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Phytochemical Screening and Antityphoid Activity of *Senna occedentalis* Aqueous Leaf Extract on *Salmonella typhi*

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Senna occidentalis is used in Northern Nigeria by the Traditional Medicine Practitioners for treatment of typhoid fever. This research was designed to evaluate the phytochemical and antibacterial activity of *Senna occidentalis* leaf extract on *Salmonella typhi*. The powdered plant sample was extracted by maceration using sterile distilled water and concentrated over water oven. The phytochemical screening was conducted using standard methods. The determination of antibacterial activity was carried out using agar well diffusion method and Ciprofloxacin (10 µg) was used as control. The phytochemical screening revealed the presence of flavonoid, tannins, saponin alkaloid, cardiac glycoside, steroid, glycoside and saponin glycoside. Anthraquinones and volatile oil were not detected. The results of the antibacterial activity revealed zone of inhibition against *Salmonella typhi* at various concentration of 30mg/ml, 60mg/ml, 90mg/ml and 120 mg/ml. The highest zone of inhibition was obtained at 120 mg/ml (15.75 ± 0.96) as against the lowest at 30 mg/ml (6.13 ± 0.48). From the results obtained Minimum inhibitory concentration were recorded at 12.50 and minimum bactericidal concentration (MBCs) 50.00. The antibacterial activity demonstrated by the extract is concentration dependent. The results showed that the plant leaf extract has antityphoid fever activity. This research recommended that, ministry of health should make it mandatory that all herbal products be subjected to scientific verifications before being sold as remedy.

Keywords: *Senna occedentalis*, Antityphoid, *Salmonella typhi* and phytochemical

1. Introduction

Antimicrobial agents are substances that interfere with the growth and metabolism of microbes (Nester *et al*., 2004). These agents depend on the normal host defenses to kill or eliminate the pathogens bacteria after its growth has been inhibited (Nester *et al*., 2004). Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhea and tuberculosis as well as increase in resistance by several bacteria to various antibiotic (Smolinski *et al.,* 2003). Unfortunately, rapid explosion in human population has made it almost impossible for modern health facilities to meet health demands all over the world, thus putting more demands on the use of natural herbal health remedies (Conco, 1999). Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as Methicillin resistant *Staphylococcus aureus, Helicobacter pylori* and MDR *Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties.

Senne occidentalis is a small tree that grow 5 - 8cm, popularly known as *Sanga sanga* by the speaking Hausa peoples, which belongs to family leguminosae, genus: Senne, species: *occidentalis* (Mitsuyama *et al*., 1987). Its annual undershrub, subglabrous, foetid, few feet high with alternate, compound, paripinnate leaves. Flowers are complete, bisexual, slightly irregular, zygomorphic, pentamerous, hypogynous, pedicelate, bractate and bracts white with pinkish tinge, thin, ovate-acuminate, caduceus and yellow. These plant distributed in Aliero bush, home or along the roads. Seeds pods long are sometimes roasted and made a coffee-like beverage (Muhammadu, 1990). Many species have been medicinally and these tropical plants have a history in natural medicine as purgative and laxatives (NRC, 2008). Historically, in the pre-antibiotic era the case fatality rate of typhoid

fever was 10-20%. Today, with prompt treatment is less than 1% (WHO, 2007). *S. occidentalis* have been of medical interest due to their good therapeutic value in folk medicine (Abo *et al.*, 1999). The availability of *S. occidentalis* in Aliero community makes the plant to be a potential source of herbal medicine for the treatment of typhoid fever by the inhabitants. Therefore, this study was aimed to determine the antibacterial activity of *S. occidentalis* leave extract against *Salmonella typhi.*

2. Materials and Methods

2.1 Study Area

Aliero local government shares common boarders with Gwandu local government areas on the Northest, Jega local government area by the southwest and by east is Tambuwal local government of Sokoto state while the northern west Birnin Kebbi local government. The local government covers a total of 412 square kilometers of land mass with a population of over 125, 785 inhabitants (NPC, 2006). The topography is flat and slightly undulating with compact, stony brown soil. Aliero has savannah vegetation. It located at latitude 110 3S, 402E.

2.2 Collection and identification of the plant

The fresh leaves of *Senna occidentalis* were collected from Fadama area within Aliero community. The plant was identified at Herbarium, Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. Plant was identified by Professor of Taxonomy Dharmendra Singh and assigned voucher number (KSUSTA/PSB/71).

2.3 Preparation of Plant Sample

Fresh leaves were washed under running tap water to eliminate dust and other foreign particles and shaded dried for 7 days. The shade-dried were pulverized into powdered using wooden pestle and mortar.

2.4 Extraction of plant Sample

Extraction of dried powdered leaves (50 g) were done used sterile distilled water and aqueous. The latex were dissolved in 100 ml of solvent. The suspended solution were left to stand for 5 days and filtered through sterile muslin cloth and re-filtered through what man No 1 filter paper as described by Omenka and Osuoba (2000). The extracts were stored at 4^{o}C until required.

