Determination of Phytochemical and Antibacterial Properties of the Leaf and Seed Extracts of *Senna occidentalis* (L.)

Ishaya, S. G.,[1*] Umaru, F. F.,[2] Samuel, K. B.,[3] Mafe, A. N.[4], Angyu, A. E.,[5] Babylon, P.,[6] Khan, N.[7] and Hammanjoda, H.[8]

[1,4]Department of Microbiology, Faculty of Science, Taraba State University, Jalingo, Nigeria.
[2,3,5,7]Department of Biological Sciences, Faculty of Science, Taraba State University, Jalingo, Nigeria.
[6]Department of Public Health, Faculty of Health Sciences, Taraba State University, Jalingo, Nigeria
[8]Department of Environmental Health, Federal Medical Centre, Yola, Nigeria.
Corresponding Author: samuelishaya29@gmail.com, +2348036700592

Abstract: *Senna occidentalis* is widely used by herbalists in northern Nigeria for the treatment of microbial diseases. This study determined the phytochemical constituents and antibacterial activities of aqueous extracts of the leaf and seed of *S. occidentalis* against some selected clinical pathogens. The Leaf and seed were reduced to powder and extracted by maceration using sterile distilled water. Extracts were concentrated using rotary evaporator and freeze drier. Phytochemical screening was conducted using standard methods while antibacterial activities were determined using the agar well diffusion method. The diameter of the zones of inhibition of bacterial growth was assessed as an index for antibacterial properties of the extracts against the selected bacteria. The phytochemical screening of the leaf and seed revealed the presence of flavonoids (33 % and 22 %), alkaloids (3.6% and 6%), saponins (1.2 % and 2.1 %), phenols (0.036 mg/mL and 0.042 mg/mL) and glycosides (2.68 mg/mL and 2.02 mg/mL) respectively, while steroids and tannins were absent in both. Both leaf and seed extracts exhibited significant (p<0.05) antibacterial activities at all the concentrations used (200mg/mL, 150mg/mL, 100mg/mL, and 50mg/mL) against the selected Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Salmonella* Typhi*, Escherichia coli,* and *Pseudomonas aeruginosa*) as compared to the standard antibiotic (Amoxicillin 10mg/mL) used as positive control. The antibacterial activities of the extracts increased significantly with increase in concentration for all organisms and the seed extracts were most active with mean inhibitory activity between 19-32mm compared to the leaf extracts with mean inhibitory activity between 12-31mm except *E. coli* where the leaf extracts were more potent (20, 25, 28, 31 mm) than the seed's (19, 22, 25, 32 mm) at concentrations ≤150 mg/mL. Hence, aqueous extracts of both plant parts possess the potential to be used as antibiotics to treat selected microbial diseases.

Keywords: Antimicrobial activities, phytochemical screening, plant extracts, *Senna occidentalis,* traditional medicine

INTRODUCTION

Medicinal plants are plants that have at least one of their parts (leaf, stem, bark or root) for therapeutic purposes (Nwali *et al*., 2010). Nature has served as a good source of medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from natural sources (Shinde *et al*., 2014). Awareness and general acceptability of the use of herbal drugs in today's medical practice is increasing. Over 70 % of the world relies on medicinal plants for primary health care and there are several reports on natural substances of plant origin which are biologically active, with desirable medicinal properties (Neuwinger, 2000; WHO, 2008; Edeoga *et al*., 2005).

Across the different regions of the world, several plants are now known to have medicinal effects and have been used over the years to treat various types of acute and chronic diseases (Cowan, 1999). Many pharmaceuticals currently available have a long history of use as herbal remedies including Aspirin, Artemisiline and Quinine. It has been reported that there are over 8000 species of known medicinal plants in Africa considered as an essential part of traditional health care systems (Nwali *et al*., 2010). More than 80 % of African population is dependent on these cheap and effective traditional medicines used against many diseases and infections (Neuwinger, 2000).

It is very common in Nigeria and other part of the world to use plants in form of crude extracts, decoction, infusion or tincture to treat common infections and chronic conditions.

