Trop J Phytochem Pharm Sci, September 2025; 4(9): 378 - 383 **ISSN 2955-1226 (Print)**
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Effect of Different Extracts and Fractions of *Senna Siamea* Leaf on *Salmonella gallinarum* and *Salmonella pullorum*

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ABSRTACT

Salmonellosis in poultry is treated with antibiotics. This often leads to antibiotic residues and antibiotic resistance, thereby posing a serious health challenge. This study aimed to evaluate the efficacy of *Senna siamea* leaf extracts and fractions against *Salmonella gallinarum* and *Salmonella pullorum* the causative agents of fowl typhoid and pullorum disease in poultry. *Senna siamea* leaf extracts were prepared by maceration of the pulverized leaves in petroleum ether. Different quantities of the marc (residue) obtained were macerated in methanol, ethanol, and water. The ethanol extract was further fractionated into chloroform, ethyl acetate, butanol, and aqueous fractions. The antimicrobial activity of the extracts and fractions were evaluated *in vitro* using the agar well diffusion assay. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts and fractions were also determined. The results showed that the three extracts (methanol, ethanol, and aqueous) demonstrated a concentration-dependent increase in inhibition of *S. pullorum* and *S. gallinarum*, with the aqueous extract exhibiting the highest inhibitory activity at 800 mg/mL with inhibition zone diameters of 17.0 ± 0.1 mm and 19.0 ± 0.1 mm against *S. pullorum* and *S. gallinarum*, respectively. Among the fractions, the ethyl acetate fraction showed the highest inhibitory activity at 200 mg/mL against *S. gallinarum* with inhibition zone diameter of 24.0 ± 0.1 mm, while the chloroform and n-butanol fractions showed the highest inhibitory activity at 200 mg/mL against *S. pullorum*. These findings suggest that *Senna siamea* leaves is a promising source of bioactive compound(s) that could be used in the treatment of fowl typhoid and pullorum disease caused by *Salmonella gallinarum* and *Salmonella pullorum*, thereby reducing the issue of antibiotics residues in meat and eggs.

Keywords: *Salmonella gallinarum*, *Salmonella pullorum*, *Senna siamea*, Fowl typhoid, Pullorum disease.

Received 16 June 2025
Revised 29 October 2025
Accepted 30 October 2025
Published online 01 November 2025

Introduction

Avian Salmonellosis remains a major problem in poultry production.[1] It is known to be one of the multidrug-resistant infectious that has been a problem in the poultry sector.[2] Salmonella species are rod-shaped Gram-negative bacteria, which are divided into *Salmonella enterica* and *Salmonella bongori*. The enterica subspecies enterica is made of more than 1400 serovars. The enterica species which include *Salmonella gallinarum* and *Salmonella pullorum*, are known to lack a flagellum (non-flagellated) and are unable to move by themselves (non-motile).[3] In poultry, the most predominant infectious diseases are Fowl Typhoid (FT) and pullorum disease (PD).[3] Fowl Typhoid, a disease in which the bacteria spread in the blood (septicemic disease) is caused by *Salmonella gallinarum*. It mainly affects matured poultry flocks, and occurs during the laying period.[2] This disease is known to cause acute systemic infection, resulting in a death rate of around 80% of infected poultry birds. Pullorum disease on the other hand, is caused by *Salmonella enterica* serovar biovar *Salmonella pullorum*. The infection may cause organ failure, resulting in serious complications and death in chicks that are newly hatched. As a result of this, poultry farmers witness high losses.[4] Clinical manifestation that characterizes chickens infected with fowl typhoid includes depression, ruffled feathers, loss of appetite, thirst, yellow diarrhoea, and lack of desire to move.

