *et al.,* 2015). The plant is also described in documented property forms, and it acts as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, and laxative, pectoral, stomachic, and vermifuge (Anibijuwon and Udeze, 2009). Carica papaya consists of many biochemically active compounds (Sharma, 2022). Javanese believe that eating papaya prevents rheumatism (Dawson, 1998). The plant kingdom synthesizes diverse active compounds, which are highly important in the control and treatment of a lot of diseases. These compounds are principally secondary metabolites. Some of the active compounds do occur singly or in combination with other inactive substances, which greatly hinder the life processes of microbes, especially the pathogenic microbes. Medicinal plants are less expensive and a renewable source of pharmacological active substances.

The aim of this study is to determine the susceptibility of *Salmonella* using pawpaw leaf extract prepared with ethanol and water at different concentrations and to explore the potentials and importance of pawpaw leaf extract for combating *Salmonella*, with implications for food safety, community health, and the development of eco-friendly antimicrobial agent.

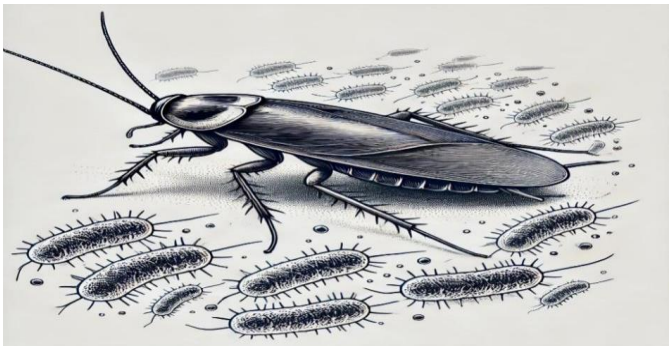

Figure 1: Cockroach as a Vector of *Salmonella*

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MATERIALS AND METHODS

Plant source

The *Carica papaya* (pawpaw) leaves were harvested from a pawpaw tree in Abeokuta, Ogun State. The leaves were aseptically collected in a sterile bag, washed in a sterile distilled water to remove contaminant and debris, and air dried at room temperature under Aseptic conditions, In succession, they were transferred to microbiological laboratory, where they are dried using the hot air oven in drying the leaves until its brown and brittle under controlled temperature. The sterilized electrical blender was used in grounding the dried leaves into a fine powder.

Collection and preparation of cockroach samples

Thirty live cockroaches were obtained from a household and transported to a microbiological laboratory in a sterile, ventilated container.

Isolation and Identification of *Salmonella*

A 10- fold serial dilution method was adopted to isolate *Salmonella* from cockroaches. Initially, these cockroaches were immersed in peptone water in a covered container, and they remained in the peptone water till they died. Then, the cockroaches were removed aseptically from the peptone water and disposed of. One mL from the 10^2 and 10^3 dilutions was pipette into the sterile petri dish respectively and a pour plate method for isolated was conducted for the cultivation of the microorganism using the prepared xylose lysine deoxycholate agar at 121 degrees Celsius for 15 minutes where 20mL of freshly prepared cooled xylose lysine deoxycholate agar were dispensed in a sterile petri dishes , and it was incubated for 24 hours.

Preparation and Application of Pawpaw leaf extract.

The leaves were harvested from a pawpaw tree and aseptically collected in a sterile bag, washed in a sterile distilled water to remove contaminant and debris, and air dried at room temperature under Aseptic conditions, In succession, they were transferred to microbiological laboratory, where they are dried using the hot air oven in drying the leaves until its dried and brittle under controlled temperature. The sterilized electrical blender was used in grounding the dried leaves into a fine powder.

Figure 2: Pawpaw tree (Miho *et al.*, 2020).

Preparation of the pawpaw leaf diluent

For the purpose of this study, distilled water and ethanol are the solvents used as the diluent. The grounded powdered pawpaw leaf was measured in four sterile bottles, which are measured at 12.5 g, 25 g, 37.5 g, and 50 g, respectively. The bottle containing 12.5 g of powdered pawpaw leaf was dissolved with 87.5 g of distilled water. The bottle containing 25g of powdered pawpaw leaf was dissolved with 75g of distilled water. The bottle containing 37.5g of powdered pawpaw leaf was dissolved with 62.5g of distilled water, and the bottle

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containing 50g of powdered pawpaw leaf was dissolved with 50g of distilled water to achieve a percentage concentration; the same measurement is applicable to ethanol. It was preserved at room temperature in a sterile environment to avoid any forms of contamination for 24 hours. After 24 hours, the diluent was sieved, and each diluent was stored in a sterile sample bottle, making a total of eight bottles (four extracts from the distilled water and four for the ethanol).