The active principle of many drugs found in plants are phytochemicals. Many of these plants contain large varieties of these phytochemicals such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds which have significant biological effects on humans (Mozumder and Hossain, 2013; Gilani *et al.,* 2009). These phytochemicals are categorized into primary and secondary metabolites. Primary metabolites include carbohydrates, protein, amino acids, and chlorophyll while alkaloids, terpenoids are some examples of secondary metabolites. The secondary metabolites are so called because they are not required for growth, respiration, transpiration or any primary function in plants (Edeoga *et al*., 2005). The major secondary metabolites include alkaloids, flavonoids, tannins, terpenoids, and steroids (Edeoga *et al*., 2005). Plants produce these phytochemical compounds to protect themselves from pathogens and predators (Poongothai *et al.,* 2011).

Several studies have demonstrated that these phytochemicals have antimalarial, antibacterial, antimutagen, antiplasmodial, anti-inflammatory, and antipyretic effects on humans and they have been responsible for the medicinal effects of plants since historical times (Oyedemi and Afolayan, 2011).

Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines, drug abuse, blind use of traditional medicine, and emergence of widespread drugs resistance (Alhassan *et al.,* 2018).

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which could likely overcome the aforementioned disadvantages. Current trends in drug development process have focused on natural sources, especially sources that are of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants (Faruq *et al.,* 2006; Neuwinger, 2000).

Senna species are commonly used as shade plants, ornaments, foods, many have important medicinal properties and are used in both traditional and modern medicines (Oyedemi and Afolayan, 2011). *S. occidentalis* is a tropical plant and an important member of the *Fabaceae family* and the *Caesalpiniaceae* subfamily. Plants belonging to this family have been extensively investigated due to their folkloric medicinal and economic uses (Musa, *et al*., 2017). Notable folkloric use of this plants includes the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of Southwestern Nigeria (Satish *et al.,* 2007); management of hypertension in Ghana; management of fever, menstrual problems, tuberculosis, diuretic anaemia, liver complaints; and it's used as a tonic for general weakness and illness (Usha *et al.,* 2007).

Several studies have demonstrated that *S. occidentalis* contains a host of phytoactive chemicals that may support its numerous applications in folk medicine. The presence of anthraquinones, flavonoids, phenols, tannins, saponins, and their antimicrobial activities have been scientifically established in this plant (El-Mahmood and Doughari, 2008; Pieme *et al.*, 2006; 2007).

Several studies have revealed that the phytoactive compounds in plants vary with region and climate (Oladeji *et al*., 2020). This variation impacts the medicinal properties of plants since the phytochemicals are responsible for them. There is, therefore, the need to determine the phytochemical and antimicrobial profiles of plants in different

regions of the world, and also establish optimal ways to extract them; hence, the need for this study.

MATERIALS AND METHODS

Collection and Preparation of Samples

S. occidentalis leaf and seed were handpicked randomly at Fufore, Adamawa State, Nigeria (9.2284 ^{o}N, 12.7135 ^{o}E). They were washed under running tap water and dried at room temperature in an oven. The dried leaf and seed were pulverized into powder using an electric grinder and stored in an airtight container at 4 ℃.

Extraction of Metabolites

Hundred grams (100g) of the pulverized leaf and seed were measured and soaked in 200 mL of distilled water. The suspended solution was left to stand for 3 days, then filtered through sterile muslin cloth and re-filtered through Whatman No 1 filter paper as described by Omenka and Osuoba (2000). The filtrates were then concentrated using rotary evaporator and freeze drier.

Phytochemical Screening

A small portion of the concentrated filtrates was subjected to qualitative phytochemical screening using the method of Sofowora (1993). The presence of alkaloids, flavonoids, steroids, phenols, tannins, saponins, and glycosides were screened for. Metabolites present in the extracts were quantified to determine their concentrations using the methods of Harborne (1998), Sofowara (1993), and Bohm and Kocipal-Abyazan (1994) respectively.

Test Organisms

Four pure clinical isolates (*Staphylococcus aureus, Salmonella* Typhi*, Escherichia coli,* and *Pseudomonas aeruginosa*) were obtained from the Microbiology Laboratory, Federal Medical Center, Yola. Isolates were adjusted to achieve turbidity equivalent to a 0.5 McFarland turbidity Standard, containing approximately 1.0 x 10^{8} CFU/mL before they were used for susceptibility testing.

