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Full Length Article

In Vitro Antibacterial Activity of Crude and Ethanolic Extracts of Some Selected Plants Against *Salmonella gallinarum*

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Abstract

This study investigated the antibacterial efficacy of crude and ethanolic extracts of selected medicinal plants including *Carica papaya*, *Azadirachta indica* (Neem), *Allium sativum* (garlic), *Moringa oleifera* (Moringa), *Zingerber officinale* (ginger) and *Aloe vera* against *Salmonella gallinarum*, a major pathogen in poultry responsible for fowl typhoid. Antibacterial activity was assessed through zone of inhibition assays and minimum inhibitory concentration (MIC) values. The ethanolic extract generally exhibited strongest antibacterial activity than their crude extracts. Papaya ethanolic leaf extract demonstrated the highest efficacy (zone of inhibition 24 mm and low MIC value) effectively inhibiting *Salmonella gallinarum* growth. In contrast, *Moringa oleifera* showed weaker antibacterial effect in both forms as indicated by the smaller zone of inhibition and higher MIC values. Notably, enrofloxacin used as reference antibiotic exhibited lower activity than *Carica papaya*, which outperformed it against *Salmonella gallinarum*. These findings suggest that ethanolic extracts enhance the bioavailability of antibacterial phytochemicals and support the potential of plant-based therapeutics. The study highlights the promising use of ethanolic plant extracts, especially C*arica papaya* as natural antibacterial agents for controlling *Salmonella gallinarum* infection in poultry.

Keywords: *Salmonella gallinarum*; Ethanol extract; Crude extract; Medicinal plants; Antibacterial activity; Broiler ceca

Introduction

Since ancient times, medicinal plants have been utilized as natural medicines to cure a wide range of illnesses. Various plants extracts are now widely used for their potential antibacterial properties in the treatment of numerous health conditions (Dagne *et al.* 2021). One of such plants is *Azadirachta indica*, commonly known as neem, which belongs to the Meliaceae family. Traditionally, different parts of the neem tree including its leaves, barks, roots, seeds and flowers have been used to manage ailments such as infections, fever, inflammatory disorders, skin disease and dental problems. Among these the leaves are particularly prized for their medicinal qualities due to having numerous biological activities including antibacterial (Lolge *et al.* 2024). *Aloe vera* is a well-known plant in the Liliaceae family that is valued for both its aesthetic qualities and therapeutic properties (Martínez-Burgos *et al.* 2022). It has long been used in traditional and indigenous medicine systems to treat a variety of illnesses (Singh *et al.* 2025). Its microbiological qualities have garnered a lot of scientific attention lately, particularly considering the growing global antibiotic resistance. Research has demonstrated that *Aloe vera* gel exhibits inhibitory effects against a range of gram positive and gram-negative bacterial pathogens, including *Vibrio parahaemolyticus, Klebsiella pneumoniae* and *Staphylococcuss aureus* (Nageshwari *et al.* 2024a), *E coli, Salmonella typhi, Helicobacter pylori, Propionibacterium acnes* and *Pseudomonas aeruginosa* (Nageshwari *et al.* 2024b). The antibacterial activity of *Aloe vera* is largely attributed to specific bioactive constituents. Compounds such as anthraquinones and saponins possess inherent antimicrobial properties, while polysaccharides contribute to immune defense by enhancing the activity of white blood cells in combating bacterial infection (Gupta 2025).

