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*Full Length Research Paper*

# Antibacterial activity of *Senna siamae* leaf extracts on *Salmonella typhi*

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**The aqueous and organic leaf extracts of the plant *Senna siamae*, traditionally used for the treatment of infectious disease, were tested for their activity against clinical isolates of *Salmonella typhi* using the disc diffusion method. The ethanol extracts showed the highest activity (zone of inhibition 10 ± 0.01 mm), followed by acetone extracts (zone of inhibition 8 ± 0.01 mm), while the aqueous extracts showed the lowest activity (zone of inhibition 3.5 ± 0.01 mm) at 40 mg/ml concentration. Preliminary phytochemical studies revealed the presence of alkaloids, saponins, tannins and glycosides. The activities of the extracts were comparable to those of ampicillin, chloramphenicol, cotrimoxazole and ciprofloxacin antibiotics (t-test; $p < 0.05$) . The antibacterial activities of the extracts against *S. typhi* did not change significantly when treated at 4, 30, 60 and 100°C for 1 h, but reduced significantly at pH 6 to 10. The MIC and MBC values of the crude extracts (1 - 3 mg/ml) were comparable to those of the tested antibiotics (0.3 - 1 mg/ml) (t-test; $p < 0.05$). Preliminary purification of the ethanol extracts with hexane, ethyl acetate, chloroform and *n*-butanol showed that the ethyl acetate fraction possessed the highest activity (zone of inhibition 15 mm), followed by *n*-butanol fraction (zone of inhibition 2 mm), while the chloroform fraction did not show any activity at 20 mg/ml.**

**Key words:** Natural bioactive compounds, *Senna siamae*, Antibacterial activity, Antityphoid drug, *Salmonella typhi*, Enteric fever, Antibiotics.

## INTRODUCTION

The bacterium *Salmonella typhi* causes typhoid fever (Doughari et al*.* 2007; Prescott et al*.* 2005). The bacterium is a gram-negative, motile, non- sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits and vegetables or via convalescent or chronic carriers (Duguid et al., 1983; Doughari, 2005). It has also been linked with zoonotic transmission via reptiles and common domestic pets (Duguid et al., 1983; Birgitta et al., 2005). Enteric fever (typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate (WHO, 2003; John et al., 2003). In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics and a rapid rise in multidrug resistance. Resistance to the first line drugs chloramphenicol, ciprofloxacin and amoxycillin has been reported (Zulfigar et al., 1994; Benoit et al., 2003). With the increase in resistance to antityphoid drugs, medicinal plants have gained popularity among both urban and rural dwellers in the treatment of the ailment. Moreover, 80% of the world population are rural dwellers and rely on medicinal plants for their daily medications, and plants have been reported to have minimal or no side effects compared to antibiotics (Bibitha et al*.,* 2002; Maghrani et al*.,* 2005). *Senna siamea* Lam. (Irwim and Barneby -*Cassia siamea* Lam) (Fabaceae, Caesalpinioideae) (Fowler, 2006) is a plant specially introduced from Asia (exotic) and commonly cultivated in fuel plantations and elsewhere in and around towns and villages. In Burkina Faso, a decoction of the leaves with lemon juice is used for the treatment of fevers (Fowler, 2006). In northern Nigeria, the tree is very popular for its local usage in the treatment of typhoid fever. The study was therefore carried out to investigate the antimicrobial property of the leaf extracts with the view to provide scientific evidence for its application as a medicinal plant.

## MATERIALS AND METHODS

Fresh leaves of *S. siamae* Lam. were collected from the wild in Yola North Local Government Area of Adamawa (9º 20´N, 12º 30´E), Nigeria and were identified and authenticated at the Department of Biological Sciences; School of Pure and Applied Sciences, Federal University of Technology, Yola; Adamawa State, Nigeria.

