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In-vitro Antibacterial Activity of Extracts from Twenty-Four Plants Species against *Salmonella typhi* in North-East Nigeria

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Abstract

Medicinal plants are used for treating different ailments caused by microorganisms. This study investigated the antimicrobial efficacy of aqueous and hexane extracts from 24 plants against Salmonella typhi strain isolated from typhoid fever patients. The efficacy of the plants extracts was evaluated in-vitro using disc diffusion method. Out of twenty four (24) plant extracts tested only thirteen plants extracts produced zone of inhibition. The result of this study revealed that Ciprofloxacin (26mm) demonstrated exceptional patency, exhibiting substantial inhibitory effects on Salmonella typhi followed by aqueous extract of Ficus sycomorus (16mm), Mangifera indica (15mm), Citrus aurantifolia (14mm) and Carica papaya (13mm) inhibition zone while the hexane extract of Gueira senegalensis (07mm) was least. Minimum Inhibitory Concentration and Minimum Bactericide Concentration range between (12.5mg/ml-50mg/ml) and (25mg/ml-100mg/ml) respectively. Ciprofloxacin, *and both the aqueous and Hexane Extracts demonstrated the bactericidal activity against Salmonella typhi. These studies justify the claim of traditional use of some of the plants extracts against Salmonella typhi in the region. The effectiveness of a combined extracts needs to be evaluated in further studies.*

Keywords: *Ficus sycomorus*, Bacteriostatic, Bactericidal, *Salmonella typhi* and aqueous extract

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INTRODUCTION

Typhoid fever is a disease caused by *Salmonella typhi*, it is significant public health concerns worldwide with approximately 21 million cases annually (World Health Organization, 2019). The increasing emergence of antibiotic-resistant *Salmonella typhi* strains has underscored the need for novel therapeutic agents (Parry, *et al.,* 2018; Mogasale, *et al.,* 2014).

Medicinal plants have been a vital source of antimicrobial compounds with many exhibiting potent activity against various pathogens Newman and Cragg, (2016). Phytochemicals or bioactive compounds derived from plants have been extensively explored for their medicinal properties including antimicrobial, anti-inflammatory, and antioxidant activities Sharma and Nogueira, (2019). Various plants have been traditionally used in folk medicine to treat typhoid fever and phytochemical screening has become a crucial step in identifying and characterizing the bioactive compounds responsible for their therapeutic effects (Singh, *et al.,* 2020; Patel and Samuel, 2018).

Antibacterial activity of plants is due to the high rich of the secondary metabolites content such as tannins, terpenoids, alkaloids, flavonoids, saponins, and Cardial glycosides (Bello, *et al.,* 2024). The *Ficus sycomorus* extracts have reported to exhibit significant antibacterial properties against various human pathogens due to the present of flavonoids, phenolic and polysaccharides contents (Josephus, *et al.,* 2024). It is also revealed that the gram-negative bacteria as an antibacterial agent are sensitive to *Mangifera indica* extracts due to the presence of phenolic (De Silva *et al.,* 2018). *Salmonella typhi* have been revealed to be sensitive to the extracts of *Eucalyptus camaldulensis* and *Gurira senegalensis* due to the presence of flavonoid and alkaloid (Bello, *et al.,* 2020). The leaf extracts of *Adensonia digitata* show antibacterial activity against different food-borne pathogens which could be attributed to their flavonoid contents to some extent (Bello, *et al.,* 2024).

Despite the widespread use of traditional medicine in North-East Nigeria, there is a significant lack of scientific evidence supporting the antimicrobial efficacy of locally use plants against *Salmonella typhi* which was the major cause of typhoid fever in the region.

This study aims to investigate the *in-vitro* antibacterial activity of extracts from twenty-four plants traditionally used in North-East Nigeria against *Salmonella typhi*. The findings of this study will contribute to a scientific validation of traditional medicine practices in the region, and ultimately inform public health strategies for the prevention and treatment of typhoid fever in North-East Nigeria.