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Citation: Otor M., Akpomie T., Usman A. Effect of different extracts and fractions of *Senna siamea* leaf on *Salmonella gallinarum* and *Salmonella pullorum*. Trop J Phytochem Pharm Sci, 2025; 4(9): 378 – 383 http://www.doi.org/10.26538/tjpps/v4i9.1

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Signs seen in the organs of dead birds include bronzed, swollen liver, with small necropsy, enlargement of the kidneys and Spleen, anemia, and inflammation of the anterior small intestine.[1] Symptoms associated with pullorum disease are similar to those of fowl typhoid. They include: loss of appetite (anorexia), moodiness, diarrhoea, and death.[5] Sometimes, some of the infected chicken could recover from the infection caused by *Salmonella pullorum*. Surviving birds may not have any clinical symptoms, but as carrier birds, they may be carrying this pathogen, which can be transmitted vertically to newly hatched chicks or other birds. These diseases have been reported to be eliminated in the commercial poultry sector in developed countries, while in developing countries like those in Asia, Africa, and Central and South America, the disease is still causing great economic loss. *Salmonella gallinarum* and *Salmonella pullorum* have a close resemblance, including the same lipopolysaccharide (O) antigens, the absence of flagella due to mutations of genes associated with flagella, and the same host birds.[6] *Salmonella gallinarum* and *Salmonella pullorum* can be differentiated by biochemical reactions like maltose, glucose, and dulcitol fermentation, and decarboxylation of ornithine.[7] DNA-based molecular technology, have been employed to identify Salmonella biovars.[7] Isolates of these bacteria can be identified and differentiated with an antibody test and/or polymerase chain reaction (PCR). Other specialized techniques such as plasmid profile analysis, pulsed field gel electrophoresis, PCR-restriction fragment length polymorphism (RFLP), multilocus variable-number tandem-repeat analysis (MVLA),[4] or ribotyping may be useful in epidemiological investigations.[8]
These diseases can be spread through the mouth during feeding and drinking of water, or by cannibalism, and through the respiratory system. Transmission of the causative agent may also occur through wounds.[4] Clinical cases have been treated with antibiotics such as amoxicillin, potentiated sulfonamide, tetracyclines, streptomycin, and fluoroquinolones.[3] Despite the antibiotic treatment effort, *Salmonella*

gallinarum and *Salmonella pullorum* has not been eliminated from poultry birds, and has also contributed to antibiotic resistance and drug residue in meat. Several vaccines, such as live *Salmonella gallinarum* 9R strain for fowl typhoid and *Salmonella pullorum* ghost vaccine, have been employed for the treatment of pullorum disease and fowl typhoid. Though many developed countries have successfully eradicated host-specific Salmonella (FT and PD) via testing and slaughtering at infected farms, the scenario is different in developing countries, where eradication is not realistic and may not be an option.

Unregulated administration of antibiotics at low doses over a long period of time for disease control and growth promotion has been linked to the antimicrobial resistance crisis globally.[9] The increase in antibiotic-resistant strains from bacteria, viruses, fungi, and protozoa and the inability of several drugs to tackle this problem have prompted a search into plant-based, natural products. Plants are known to be the primary source of secondary metabolites (bioactive compounds) with potential as a suitable agent for the development of nutritional and pharmaceutical drugs.

The efficacy of medicinal plants is attributed to the ability of their bioactive compound to interact with pathogens in several ways, unlike most antibiotics, with a single target site. The activities of these secondary metabolites from medicinal plants can disrupt the cell membrane of a pathogen, and also, stimulate the immune system of the host, protect the intestinal wall of the host from being colonized, and help to promote the growth of beneficial bacteria. These have resulted in increasing research into medicinal plant-derived natural products as an alternative to synthetic drugs. Plants produce a vast array of natural products or bioactive compounds which are known to exhibit more complex reaction patterns compared to synthetic drugs, which have been observed as flat, rigid molecules with a high degree of aromatic character.[10] Natural products also have a high number of ring systems and chiral centers, making them to have more sterically complex structures. They also have lower numbers of nitrogen, halogen, and sulfur atoms, and higher oxygen content compared to synthetic drugs. Other properties include higher molecular weights, lower hydrophobicity, and increased polarity compared to synthetic drugs. These observable structural characteristics have enabled natural products to provide highly selective and specific biological activities.[11]