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**Preparation of crude and organic extracts**

The plant extracts were prepared using the method of Ahmad and Beg (2001) with minor modifications. The fresh leaves were shade-dried to constant weight for 5 days, coarsely powdered using mortar and pestle and further reduced to powder using an electric blender, and stored in closed bottles. The powdered samples (25 g) were then extracted using distilled water, acetone (BDH Chemicals Ltd, Poole, England) and ethanol (BDH Chemicals Ltd, Poole, England) by allowing to sediment at room temperature (30 - 32°C) for 72 h with manual agitation of the flask using a sterile glass rod after every 24 h. After 72 h, each of the extracts was filtered using a clean sterile muslin cloth and then using Whatman filter paper. The filtrates were then concentrated in vacuum at 40°C and stored at 4°C for further use.

**Test bacteria**

The - lactamase possessing bacteria *S. typhi* was isolated from clinical samples of clotted blood obtained from the Federal Medical Centre, Yola and serotyped according to WHO (2003). The isolates were maintained on MacConkey agar (Oxoid) at 4°C.

**Determination of -lactamase activity**

For determination of bacteria -lactamase activity the iodometric test, as described by Livermore and Brown (2001), was used. In this method, 6 mg/ml of benzyl penicillin in 0.1 M phosphate buffer pH 6.0 was distributed in 0.1 ml quantities in test tubes. A heavy suspension of the test organism from nutrient agar plates was introduced into the test tubes. The suspensions were held at ambient temperature (32ºC) for 30 - 60 min, and then 2% iodine (20 µl) in 53% aqueous potassium iodide (w/v) was added. Positive test for -lactamase activity was demonstrated by decolorization of the iodine within 5 min. Positive and negative controls were run concurrently.

**Preliminary phytochemical analysis**

Preliminary phytochemical studies to determine the presence of alkaloids, anthraquinones, saponins, tannins and phlobatamins were carried out using the method described by Odebiyi and Sofowora (1999).

**Antibiotic susceptibility testing**

Antibacterial activity of the aqueous and organic extracts of the plant sample was evaluated by the paper disc diffusion method on Mueller Hinton agar (MHA) plates (Kabir et al., 2005; Aida et al., 2001). Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto MHA (Oxoid) plates (diameter: 15 cm). Sterile filter paper discs (diameter 6 mm) soaked in a known concentration of extract (2, 5, 10, 20 and 40 mg/ml per disc in each case) in DMSO were applied over each of the culture plates previously seeded with the 0.5 McFarland turbidity standard cultures of the test bacteria. The cultures were then incubated at $37^{O}$C for 18 h. Paper discs soaked in a known concentration (5 mg/ml per disc) ampicillin, amoxicillin, ciprofloxacin, chloramphenicol and cotrimoxazole in distilled water, as standard antibiotics, were used for comparison. Filter paper discs dipped into sterile distilled water and afterwards dried were used as controls. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc.

For each extract, three replicate trials were conducted against each organism.

**Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

Determination of the MIC and the MBC was carried out as described by Gupte (2006) with slight modification. The minimum inhibittory concentration of the extracts was determined for the test organisms in triplicates at varying concentrations of 20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0 and 0.5 mg/ml. To obtain these concentrations, varying concentrations (1 ml) of the extracts containing double strength of the concentrations (40.0, 36.0, 30.0, 20.0, 16.0, 10.0, 2.0 and 1.0 mg/ml) were constituted in different test tubes, and 1 ml of nutrient broth was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin, chloramphenicol and cotrimoxazole). A tube containing nutrient broth only was seeded with the test organism, as described above, to serve as control. All the culture tubes were then incubated at $37^{O}$C for 24 h. After incubation, they were examined for bacterial growth by observing-measuring turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any bacterial growth, and inoculated on sterile nutrient agar by streaking. Nutrient agar plates were streaked with the test organisms only to serve as control. The plates were then incubated at $37^{O}$C for 24 h. After incubation the concentration at which no visible bacterial growth was observed was noted as the MBC.