MATERIALS AND METHODS

Preparation of Plant Extracts

All the twenty (24) four plants species collected from the study areas (Giere, Numan and Mubi North) in Adamawa, (Kaga, Biu and Munguno) in Borno, (Bauchi, Itas/Gadau and Misau) in Bauchi, (Akko, Kwami and Kaltungo) in Gombe, (Sardauna, Jalingo and Ibbi) in Taraba and (Potiskum, Gashua and Bunu-Yadi) in Yobe using simple random sampling were subjected to *in-vitro* antibacterial activity. The plant samples (leaves) were cleaned with distilled water, air-dried for ten days at room temperature, and then ground into a powdery consistency using mortar and pistle. The dried plant samples were grounded into powdery form using a mortar and pestle. The method of extraction was used in accordance with the method of (Zigau and Bello, 2020) with a little modification. 50gram of plants part powder was introduced into 500 ml of distilled water (polar) and hexane (non-polar) respectively. Upon reaching saturation, the solutions were filtered using Whatman No. 1 filter paper. The resulting extracts were then obtained at 37^{o}C using a water bath and store for future research.

Isolation of *Salmonella typhi*

The *Salmonella typhi* was isolated from the patient's stool from the Microbiology Department at Specialist Hospital Potiskum, Yobe State Nigeria. 1gram from the stool was introduced into test tubes of selenite F Broth for the multiplication of the bacteria and incubated overnight on a prepared deoxycholate citrate (DCA) and Macconkey plates respectively. The blackish and yellowish color on a prepared deoxycholate citrate (DCA) and Macconkey plates respectively suspect the present of *Salmonella typhi* as shown in Plate 1 and 2. The isolated bacteria was picked and stored in a sterilized container and also sub-cultured for confirmation (biochemical test) (Iliyasu *et al.*, 2019).

Biochemical Test of the Isolates (*Salmonella typhi*)

The methods used by Matthew (2014) and Cheesbrough (2006) were adapted for biochemical test of *Salmonella typhi*. Biochemical test including mobile test, gram staining, indole test, triple sugar iron agar and Urease activity were performed.

Motility

Salmonella typhi was grown in nutrient broth and small amount was introduced on a microscope slide and covered with cover slide after few drops of formalin were added. The specimen was observed at 40× lens. The present of movable organism with rod shape, bright color against the dark background, flagella, circular, smooth body and movements indicate that the microorganism was motile.

Indole test

The test tube containing tryptone broth was incubating with a loopful of *Salmonella typhi*, the Incubate the incubated broth at 37^{o}C for 24hour. Three to four (3-4) drops of Kovacs' reagent were introduced to the incubated broth. Positive result occurs when the Kovacs' reagent turns pink-red and the negative result occurs when the Kovacs' reagent remains orange-yellow (unchanged).

Triple Sugar Iron

A Small sample of bacterial growth was collected from the prepared agar and used to inoculate the agar slant. The agar was stabbed once and the tube was loosely capped and incubated at 360^{C}. The cultures were examined for gas production and H_2S formation, indicated by bubbles, crack, or black precipitate in the agar, over various time periods (24-48 hours or 5-7 days).

Urease test

The surface of the urea agar slants were used to incubate the bacteria up to forty eight hours at 37^{o}C. The change in color from the plain to pink in the tubes were observed

Sensitivity Test (*in-vitro*)

The disc diffusion methods described by Deborah *et al.* (2023) and Umar *et al.* (2021) were employed to assess antimicrobial activity. The method involved inoculating freshly prepared Muller Hinton agar plates with 0.1 ml of test organisms using the streaking method. Sterile discs impregnated with plant extracts at various concentrations (100, 200, and 300 mg/ml) were placed on the agar surface. The plates were incubated at 37°C for 24-48 hours. The same procedure was carried out using Ciprofloxacin and distilled water which served as positive and negative controls respectively. Zones of inhibition were measured and recorded in millimeters, with each plant extract tested in triplicate.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined using the method described by Garba *et al.* (2014) and Umar *et al.* (2021). Serial broth dilution was performed by introducing 2 ml of sterilized nutrient broth into six tubes, each containing a different concentration of extract (100, 50, 25, 12.5, and 6.25 mg/ml). Each tube received 0.025 ml of extract and was mixed thoroughly. The tubes were then inoculated with 0.5 ml of *Salmonella typhi* and incubated at 37°C for 24 hours. Turbidity was observed to determine the MIC, with the lowest concentration of extract that inhibited bacterial growth considered the MIC.