Many studies have been conducted on *Senna siamea* (family Fabaceae) for its anti-salmonella activity. Both the aqueous and ethanol extracts of *Senna siamea* have been reported to inhibit the growth of *Salmonella typhi*.[12] Similar activity has been reported against *Salmonella spp*.,[13] but none against *Salmonella gallinarum* and *Salmonella pullorum*. This study therefore aims to evaluate the effect of some crude extracts and fractions of ethanol extract of *Senna siamea* leaves on *Salmonella gallinarum* and *Salmonella pullorum* as a preliminary step towards the isolation and identification of the bioactive compounds responsible for the anti-salmonella activity of the plant.

Materials and Methods

Plant collection and identification

Senna siamea leaves (Figure 1) was harvested in March 2024 from its natural habitat in National Veterinary Research Institute Vom, Plateau State, Nigeria (Latitude 9.7300o N and longitude 8.7874o E). The plant was identified by Mr. O.E Agyeno at the Herbarium of the Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria where a voucher specimen number JUHN23000627 was assigned.

Figure 1: *Senna siamea* leaves

Drying and pulverization of plant material

Senna siamea leaves were rinsed in clean water and dried at room temperature with good ventilation to avoid fermentation by microbes and loss of beneficial bioactive compounds as a result of unwanted biochemical processes of plant secondary metabolites.[14] The dried leaves were pulverized using a wooden mortar and pestle and stored in a clean polythene bag.

Extraction of plant material

Powdered *Senna siamea* leaves (1 kg) was macerated in 1.5 L of 100% petroleum ether at room temperature for 48 h. The extract was filtered and the marc (residue) was dried. The marc was divided into three portions. A 500 g portion of the marc was extracted by maceration in 1 L of absolute ethanol at room temperature for 48 h. The extract was filtered through a 150 µm sieve, then re-filtered through Whatman No. 1 filter paper.[15] Similarly, 250 g and 200 g portions of the marc were macerated in 1 L each of absolute methanol and water, respectively. The methanol and ethanol extracts were air dried, while the aqueous extract was dried at 60°C in a Carbolite hot air oven (model PIN200). The extracts were weighed and their percentage yields were calculated using the following formula (Equation 1):

$$Percentage\ yield = \frac{\text{weight of extract obtained}}{\text{weight of the sample extracted}} \quad \text{..... (Eq, 1)}$$

Phytochemical screening

The phytochemical composition of the extracts was evaluated following the procedure previously reported by Otor *et al.*, (2023).[16]

Fractionation (liquid-liquid extraction)

The ethanol extract was subjected to fractionation by liquid-liquid extraction. Briefly, 10.58 g of the crude ethanol extract was reconstituted with 200 mL of distilled water in a 1000 mL conical flask, covered with a stopper, and allowed to dissolve properly by agitation. The extract solution was poured into a separating funnel, followed by the addition of 100 mL chloroform. The mixture was agitated for 3 minutes, then allowed to stand for separation into two phases. The lower chloroform phase was collected into a volumetric flask, and the upper aqueous layer was collected into another volumetric flask. The aqueous layer was poured back into the separating funnel, and the process was repeated until a clear chloroform layer was obtained. The aqueous layer was returned to the separation funnel, and fractionated successively with ethyl acetate and n-butanol following the same procedure as described for chloroform. The chloroform, ethyl acetate, and butanol fractions were air dried, while the aqueous fraction was oven dried at 50°C.[14]

Preparation of bacterial inoculum and biochemical test

Three colonies from a 24 h pure culture were collected from microbiology laboratory of Central Diagnostic National Veterinary Research Institute Vom, Plateau State, Nigeria. The colonies were suspended in 5 mL of sterile saline solution. The solution was mixed thoroughly to obtain a homogeneous suspension.

