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

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Authors' contributions

This work was carried out in collaboration among all authors. Author ZMK collected and analyzed the clinical specimens. Author MYI designed the study, prepare and type the manuscript. Authors MNY and AHI performed the phytochemical analysis. Authors MHA, AS and HSM scrutinized the manuscripts, organize the data and perform statistical analysis. Author AFU is the general overseer who supervised the entire work and led the research team. All authors read and approved the final manuscript.

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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 by herbalists and traditional healers 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 *Tamarindus indica* fruit pulp against multidrug resistant *Salmonella typhi*.
Methods: A total of 168 samples of stool and blood from patients with suspected cases of typhoid

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fever were collected and only three (3) *Salmonella typhi* isolates were recovered (from the 168 samples) 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 *Tamarindus indica* fruit pulp via percolation method. The extracts were tested for the antibacterial activity against the clinical isolates of *Salmonella typhi.*

Results: All the isolates (100%) were resistant to Ampicillin, Amoxicillin, while two (66.7%) to Erythromycin and Tetracycline and sensitive (100%) to Streptomycin, Gentamycin, Nalidixic acid Ciprofloxacin, Cotrimoxazole, Augmentin and Chloramphenicol, The phytochemical screening o of both plants extracts showed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins, carbohydrates and cardiac glycosides. The result shows that aqueous and ethanolic extracts of combined *Tamarind* 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 on *S. Typhi*, hence, possess antimicrobial potentials that it can be used in treatment of typhoid.

Keywords: Multidrug resistance; antibacterial activity; Senna siamea; Tamarind; extracts; antibiotics; Salmonella typhi.

1. 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 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 also used to reduce sugar level in the blood. It was also reported by Aliyu [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 [6]. 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.

2. MATERIALS AND METHODS

2.1 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 the departmental herbarium and deposited for future reference as employed by Abdulrazak et al. [8].

2.2 Sample Processing and Extraction

The leaves of *Senna siamea* and *Tamarindus indica* fruit pulp were handpicked, washed thoroughly under running tap water, dried under shed, 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 (for 5 days) until used for bioassay as described by Ogundare et al. [9].

2.3 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].

2.4 Preparation of Stock Solution

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

2.5 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 [14]. 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 400 mg/ml, 200 mg/ml, 100 mgml, 50 mg/ml and 25 mg/ml respectively in each case of ethanol and water extracts. Positive control of Chloramphenicol (30 µg) 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 ($1.5x10^8$ 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 (400 µg/ml, 200 µg/ml, 100 µg/ml and 50 µg/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 37^0C [15].

2.6 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) [16]. 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^oC. 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.

2.7 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. [16].

2.8 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≤0.05).

3. RESULTS AND DISCUSSION

3.1 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 [17,18,19] 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.

3.2 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.

Table 1. Phytochemical constituents of tamarind fruit pulp and *Senna siamea* extracts

	Tamarind		***Senna siamea***	
Phytochemicals	**Water**	**Ethanol**	**Water**	**Ethanol**
Alkaloids	++	-	++	+++
Saponins	+	++	+++	-
Tannins	-	-	+++	++
Flavonoids	+++	+++	+++	+++
Carbohydrates	+++	+++	++	++
Steroids	-	+	-	++
Terpenes	-	-	-	-
Anthraquinones	-	-	-	+
Cardiac glycosides	+	++	++	++

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

Table 2. Distribution of *Salmonella* isolates according to antibiotic susceptibility pattern

	No. (%) of *Salmonella* isolates (n=03) and Susceptibility pattern	
Antibiotics(µg)	**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 [20]

Table 3. MIC and MBC of *Tamarind*, *Senna siamea* and Combined *Tamarind* and *Senna siamea* extracts against *Salmonella typhi*

	Tamarind		*Senna siamea*		Combined	
Extractions	**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

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

		Mean zone of inhibition (mm)	
Plant extracts	**Conc. (mg/ml)**	**Water**	**Ethanol**
Tamarind and *Senna siamea*	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 ± standard error of three replicates. Mean followed by the same superscripts in a column are not significantly different from each other.

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 [21, 22]. The result here 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. [23].

3.3 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 (2.50 µg/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.

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 (10.00 µg/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 (20.00 µg/ml) against *Salmonella typhi* when compared to separate extracts.

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 [19] and Mohammed [14], who reported that anti-pseudomonas activity of *Senna siamea* was due to presence of the phytochemical, tannin.

4. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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