Minimum Bactericidal Concentration

Plant extract concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml were prepared to determine the minimum bactericidal concentration (MBC). The MBC was determined through broth dilution testing, where the contents of the minimum inhibitory concentration (MIC) tube were sub-cultured onto antibacterial-free agar. The lowest concentration exhibiting no visible bacterial growth was considered the MBC, as described by Garba *et al.* (2014) and Umar *et al.* (2021).

Data Analysis

Data were subjected to two-way ANOVA using SPSS (version 2021), with results deemed significant at $p<0.05$.

Results

Results of Isolation of *Salmonella typhi* from stool

After incubation of the isolated bacterial for twenty four hours at 37^{o}C, appearance of the blackish and yellowish color on Deoxycholate citrate Agar (DCA) and Macconkey plates respectively were observed as shown in plate 1 and plate 2.

Plate 1: Isolation of *S. typhi* on DCA

Plate 2: Isolated of *S. typhi* on Maconkey

Plate 3: the *S. typhi* sample

Table 1: Results of the Biochemical Test of the isolated *Salmonella typhi*

Isolate bacteria	Gram staining	Motile test	Indole test	H_2S	Triple sugar iron	Urease
Salmonella typhi	Negative	Motile	Negative	Positive	Alkaloid/Acid	Negative

Table 2: Zone of inhibitions in millimeters of the aqueous, hexane, positive and negative control of Twenty Four Plants leaves on *Salmonella typhi*

S/N	Plant species	Solvents	300mg/ml	200mg/ml	100mg/ml
1	*Adansonia digitata*	Aqueous extract	00.00	00.00	00.00
		Hexane extract	00.00	00.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
2	*Allium cepa*	Aqueous extract	00.00	00.00	00.00
		Hexane extract	00.00	00.00	26.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
3	*Allium sativum*	Aqueous extract	11.00	10.00	08.00
		Hexane extract	09.00	08.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
4	*Azadrachta indica*	Aqueous extract	10.00	09.00	08.00
		Hexane extract	00.00	00.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
5	*Calotropis procera*	Aqueous extract	10.00	08.00	00.00
		Hexane extract	00.00	00.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
6	*Carica papaya*	Aqueous extract	13.00	11.00	10.00
		Hexane extract	12.00	10.00	08.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
7	*Citrus aurantifolia*	Aqueous extract	14.00	12.00	10.00
		Hexane extract	12.00	10.00	08.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
8	*Eucalyptus camaldulensis*	Aqueous extract	10.00	09.00	08.00
		Hexane extract	00.00	00.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	13.00	00.00	20.00
9	*Ficus sycomorus*	Aqueous extract	16.00	14.00	12.00
		Hexane extract	14.00	12.00	10.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
10	*Ficus thoningii*	Aqueous extract	00.00	00.00	00.00
		Hexane extract	00.00	00.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
11	*Furcraea foetide*	Aqueous extract	00.00	00.00	00.00
		Hexane extract	00.00	00.00	26.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	20.00
12	*Gueira senegalensis*	Aqueous extract	10.00	08.00	07.00
		Hexane extract	00.00	00.00	00.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00
13	*Khaya senegalensis*	Aqueous extract	00.00	00.00	00.00
		Hexane extract	00.00	00.00	26.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	20.00
14	*Mangifera indica*	Aqueous extract	15.00	14.00	12.00
		Hexane extract	12.00	11.00	10.00
		Ciprofloxacin	26.00	23.00	20.00
		Negative control	00.00	00.00	00.00