**Effect of temperature and pH on antibacterial activity of extracts**

Tests were carried out as described earlier (Doughari, 2006). 5 ml of 60 mg/ml of acetone extracts were constituted in test tubes and treated for 1 h at $4^{O}$C in the refrigerator and at 30, 60 and $100^{O}$C in a water bath, and further tested for antibacterial activity.

To determine the effect of pH, pH of plant acetone extracts were adjusted to different pH ranges from 3 to 10 using 1 N HCl and 1 N NaOH solutions, respectively, in series of test tubes for 1 h. The plant acetone extracts were afterwards neutralized to pH 7 and tested for antibacterial activity.

**Preliminary purification of *S. siamae* extracts**

Preliminary purification of the ethanol fractions was carried out using hexane (BDH Chemicals Ltd, Poole, England), chloroform (BDH Chemicals Ltd, Poole, England), ethyl acetate (BDH Chemicals Ltd, Poole, England) and *n*-butanol (BDH Chemicals Ltd, Poole, England) as described by Farah et al. (1985). 50 mg of ethanol extracts was mixed with 30 ml of hexane in a separator and allowed to soak for 5 min, and then shaken vigorously to mix. The hexane soluble fraction (fraction A) was drained out into a sterile Petri dish. 30 ml of chloroform was then added to the hexane insoluble fraction, allowed to soak for 5 min and shaken to mix. The chloroform soluble fraction (fraction B) was also separated into a clean sterile Petri dish. The same procedure was continued with ethyl acetate and *n*-butanol to obtain fractions C and D, respectively. The *n*-butanol insoluble fraction was fraction E. All the fractions were allowed to evaporate to dryness and then used for antibacterial susceptibility testing.

**Determination of antibacterial activity of the fractions obtained**

Each of the dried fractions was dissolved in methanol (1 ml) to a final concentration of 20 mg/ml, impregnated on filter paper discs (20 mg/ml per disc) and used to determine their antibacterial activities against *S. typhi*, respectively.

**Table 1.** Antibacterial activity of leaf extracts of *S. siamae* against *S. typhi*.

| Extract/Antibiotic | Antibacterial activity - zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Concentrations (mg/ml) | | | | | |
| | 40 | 30 | 20 | 10 | 5 | 2 |
| Acetone | 8.0 ± 0.0 | 7.3 ± 0.08 | 7.5±0.4 | 5.5 ± 0.02 | 1.8 ± 0.02 | - |
| Ethanol | 10.0 ± 0.2 | 8.0 ± 0.0 | 8.0 ± 0.02 | 4.5± 0.04 | 3.3 ± 0.2 | - |
| Water | 3.5± 0.01 | 2.5 ± 0.2 | 1.0±0.0 | - | - | - |
| Ampicillin | 8.0 ± 0.01 | 6.0 ± 0.05 | 4.2 ± 0.05 | 4.0 ± 0.0 | 2.0 ± 0.0 | - |
| Amoxycillin | 10.5 ± 0.2 | 9.0 ± 0.03 | 7.0±0.6 | 4.0 ± 0.0 | 2.1 ± 0.7 | 2.5 ± 0.0 |
| Ciprofloxacin | 30.0 ± 0.0 | 28.0 ± 0.0 | 26.0 ± 0.0 | 23.0 ± 0.0 | 21.0 ± 0.4 | 10.0 ± 0.3 |
| Cotrimoxazole | 14.0 ± 0.02 | 12.3 ± 0.5 | 9.0 ± 0.8 | 7.0 ± 0.0 | 4.0 ± 0.0 | 4.0 ± 05 |
| Chloramphenicol | 16.0 ± 0.01 | 14.0 ± 0.01 | 13.2±0.05 | 12.0 ± 0.0 | 11.0 ± 0.0 | 6.0 ± 0.0 |