Carica papaya, commonly known as papaya, is a tropical plant that has been traditionally valued for its medicinal benefit (Yogiraj *et al.* 2014). Many parts of plants, especially leaves and seed have been utilized extensively in traditional medicine because of their purported ability to

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promote health (Krishna *et al.* 2008). Its wide range of bioactive substances such as flavonoids, alkaloids, papain, and carpaine, are thought to have therapeutic potential (Kaur *et al.* 2019). This plant has gained increasing interest in both conventional medical procedures and contemporary pharmaceutical research (Nafiu *et al.* 2019). Ginger or *Zingiber officinale*, is a member of the Zingiberaceae family and has long been used as a medicinal herb (Banerjee *et al.* 2011). According to Mao *et al.* (2019), ginger has long been used to alleviate ailments. Clinical investigations have demonstrated that ginger-based formulation enhances clinical and preclinical outcomes and speed symptom recovery in individuals suffering from severe acute respiratory syndrome caused by COVID-19. *Zingiber officinale* and its active ingredients have also been reported to possess antimicrobial activity (Aaisha *et al.* 2020). Garlic has been reported as one of the best natural treatments for bacterial infections because of its strong antibacterial qualities (Magryś *et al.* 2021). Likewise, *Moringa oleifera* has been reported to possess antibacterial properties attributed to its gallic acid, isothiocyanate, niazimicin, tannins, saponins, alkaloids and flavonoids content (Ndhlala *et al.* 2014). Of them, chemical 4 (α-L-rhamnopyranosyl-oxy-isothiocyanate) has been found to be especially effective at preventing the development of bacteria by interfering with important enzymatic processes (Bukar *et al.* 2010). The present study was carried out to study the antibacterial activity of crude and ethanolic extract of *Carica papaya, Azadirachta indica, Allium sativum, Moringa oleifera, Zingiber officinale* and *Aloe* vera against *Salmonella gallinarum* isolated from Broiler Ceca.

Material and Methods

Sample collection

A total 120 samples of small intestine were collected from the district Sanghar in polythene bags containing normal saline and then brought to Central Veterinary Diagnostic Laboratory Tandojam for further processing.

Isolation and identification of *Salmonella gallinarum*

Salmonella Shigella agar (S.S agar) was prepared for culture of *Salmonella* and then intestinal contents were diluted with normal saline, after that sample were streaked with the help of wire loop on already prepared media and incubated at 37°C for 24 hours to obtain primary culture. The bacterial colonies were further incubated in nutrient broth for the antibiotic sensitivity test.

Morphological characterization by Gram's staining method

A small colony among the representative *Salmonella gallinarum* colonies was picked up from SS plates with a bacteriological loop, smeared on separate glass slide and fixed by gentle heating. Crystal violet was applied on each smear to stain for two minutes and then washed with running water. Few drops of Gram's iodine were then added to act as mordent for one minute and again washed with running tap water. Then acetone alcohol was added (as decolorizer) for few seconds. After washing with water, safranin was added as counter stain allowing to stain for 2 minutes. Then the slides were washed with water, blotted and dried in air and then examined under microscope with high power objective (100X) using immersion oil.

Collection of plant material

The ginger, garlic, leaves of Papaya, neem and moringa plants were collected from local agriculture farm in the vicinity of Tandojam. The plants leaves were washed with tap water and dried at room temperature. The powder was obtained from the leaves of the plants by grinding through electrical grinder and finally by filtration through muslin cloth.

Crude and ethanol extraction of plants

The powder (25 g) of plants was weighed and put into 100 ml of 95% of ethanol and 100 mL distilled water separately. The mixture was kept in conical flask for 24 h with constant interval shaken. After that mixture was filtered using Whatman No. 1 filter paper. Filtered solution was kept open for evaporation of ethanol to obtain concentrated dried extract and finally 25% of each was made. The dried samples were kept at 4°C in refrigerator until further use.

Antibacterial susceptibility test

The antibacterial susceptibility test was performed by disc diffusion method. The discs (ginger, garlic, leaves of papaya, neem and moringa) were prepared by soaking in 5 μL (1.25 mg) and 10 μL (2.50 mg) of plants extract. The bacterium was cultured on Muller Hinton agar. The soaked discs were placed on the cultured medium and pressed gently with sterilized forceps. Whereas, the enrofloxacin discs containing 5 µg concentration was purchased from Basingstoke, Hampshire England Company. The petri dishes were kept in an incubator at 37°C for 24 h to check the susceptibility of organism against the treatment by measuring the zone of inhibition.

