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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LEAVES EXTRACT OF SENNA OCCIDENTALIS (*L.*)

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ABSTRACT
Studies were carried out to determine the phytochemicals and the antibacterial activity of *Senna occidentals,* it is a shrub that grows between 5 to 8cm in height, it is an important member of the plant family *Fabaceae* and the subfamily *Caesalpiniaceae* and commonly found in the tropics. *S. occidentalis* leaves were collected in September, 2017 at flowering stage, the sample was air-dry at room temperature, the dried leaves were powdered and stored in an air-tight container for further use. The ethanolic, petroleum Ether and aqueous extracts were made by transferring 50g of the powder into 150ml of the solvents respectively and allowed to soak for four days; it was filtered using a filter paper. The results obtained from the research reveals that *S.occidentalis* contains certain phytochemicals such as anthraquinones, flavonoids, alkaloids, saponins, terpenoids, and Tannins which are responsible for the antibacterial activity of the plant; suggesting its potential use in the production of a new brand of antibiotic .

Keywords: *Senna occidenatalis, Phytochemical Screening, Extracts, Antibacteria*

INTRODUCTION

Medicinal plants have been used over the years to treat various type of acute and chronic diseases (Cowan, 1999). Medicinal plants contain a lot of bioactive constituents or phytochemical compounds which are secondary metabolites so called because they are not required for growth, respiration, transpiration or any primary function in plants (Edoga, and Mbaebie, 2005). The major secondary metabolites including alkaloids, carbohydrates, flavonoids, tannins, terpenoids, and steroids (Edoga, and Mbaebie, 2005). Plant initially produces these phytochemical compounds to protect themselves from pathogens and predator (Poongothai *et al.,* 2011). 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 of new antimicrobial agents. Current trends in drug development process have focused on natural sources, especially sources of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants (Faruq *et al.,* 2006)

Local healers in Jos and Niger State, Nigeria, have reported that the infusion of the leaves of *S. occidentalis* is used as an effective treatment for typhoid. The potential of the leaf extract of *S. occidentalis* may be related to its antioxidant activity (Saidu *et al 2017*). *S. occidentals* is a shrub that grows between 5 to 8cm in height, it is an important member of plant family *Fabaceae* and the subfamily *Caesalpiniaceae* and commonly found in the Tropics (Musa, *et al*., 2017). Plants belonging to the family have been extensively investigated because of their rich medicinal (anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-plasmodial, anti-rheumatic and hepatoprotective) and economic uses. Due to the prevalent claims of typhoid fever infections around Katsina Sate, Nigeria and high patronage of traditional medicines, different mixtures are being used to treat typhoid fever. Plants like *S. occidentalis* are very popular for the treatment of typhoid fever (Faruq *et al.,* 2006).

The leaves of the plant are used for the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of southwestern Nigeria. In addition to this, the leaves are also known to be effective against jaundice, headache and toothache (Satish *et al.,* 2007).

It is an ayurvedic plant with huge medicinal importance. Leaves of *S. occidentalis* plant have ethno medicinal importance as paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection (Sharma *et al.*, 2010). Despite its great importance, *S.* occidentalis plant is one of the most toxic plants of veterinary interest as regards contamination of animal rations. Its poisoning effects include ataxia, diarrhea, myoglobinuria and sternal recumbency leading to death depending on the animal. Pieme *et al.*, (2006), have shown that histopathological tests of animals fed with the plant revealed that the heart and liver were the main organs affected with myocardial necrosis and centrolobular development. *S.* occidentalis is one of the most important species of the genus *Senna* which is rich in anthraquinones and polyphenols. The leaves of *S.* occidentalis have been qualitatively analysed for the presence of primarily five pharmacologically active anthraquinones: rhein, aloe-emodin, chrysophanol, emodin and physcion as well as the flavonoid, kaempferol (El-Mahmood and Doughari, 2008). Idu *et al.* (2007) observed that, preliminary phytochemical analysis of *S. occidentalis* showed the presence of phenols, tannins, anthraquinones, saponins and flavonoids. Sharma *et al.*, (2010) also reported in their study that Preliminary phytochemical screening of alcoholic extract of *S. obtusifolia* revealed the presence of anthraquinone glycosides, Phenolic compounds; Saponin glycoside while aqueous extract showed presence of glycosides and Phenolic compounds, Saponin glycoside.

Pieme *et al.*, (2006) observed that, the ethanol-aqueous extract of *S. occidentalis* showed moderate antibacterial and antifungal activity. The present study was aimed at determining the phytochemical constituents of the plant and evaluation of its antibacterial activity against a gram positive and gram negative bacteria with a view of designing a new class of antibiotic from plant source to arrest the problem of resistance to some antibiotics.