Evaluation of Antibacterial Activity

The antimicrobial activities of the leaf and seed extracts of *S. occidentalis* were tested against the selected clinical pathogens using the agar well diffusion method (Valgas *et al.,* 2007).

Thirty-eight grams (38g) of Mueller-Hinton (MH) agar was suspended in 1 litre of distilled water. The medium was completely dissolved by heating it with constant stirring and then boiled for 1 minute. The mixture was autoclaved at 121°C for 15 minutes and cooled at room temperature. Cooled Mueller-Hinton agar was poured into sterile petri dishes on a level laboratory bench and a uniform depth of 4 mm was obtained. The petri dishes were then left to solidify at room temperature. Bacterial inoculum was uniformly spread using sterile cotton swab on the sterile petri dish MH agar. Wells were then bored into the agar medium using a sterile 6 mm cork borer. Hundred microlitres (100 uL) of various concentrations (50 mg/mL, 100mg/mL, 150mg/mL, and 200 mg/mL) of the extracts, distilled water, and 10mg/mL of Amoxicillin were dispensed into designated wells without allowing it to spill on the surface of the medium. The distilled water and Amoxicillin served as negative and positive controls respectively.

The plates were allowed to stand on the laboratory bench for 1 hour before incubating them at 37 ℃ for 24 hours and the diameter of the zones of inhibition were measured and interpreted as the index of antibacterial activity. The antimicrobial sensitivity test was conducted in triplicates. The effect of the extract on bacteria was compared with that of a standard antibiotic (10mg/mL of Amoxicillin).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the method of Usman *et al.* (2007) for 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL of the extracts. Briefly, 4 mL of nutrient broth was dispensed into 4 sterile test tubes each for the leaf and seed extracts respectively; 1 mL of the different concentration of the extracts were placed into each tube and mixed thoroughly; and then inoculated with 1 µL of the standardized inoculants.

A tube containing nutrient broth only was seeded with the test organism and another tube containing nutrient broth and extract only served as organism and extract control respectively. All the tubes were then incubated at 37 ℃ for 24 hours and then examined for growth by optically observing for turbidity. The dilutions that showed no sign of visible turbidity were taken as the MICs.

Determination of Minimum Bactericidal Concentration (MBC)

The test tubes of the MIC that showed no visible turbidity were sub cultured on MH agar plates and incubated at 37 ℃ for 24 hours. The plates that showed no visible growth were the MBCs.

Data Analysis

The data collected were expressed as percentages and mean. Means were compared using Analysis of Variance (ANOVA) and Student's t-test at 0.05 level of significance.

RESULTS

Phytochemical Screening

The phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, phenols, and glycosides in both seed and leaf, while steroids and tannins were absent in both (Table 1). However, the phytochemical constituents of the leaf and seed extracts showed no significant difference at the 5% confidence level.

Antibacterial Properties of the Leaf and Seed Extracts of *Senna occidentalis*

The test organisms were susceptible to both extracts at all concentrations but were more susceptible at higher concentrations, when compared with the positive control at all concentrations except *P. aeruginosa* that was not susceptible to 10mg/mL of Amoxicillin. The seed extracts were more potent against the test organisms at all concentrations except for *E. coli* against which the leaf extracts (20, 25, 28, 31 mm) were more potent than the seed's (19, 22, 25, 32 mm) at concentrations ≤150mm/mL (Table 2).

The MIC of the leaf extracts against all the selected organisms was 12.5mg/mL. This varied among organisms in the case of the seed extracts where *E. coli* and *P. aeruginosa* had MICs of 12.5 mg/mL, *Salmonella* Typhi of 25 mg/mL and *S. aureus* of 6.255 mg/mL (Figure 1).

The MBC of the leaf extracts against all the selected organisms was 25 mg/mL. This varied among organisms in the case of the seed extracts where *E. coli* and *P. aeruginosa* had MBCs of 25 mg/mL, *Salmonella* Typhi of 50 mg/mL and *S. aureus* of 12.5 mg/mL (Figure 1).