Antibacterial susceptibility test through MIC

In current study, the crude and ethanol extract of herbs and enrofloxacin were used to determine the antibacterial susceptibility test. It was observed by micro broth dilution method on Muller Hinton medium. For minimum inhibitory concentration (MIC), a dilution of 1:1000 medium was prepared. The dilution ranging from 250-0.48 mg/mL was prepared from plants, and 0.5 McFarland standard was followed for MIC. In well numbers 1 to 10 (50 μL of bacterial culture broth + 50 μL Muller Hinton broth + plants

20 µL) was added. While in A11 only bacterial culture broth and 100 µL and A12 was dispensed with Muller Hinton medium 100 µL as control. Then the MIC plates were incubated at 37°C for 24 h. Consequently, the break points were recorded to observe minimum inhibitory activity of herbs and enrofloxacin by observing transparency and turbidity of the medium.

Statistical analysis

Data were entered and coded in a Microsoft Office Excel 2016 spread sheet. Analysis was performed using statistical software *i.e.*, Statistix 8.1 version, USA through factorial design analysis of variance (ANOVA). Further for critical comparison among the mean values the Least Significant Difference (LSD) test was applied.

Results

A total of 120 samples of ceca were collected and subjected for pathogen testing, where over total samples, 44 were found positive for *Salmonella gallinarum* while remaining 76 number of samples noted to be negative (Fig. 1). Pathogen was identified based on their morphology, cultural characteristics and staining reactions.

Antibacterial susceptibility test through disc diffusion

In present study the crude and ethanolic extract of *Aloe vera*, papaya, ginger, neem, garlic and moringa each at concentration of 1.25 and 2.5 mg were used to compare the efficacy against *Salmonella gallinarum*. The results in Fig. 2 indicate a significant decrease in antibacterial activity across all groups when tested with a single concentration (1.25 mg) of crude extract of plants. Papaya showed significantly higher zone of inhibition (13.00 mm), followed by garlic (10.00 mm), *Aloe vera* (8.00 mm), ginger (7.00 mm) and moringa (6.00 mm). While at double concentration (2.5 mg) shown in Fig. 3 the papaya leaves extract again exhibited the greatest zone of inhibition (20.00 mm), followed by neem (19.00 mm), garlic (17.00 mm), *Aloe vera* (15.00 mm) and ginger (14.00 mm) but moringa appeared to be the least in antibacterial activity. When ethanolic extract was compared with the pure plants extract at single concentration (1.25 mg), the results presented in Fig. 4 show that the extract of papaya and neem have inhibited the growth of organism in a similar fashion ($P > 0.05$) and made the zone of 12.60 and 12.00 mm in diameter, respectively. But it was markedly higher than that of garlic (11.00 mm) followed by *Aloe vera* (10.00 mm), ginger (8.00 mm) and moringa (7.00 mm), while later both zones of inhibition were noted statistically similar ($P > 0.05$).

While the concentration of plants extract was used as double as 2.5 mg, the results shown in Fig. 5 indicate that the papaya leaves extract again inhibited the growth remarkably ($P < 0.05$) significant (zone size; 24.33) compared to that of neem (23 mm) garlic (zone size; 20 mm), *Aloe vera* (zone size; 17 mm) and ginger (zone size; 16 mm), while moringa exhibited the lowest zone of inhibition (11 mm). However, the extract of *Aloe vera* and ginger displayed the zone of inhibition statistically similar ($P > 0.05$) to each other. Reaffirming the weaker antimicrobial potential of moringa, the standard antibiotic enrofloxacin demonstrated 14 mm zone of inhibition.