Plate 1: *an image of Senna occidentalis plant taken at Fudma take off campus premises*

METHODOLOGY

Plant sample collection

S. occidentalis leaves were collected from the premises of Federal University, Dutsinma Katsina Nigeria in September, 2017 at flowering stage. The plant was identified in Department of Biological Science, Federal University Dutsinma Nigeria by Mr.Abdulaziz Bashir Kutawa and authenticated by Prof. Bem. A.A using standard chart, sample was air-dried at room temperature, the dried leaves were powdered and stored in an air-tight container for future use. The ethanolic, pet. Ether and aqueous extracts were made by transferring 50g of the powder into 150ml of the solvents respectively and allowed to soak prior to phytochemical screening

PREPARATION OF EXTRACT

S.occidentalis leaves were collected at the Federal University Dutsinma premises. They were washed and air dried for a week before they were milled into powder. The powder was stored in an air tight container Fifty grams (50g) of the powdered leaves of *S occidentalis* was extracted in cold water using 60% Ethanol, Distill water and for 4 days following the method of Sharma *et al.*, (2010). The mixture was then filtered and the filtrate was dried *in vacuo* using a rotary evaporator. The yield collected was 85 g

Prelinimary phytochemical Screening

The aqueous extracts of *S. occidentalis* leaves were used for preliminary phytochemical analysis using standard methods;

alkaloids, Saponins, flavonoids (Sofowora, 1993); anthraquinones and tannins (Harbone, 1973). For the quantitative determination of some phytochemicals, the method of Trease and Evans (1989), was used for flavonoids, while alkaloids, saponins and tannins were analyzed using that of Wasagu *et al.* (2005).

Tannins Test

1 g of the extract was dissolved in 25 ml of distilled water and filtered, 2 to 3 drops of 10% of FeCl3 was added to 3 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins. To another conical flask, 3 ml of the filtrate was added 2 ml of bromine water. A precipitate was taken as positive for tannins.

Flavonoids Test

0.4 g of the extract was dissolved in 3 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of three (3) drops of concentrated HCL. The occurrence of a red or orange colouration was indicative of the flavonoids.

Anthraquinones (Borntrager's Test)

Two gram (2g) of the plant extract was dissolved in petroleum ether and filtered. Aqueous ammonia was then added to the filtrate, formation of pink colouration was taken as indication for the presence of anthraquinones in the plant extract (Trease and Evans, 1989)

Preparation of bacterial isolate

The following organisms were used for the experiment, *Escherichia coli* (Gram negative organism) and *Staphylococcus aureus* (Gram positive organism). The *E.coli* was isolated from a water sample collected from Dutsin-Ma dam and was taken to the Biological Sciences Laboratory, Federal University Dutsin-Ma. The sample was cultured by spreading of the water sample on a nutrient agar using a glass rod. *S. aureus* was collected from the skin surface and door handle using a swab stick which was then smeared on an already prepare nutrient agar (Faruq *et al.,* 2006). Both media were incubated for five days at 37◦C to get a pure culture which was used for the research.

Sensitivity test

The antibacterial activities of the fractions were determined using agar-well diffusion method described by Russell and Furr (1977) and Irobi *et al*. (1994), with little modification. The bacterial isolates were first grown in nutrient broth before use and later standardized using Mac Farland test. One hundred microliter (100 µL) of the standardized bacterial suspension was evenly spread on Mueller-Hinton agar medium using a sterile glass spreader. Wells were then bored into the agar medium using a sterile 6 mm cork borer and were filled with the solution of the fractions taken care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the medium. The plates were later incubated in an incubator at 37°C for 24 h after which they were examined for zones of inhibition. The effects of the fractions on the test isolates were compared with that of the standard antibiotic, streptomycin (Sathya *et al*., 2012).

Determination of the minimum inhibitory concentrations (MICs)

Minimum Inhibitory Concentration is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. It`s also defined as the lowest concentration where no visible turbidity was observed in the test tubes. The concentrations were determined as earlier described by Vollekova *et al.* (2000) with some modification by Usman *et al.* (2004). The MIC was determined for microorganisms that showed sensitivity to the test extracts. After 24 h incubation at 37 °C, the tubes were observed for turbidity.

different concentrations of the solution was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to give final concentrations regime of 10 to 0.079 mg/ml. The medium was then poured into Petri dishes and allowed to set. The surfaces of the media were allowed to dry before streaking with 18 h old bacterial isolates. The plates were later incubated in an incubator at 37°C for up to 72 h after which they were examined for the presence or absence of growth. The Minimum inhibitory concentration was taken as the lowest concentration that will prevent the growth of the test bacterial isolates (Sathya *et al*., 2012).