15	*Moringa oleifera*	Aqueous extract Hexane extract Ciprofloxacin Negative control	00.00 00.00 26.00 00.00	00.00 00.00 23.00 00.00	00.00 00.00 20.00 00.00
16	*Musa paradisiaca*	Aqueous extract Hexane extract Ciprofloxacin Negative control	00.00 00.00 26.00 00.00	00.00 00.00 23.00 00.00	00.00 00.00 20.00 00.00
17	*Olea europaea*	Aqueous extract Hexane extract Ciprofloxacin Negative control	00.00 00.00 26.00 00.00	00.00 00.00 23.00 00.00	00.00 00.00 20.00 00.00
18	*Psidium guajava*	Aqueous extract Hexane extract Ciprofloxacin Negative control	12.00 00.00 26.00 00.00	10.00 00.00 23.00 00.00	08.00 00.00 20.00 20.00
19	*Saccharum officianarum*	Aqueous extract Hexane extract Ciprofloxacin Negative control	00.00 00.00 26.00 00.00	00.00 00.00 23.00 00.00	00.00 00.00 20.00 00.00
20	*Senna Ciamea*	Aqueous extract Hexane extract Ciprofloxacin Negative control	12.00 00.00 26.00 00.00	10.00 00.00 23.00 00.00	08.00 00.00 20.00 00.00
21	*Senna ocidentalis*	Aqueous extract Hexane extract Ciprofloxacin Negative control	10.00 00.00 26.00 00.00	09.00 00.00 23.00 00.00	08.00 00.00 20.00 00.00
22	*Senna tora*	Aqueous extract Hexane extract Ciprofloxacin Negative control	00.00 00.00 26.00 00.00	00.00 00.00 23.00 00.00	00.00 00.00 20.00 00.00
23	*Syzygium guineese*	Aqueous extract Hexane extract Ciprofloxacin Negative control	00.00 00.00 26.00 00.00	00.00 00.00 23.00 00.00	00.00 26.00 20.00 20.00
24	*Tamerindus indica*	Aqueous extract Hexane extract Ciprofloxacin Negative control	10.00 00.00 26.00 00.00	08.00 00.00 23.00 00.00	00.00 26.00 20.00 20.00

Some of the efficacy of the aqueous extract of the most effective plant against *Salmonella typhi*

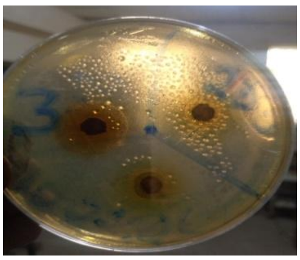
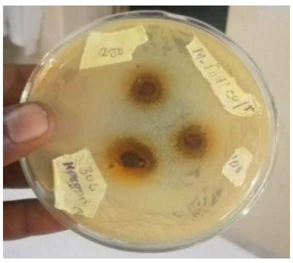
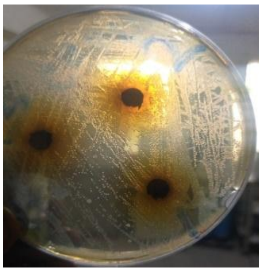


Plate 1: *Ficus sycomorus* **Plate 2:** *Mangifera indica* **Plate 3:** *Citrus aurantifolia* **Plate 4:** *Carica papaya*

Some of the ineffective aqueous extract at 100mg/ml, 200mg/ml and 300mg/ml against *Salmonella typhi*

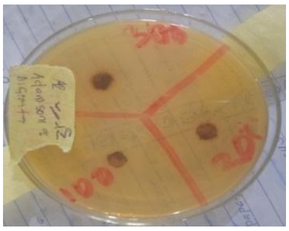

Plate 1: *Adansonia digitata*

Plate 2: *Senna tora*

Plate 3: *Allium cepa*

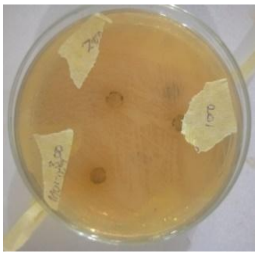

Plate 4: *Syzygium guineese.*

Table 3: MIC and MBC of the aqueous and hexane extracts of plants leaves against *Salmonella typhi*