**Key:** - → no measurable zone.

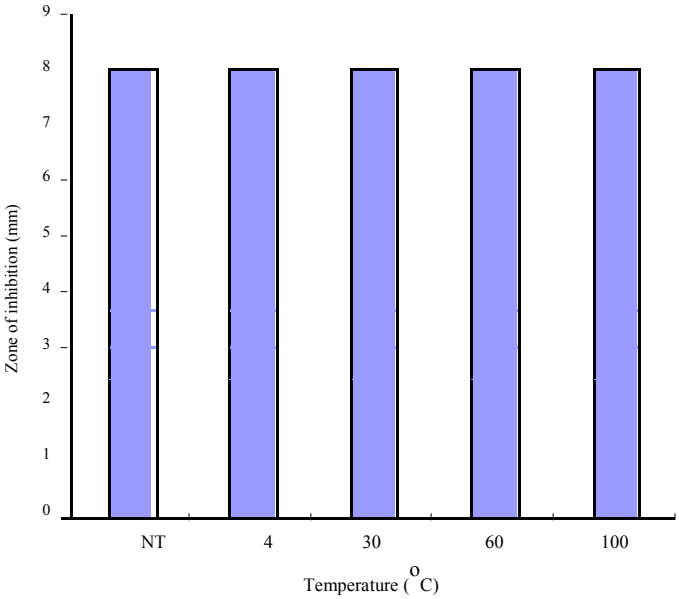


**Figure 1.** Effect of temperature treatment of ethanol leaf extracts of *S. siamae* at 4, 30, 60 and 100 °C for 1 h on antibacterial activity. NT = nontreated (Control – room temperature).

## RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed that the leaf extracts of *S. siamae* possesses alkaloids, saponins, tannins and glycosides (data not shown). Phytoconstituents such as saponins, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plantsagainst bacterial and fungal infections (Gonzalel and Mather, 1982; Okwute, 1992). The leaf extracts showed antibacterial activity against *S. typhi* at all the tested concentrations (2 - 40 mg/ml) (Table 1). The ethanol extracts demonstrated the highest activity (zone of inhibition 10 ± 0.01 mm), followed by acetone extracts (zone of inhibition 8 ± 0.01 mm), while the aqueous extracts of the plant showed the lowest activity (zone of inhibition 3.5 ± 0.01 mm) at 40 mg/ml. The demonstration of higher activity by the organic solvents may be an indication that the phytoconstituents are more soluble in them than the aqueous

**Table 2.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of leaf extracts of *S. siamae* and model antibiotics.

| Extract/Fraction | MIC (mg/ml) | MBC (mg/ml) |
|---|---|---|
| *S. siamae* | 1.0 | 1.3 |
| Ampicillin | 1.0 | 1.0 |
| Amoxycillin | 0.7 | 0.7 |
| Ciprofloxacin | 0.4 | 0.5 |
| Cotrimoxazole | 0.2 | 0.2 |
| Chloramphenicol | 0.3 | 0.5 |

solvent; acetone particularly is known to be selective for tannins (Marjorie, 1999). Lack of significant antibacterial activity by the aqueous extracts may explain why the local usage of the plant for the treatment of typhoid is prolonged, probably to achieve the needed effective dosage level. In this study we compared antibacterial activity of *S. siamae* leaf extras against the test bacteria with activities of model antibiotics. The higher antibacterial activity of model antibiotics is not surprising, since the antibiotics are in a refined state. The standard antibiotics (ampicillin, amoxycillin, ciprofloxacin, cotrimoxazole, chloramphenicol) used in this study are first line drugs employed in the treatment of typhoid fever (Prescott et al., 2005). The effect of temperature (Figure 1) on the antibacterial activity of the ethanol extracts on *S. typhi* showed that after treatment of the extracts at 4, 30, 60 and 100°C for 1 h, the antibacterial activity remained unchanged. With an increase in pH, the activity reduced significantly at pH 8 and 10 (Figure 2). This may be an indication that the bioactive components of this plant are