***Salmonella* Typhi**

This Gram-negative bacterium was minimally inhibited by the leaf and seed extracts at 12.5 mg/mL and 25 mg/mL respectively. The leaf extracts totally inhibited the organism at 25 mg/mL while the seed extracts totally inhibited the organism at 50 mg/mL (Table 3). Both extracts were potent against this organism but the seed extracts were more potent (p = 0.001) compared to the leaf (p = 0.010) while the seed extracts were more potent compared to the positive control (Table 2).

Staphylococcus aureus

This Gram-positive bacterium was minimally inhibited by the leaf and seed extracts at 12.5mg/mL and 6.25mg/mL respectively. Both leaf and seed extracts totally inhibited the organism at 25mg/mL and 12.5mg/mL respectively (Table 3). Both extracts were potent against this organism but the seed extracts were more potent (p = 0.001) compared to the leaf's (p = 0.003) while both extracts were more potent compared to the positive control (Table 2).

Escherichia coli

This Gram-negative bacterium was minimally inhibited by the leaf and seed extracts at 12.5mg/mL and totally inhibited at 25 mg/mL (Table 3). Both extracts were potent against the organism but the leaf extracts were more potent (p = 0.002) compared to the seed's (p = 0.003) while both extracts were more potent compared to the positive control (Table 2).

Pseudomonas aeruginosa

This Gram-negative bacterium was minimally inhibited by the leaf and seed extracts at 12.5 mg/mL and totally inhibited at 25 mg/mL (Table 3). Both extracts were equally potent against this organism (p = 0.001). However, this organism is not susceptible to the positive control (Table 2).

Table 1: Phytochemical constituents of leaf and seed extract of *S. occidentalis*

Metabolite	Leaf	Seed
Alkaloid	+(3.6%)	+(6%)
Flavonoid	+(33%)	+(22%)
Glycoside	+(2.68mg)	+(2.02mg)
Tannin	-	-
Saponin	+(1.2%)	+(2.1%)
Steroid	-	-
Phenol	+(0.036mg)	+(0.042mg)

Key: +: Present; **-:** Absent; Figure in % and mg**:** Quantity of metabolites.

Table 2: The mean of the diameter of the zone of inhibition of the leaf and seed extracts of *S. occidentalis* against selected clinical pathogens

Organisms	Plant extracts	Mean of the diameter of the zones of inhibition (mm) for the various concentrations (mg/mL) of extracts used				*p-value*	Controls	
		50	100	150	200		+AMC	-dH$_2$O
Salmonella Typhi	Leaf	12	20	24	28	0.003	22	-
	Seed	21	23	28	30	0.001		
S. aureus	Leaf	18	21	26	30	0.010	20	-
	Seed	20	24	26	30	0.001		
E. coli	Leaf	20	25	28	31	0.002	18	-
	Seed	19	22	25	32	0.003		
P. aeruginosa	Leaf	22	25	28	29	0.001	0	-
	Seed	22	25	30	31	0.001		

Table 3: Inhibition Concentration (IC) of the leaf and seed extracts of *S. occidentalis* against bacterial pathogens.

Organisms	Plant Extracts	Inhibition Concentrations (mg/mL)				
		3.12	6.25	12.5	25	50
Salmonella Typhi	Leaf	+	+	δ	β	-
	Seed	+	+	+	δ	β
S. aureus	Leaf	+	+	δ	β	-
	Seed	+	δ	β	-	-
E. coli	Leaf	+	+	δ	β	-
	Seed	+	+	δ	β	-
P. aeruginosa	Leaf	+	+	δ	β	-
	Seed	+	+	δ	β	-

