

Antibacterial Activity of Composite Mixture of *Senna siamea* Leaves and Tamarind pulp Extracts on Multidrug Resistant *Salmonella typhi*

ABSTRACT

Background: For many years, antimicrobial chemotherapeutic approach have been challenged by drugs resistant bacteria such as *Salmonella* associated with gastrointestinal infections. To overcome these effects, several attempts were in progress, using some plants parts to treat Typhoid fever in Nigeria.

Aim: The aim of this work was to determine the antibacterial activity of *Senna siamea* leaves and Tamarind fruit pulp against multidrug resistant *Salmonella typhi*.

Methods: A total of 168 samples of stool and blood from patients with suspected cases of typhoid fever were collected and only three (3) *Salmonella typhi* were recovered by conventional microbiological techniques. The isolates were screened for multidrug resistant properties according to Kirby -Bauer disc diffusion method. Water and ethanol were used to extract phytochemical components from powdered leaves of *Senna siamea* and Tamarind fruit pulp via percolation method. The extracts were tested for the presence of bioactive compounds and antibacterial activity against the clinical isolates of *Salmonella typhi*.

Results: All the isolates (100%) 100% of the isolates were resistant to Ampicillin and Amoxicillin, while two (66.7%) were resistant to Erythromycin and Tetracycline. In addition to that and all isolates were sensitive (100%) to Streptomycin, Gentamycin, Nalidixic acid Ciprofloxacin, Cotrimoxazole, Augmentin and Chloramphenicol. The phytochemical screening showed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins, carbohydrates and cardiac glycosides. The result shows that aqueous and ethanolic extracts of combined *Tamarindus indica* and *Senna siamea* were more active (2.50µg/ml) against *Salmonella typhi* when compared to individual extracts.

Conclusion: This study shows that aqueous and ethanol extracts of both plants exhibited activity against *S. Typhi*, hence, possesses antimicrobial potentials that it can and could be used in the treatment of typhoid.

Key Words: Multidrug resistance, antibacterial activity, *Senna siamea*, *Tamarindus indica*, extracts, antibiotics, *Salmonella typhi*.

INTRODUCTION

Enteric fever (typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate [1]. *Salmonella* species is a genus of the enteric pathogens consisting of two species; *Salmonella enterica* and *Salmonella bongori* which cause diseases in broad range of hosts. *Salmonella bongori* is predominantly associated with infections in reptiles although it has been isolated in human infections [2]. Among the *Salmonella enterica* sub-species, *Salmonella enterica* subsp. *enterica* is the only one that can infect mammals and being is associated with human diseases [3].

A traditional claim have cited *Senna siamea* Lam (Kassod tree - English; Malga/marga - Hausa) to be used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, and is

Comment [S1]: Attempts by who? This section is vague. State if the attempts are made by herbalist, if it is used in ethno-medicine by the locals.

Comment [S2]: There is a disconnect between the background and the aim. Elaborate the background to mention the use of *Senna* and *Tamarind* for treatment of certain diseases. That will improve the transition to the aim.

Comment [S3]: What is the botanical name. Be consistent as you used a botanical name for the other plant.

Comment [S4]: Does this mean three of the 168? Or another 3 samples. The highlighted section is vague.

Comment [S5]: This should be the first sentence in your method as it is the first process of deriving the extract before using it for the diffusion method.

Comment [S6]: Did you mean phytochemical screening? I think it will be better put that way.

Comment [S7]: Phytochemical screening of which of the plants? You need to state clearly which one (*Senna* or *Tamarind*).

Comment [S8]: So what did this dose achieve? Was it 100% bacterial killing?

Comment [S9]: It will be interesting for you to include the results for the individual extracts and at what dose they worked.

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also used to reduce sugar level in the blood. It was also reported by [4] that *S. siamea* is ethnomedicinally used as laxative, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes and rhinitis. Thus, it is necessary to further evaluate the pharmacological potential use of *S. siamea* leaves for the treatment of many other diseases. *Tamarindus indica* (fruit pulp) is one such widely used medicinal plant as anti-pyretic, antiscorbutic, laxative, carminative and remedy for biliousness and bile disorder and the leaves have antihelmintic and vermifuge properties, destroying intestinal parasites [5]. With the increase in resistance to anti-typhoid drugs, and indiscriminate use of antibiotics and rapid rise in multi-drug resistance to *Salmonella typhi*, medicinal plants have gained popularity among both urban and rural dwellers in the treatment of the ailment. Thus plants have been reported to have minimal or no side effects compared to antibiotics [6,7]. It is therefore imperative to continue to screen suspected potential plants for a promising candidate against the organism. This in the end might justify the use of these plants in traditional practices for the treatment of typhoid fever.