Microbiological analysis

After the cultivation of the organism on the Xylose lysine deoxycholate agar (XLD agar) in a plate that was incubated for 24 hours, the morphology of the growth of the microorganism shows red colonies with black centers. Gram staining procedure was employed for the identification of the organism that grew on the XLD agar plate under the microscope, which showed some characteristics features of *Salmonella* such as rod shape, non-clustering, colonies are independent, i.e., they are not forming chains.

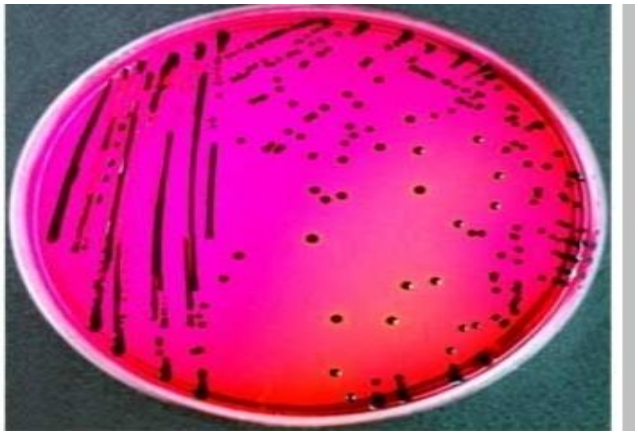

Figure 3: *Salmonella* on XLD.

Determination of Antimicrobial Susceptibility Test

The Muller Hinton agar is used to determine the antimicrobial susceptibility test for microorganisms. it is prepared in the autoclave for 121 degrees Celsius at 15psi. Muller-Hinton agar is a type of growth medium used in microbiology to culture bacteria isolated and test their susceptibility to antibiotics. MH agar may be used in the laboratories for the rapid presumptive identification of C. albicans as an alternative method for germ tube testing (Mattie 2014). The microorganism (*Salmonella)* is inoculated on the MH AGAR PLATE by creating a lawn on the plate. Then a sterilized browler of 2-3mm was used to make small holes in the middle of each of the eight plates, respectively, and 0.5 ml of the diluent (ethanol and distilled water) was introduced into the hole. Then it was incubated for 24 hours at 37 degrees Celsius.

Figure 4: Mueller Hinton Agar Jar

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Statistical Analysis

We performed series of statistical analyses to evaluate the relationship between extract type (Ethanol or Water), solvent percentage, concentration, and the presence of a zone of inhibition. First, a Chi-Square test was conducted to examine the association between extract type and the zone of inhibition(Singhal and Rana, 2015). Following this, three separate logistic regression models were developed to assess how solvent percentage and concentration influenced the zone of inhibition and the Odd ration was reported with the 95% confident interval (Table 1)(Nepal et al., 2015). To further investigate trends, a Cochran-Armitage test for trend was performed to examine a potential linear relationship between increasing solvent percentage and concentration and the zone of inhibition. Finally, Wilcoxon rank-sum tests were conducted to compare the solvent percentage and concentration between the two groups (Yes and No for zone of inhibition)(Haynes, 2013). These tests were chosen because they do not require assumptions about the data's distribution, making them suitable for comparing medians between independent groups. The analysis was done using R programming software.

Table 1: Included and excluded predictor variables across three logistic regression

	Model One	Model Two	Model Three
Solvent Percentage	√	X	√
Extract	√	√	√
Concentration	X	√	√

RESULTS

The analysis focused on the effects of extract type, solvent percentage, and concentration on the inhibition zone. Statistical methods, including chi-square tests, logistic regression, and trend analysis, were employed to evaluate the relationships between these factors and microbial inhibition.

Table 2 shows that water extracts had no antimicrobial effect across all concentrations. Ethanol extracts inhibited Salmonella at lower concentrations (12.5 g and 25 g) but lost effectiveness at higher concentrations (37.5 g and 50 g), indicating ethanol enhances *Carica papaya's* antimicrobial activity at specific levels.