Determination of minimum bacteriacidal concentrations (MBCs)

The MBC of the extract was determined using Olorundare *et al.* (1992) with little modification. Samples were taken from plates with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar plates and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

RESULTS

The table below provides information of the phytochemicals present in the different extracts of the plant, the result shows that tannins, anthraquinones and flavonoids were present in all the extracts, however, saponins and alkaloids were absent in ethanolic and aqueous extracts respectively. Table 2, 3 and 4. Shows the zone of inhibition of the different extracts

Table 1. Result for the Phytochemical screening of the leaves extracts

Phytochemical compound	Plant extracts		
	Pet.ether	Ethanol	Water
Tannins	+	+	+
Anthraquinones	+	+	+
Saponins	+	-	+
Flavonoids	+	+	+
Alkaloids	+	+	-

Table 2. Effect of different concentration of ethanol extract on *Staphylococcus aureus and Escherichia coli.*

ZONE OF INHIBITION (CONC. (%))		
Conc.	**E. *coli***	***S. aureus***
10	0	0
20	12	5
40	19	15
60	22	17
80	27	27
100	32	30

Table 3. Effect of different concentration of aqueous extract on *Staphylococcus aureus and Escherichia coli*.

ZONE OF INHIBITION (mm)		
CONC. (%)	**S. *aureus***	***E. coli***
10	0	0
20	2	6
40	5	12
60	11	14
80	16	17
100	21	22

Table 4. Effect of different concentration of petroleum ether extract on *Staphylococcus aureus and* Escherichia *coli.*

ZONE OF INHIBITION (mm)		
CONC. (%)	***S. aureus***	***E. coli***
10	0	0
20	18	12
40	25	17
60	28	27
80	33	31
100	37	40

Table 5. presents result for the minimum inhibitory concentrations of three solvents required to inhibit the growth of the bacteria

Table 5. Minimum inhibitory concentration (MIC).

Microorganisms	**Zone of inhibition**		
	Pet.ether	**Ethanol**	**Aqueous**
Escherichia coil	1.25	1.56	0.250
Staphylococcus aureus	2.73	1.22	1.04

(Key pet. ether = petroleum ether, mm =millimeter).

Table 6. presents the result for the minimum bacteriacidal concentrations of the three extracts the values are the least amount of the extract(concentration) needed to kill the bacteria

Table 6. Minimum bacteriacidal concentration (MBC).

Microorganisms	**Zone of inhibition(mm)**		
	Pet.ether	**Ethanol**	**Water**
Escherichia coil	0.31	2.50	2.250
Staphylococcus aureus	0.313	0.157	5.00

(Key pet. ether = petroleum ether, mm =millimeter).

DISCUSSION

Results obtained (table.1) revealed the presence of anthraquinones, tannins, flavonoids while alkaloids and saponins were not detected in the water and ethanolic extracts respectively which was similar to the findings of Pieme *et al*., (2006). It was also observed from our investigations on three fractions (Pet. ether, ethanol and water extract) of *S. occidentalis* that all the three extracts exhibited antibacterial activities at a concentration of 100 mm. The zones of inhibition exhibited by the Petroleum ether extract against Gram-positive bacteria ranged between 12 and 40 mm(table 2,3 and 4), while it was between 18 and 37 mm for Gram-negative bacteria as shown in table . On the other hand, zones of inhibition exhibited by water fraction of the extract against Gram-positive bacteria ranged between 6 and 22 mm(table 5 and 6), while the zones are between 5 and 30 mm against the Gram-negative bacteria. The standard antibiotic streptomycin, used as positive control exhibited zones of inhibition ranging between 15 to 27 and 14 to 30 mm.

Thus, the study upholds the claims of traditional healers on the usefulness in the treatment of typhoid fever of *S. occidentalis* in traditional remedies for the disease caused by these pathogens.

The MICs of petroleum ether, ethanol and water were also determined. The MIC exhibited by the three extracts against the bacterial isolates ranged between 0.156 and 2.73 which is similar to the findings of (Akinpelu and Kolawole 2004).

The MBCs of the extracts were also determined and found to range from 1.57 to 5.00 which is in line with the findings of (Sathya *et al.,* 2012).

CONCLUSION

The results obtained from the research reveals that *S. occidentalis* contains certain phytochemical such as anthraquinones, flavonoids, alkaloids, saponins, terpenoids, and Tannins

These phytochemical confers antibacterial activity to the plant, hence has the potential to be used in the production of a new brand of antibiotics from plant source.

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