Key: +: Growth, δ**:** MIC, β**:** MBC **-:** No Growth

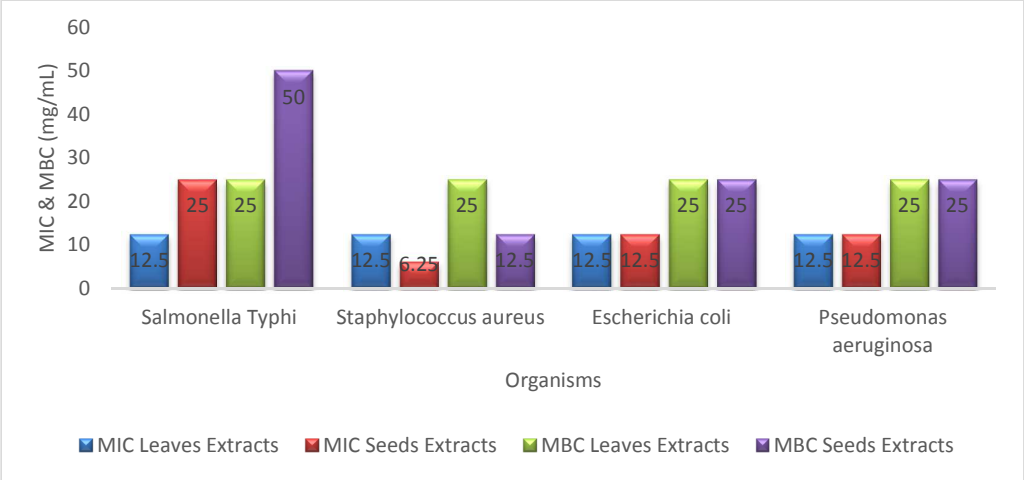

Figure 1: Minimum Inhibition Concentration and Minimum Bactericidal Concentration (MIC & MBC) of the leaf and seed extracts of *S. occidentalis* against selected clinical bacterial pathogens

DISCUSSION

The phytochemical screening of the leaf and seed extracts of *Senna occidentals* revealed the presence of flavonoids (33 % and 22 %), saponins (1.2 % and 2.1 %), alkaloids (3.6 % and 6%), phenols (0.036mg and 0.043 mg) and glycosides (2.68 mg and 2.02 mg) respectively, while tannins and steroids were not detected in the samples. This finding disagrees with the work of Yadav *et al.* (2010), Aja *et al.* (2017), Sadiq *et al.* (2012), and Naka *et al.* (2020), who in addition to the phytochemicals revealed in this study reported the presence of tannins, cardiac glycosides, and anthraquinone in ethanolic and aqueous extracts; tannins, steroids, and terpenoids in the leaf extracts; tannins, cardiac glycosides, and anthraquinone in aqueous and ethanolic extracts; tannins, cardiac glycosides, steroid glycoside and saponin glycosides in aqueous leaf extracts respectively. There was also disparity in the presence and quantity of phytochemicals reported in this study and that of Ishaku *et al.* (2016), who reported the presence of tannins (9.2 %), sterols (1.64 %), saponins (4.6 %), alkaloids (10.6 %), terpenoids, and cardiac glycosides and the absence of flavonoids, glycosides, and phenols in ethanolic leaf extracts, metabolites that were found in abundance in this study. Unlike the other studies that reported the presence of anthraquinones in extracts of *S. occidentalis*, this metabolite was not detected in this study and that of Naka *et al.* (2020).

The differences in the presence and quantity of these compounds detected in these studies could be due to the differences of extraction methods and the environmental conditions in which the plants grew (Pavarini *et al.*, 2012; Ramakrishna and Ravishankar, 2011).

Sofowora (1993) reported that phytoactive compounds are well known for their wide pharmacological activities against bacteria and fungi. This report agrees with the significant antibacterial activities of the plant extracts against *Salmonella* Typhi, *S. aureus*, *E. coli,* and *P. aeruginosa.* Naka *et al.* (2020) and Sadiq *et al.* (2012) demonstrated the same in their studies when they reported the antibacterial activities of ethanolic and aqueous extracts of *S. occidentalis* against selected bacteria.

The presence of these phytochemicals is of great medical implication. Several studies have reported the astringent, antimalarial, antioxidant, anticancer, anti-inflammatory, antiviral, antifungal, antibacterial, anti-trypanosomal and antileishmanial properties of these metabolites (Naka *et al.*, 2020; Musa *et al.*, 2017; Sadiq *et al.*, 2012; Arya *et al.,* 2010; Yadav *et al.*, 2010; Edeoga *et al.,* 2009;

Nuhu and Aliyu, 2008; Chukwujeku *et al.,* 2006; Faruq *et al.,* 2006; and Just *et al*., 1998). These reports support the use of *S. occidentalis* in the management of diseases, its usefulness in traditional medicines as well as its relevance in folklore medicine and in the management of infectious and oxidative stress related diseases. This study corroborates these reports and strengthens the position of traditional medicine by establishing the presence of essential metabolites that are of medicinal value and also demonstrating their antibacterial properties at different concentrations.