S/N	Plant species	MIC	MBC
1	***Carica papaya***		
	Aqueous extract	12.5	25
	Hexane extract	25	50
2	***Ficus sycomorus***		
	Aqueous extract	12.5	25
	Hexane extract	25	50
3	***Mangifera indica***		
	Aqueous extract	12.5	25
	Hexane extract	25	50
4	***Allium sativun***		
	Aqueous extract	12.5	25
	Hexane extract	25	50
5	***Citrus aurantifolia***		
	Aqueous extract	12.5	25
	Hexane extract	25	50

Keys: MIC = Minimum Inhibitory Concentrations, MBC = Minimum Bactericide Concentrations

Table 4: MIC and MBC of aqueous extracts of plants leaves against *Salmonella typhi*

S/N		Plant species	MIC	MBC
1		*Eucalyptus cameldulensis*	25	50
2		*Senna Ciamea*	25	50
3		*Azadrachta indica*	25	50
4		*Guiera senegalensis*	25	50
5		*Senna ocidentalis*	50	100
6		*Psidium guajava*	25	50
7		*Calotropis procera*	50	100
8		*Tamerindus indica*	50	100

Keys: MIC = Minimum Inhibitory Concentrations, MBC = Minimum Bactericide Concentrations

DISCUSSION

Antimicrobial Activity

Different concentration (300mg/ml, 200mg/ml and 100mg/ml) of the aqueous and hexane extracts of twenty four (24) screened plants leaves were tested against *Salmonella typhi*, but only thirteen (13) of the plant extracts had the efficacy against *Salmonella typhi*. Ciprofloxacin concentrations were highly sensitive against *Salmonella typhi* compare to aqueous and hexane extracts. This result was similar with the previous finding of (Josephus, *et al.*, 2024; Bello *et al.*, 2020). The aqueous extract of *Ficus sycomorus* had (16mm) zone of inhibition; which demonstrated the inhibitory effects on the growth of *Salmonella typhi*. This result justifies the previous finding of (Josephus, *et al.,* 2024). While the hexane extract of *Ficus sycomorus* had (14mm) zone of inhibition at 300 mg/ml. This result is similar to the finding of (Josephus, *et al.,* 2024). The efficacy of the aqueous extract of (15mm) *Mangifera indica* demonstrated a broad zone of inhibition; this indicated that the aqueous extract of *Mangifera indica* was sensitive to the *Salmonella typhi* and the extracts have been shown to exhibit significant antimicrobial activity against *Salmonella typhi*. This result is corroborated with the previous finding of (Abdul-Hannan, *et al.,* 2013). While the hexane extract of *Mangifera indica* (12mm) was also sensitive to the *Salmonella typhi* at 300 mg/ml. This result was similar to the finding of (Abdul-Hannan, *et al.,* 2013). The Antibacterial activity revealed that the aqueous extract of *Citrus aurantifolia* demonstrated (14mm), which indicated the aqueous extract of *Citrus aurantifolia* was sensitive against *Salmonella typhi*. This result is corroborated with the previous finding of (Bello *et al.,* 2020). While the hexane extract of *Citrus aurantifolia* (12mm) was also sensitive to the *Salmonella typhi* at 300 mg/ml. This result is similar to the previous finding of (Bello *et al.,* 2020). *Carica papaya* aqueous and hexane extracts had (13mm) and (12mm) respectively at 300mg/ml; this shows that the aqueous and hexane extracts of *Carica papaya* were sensitive against *Salmonella typhi*. This result was similar to the previous finding of (Adeyemo and Omolade, 2021). Seven (7) plants (*Allium sativum, Senna ocidentalis, Eucalyptus camaldulensis, Azadrachta indica, Cassia siamea, Psidium guajava* and *Gueira senegalensis*) demonstrated the antibacterial activity at all aqueous extracts concentrations but only *Allium sativum* revealed the zone of inhibition at 300mg/ml and 200mg/ml of hexane extract. This result justifies the previous finding of (Aondover, *et al.,* 2024; Bello *et al.,* 2020). Antibacterial effects were observed in the aqueous extracts of *Psidium guajava* and *Cassia siamea* revealed the zone of inhibitions of (12mm) respectively while the *Salmonella typhi* was resistant to the hexane extract of *Psidium guajava* and *Cassia siamea*. This result justifies the previous finding of (Aondover, *et al.,* 2024; Bello *et al.,* 2020). The results of the aqueous extracts of *Eucalyptus camaldulensis, Guiera senegalensis, Senna ocidentalis,* and *Azadrachta indica* had (10mm) at 300mg/ml against *Salmonella typhi* but the *Salmonella typhi* was resistant to the hexane extract of *Senna ocidentalis, Eucalyptus camaldulensis, Azadrachta indica* and *Guiera senegalensis*. This shows that the aqueous extracts were sensitive to the *Salmonella typhi.* This finding justifies the previous finding of (Bello *et al.,* 2020). The efficacy of the aqueous extracts of *Calotripis procera* and *Tamerindus indica* was 10mm and 8mm at 300mg/ml and 200mg/ml against *Salmonella typhi* but the *Salmonella typhi* was resistant to the hexane extract of *Calotripis procera* and *Tamerindus indica*. This result justifies the previous finding of (Adeyemo, and Omolade, 2021; Priscila, *et al.,* 2007). *Salmonella typhi* was resistant to the aqueous and hexane extracts of the *Furcraea foetide, Senna tora, Syzygium guineese, Khaya senegalensis, Adansonia digitata, Moringa oleifera, Olea europaea, Allium cepa, Ficus thoningii, Furcraea foetide, Saccharum officianarum,* and *Khaya senegalensis*. This result is in contrast with the previous finding of (Bello, *et al.,* 2024) on *Adansonia digitata.* The ineffectiveness of these plant extracts could be attributed to various factors, including soil composition, seasonal variations, plant age, climate and geographical location, as also suggested by (Kleiton, *et al.,* 2023).