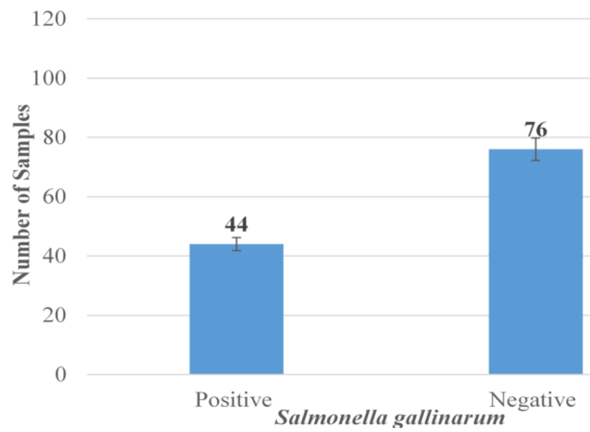
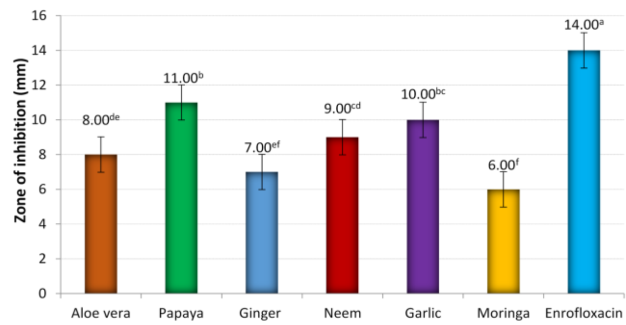
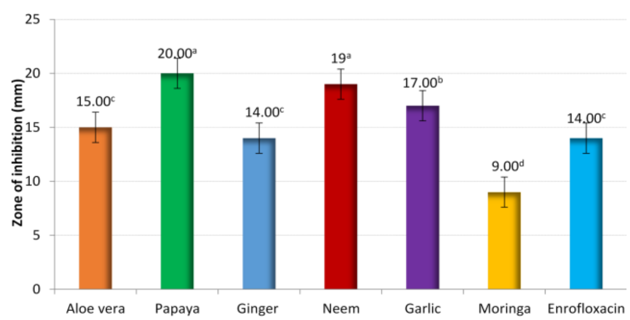


Fig. 1: Number of positive and negative ceca samples for *Salmonella gallinarum*



LSD (0.05) = 1.8414; SE± = 0.8452
Mean values with different superscripts are significantly different ($P < 0.05$) from one another

Fig. 2: Average zone of inhibition (mm) using crude plant extract at concentration of 1.25 mg against *Salmonella gallinarum*



LSD (0.05) = 1.7143; ±SE = 0.7868
Mean values with different superscripts are significantly different ($P < 0.05$) from one another

Fig. 3: Average zone of inhibition (mm) using crude plant extract at concentration of 2.5 mg against *Salmonella gallinarum*

Antibacterial susceptibility test through minimum inhibitory concentration (MIC)

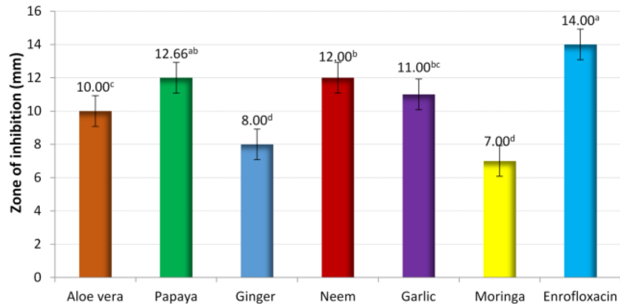
The MIC test was performed to determine the antibacterial activity of six different plants extract each at concentration of 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9, 0.97 and 0.48 mg/mL in comparison to enrofloxacin against *Salmonella gallinarum*, and the results are present in Table 1. It was observed that the MIC values varied significantly among the tested plants extracts. The Group 2 which correspond to papaya extract exhibit the lowest MIC 15.62 mg/mL indicating the strongest antibacterial activity (40%) in contrast to other plants extract. Further, the papaya plant extract had affected the bacterial activity significantly than the other plant extracts like *Aloe vera*, ginger, neem, garlic and moringa at each MIC from 250 to 0.97 mg/mL. Moreover, the extract of this plant was found to be effective in antibacterial activity even at lowest MICs like 3.9, 1.9 and 0.79 mg/mL where it showed antibacterial effect as 16, 8 and 2%, respectively in comparison to other plant extracts. Subsequently, the extract of plant neem remained next to papaya for antibacterial activity at each MIC and showed 88, 81, 68, 52, 35, 22, 10 and 2% at concentration of 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, and 1.9 mg/mL, respectively. While Group 6 which corresponds to Moringa extract showed the relativity to weaker antibacterial activity (*i.e.* 58%) at highest MIC (125 mg/mL) compared to other all plant extracts, the smaller the minimum inhibitory concentration (MIC), the stronger the antibacterial activity.