The extracts demonstrated a good antibacterial activity at all concentrations against the test organisms with significant increase in activity with increase in concentration, and the seed extracts were more potent than the leaf extracts at all concentrations except for *E. coli* where the leaf extracts were more potent than the seed's. These findings agree with the works of Sadiq *et al.* (2012) and Naka *et al.* (2020), who reported that, antibacterial activity of *Cassia occidentalis* leaves of ethanol and water extracts against test organisms increased when used in higher concentration. It was observed that the MICs of the extracts were lower than the MBCs with a ratio of 2 across all concentrations of the extracts and organisms. According to Noumedem *et al.* (2013), an extract is bactericidal when the ratio of MBC/MIC ≤ 4 and bacteriostatic when this ratio is >4. Thus, the extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations.

Salmonella Typhi is least susceptible to both extracts across all concentrations when compared to other test organisms with mean of the diameter of zones of inhibition of 12 mm and 21 mm at 50 mg/mL, 20 mm and 23 mm at 100 mg/mL, 24 mm and 28 mm at 150 mg/mL, 28mm and 30 mm at 200mg/mL; and MIC and MBC of 12.5mg/mL and 25 mg/mL, and 25 mg/mL and 50 mg/mL for leaf and seed extracts respectively. This trend is similar to that reported by Naka *et al.* (2020): mean of the diameter of zone of inhibition of 15.75±0.95 mm at 120 mg/mL, 13.25±0.64 mm at 90 mg/mL, 8.88±0.54 mm at 60 mg/mL, 6.13±0.48 mm at 30 mg/mL; MIC and MBC of 12.50 mg/mL and 50 mg/mL respectively of the leaf extract.

P. aeruginosa is most susceptible to both extracts at concentrations ≤150 mg/mL with mean of the diameter of zones of inhibition of 22 mm and 22 mm at 50 mg/mL, 25 mm and 25 mm at 100 mg/mL, 28 mm and 30 mm at 150 mg/mL, 29 mm and 31 mm at 200 mg/mL for leaf and seed extracts respectively; and the same MIC and MBC of 12.5 mg/mL and 25 mg/mL for both extracts.

S. aureus is more susceptible to the seed extracts at lower concentrations with MIC and MBC of 6.25 mg/mL and 12.5 mg/mL, the lowest in the study. This is contrary to the findings of Sadiq *et al.* (2012), who reported that *S. aureus* was resistant to both ethanol and water extracts of *S. occidentalis*.

E. coli is more susceptible to the leaf extracts at concentrations ≤150mg/mL with mean of the diameter of the zones of inhibition of 20 mm at 50 mg/mL, 25 mm at 100 mg/mL, 28 mm at 150 mg/mL, and 31 mm at 200 mg/mL compared to the seed extracts with mean of the diameter of the zones of inhibition of 19 mm at 50 mg/mL, 22 mm at 100 mg/mL, 25 mm at 150 mg/mL, and 32 mm at 200 mg/mL. However, their MICs and MBCs are the same: 12.5 mg/mL and 25 mg/mL. The extracts in this study seem to be more potent against *E. coli* compared to that reported by Ishaku *et al.* (2016). Ishaku, reported a mean of the diameter of the zones of inhibition of 34±0.50 mm at 1000 mg/mL, 20±1.00 mm at 500 mg/mL, 17±1.50 mm at 250 mg/mL.

The antibacterial properties of the leaf and seed extracts of *S. Occidentalis* compared favorably with the positive control with increase in concentration except for *P. aeruginosa* that was not sensitive to 10mg/mL of the positive control. Thus, the potency of the extracts against bacteria is dependent on concentration and bacterial susceptibility to the active ingredients.