Table 2: Effect of Concentration on Zone of Inhibition by Extract Type

Extract Type	**Concentrate(g)**	**Percentage of solvent (%)**	**zone of inhibition**
Water	12.5	87.5	**X**
	25	75	**X**
	37.5	62.5	**X**
	50	50	**X**
Ethanol	12.5	87.5	√
	25	75	√
	37.5	62.5	**X**
	50	50	**X**

Table 3 indicate that there is no significant association between the type of extract (Ethanol vs. Water) and the zone of inhibition (Yes and No). The Cochran-Armitage test (Table 4) validated this result with $p > 0.05$ indicating no significant trend between concentration, solvent percentage and the zone of inhibition. The

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Wilcoxon Rank-Sum test(Table 5) showed no significant differences in the zone of inhibition based on concentration (W = 10, p = 0.232) or solvent percentage (W = 2, p = 0.232).

Table 3: Association between the extract and zone of inhabitation

Test	Estimate	P values
Chi -square	0.67	0.4142

Table 4: Cochran-Armitage Test for Trend Evaluating the Relationship on Zone of Inhibition

	Cochran-Armitage	P values
Concentration	1.4606	0.144
Solvent percentage	-1.4606	0.144

Table 5: Wilcoxon Rank-Sum Test Results Assessing Differences on Zone of Inhibition

	W	P values
Concentration	10	0.232
Solvent percentage	2	0.232

Models M[1] and M[2] show no significant effect(Table 6), with both Wald ($p > 0.05$) and Likelihood Ratio tests ($p > 0.05$) indicating no statistically significant association. However, Model M[3] reveals a significant Wald test result ($p < 0.05$), suggesting a significant impact of the factors on the zone of inhibition. The Likelihood Ratio test for Model M[3] was not significant ($p > 0.05$), indicating that this specific test did not support the conclusion reached by the Wald test.

Table 6: The impact of solvent percentage and extract type on the zone of inhibition

Model	Wald Test	Likelihood Ratio Test
M[1]	2.944(p>0.05)	3.786(p>0.05)
M[2]	2.944(p>0.05)	3.786(p>0.05)
M[3]	3562.96(p<0.05)	0.032(p>0.05)

For Model M[1], the Chi-square values for the intercept and solvent percentage were 1.866 and 1.955, respectively, indicating no significant association with the zone of inhibition. In Model M[2], the Chi-square value for the intercept was 1.592, and the effect of solvent percentage was not considered. For Model M[3], the Chi-square values for the intercept, solvent percentage, and extract type were all low (close to 0.022), suggesting no significant effect of these variables on the zone of inhibition. Extract Ethanol was used as the reference category in all models, and Concentration was included only in Model M[3] with a Chi-square value of 0.022.

Table 7: Chi-square results for the logistic regression model assessing solvent percentage and extract type on zone of inhibition

	M[1]X^2	M[2]X^2	M[3]X^2
Intercept	1.866	1.592	0.022

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Solvent Percentage	1.955		0.022
Extract Ethanol	ref	ref	ref
Extract Water	2.372	2.372	0.022
Concentration		1.955	0.022

Table 8 presents the odds ratios (OR) for the factors influencing the zone of inhibition across three models (M1, M2, M3). In Model M1, Solvent percentage (OR: 1.097, CI: 0.97 to 1.64), indicating a slight increase in the likelihood of zone inhibition as solvent percentage increases. The Extract Water (OR: 0.062), indicating a significantly lower likelihood of inhibition compared to the reference extract (Ethanol). In Model M2, with Extract Water's (OR: 0.06), indicating a reduced likelihood of inhibition. Solvent percentage and Concentration were not significant in this model. In Model M3, The Extract Water variable also shows no significant impact, with an OR close to 1 (0.999), suggesting that it does not significantly influence the zone of inhibition. Similarly, Concentration shows a minimal effect with an OR of 1.00, indicating no change in the likelihood of inhibition based on

Table 8: Factors influencing zone of inhibition

	OR[M1]	OR[M2]	OR[M3]
Intercept	0.002[0.00 to 10.13]	18.12[0.26 to 9,116,369]	1.00[0.00 to 8.40]
Solvent Percentage	1.097[0.97 to 1.64]		0.999[0.97 to 1.08]
Extract Ethanol	Ref	Ref	Ref
Extract Water	0.062[0.00 to 1.89]	0.06[0.61 to 1.03]	0.999[0.99 to 1.00]
Concentration		0.91[0.00 to 1.89]	1.00[0.99 to 1.00]