heat stable but labile in alkaline conditions. The result (Table 2) shows that the MIC and MBC values of *S. siamae* extracts were comparable to standard antibiotics (ampicillin, amoxicillin), but higher than those of cotrimoxazole, chloramphenicol and ciprofloxacin (t-test; $p < 0.05$ – using statistical software package – SPSS). The high MIC and MBC values of some of the antibiotics are an evidence of (possible) drug resistance of the test organism. Results also showed slightly higher MBC values indicating that the extracts may be bactericidal in action (Jigna et al., 2006) . Table 3 shows the antibacterial activity of fractions of *S. siamae* leaf extracts after preliminary purification using polar solvents (hexane, chloroform, ethyl acetate and butanol).

The result showed that all of the fractions obtained (fractions A, B, C, D and E) have acidic pH (pH 4 - 4.5). Comparing all four fractions, the ethyl acetate fraction showed the highest antibacterial activity against *S. typhi* (zone of inhibition 15 mm), followed by the butanol fraction

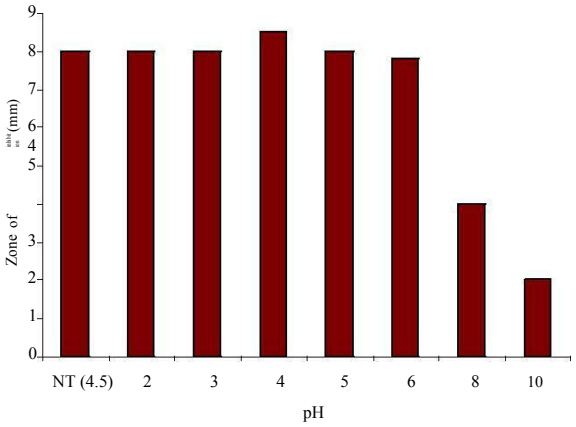


**Figure 2.** Effect of pH treatment of ethanol leaf extracts of *S. siamae* on antibacterial activity. NT = non-treated extract (pH 4.5).

**Table 3.** Antibacterial activity of crude and fractionated ethanol leaf extracts of *S. siamae* (at 20 mg/ml concentration).

| Extract/fraction | pH | Antibacterial activity - zone of inhibition (mm) |
|---|---|---|
| Crude extract | 3.8 | 4.5 |
| Fraction A | 4.4 | 1.0 |
| Fraction B | 4.4 | - |
| Fraction C | 4.3 | 15.0 |
| Fraction D | 4.5 | 3.0 |
| Fraction E | 4.0 | - |

**Key:** Fraction A → hexane fraction; fraction B → chloroform fraction; fraction C → ethyl acetate fraction; fraction D → butanol fraction; fraction E → butanol insoluble fraction; - → no measurable zone.

(zone of inhibition 3 mm) and the hexane fraction (zone of inhibition 1 mm) at 20 mg/ml. The chloroform fraction did not show any activity. Kyatyayani et al. (2002) sho-wed that ethyl acetate solubles of acidified mother liquor and buffer solubles of neutral mother liquor of stem bark of *Alangium salvifolium* showed significant antibacterial activity against some gram-positive and gram-negative bacteria and fungi. It may thus be concluded that solubility of active phytoconstituents in ethyl acetate may be responsible for the high activity of ethyl acetate fractions against *S. typhi.*

## Conclusion

The demonstration of antibacterial activity by *S. siamae* leaf extracts against *S. typhi* has provided a scientific basis for its local usage as a medicinal plant in the treatment of typhoid fever. The fact that antibacterial activity of the extracts increased after purification using polar solvents is evidence that the bioactive compounds in the plant *S. siamae* are promising source of a potent antityphoid drug that will be effective, cheap and accessible to all. Therefore, further research to refine, detect and characterize bioactive compounds and its effect on *S. typhi*, including toxicological studies, needs to be carried out.

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