CONCLUSION

This study revealed the presence of phytochemicals that are known for their medicinal values and demonstrated their antibacterial properties in aqueous extracts of the leaf and seed of *S. occidentalis*. The antibacterial properties increased with increase in concentration and was found to be dependent on the solvent used for extraction, the concentration used, and the susceptibility of organisms. Hence, *S. occidentalis* possess the potential to be used for the treatment of gastrointestinal fever, urinary tract infection, wound and boil infections, a potential already being exploited by herbalists in Northern Nigeria, considering the potency of the metabolites present, which inhibited the growth of the gastrointestinal bacteria.

REFERENCES

Aja, P., Okechukwu, P., Kennedy, K., Ibere, J., & Ekpono, E. (2017). Phytochemical analysis of *Senna occidentalis* leaves. *IDOSR J Appl Sci*, *2*(1), 75-91.

Alhassan, A., Ahmed, Q., Latip, J., Shah, S., Sarian, M., Wahab, R., Taher, M., Abdullahi, M. & Khatib, A. (2018). Phytoconstituents from *Vernonia glaberrima* Welw. Ex O. Hoffm. leaves and their cytotoxic activities on a panel of human cancer cell lines. *South African Journal of Botany*, *116*:16-24.

Arya, V., Yadav, S., Kumar, S., & Yadav, J. (2010). Antimicrobial activity of *Cassia occidentalis* L (leaf) against various human pathogenic microbes. *Life Sciences and Medicine Research*, *9*(1):1-10.

Bohm, B. A., & Koupai-Abyazani, M. R. (1994). Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and *V. calycinum* (Ericaceae). Pac Sci *48*(4): 458-463

Chukwujekwu, J., Coombes, P., Mulholland, D., & Van Staden, J. (2006). Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*. *South African Journal of Botany*, *72*(2):295-297.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4):564-582.

Edeoga, H. O., Okwu, D., & Mbaebie, B. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, *4*(7):685-688.

El-Mahmood, A., & Doughari, J. (2008). Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. *African Journal of Pharmacy and Pharmacology*, *2*(7):124-129.

Faruq, U., Hassan, L., Manga, B., Uba, A., Olatunji, M., Tal, M., & Isiak, F. (2006). Anti-bacterial activity of *Cassia occidentalis* on *Salmonella* Typhi. Proceedings of the 28th Annual International Conference Chemicals Society of Nigeria,

Gilani, A. H., Shah, A. J., Zubair, A., Khalid, S., Kiani, J., Ahmed, A. & Ahmad, V. U. (2009). Chemical composition and mechanisms underlying the spasmolytic and bronchodilatory properties of the essential oil of *Nepeta cataria* L. *Journal of Ethnopharmacology*, *121*(3):405-411.

Harborne, A. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. *Springer Dordrecht, 3*(14):302

Ishaku, G., Arabo, A., Effa, E., Adedayo, A., Uwem, U., & Godwin, E.-U. (2016). Physicochemical Characterization and Antibacterial Activity of Senna occidentalis Linn. *Journal of Chemistry and Chemical Sciences*, *6*, 9-18.

Just, M. J., Recio, M. C., Giner, R. M., Cuéllar, M. J., Máñez, S., Bilia, A. R., & Ríos, J.-L. (1998). Anti-

inflammatory activity of unusual lupane saponins from *Bupleurum fruticescens*. *Planta Medica*, *64*(05):404-407.

Mozumder, S., & Hossain, M. (2013). Effect of seed treatment and soaking duration on germination of *Eryngium foetidum* L. seeds. *International Journal of Horticulture*, *3*(10):46-51.

Musa, D., Aliero, A., & Bashir, K. (2017). Preliminary Allelopathic Activity of Aqueous Leaf Extract of *Senna occidentalis* on the Germination and Early Seedling Growth of Cowpea (*Vigna unguiculata*). *International Journal of Current Science and Studies*, *1*(1):23-25.

Naka, K., Obaroh, I., Naka Keta, J., Aminu, M., John, P., & Joseph, G. G. (2020). Phytochemical Screening and Antityphoid Activity of *Senna occidentalis* Aqueous Leaf Extract on *Salmonella* Typhi. *CaJost,* 2020, 2, 137-141

Neuwinger, H. D. (2000). *African traditional medicine: a dictionary of plant use and applications with supplement: search system for diseases*. Medpharm Scientific, Stuttgart, Germany, 589 pp.