A microbact plate was placed on a holding tray, and with the aid of a sterile Pasteur pipette, 100 µL of the bacterial suspension was dispensed into each well in separate rows of the microplate. Sterile mineral oil was used to overlay the substrates using a Pasteur pipette. The inoculated rows were sealed with an adhesive and labelled appropriately. The plates were incubated at 37°C for 24 h.

The Salmonella was identified biochemically according to the procedure reported by.[17]

Antimicrobial activity screening

Isolates of *Salmonella gallinarum* and *Salmonella pullorum* were obtained from microbiology laboratory of Central Diagnostic National Veterinary Research Institute Vom, Plateau State, Nigeria.

The anti-*Salmonella* (*gallinarum* and *pullorum*) activity of the crude extracts and fractions was determined using the agar well diffusion method.[18] A freshly prepared broth was poured into a petri dish and

allowed to solidify at room temperature. The plates were incubated at 37℃ for 24 h before being used, to ensure that there is no contamination of the plates. Aliquot (1 mL) of the already prepared isolate was inoculated into the broth by evenly distributing it by gentle swirling of the plate. Four wells were made on the broth in the plates. The wells were filled with about 1 mL of the extract (200, 400, and 800 mg/mL). Dimethyl sulfoxide (DMSO) was used as a control since it was used to dissolve the extract. The petri dish was incubated at 37℃ for 24 h.[18] The experiment was done in triplicate. After 24 hours, the antimicrobial activity of the extracts and fractions was assessed by measuring the zone of inhibition. Streptomycin (200, 400, 800 mg/mL) was used as a standard drug.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC were determined by preparing four concentrations of the extracts and fractions. For the crude extract, a 400 mg/mL concentration was prepared, and three other concentrations; 200, 100, and 50 mg/mL, were prepared by serial dilution. A loopful of isolate was inoculated into the extract in the test tube and incubated at 37℃ for 24 h. The test tubes were observed for turbidity at different concentrations for MIC. MBC was determined by re-culturing the inoculum from each of the test tubes in a petri dish containing a broth and incubating at 37℃for 24 h. The plates were observed for growth, and the MBC was recorded as the least concentration of extract in which there was no growth of the bacteria.[16]

Statistical analysis

Data were presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS IBM version 23. Data were subjected to one-way analysis of variance (ANOVA), and differences between mean values were regarded as significant at $p \leq 0.05$.

Results and Discussion

Extraction yields

The quantities of the extracts and fractions obtained from the leaves of *Senna siamea* are presented in Table 1. A total of 1000 g of the pulverized leaves was defatted with petroleum ether. Approximately 4 g of fat was obtained, while 16.49 g of ethanol extract corresponding to a percentage yield of 3.29% was obtained from 500 g of the marc. The methanol and aqueous extracts yielded 11.10 and 22.21 g, respectively. The percentage yield (4.44%) for the methanol extract was lower than values reported from previous studies for this plant.[19,20]. The percentage yield (11.10%) of the aqueous extract was also lower compared to what was previously reported.[21]

Table 1: Yield of crude extracts and fractions of *Senna siamea* leaves

Extract	Weight of powdered sample/residue (g)	Yield (g)	Percentage yield (%)
Petroleum ether	1000	4.21	0.42
Ethanol	500	16.49	3.29
Methanol	250	11.10	4.44
Aqueous	200	22.21	11.10
Fraction			
Chloroform	10.58	5.021	47.50
Ethyl acetate	10.58	0.50	4.69
Butanol	10.58	0.65	6.19
Aqueous	10.58	4.39	41.48

On subjecting 10.58 g of crude ethanol extract to partitioning in chloroform, ethyl acetate, and n-butanol, the following percentage yields were obtained; 47.45% for chloroform fraction, 4.69% for ethyl acetate fraction, 6.19% for butanol fraction, while the remaining aqueous fraction gave a percentage yield of 41.48%.