Comment [S10]: Check Vancouver referencing and reference appropriately. You should state author in this case with the number at the end.

Comment [S11]: This is accurate.

Comment [S12]: Use a citation to support this claim.

MATERIALS METHODS

Plants Sample Collection

Fresh mature leaves of *Senna siamea* lam (Kassod tree) were collected from Biological Sciences Department, Abubakar Tafawa Balewa University (ATBU), Bauchi and Tamarind fruit pulp was bought from Bauchi central market. The samples were identified at a herbarium in the Department of Biological Sciences, ATBU. A voucher number was allocated to the samples and a specimen was deposited at the departmental herbarium for future reference as employed by Abdulrazak *et al.* [8].

Comment [S13]: State the voucher number. Also was a voucher number allocated for the tamarind pulp?

Sample Processing and Extraction

The leaves of *Senna siamea* and *Tamarindus indica* fruit pulp were handpicked, washed thoroughly under running tap water, ~~dried under shade~~ dried, ground into fine powder and sieved. Five hundred grams (500g) each of the ground powder was extracted by percolation, divided into two and each part soaked in ethanol (95% w/v), and water respectively in different conical flasks for 72 hours, placed in Gallenkamp rotary shaker at 65 revolutions per minute (R110) (Allex manufacturing limited). The contents were then homogenized and filtered using Whatman filter paper No.1. The filtrates were concentrated using a Buchi Rotavapor R-200. The resultant filtrates were then stored in a labeled sterile container, dried at room temperature until used for bioassay as described by Ogundare *et al.* [9].

Comment [S14]: Check this citation method.

Preliminary Phytochemical Screening of the Extracts

The extracts were subjected to preliminary phytochemical tests namely; tests for alkaloids, Anthraquinones, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, and terpenoids to determine the active constituents present in the different extracts using standard procedures as described by Sofowora [10], Harborne [11] and Trease [12].

Comment [S15]: How long did it take to dry the extract at room temperature. More clarity should be shed about the drying process.

Preparation of Stock Solution

Stock solutions were prepared from the plant extracts by weighing 0.2g of *Senna siamea* and 0.2g of *Tamarindus indica* combined to form 0.4g each in 1ml of Dimethyl Sulfoxide (DMSO) for ethanolic extracts and 1m of water for aqueous extracts, making a solution of 400mg/ml from where serial dilution of different concentrations were made (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml). This was done for each of the plant extracts as employed by Mohammed *et al.* [13].

Comment [S16]: What is the justification for DMSO? Did you consider the possible toxicity of DMSO to the isolates?

Preparation of Susceptibility Test Discs Using the Plant Extracts

Discs of 6 milliliters were prepared from Whatman no.1 filter paper. The discs prepared were filled in different McCartney bottles. The discs were then sterilized by autoclaving at 121°C for 15 minutes and dried in hot air oven at 50°C [23]. Forceps used for picking the discs were

sterilized using a spirit lamp and left to cool. The Forceps were then used in picking the sterilized discs and placing them in the various concentrations made from the stock solutions of the extracts (*Senna siamea* and *Tamarindus indica*) singly and in combined from 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml respectively in each case of ethanol and water extracts. Positive control of Chloramphenicol (30µg) and negative control with 4% Dimethyl sulphoxide were also impregnated on discs.

Agar well diffusion method was employed for the antimicrobial susceptibility test. Mueller Hilton agar was prepared according to manufacturer's specification. Using sterile swab stick, standardized inocula (1.5×10^8 cells/mL) of each isolate was swabbed onto the surface of the agar. Four wells of 6mm each were made in each plate using a sterile cork-borer. The wells were filled with 0.1ml of diluted concentrations (400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml) of the extracts with the aid of sterile pipettes per well. Standard antibiotic (Amoxycillin) was used as positive control while sterile distilled water as negative. The plates were allowed to stand for 15 minutes on a table to allow pre-diffusion of the extracts. Diameters of zones of inhibition were measured using plastic ruler after 24 hours of incubation at 37°C [14].