Noumedem, J. A. K., Mihasan, M., Lacmata, S. T., Stefan, M., Kuiate, J. R., & Kuete, V. (2013). Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria. *BMC Complementary and Alternative Medicine*, *13*(1), 26. https://doi.org/10.1186/1472-6882-13-26

Nuhu, A., & Aliyu, R. (2008). Effects of *Cassia occidentalis* aqueous leaf extract on biochemical markers of tissue damage in rats. *Tropical Journal of Pharmaceutical Research*, *7*(4):1137-1142.

Nwali, B., Okaka, A., Ibiam, U., & Aja, P. (2012). Phytochemical composition of *Bryophyllum pinnatum* leaves. *International Journal of Advanced Biological Research*, *2*(4):614-616.

Oladeji, O. S., Odelade, K. A., & Oloke, J. K. (2020). Phytochemical screening and antimicrobial investigation of *Moringa oleifera* leaf extracts. *African Journal of Science, Technology, Innovation and Development*, *12*(1):79-84.

Omenka, C., & Osuoha, J. (2000). Antimicrobial potency of Grapefruit seed extract on five selected pathogens. *Nigerian Journal of Microbiology*, *14*(2):39-42.

Oyedemi, S. O., & Afolayan, A. J. (2011). Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Medicine*, *4*(12):952-958.

Pavarini, D. P., Pavarini, S. P., Niehues, M., & Lopes, N. P. (2012). Exogenous influences on plant secondary metabolite levels. *Animal Feed Science and Technology*, *176*(1-4):5-16.

Pieme, C., Penlap, V., Nkegoum, B., Taziebou, P., Tekwu, E., Etoa, F., & Ngongang, J. (2006). Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Ceasalpiniaceae). *African Journal of Biotechnology*, *5*(3):283-289.

Poongothai, A., Sreena, K., Sreejith, K., Uthiralingam, M., & Annapoorani, S. (2011). Preliminary phytochemicals screening of *Ficus racemosa* Linn. Bark. *Int J Pharm Bio Sci*, *2*(2):431-434.

Ramakrishna, A., & Ravishankar, G. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav* 6: 1720–1731. In.

Sadiq, I., Shuaibu, M., Bello, A., Isah, S., Izuagie, T., Nasiru, S., & Kamaru, M. (2012). Phytochemistry and antimicrobial activities of *Cassia*

occidentalis used for herbal remedies. *Methods*, *18:*19-28.

Satish Kumar, V., Dasika, M. S., & Maranas, C. D. (2007). Optimization based automated curation of metabolic reconstructions. *BMC Bioinformatics*, *8*(1):1-16.

Shinde, P., Wagh, K., Patil, P., & Bairagi, V. (2014). Pharmacognostic standardization and antibacterial potential of aerial herbs of *Portulaca grandiflora* Hooker (Portulaceae). *World Journal of Pharmaceutical Sciences*, *2*(12):1871-1885.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa* Spectrum books LTD. Ibadan, Nigeria, *289.*

Usha, T., Middha, S. K., Brahma, B. K., Narzary, D., & Goyal, A. K. (2017). In silico and in vivo based scientific evaluation of traditional anti-diabetic herb *Hodgsonia heteroclita. Bangladesh Journal of Pharmacology*, *12*(2):165-166.

Usman, H., Abdulrahman, F., & Ladan, A. (2007). Phytochemical and antimicrobial evaluation of *Tribulus terrestris* L. (Zygophylaceae). Growing in Nigeria. *Res. J. Bio. Sci. Medwell Journals*, *2*(3):244-247.

Valgas, C., Souza, S. M. d., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, *38*, 369-380.

World Health Organization. (2008). Traditional medicine. Media Centre. In. Available from: http://www.who.int/mediacentre/factsheet/fs/34/en/

Yadav, J., Arya, V., Yadav, S., Panghal, M., Kumar, S., & Dhankhar, S. (2010). *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*, *81*(4): 223-230.