2.5 Phytochemical analysis

The preliminary qualitative phytochemical screening of *Senna occidentalis* leaf extract was carried out to test for the presence of saponins, alkaloids, glycosides, flavonoids, tannins, volatile oil, steroid, Anthraquinones and cardiac glycoside using standard qualitative procedures (AOAC, 2000).

2.6 Collection of *Salmonella typhi*

The *Salmonella typhi* was collected from stock cultures from the Department of Microbiology laboratory, Faculty of Science Usman Danfodiyo University Sokoto. The isolates were maintained on nutrient agar slant at 4°c until further use.

2.7 Antibiotics Susceptibility Testing of the Test Organism

Antimicrobial disc tests of the isolates were performed according to the recommendations of the National Committee Laboratory Standards (2000) using the ciprofloxacin (l0µg), antibiotics resistance was interpreted by diameter of inhibition zones around the antibiotic discs.

2.8 Antibacterial Susceptibility Testing of the Extracts with Test Organism

The inocula were prepared by inoculating the test organism in nutrient broth and they were incubated for 24 hours at 37^{o}C. The cultures were diluted to 0.5 McFarland turbidity standard after the incubation while 0.2 milliliter of the cultures was further diluted in normal saline and were inoculated onto solidified nutrient agar using glass rod by spreading technique. The ability of the various extracts to inhibit the growth of the clinical test organism were determined using the agar well technique. The inoculated nutrients agar plates were allowed to dry. After which, wells were bored on the surface of inoculated agar plates using 4 mm cork borer. 0.2 ml of the different concentration of each extracts were transferred into the well using Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zones of inhibition from overlapping. The plates were incubated at 37^{o}C for 24 hours (Omenka and Osuoba, 2000).

2.9 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the aqueous were determined on the test organism in triplicates at varying concentrations of 30, 60 and 90 mg/ml. One milliliter (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 to the tubes. A tube containing nutrient broth only were seeded with the test organism to serve as control. All the tubes were then incubated at 37^{o}C for 24 hours and then examined for growth by observing for turbidity.

2.10 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the *Senna occendentalis* extract on the clinical bacteria isolate was carried out according to method described by Omenka and Osuoba (2000). Briefly, 1 ml bacterial culture were pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and were sub-cultured onto nutrient agar and incubated at 37^{o}C for 24 hours. After incubation the concentration at which there was no single colony of bacteria was taken as MBC.

2.11 Statistical Analysis

The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical package and the results were expressed as mean ± standard deviation of three replicates with Tukey's multiple comparison testing to determine the significant differences at P< 0.05 between the control and experimental groups.

3. Results and Discussion

3.1 Results

The phytochemical analysis of the leaf extract of *S. occedentalis* revealed the presences of flavonoid, tannins, saponins, alkaloids, cardiac glycosides, steroid glycoside and saponin glycoside while, anthraquinones and volatile oil were not detected in the sample as presented in (Table 1).

The extract of the leaf was tested against *Salmonella typhi,* the results of the test showed higher activity against the test organism at higher concentration (120 mg/ml) with zone of inhibition of 15.75±0.95. The lowest zone of inhibition (6.13±0.48) was observed at 30 mg/ml as seen in (Table 2). However, the control drug Ciprofloxacin were found to exhibits antimicrobial activity against test organism (*Salmonella typhi*) at 38.00 ± 0.00 as shown in (Table 2). MBC of the aqueous extracts against *S. typhi* were presented in (Table 3).

Table 1. Phytochemical Analysis of *Senna occedentalis* Aqueous Leaf Extract

Phytochemical constituents	Results
Flavonoid	+
Tannins	+
Saponin	+
Alkanoid	+
Cardiac glycoside	+
Steroid	+
Anthraquinones	N.D
Glycoside	+
Saponin glycoside	+
Volatile oil	N.D

Key + = presence; N.D = Not detected

Table 2. Diameter of mean zone of inhibition on *Salmonella typhi*

Organism	Concentration (mg/ml)	Mean diameter zone of inhibition (mm)
Salmonella typhi	30	6.13±0.48
Salmonella typhi	60	8.88±0.54
Salmonella typhi	90	13.25±0.64
Control	Ciproflox (10µg)	38.00 ± 0.00