Phytochemical constituents of Senna siamea leaves

The phytochemical screening of the crude powdered leaves, aqueous, methanol, ethanol, and petroleum ether extracts of *Senna siamea* leaves revealed the presence of tannins, saponins, cardiac glycosides, flavonoids, alkaloids, and steroids in all the extracts except the petroleum ether extract in which none of the phytochemicals was detected (Table 2). This finding is similar to that reported in some previous studies except for the absence of one or two phytochemicals.[3,20,22] The absence of some of these phytochemicals may be due to differences in the methods of extraction or abiotic environmental factors at the location of plant collection. Environmental factors such as light, climate, edaphic and biotic interaction have been reported to affect the type and quantities of plants' secondary metabolites.[23]

Table 2: Phytochemical constituents of *Senna siamea* leaves

Phytochemical	**Result**				
	Crude powdered leaves	**Aqueous extract**	**Methanol extract**	**Ethanol extract**	**Petroleum ether extract**
Tannins	+	+	+	+	-
Saponins	+	+	+	+	-
Cardiac glycosides	+	+	+	+	-
Flavonoids	+	+	+	+	-
Alkaloids					
Steroids	+	+	+	+	-

Key: (+) Detected; (-) Not detected

Biochemical test result

The result of the biochemical tests on the organisms are shown in Table 3. Gram staining, motility, gas from glucose, inositol, lactose, salicin, indole, urease, oxidase, adonitol, and ornithine decarboxylase were all negative for *Salmonella gallinarum*, while rhamnose, trehalose, xylose, arginine dihydrolase, dulcitol, maltose, mannitol, arabinose, and lysin decarboxylase were found to be positive.

Table 3: Biochemical test result on *Salmonella gallinarum* and *pullorum*

Biochemical test	**Result**	
	Salmonella. gallinarum	***Salmonella pullorum***
Gram staining	Negative red	Negative red
Motility	-	-
Citrate	Delayed	Delayed
Gas from glucose	-	+
Adonitol	-	-
Arabinose	+	+
Dulcitol	+	-
Inositol	-	-
Lactose	-	-
Maltose	+	Delayed
Mannitol	+	+
Rhamnose	+	+
Salian	-	-
Trehalose	+	+
Xylose	+	+
Indole	-	-
Urease	-	-
Oxidase	-	-
Arginine dihydrolase	+	+
Lysine decarboxylase	+	+
Ornithine decarboxylase	-	+

Key: (+) Reactive, (-) Not reactive

However, the citrate test was delayed for more than 24 hours. Although these parameters cannot be used as a confirmatory test, they indicated that the test organism is *Salmonella gallinarum*. On the other hand, Gram staining, adonitol, dulcitol, inositol, lactose, salicin, indole, urease, and oxidase were all found to be negative in the biochemical test for *Salmonella pullorum*. The test was positive for gas production by fermentation of D-glucose, arabinose, mannitol, rhamnose, trehalose, arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase, while the citrate and maltose tests were delayed for more than 24 hours. These findings are comparable to that reported by [24] for *Salmonella pullorum* from broiler chicken. From the results of the biochemical test, the two species of salmonella used in this study were identified as *Salmonella gallinarum* and *Salmonella pullorum*. They were distinguished from one another by the fact that *Salmonella pullorum* decarboxylates ornithine, while *Salmonella gallinarum* ferments dulcitol.

Antimicrobial activity of extracts and fractions of Senna siamea leaves

Tables 4 – 7 presents the antimicrobial activity of crude extracts and fractions of *Senna siamea* leaves. The results showed that the three crude extracts (methanol, ethanol, and aqueous) inhibited the growth of *S. pullorum* and *S. gallinarum* in a concentration-dependent manner. The aqueous extract exhibited the highest inhibitory activity against *S. pullorum*, with inhibition zone diameter of 17.0 ± 0.1 mm at 800 mg/mL, followed by the methanol and ethanol extracts, with inhibition zone diameters of 16.0 ± 0.1 and 15.0 ± 0.1 mm, respectively at the same concentration. For *S. gallinarum*, the ethanol and aqueous extracts exhibited similar inhibitory activity, with inhibition zone diameter of 19.0 ± 0.1 mm each, which was significantly higher than that of the methanol extract, which showed inhibition zone diameter of 15.0 ± 0.1 mm at 800 mg/mL (Table 4). Overall, the inhibitory activities of the extracts against both organisms were significantly lower than that of the standard drug streptomycin, a broad-spectrum antibiotic.