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was carried out on sensitive dilutions. Broth dilution method was used for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [15]. The extracts were serially diluted to give a concentration of 400, 200, 100, 50 and 25 mg/ml in test tubes. Dilution of the plant extracts was incorporated in nutrient broth in 1:-1 ratio and was seeded with 0.1ml of standard suspension of the test organisms.

Tube containing broth and extracts without inocula serve as a positive control while tubes containing broth and inocula without extract serve as negative control. Tubes were incubated for 24hrs at a temperature of 37°C. After incubation the tubes were then examined for least concentration without turbidity. The test tube with least concentration which showed no turbidity indicates the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration, (MBC) was determined by inoculating samples from the MIC tubes that visibly showed no bacterial growth on Mueller Hinton agar plates separately and was incubated for further 24 hours. The highest dilution that shows no single bacterial colony was taken as the minimum bactericidal concentration (MBC) as reported by Baker *et al.* [15].

Statistical Analysis

The data obtained was subjected to descriptive statistics and inferential statistical analysis to determine their significance at 5% level using Statistical Package for Social Sciences (SPSS) version 23. Means were separated using Duncan Multiple Range Test (DMRT) ($P \leq 0.05$).

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The plants extracts of *Senna siamea* leaves and Tamarind fruit pulp were qualitatively tested for the presence of numerous secondary metabolites as presented in Table 1.

The phytochemical analysis showed that the leaf extract of *Senna siamea* and tamarind fruit pulp possess alkaloids, flavonoids, tannins and steroids. Phytoconstituents have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections [16,17,18] reported that tannins and alkaloids are nature products that have medicinal properties. He also reported that some remedial values of tannins include application on burn to heal injury

and cuts to stop bleeding. Moreover it stops infections on the skin surface whereas internally tannins continue to heal the wound.

Comment [S17]: This should be in the discussion section not results. Simply present and interpret results in the results section.

Table 1: Phytochemical Constituents of Tamarind fruit pulp and *Senna siamea* Extracts

Phytochemicals	Tamarind		<i>Senna siamea</i>	
	Water	Ethanol	Water	Ethanol
Alkaloids	++	-	++	+++
Saponins	+	++	+++	-
Tannins	-	-	+++	++
Flavonoids	+++	+++	+++	+++
Carbohydrates	+++	+++	++	++
Steroids	-	+	-	++
Terpenes	-	-	-	-
Anthraquinones	-	-	-	+
Cardiac glycosides	+	++	++	++

Key: (+) low present, (++) moderately present, (+++) highly present and (-) absent

Antibiotic Susceptibility Pattern of *S. typhi* Isolates

Emergence and persistence of antibiotics resistance is propelled by different factors such as indiscriminate use of drugs and variable antimicrobial efficacy and pose significant challenges in the control of infectious diseases. In this study, all the salmonella isolates (100%) were resistant to Ampicillin, Amoxicillin, while two (66.7%) to Erythromycin, and Tetracycline. The isolates were sensitive to Streptomycin, Gentamycin and Nalidixic acid, Ciprofloxacin, Cotrimoxazole, Augmentin and Chloramphenicol.

Comment [S18]: This should be in discussion. See comment above

Table 2: Distribution of *Salmonella* isolates according to antibiotic susceptibility Pattern

Antibiotics(µg)	No. (%) of <i>Salmonella</i> isolates (n=03) and Susceptibility pattern	
	Sensitive	Resistant
Ampicillin (10)	00(0.0)	03(100)
Amoxycillin (30)	00(0.0)	03(100)
Streptomycin(30)	00(0.0)	00(0.0)
Gentamycin(10)	00(0.0)	00(0.0)
Erythromycin(30)	01(33.3)	02(66.7)
Tetracycline(30)	01(33.3)	02(66.7)
Ciprofloxacin(5)	03(100)	00(0.0)
Cotrimoxazole(30)	03(100)	00(0.0)
Nalidixic acid(10)	03(100)	00(0.0)
Chloramphenicol(30)	03(100)	00(0.0)
Augmentin(30)	03(100)	00(0.0)

Zones of inhibition interpreted according to CLSI [19]

Comment [S19]: It is unclear how these doses were achieved based on your methods description

There may be emergence of multiple drug resistant (MDR) strain of *S. Typhi* in the study area. This may be as a result of over dependence or uncontrolled use of available antibiotics and /or inaccurate or inconclusive diagnosis resulting in the development and spread of resistant strains of *S. Typhi*. The standard antibiotic (Amoxycillin, Ciprofloxacin and Chloramphenicol used in this study are the first line drugs used in the treatment of typhoid fever [20]. The result here

Comment [S20]: This should be in the discussion section.

shows that the isolates were resistant to erythromycin, ampicillin and tetracycline. *S. Typhi* with resistance to these antibiotics are considered multidrug resistant which are in agreement with the findings of Omulo *et al.* [21].