Different concentrations of ethanolic plants extract were also used to determine the minimum inhibitory concentration (MIC) against *Salmonella gallinarum.* The result in Table 2 indicates that the MIC values varied significantly ($P < 0.05$) among the tested plants extracts. Group 2 (papaya) indicated strongest antibacterial activity at lowest MIC value of 15.62 mg/mL. In contrast Group 6 (Moringa extract) showed the highest MIC value of 125 mg/mL, indicating relativity weaker antibacterial activity, however it revealed weaker antibacterial activity either at 61.5, 31.25, 15.6 or 7.8 mg/mL in comparison to all other plant extracts. While Group 2 (papaya extract) further proved higher antibacterial activity at almost all concentrations like 61.5, 31.25, 15.6,7.8, 3.9, 1.9 and 0.97 mg/mL compared to other groups as Group 1 (*Aloe vera*), 3 (Ginger), 4 (Neem), 5 (Garlic), 6 (Moringa) and 7 (Enrofaxicin). Further Table 2 shows that the Group 4 (Neem extract) found to be the next stronger in antibacterial activity at 61.5, 31.25, 15.6 or 7.8 mg/mL, followed by Group 5 (Garlic extract) and Group 1 (*Aloe vera* extract). The plant extract *Aloe vera* (Group 1) remained the medium in antibacterial activity in contrast to other plants extract.

Discussion

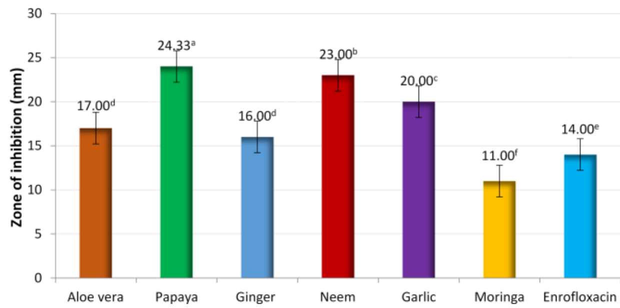
Prevalence of Salmonella

In current study out of total 120 samples of ceca collected and subjected for pathogen testing 44 were found positive while remaining 76 number of samples were negative for *Salmonella gallinarum.* The overall prevalence rate was found as 36.6% in distric Sanghar. This is only slightly lower than the 41.8% sero-prevalence reported by Shoaib *et al.* (2020) in Rawalpindi, underscoring that *Salmonella gallinarum* remains a major concern in regional poultry. On broader scale global meta analysis data shown an average prevalence of about 8.5% over many years (Zhou *et al.* 2022).