The zone diameter of zone of inhibition is presented as mean ± SD

Table 3. Minimum inhibition concentration and Minimum bactericidal concentration

Test organism	MIC (mg/ml)	MBC (mg/ml)
	Aqueous	
Salmonella typhi	12.50	50.00

3.2 Discussion

This present study on the phytochemical constituents of the leaf extract of *Senna occedentalis* revealed the presences of flavonoid, tannins, saponins, alkaloids, cardiac glycosides, steroid glycoside and saponin glycoside while, anthraquinones and volatile oil were not detected in the sample as seen in (Table 1). This statement disagrees with the research of Sadiq *et al*. (2012) who reported that *Cassia occedentalis* of the ethanol and water extract revealed the presence of tannins, cardiac glycosides, saponins, and Anthraquinone while the fractions revealed the presence of tannins, terpenoids and anthraquinones. These differences of some compounds that where not

detected in this present study could be due to the differences of methods use, environmental conditions were this plant growth. However, according to the Trease and Evans (1993) reported phytoactive compound are well known for their wide pharmacological activities ranging from antibacterial and antifungal. Also, the presence of tannin, alkaloids in aqueous extract of *Senna occidentalis* indicate that the plant is an astringent, which help in the wound healing and anti-malaria (Safary *et al.,* 2009). Likewise, alkaloids are known to possess anti-malaria prevent motion sickness, and as components of pre-anesthetic drug. This are administered either as pure compound or as extract and have often served as model structures for synthetic drugs (Safary *et al.*, 2009). Plant containing saponin are believed to have antioxidant, anticancer, anti-inflammatory and anti-viral properties saponins help humans to fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knockout some kind of tumor cells. Particularly lung and blood cancers. These compounds served as natural antibiotics which help the body to fight infections and microbial invasion (Safary *et al.,* 2009).

The extract of the leaf was tested against *Salmonella typhi* as a test organism and the results obtained indicated a significant effect against the test organism but higher effect was recorded at 120mg concentration which showed (15.75±0.95) and the lower effect was observed at 30mg/ml with (6.13±0.48) zone of inhibition as showed in (Table 2). This finding agree with study of Sadiq *et al*. (2012) and Keta *et al*. (2019), who suggested that, antibacterial activity of *Cassia occidentalis* leaves of ethanol and water extract against test organisms were increase when used in higher concentration. The control drug (Ciprofloxacin) was found to exhibits antibacterial activity against the *Salmonella typhi* as Gram negative bacteria. Thus the study suggests that the inhibition of bacterial growth activity of the extracts is dose dependent.

The finding of the study showed that *Senna occidentalis* aqueous leaf extract has antibacterial against *Salmonella typhi*, with maximum mean zone of inhibition. *Salmonella typhi* has been incriminated in causing *Salmonellsis* which is characterized by gastric initial upset, diarrhea, fever and occasionally death. The antimicrobial activity may be attributed to the presence of volatile oil which contains more bioactive compounds, such as flavanoid, saponin, phenol acid etc. that have been reported to show antimicrobial activity (Edeoga *et al.,* 2009). However, the high activity of the extract recorded on *Salmonella typhi* is very necessary at 30, 60 and 90 mg/ml concentrations this could be due to the bioactive compounds in the leaf. Similarly, the antimicrobial activity of queues extract of standard drug (ciprofloxacin) against *salmonella typhi* at 90 mg/ml shows complete mortality of *S. typhi*.

Based on the results, the extracts showed a greater antimicrobial activity against *S. typhi* with an MBC concentration of 12.00 and an inhibition zone of 50.00mm been the largest. The activity on *S. typhi* was different as zones of inhibition were gotten which was similar to results gotten by Onyeaba *et al*. (2015) 90% v/v concentration was shown to kill *E.coli*. This could be as a result of two possible lab errors, either the bacterial lawn was too thick which rendered the extract inactive or no cells were transferred from the MIC tube to the MBC plates.

A similar trend to the MICs of extracts was also observed for the MBCs of extracts on the test organism, which depicts the lowest concentration of extract that did not allow any bacterial growth on the surface of the agar plates (i.e. the lowest concentration that kills all the bacteria). It was however observed that MICs of the extracts were lower than the MBCs, suggesting that the extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations. This supports the report of Aliyu *et al.* (2008), which evaluated the antibacterial activity of 12 medicinal plants used in Northern Nigerian traditional medicine against 25 hospital isolates of methicillin resistant *Staphylococcus aureus* (MRSA) using disc diffusion and broth dilution assays. According to Noumedem *et al.* (2013), an extract is bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when this ratio is >4. It therefore appeared that all except the second aqueous extract were bactericidal against the susceptible bacteria. The aqueous extract showed a bactericidal effect within the concentrations used for this study.

These observations are likely to be the result of the differences in the cell wall structure between Gram-negatives and Gram-positive bacteria, with Gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics (Tortora *et al.*, 2001). According to Nester *et al.* (2004), the cell walls of Gram-negative bacteria (*E. coli* and *S. typhi*), contain an outer membrane and lipid bilayer embedded with proteins and porins (carrier proteins).

4. Conclusion

This research concludes that the potency of the *Senna occiedentalis* is dependent on the solvent of extraction and the dose administered, which may vary with target organisms or infections. Also *S. occidentalis* could be useful herbal remedy in treatment of Typoid due to the present of these compounds obtained in the study. This justifies the claim that the *S. occendentalis* are efficacious in the management of diseases particularly related to *S. typhi*, such as gastroenteritis, meningitis, diarrhoea, typhoid fever, pneumonia, boils, wound and urinary tract infections.

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Conflict of interest

The authors declare no conflict of interest.

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