MIC and MBC of Tamarind and Senna Siamea Extracts against Salmonella Typhi

The minimum inhibitory and bactericidal concentrations of Tamarind, *Senna siamea* and Combined *Tamarind* and *Senna siamea* extract against *Salmonella typhi*, the results (Table 3) shows that water and ethanol of combined *Tamarind* and *Senna siamea* extracts were more active (2.50µg/ml) against *Salmonella typhi* when compared to other extracts. However, the antibiotic used as a positive control (ciprofloxacin) was more active against *Salmonella typhi* when compared to all the extractions.

Table 3: MIC and MBC of Tamarind, Senna siamea and Combined Tamarind and Senna siamea Extracts against Salmonella typhi

Extractions	<i>Tamarind</i>		<i>Senna siamea</i>		Combined	
	MIC	MBC	MIC	MBC	MIC	MBC
Water	20.00	40.00	20.00	40.00	10.00	20.00
+VE control	2.00	6.00	2.00	5.00	2.00	5.00
-VE control	0.00	0.00	0.00	0.00	0.00	0.00
Ethanol	20.00	40.00	20.00	40.00	10.00	20.00
+VE control	2.00	5.00	2.00	5.00	2.00	5.00
-VE control	0.00	0.00	0.00	0.00	0.00	0.00

The result in Table 3 presented the minimum inhibitory and bactericidal concentrations of *Tamarind*, *Senna siamea* and Combined *Tamarind* and *Senna siamea* against *Salmonella typhi*. The result further shows that the water and ethanol of combined *Tamarind* and *Senna siamea* extract was more active (10.00µg/ml) against *Salmonella typhi* when compared to other extracts. However, the antibiotic used as a positive control (ciprofloxacin) was more active against *Salmonella typhi* when compared to all the extractions. Hence, combined *Tamarind* and *Cassia siamea* was bactericidal in lower concentration (20.00µg/ml) against *Salmonella typhi* when compared to separate extracts.

Table 4: Mean zone of inhibition of Tamarind and Senna siamea leaves extract against S. typhi

Plant extracts	Conc. (mg/ml)	Mean zone of inhibition (mm)	
		Water	Ethanol
<i>Tamarind</i> and <i>Senna siamea</i>	400	5.00 ^a	7.33 ^a
	200	4.00 ^b	5.33 ^b
	100	2.67 ^c	3.67 ^c
	50	2.00 ^d	1.67 ^d
	25	0.33 ^d	1.33 ^e
	+ve Ctrl	16.67 ^a	16.67 ^a
	-ve Ctrl	0.00 ^e	0.00 ^f
	SE	0.33	0.36

Each value is a mean of \pm standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

The minimum inhibitory Conc. of the extracts of plants with high activity against *S. Typhi* usually have a lower MIC and MBC values this is exhibited by both extracts of aqueous and ethanol while those with lower activity showed MIC and MBC of higher concentration. The findings were similar to previous study in Bayero University Kano, Nigeria by Philips [18] and Mohammed [23], who reported that anti-pseudomonas activity of *Senna siamea* was due to presence of the phytochemical, tannin.

Comment [S21]: Suggest creating a discussion section and separating results from the discussion. Elaborate more on the phytochemical compounds linking to wider literature.

CONCLUSION

Based on the finding of this research, aqueous and ethanol extracts of both plants exhibited activity on *S. Typhi*, hence, possess antimicrobial potentials. This provides a scientific basis for its local usage in folklore medicine for the treatment of typhoid fever. This means that if the concentration of the extracts could be further standardized, can meet up against future challenges of commercially manufactured drugs resistance and be used as an alternative therapeutic agents.

Comment [S22]: The phytochemical tests has not been included in the results.

NOTE:

The study highlights the efficacy of "folklore medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Comment [S23]: Inconsistent reference format. You have commas in some and full stop in others. Et al should not be used. Present all authors in reference.

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