LSD (0.05) = 1.6922; ±SE = 0.7766
Mean values with different superscripts are significantly different ($P < 0.05$) from one another

Fig. 4: Average zone of inhibition (mm) using ethanolic plant extract at concentration of 1.25 mg against *Salmonella gallinarum*



LSD (0.05) = 1.2276; ±SE = 0.5634
Mean values with different superscripts are significantly different ($P < 0.05$) from one anther

Fig. 5: Average zone of inhibition (mm) using ethanolic plant extract at concentration of 2.5 mg against *Salmonella gallinarum*

Zone of inhibition by selected plants

The key findings of current study on antibacterial activity of crude plants and ethanolic extract of plants against *Salmonella gallinarum* revealed that ethanolic extract of selected plants generally exhibited higher antibacterial activity against *Salmonella gallinarum* compared to crude extracts. These observations align with prior study which has consistently demonstrated that ethanolic extracts of medicinal plants exhibited strong antibacterial activity compared to crude forms (Alzohairy 2016). Among the tested samples the ethanolic extract of papaya leaves displayed the most pronounced antibacterial effect with a zone of inhibition measuring 24 mm at double concentration significantly

Table 1: Minimum inhibitory concentration (MIC) of pure extract of plants against *Salmonella gallinarum*

Groups		Concentrations (mg/mL)										Control	Medium
		250	125	61.5	31.25	15.6	7.8	3.9	1.9	0.97	0.48		
1	Percentage	84^{cd}	74^{c}	56^{c}	43^{d}	29^{d}	14^{c}	4^{c}	0^{d}	0^{b}	0^{0}	0^{0}	0^{0}
2		90a	84^{a}	72^{a}	56^{a}	40^{a}	28^{a}	16^{a}	8^{a}	2^{a}	0^{0}	0^{0}	0^{0}
3		81^{de}	71^{c}	52^{d}	35^{e}	21^{e}	11^{d}	3^{c}	0^{d}	0^{b}	0^{0}	0^{0}	0^{0}
4		88^{ab}	81^{ab}	68^{b}	52^{b}	35^{b}	22^{b}	10^{b}	2^{b}	0^{b}	0^{0}	0^{0}	0^{0}
5		86^{bc}	79^{b}	67^{b}	49^{c}	32^{c}	20^{b}	9^{b}	2^{bc}	0^{b}	0^{0}	0^{0}	0^{0}
6		73^{f}	58^{e}	40^{e}	27^{f}	15^{f}	5^{e}	1^{d}	0^{d}	0^{b}	0^{0}	0^{0}	0^{0}
7		79^{e}	67^{d}	58^{c}	43^{d}	28^{d}	12^{cd}	3^{c}	1^{c}	0^{b}	0^{0}	0^{0}	0^{0}

1 = *Aloe vera*, 2 = Papaya, 3 = Ginger, 4 = Neem, 5 = Garlic, 6 = Moringa, 7 = Enrofloaxcin
Mean values with different superscripts are significantly different ($P < 0.05$) from one another

Table 2: MIC of ethanolic extract of plants against *Salmonella gallinarum*

Groups		Concentrations (mg/mL)										Control	Medium
		250	125	61.5	31.25	15.6	7.8	3.9	1.9	0.97	0.48		
1	Percentage	85^{d}	74^{d}	59^{c}	51^{d}	40^{d}	23^{d}	14^{d}	6^{c}	2^{b}	0^{b}	0^{0}	0^{0}
2		96^{a}	89^{a}	77^{a}	65^{a}	52^{a}	39^{a}	28^{a}	16^{a}	8^{a}	2^{a}	0^{0}	0^{0}
3		80^{e}	68^{c}	53^{e}	42^{e}	35^{e}	18^{e}	11^{e}	2^{d}	0^{c}	0^{b}	0^{0}	0^{0}
4		92^{b}	82^{b}	69^{b}	58^{b}	47^{b}	32^{b}	20^{b}	9^{b}	2^{a}	0^{b}	0^{0}	0^{0}
5		89^{c}	79^{c}	64^{c}	56^{c}	43^{c}	29^{c}	16^{c}	7^{c}	2^{b}	0^{b}	0^{0}	0^{0}
6		72^{f}	58^{f}	43^{f}	35^{f}	28^{f}	16^{e}	7^{f}	2^{d}	0^{c}	0^{b}	0^{0}	0^{0}
7		79^{e}	67^{e}	58^{d}	43^{e}	28^{f}	12^{f}	3^{g}	1^{d}	0^{c}	0^{b}	0^{0}	0^{0}