MIC and MBC of the Aqueous and hexane Extract of the Screened Plants on *Salmonella typhi*

The MIC and MBC of aqueous and hexane extracts of the screened plants leaves on *Salmonella typhi* were revealed Tables 3 and 4. The lowest antibacterial activity was observed in ciprofloxacin on *Salmonella typhi*. Aqueous extracts of *Ficus sycomorus, Mangifera indica, Citrus aurantifolia, Carica papaya* and *Allium sativum* showed MIC and MBC of 12.5mg/ml and 25 mg/ml respectively against *Salmonella typhi*, while hexane extract was 25 mg/ml (MIC) and 50mg/ml (MBC) (Aondover, *et al.,* 2024)**.** The MIC and MBC values of the aqueous extracts of

Psidium guajava, Azadrachta indica, Cassia siamea and *Eucalyptus camaldulensis* against *Salmonella typhi* were 25mg/ml and 50 mg/ml respectively (Adeyanju, *et al.,* 2011; Priscila, *et al.,* 2007: Bello *et al.,* 2020). MIC and MBC of aqueous extracts of *Senna occidentalis* and *Guiera senegalensis* against *Salmonella typhi* were (50mg/ml and 100 mg/ml) respectively. This finding was similar to the previous finding of (Bello *et al.,* 2020). MIC and MBC of the aqueous extracts of *Calotripis procera,* and *Tamerindus indica* against *Salmonella typhi* were (100 mg/ml and 100mg/ml) respectively. The bacteria were killing only when the extracts had higher MBC to inhibit their growth. Hence, the outcome of this investigation corroborates the respondent's perception of crude extracts as a viable remedy for typhoid fever

Conclusion

The leaves of the *Ficus sycomorus Mangifera indica, Citrus sinensis* and *Carica papaya* have shown promise as a potential antibacterial agent against *Salmonella typhi* more than other screened plants. MIC and MBC of the *Ficus sycomorus, Mangifera indica* and *Carica papaya* demonstrated the bactericidal activity but *Psidium guajava, Azadrachta indica, Cassia siamea*, *Eucalyptus camaldulensis, Allium sativum, Senna occidentalis* and *Guiera senegalensis* demonstrates bacteriostatic activity.

Recommendations

It is recommended that the extracts from *Ficus sycomorus, Mangifera indica, Citrus aurantifolia* and *Carica papaya* leaves demonstrated antibacterial activity against *Salmonella typhi*. Therefore, the plants could serve as sources medicine for typhoid fever after further confirmatory studies.

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