DISCUSSION

The study investigated the antibacterial efficacy of pawpaw *(Carica papaya)* leaf extracts, prepared in distilled water and ethanol, against *Salmonella* spp. The observed results suggest that the solvent used for extraction plays a critical role in determining the antimicrobial potency of the pawpaw leaf. From the results obtained, study showed that the plate labeled with 12.5g of powdered pawpaw leaf filled with 0.5 ml of extract obtained from the pawpaw leaf soaked in 87.5g of distilled water showed no zone of inhibition. The plate labeled 25g of powdered pawpaw leaf filled with 0.5ml of extract obtained from pawpaw leaf soaked in 75g of distilled water showed no zone of inhibition, as did the remaining two plates (37.5g and 50g). Furthermore, the plate labeled with 12.5g of powdered pawpaw leaf filled with 0.5 ml of extract obtained from the pawpaw leaf soaked in 87.5g of ethanol showed a zone of inhibition and The plate labeled 25g of powdered pawpaw leaf filled with 0.5ml of extract obtained from pawpaw leaf soaked in 75g of ethanol showed a zone of inhibition, but the remaining two plates labeled (37.5g and 50g) were observed not to have a zone of inhibition. It is evident that pawpaw leaf extracts prepared with different solvents and concentrations had varying effects on the susceptibility of *Salmonella*. Specifically, pawpaw leaf soaked in distilled water exhibited no antimicrobial activity across all tested concentrations. This absence of inhibition indicates that distilled water as a solvent does not effectively extract the bioactive compounds necessary to impact *Salmonella* growth. This finding is consistent with previous studies suggesting that water-based extracts may not always capture the full range of antimicrobial components present in certain plant materials (Eloff, 2019).

In contrast, ethanol as a solvent demonstrated significant effectiveness in extracting bioactive compounds from pawpaw leaves, especially at higher concentrations (Asghar et al., 2016). The ethanol-soaked pawpaw leaf

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extracts at 75g and 87.5g concentrations showed measurable zones of inhibition against *Salmonella*, with inhibition zones of 7.0mm and 8.9mm, respectively agreing with a study conducted by Dada *et al*.(2019). These results suggest a dose-dependent relationship between the concentration of the ethanol extract and its antimicrobial effect. Ethanol's higher solvency allows it to dissolve and extract more phytochemicals, such as alkaloids, flavonoids, and phenols, which are likely responsible for the observed antimicrobial activity against *Salmonella* (Ali and Oyeyi, 2018, Altemimi et al., 2017).).

The increased inhibition at higher concentrations of ethanol extract points to the potential of pawpaw leaves as a source of natural antimicrobial agents against *Salmonella*. However, the absence of inhibition in water-based extracts highlights the importance of solvent choice when assessing the antimicrobial properties of plant materials. These findings contribute to a growing body of evidence that supports the selective effectiveness of plant-based treatments and the critical role of solvent choice in maximizing their efficacy.

The logistic regression analysis revealed key insights into the role of these factors. Odds ratio estimates showed that solvent percentage had a minimal, non-significant effect on inhibition (OR = 1.097, 95% CI: 0.97 to 1.64), while the Water extract had consistently lower odds of inhibition compared to Ethanol (OR = 0.062 in M[1]). This highlights Ethanol as the more effective solvent for enhancing antimicrobial activity. Concentration emerged as an influential factor in Model M[3] ($p < 0.05$), emphasizing its potential to significantly impact microbial inhibition when combined with other variables.

CONCLUSION AND RECOMMENDATION

Conclusion

The method used in the preparation of the diluent from the pawpaw leaf was demonstrated, and the evaluation of the antimicrobial susceptibility test was performed on the Muller Hinton agar in which it was observed that the concentration of the two highest grams of the ethanol diluent showed a zone of inhibition, which proved that those two concentrations are susceptible to salmonella.

Recommendations

Since a lot of problems caused by salmonellosis affecting both humans and animals (e.g. poultry birds) as well as loss of income for livestock farmers, mortality, consumption of infected food by cockroaches leading to a high rate of sickness, etc., public enlightenment should be given to the general public on exhibiting a sanitary environment in order to reduce the vector (cockroach) spreading this *Salmonella* infection. Further research should be done to determine the component highly present in pawpaw leaf and ethanol in higher concentrations that are capable of inhibition and produce drugs to reduce a high rate of sickness such as diarrhoea and bloody faeces.

Specifically, the odds ratios suggested that concentration might play a more significant role in the likelihood of inhibition than either the solvent percentage or extract type. However, the overall lack of consistent significant results across different tests points to the need for further research to better understand the complex interactions between these factors. A larger sample size, different solvent types, and varying concentrations may provide more robust findings in future studies.

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