1 = *Aloe vera*, 2 = Papaya, 3 = Ginger, 4 = Neem, 5 = Garlic, 6 = Moringa, 7 = Enrofloxacin
Mean values with different superscripts are significantly different ($P < 0.05$) from one another

surpassing its crude extract which exhibit 18 mm zone of inhibition. The results of current study are in agreement with the finding of Nayak and Khasim (2015), who reported strong antibacterial activity of papaya leaf extract against salmonella spps. A similar trend was observed with neem and garlic extracts, where ethanolic extract demonstrated more potent antibacterial activity then crude extract (Subapriya and Nagini 2005). Notably, both crude and ethanolic extracts showed concentration dependent antibacterial activity with double concentration generally exhibit larger zones of inhibition than single concentrations, supporting previous observation of dose response antimicrobial activity in plants extracts (Parekh and Chanda 2007). According to Clinical and Laboratory Standards Institute ethanolic extract of papaya leaves was classified as susceptible with a zone of inhibition of 24 mm outperforming its crude extract counterpart which was categorized as Intermediate classifications of their crude extracts. The majority of crude extract including those from *Aloe vera*, ginger and moringa were classified as resistant, highlighting their relatively limited antibacterial potential in current study, these finding has also been documented in earlier work (Cowan 1999). The enrofloxacin had a zone of inhibition 14 mm also classified as resistance, the ethanolic extract of papaya leaves not only surpasses the efficacy of most crude extracts but also outperforms enrofloxacin, highlighting its potential as a potent natural antibacterial agent against salmonella. These finding highlight the superior antibacterial activity of ethanolic plants extract over crude extracts against Salmonella.

Minimum Inhibitory concentration of selected plants

A comparative analysis of the minimum inhibitory concentration values of crude and ethanolic plants extract against *Salmonella gallinarum* reveals that ethanolic extract generally exhibit stronger antibacterial activity, and these findings consistent with previous studies that have demonstrated significantly lower MIC value for ethanolic plant extracts indicating higher efficacy compared to crude or aqueous forms (Parekh and Chanda 2007). Among the tested plants the ethanolic extract of papaya demonstrated the most potent antibacterial activity, with a notably low MIC value such observations are supported by Nayak and Khasim (2015). Who also reported strong antimicrobial effect of papaya ethanolic leaf extracts against salmonella spps. These results suggest that ethanol enhances the bioavailability and extraction efficacy of active phytochemicals responsible for antibacterial effect (Cowan 1999). While moringa extracts exhibited weaker antibacterial activity in both crude and ethanolic forms in contrast to all other plant extract as indicated by their higher MIC values. These out comes align with the findings reported by Rehman *et al.* (2009) who noted that moringa extract has only moderate or low antibacterial potential against Gram negative bacteria such as *Salmonella*.

Conclusion

In general, it was concluded that ethanolic extracts of selected plants generally exhibited higher antibacterial activity against *Salmonella gallinarum* compared to crude extracts. Particularly the ethanolic extract of papaya leaves presented the most pronounced antibacterial effect with high size of zone at double concentration markedly greater than its crude extract which shows lower zone of inhibition. Over total tested plants the ethanolic extract of papaya again confirmed the most potent antibacterial activity with significantly lower MIC value.

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Author Contributions

RB and SB conceived and designed the study. RB contributed to the analysis of data. SGM completed the write up. ASM participated in overall helped during the study period.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data in this work will be accesssible upon reasonable request to the corresponding author

Ethical Approval

Not applicable to this paper.

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