Table 4: Antimicrobial activity of crude extracts of *Senna siamea* leaves

Extract conc. (mg/mL)	Test organism	Zone of inhibition (mm)				*P-value*
		Methanol extract	Ethanol extract	Aqueous extract	Streptomycin	
200	*S. pullorum*	$13.0^{a} \pm 0.1$	$9.0^{c} \pm 0.1$	$11.0^{b} \pm 0.1$	30.0 ± 0.8	0.000**
	S. gallinarum	$9.0^{c} \pm 0.1$	$10.0^{b} \pm 0.1$	$14.0^{a} \pm 0.1$	28.0 ± 0.1	
400	*S. pullorum*	$14.0^{b} \pm 0.1$	$13.0^{c} \pm 0.1$	$15.0^{a} \pm 0.1$	30.0 ± 0.8	0.000**
	S. gallinarum	$14.0^{c} \pm 0.1$	$15.0^{b} \pm 0.1$	$18.0^{a} \pm 0.1$	28.0 ± 0.1	
800	*S. pullorum*	$16.0^{b} \pm 0.1$	$15.0^{c} \pm 0.1$	$17.0^{a} \pm 0.1$	30.0 ± 0.8	0.000**
	S. gallinarum	$15.0^{b} \pm 0.1$	$19.0^{a} \pm 0.1$	$19.0^{a} \pm 0.1$	28.0 ± 0.1	

Values are Mean ± SD. Different superscript letters on the same row indicate significant difference at $p \leq 0.05$. ****:** The extract and the standard are significantly different

The fractions (chloroform, ethyl acetate, butanol, and aqueous) obtained from the liquid-liquid partitioning of the ethanol extract showed inhibitory activity against *S. pullorum* and *S. gallinarum*, but with varying zones of inhibition at different concentrations. The ethyl acetate fraction had the highest inhibitory activity against both *S. gallinarum* and *S. pullorum*, with inhibition zone diameters of 16.0 ± 0.1 and 24.0 ± 0.1 mm at 100 and 200 mg/mL, respectively against *S. gallinarum*, and 12.0 ± 0.1 and 15.0 ± 0.1 mm at 100 and 200 mg/ml, respectively against *S. pullorum* (Table 5). When compared to the crude extracts, the ethyl acetate fraction significantly higher activity. However, comparison with the standard drug (streptomycin) indicated a significantly lower activity.

Table 5: Antimicrobial activity of fractions of *Senna siamea* leaves

Extract conc. (mg/mL)	Test organism	Zone of inhibition (mm)					*P-value*
		Chloroform fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction	Streptomycin	
100	*S. pullorum*	$0.0^{a} \pm 0.0$	$12.0^{b} \pm 0.1$	$14.0^{c} \pm 0.1$	$13.0^{b} \pm 0.1$	30.0 ± 0.8	0.00**
	S. gallinarum	$0.0^{a} \pm 0.0$	$16.0^{c} \pm 0.1$	$10.0^{b} \pm 0.1$	$0.0^{a} \pm 0.0$	28.0 ± 0.1	
200	*S. pullorum*	$17.0^{b} \pm 0.1$	$15.0^{a} \pm 0.1$	$17.0^{b} \pm 0.1$	$15.0^{a} \pm 0.1$	30.0 ± 0.8	0.00^{**}
	S. gallinarum	$0.0^{a} \pm 0.0$	$24.0^{c} \pm 0.1$	$10.0^{b} \pm 0.1$	$0.0^{a} \pm 0.0$	28.0 ± 0.1	

Values are Mean ± SD. Different superscript letters on the same row indicate significant difference at $p \leq 0.05$. ****:** The extract and the standard are significantly different

Table 6 presents the minimum inhibitory concentration (MIC), which is the lowest concentration of an antimicrobial agent that can prevent visible growth of a microorganism.[25] This is used to determine the susceptibility of an organism to antibacterial or antibiotics.[26] The MIC for the methanol, ethanol, and aqueous extracts were 50, 50, and 100 mg/mL, respectively against *S. pullorum* and 100 mg/mL each against *S. gallinarum*. For the fractions, the chloroform, ethyl acetate, butanol, and aqueous fractions produced MICs of 25, 50, 100, and 50 mg/mL against *S. pullorum* and 100, 50, 100, and 50 mg/mL against *S. gallinarum*.

Table 7 shows the minimum bactericidal concentration (MBC), which is the lowest concentration of the extract that can kill the bacteria.[27] Four concentrations were prepared from 400 mg/mL of methanol, ethanol, and aqueous extracts. Another four concentrations were prepared from 100 mg/mL of chloroform, ethyl acetate, butanol, and aqueous fractions. There was no growth of *S. pullorum* on the culture plate at 100 mg/mL for the crude extracts. For *S. gallinarum*, the MBC was determined to be 200 and 100 mg/mL for ethanol and methanol extracts, but 400 mg/mL for aqueous extract, indicating that, at these concentrations, the extract kills the bacteria, not just inhibiting their growth. The MBCs of the chloroform, ethyl acetate, butanol, and aqueous fractions were 50 mg/mL each for *S. pullorum*, while for *S. gallinarum*, the MBCs were 100, 50, 100, and 50 mg/mL for chloroform, ethyl acetate, butanol, and aqueous fractions, respectively. The selection of ethanol for fractionation by liquid-liquid extraction was based on the MBC results of the crude extracts in which 400 mg/mL of aqueous extract was required to kill the bacteria which was higher than that of ethanol extract.

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

Table 6: Minimum Inhibition Concentration (MIC) of the crude extracts and fractions of *Senna siamea* leaves

Extract	MIC (mg/mL)	
	S. pullorum	***S. gallinarum***
Methanol	50	100
Ethanol	50	100
Aqueous	100	100
Fractions		
Chloroform	25	100
Ethyl acetate	50	50
Butanol	100	100
Aqueous	50	50

Table 7: Minimum Bactericidal Concentration (MBC) of crude extracts and fractions of *Senna siamea* leaves

Extract	**Test organism**	**Concentration (mg/mL)**						**MBC (mg/mL)**
		400	**200**	**100**	**75**	**50**	**25**	
Methanol	*S. pullorum*	-	-	-		+		100
	S. gallinarum	-	-	+		+		100
Ethanol	*S. pullorum*	-	-	-		+		100
	S. gallinarum	-	-	+		+		200
Aqueous	*S. pullorum*	-	-	-		+		100
	S. gallinarum	-	+	+		+		400
Fractions								
Chloroform	*S. pullorum*			-	-	-	+	50
	S. gallinarum			-	+	+	+	100
Ethyl acetate	*S. pullorum*			-	-	+	+	50
	S. gallinarum			-	-	+	+	50
Butanol	*S. pullorum*			-	-	+	+	50
	S. gallinarum			-	+	+	+	100
Aqueous	*S. pullorum*			-	-	+	+	50
	S. gallinarum			-	-	+	+	50

Key: (+) Growth, (-) No growth

Conclusion

The findings from the present study showed that the extracts of the leaves of *Senna siamea* have antibacterial activity against *Salmonella gallinarum* and *Salmonella pullorum*, with the aqueous extract exhibiting the highest activity. Of all the fractions (chloroform, ethyl acetate, butanol, and aqueous), the ethyl acetate fraction showed the highest activity against the two Salmonella species. This indicates that *Senna siamea* leaves is a potential source of bioactive agents that can be used for the treatment of salmonellosis in poultry. Future study should focus on the isolation of bioactive compound(s) responsible for the observed activity against *Salmonella gallinarum* and *Salmonella pullorum*.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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