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Phytochemical Evaluation, Antimicrobial Activities and Mineral Analysis of *Senna Occidentalis* Leaves

Abbas Ibrahim*[1], Ahmad Abdullahi Abubakar[1], Abdul-alim Muhammad Isa[2], Muhammad Kabir Umar[1], Nura Musa Umar[3], Abubakar Chadi Sadiq[1]

[1]Department of Chemistry, Bauchi State University, Gadau, PMB 65 Gadau, Bauchi State, Nigeria
[2]Department of Pure and Applied Chemistry, Usman Dan fodio University, PMB 2436, Sokoto State, Nigeria
[3]Department of Science Laboratory Technology, Federal Polytechnic, PMB 0231, Bauchi State, Nigeria

Abstract The aim of this study is to determine the appropriate solvent that is effective among ethanol, acetone and aqueous extracts of *Senna occidentalis* leaves respectively, examined for their antimicrobial activities against selected clinical organisms including two gram positive organisms (*Staphylococcus aureus* and *Streptococcus pneumonia*) and two gram negative organisms (*Escherichia coli* and *pseudomonas aeruginosa*).the mineral composition of theplant leaves was also determined. Leaves of *Senna occidentalis* plant were collected in a clean container in Gadau and its environ Bauchi State, Nigeria. About 250g of the plant prepared in powdered form were separately soaked in 400ml of 95% Ethanol, acetone and Distilled water in 500ml reagent bottles and stoppered. These were allowed to stand for 24 days to permit full extraction of the active ingredients. Plant filtrates were used for the phytochemical screening for saponins, alkaloids flavonoids, sterols and other polyphenols. The extracts were equally tested on the microbial isolates cultured on Mueller Hinton agarusing an agar-disc diffusion method. Standard procedure was also used for the determination of the mineral composition of the plant. Phytochemical screening shows that the plant leaves contain some secondary metabolites of therapeutic importance like saponins, alkaloid, flavonoids and sterols which forms their bioactive components. The ethanolic extract of *Senna occidentalis* is significantly more active against the growth of E. coli with the 3000µg/ml concentration giving the highest measurement of zone of inhibition (35).While the minerals component from this plant leaves are phosphorus (3.35±0.02), iron (2.44±0.01), zinc(1.75 ± 0.02),sodium (1.71 ± 0.01), calcium (0.77 ± 0.01) and potassium (0.66± 0.01). This research validates the traditional uses of *Senna occidentalis* leaves for treatment of some diseases.

Keywords Phytochemical; Antimicrobial; *Senna occidentalis*; mineral; organism

Introduction

Plants are important source of drugs; especially in traditional medicine [1]. It is a common practice in Nigeria and other parts of the world to use plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. According to WHO, over 70 % of the world populations rely on medicinal plants for primary health care and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antimicrobial and antioxidant properties [2].

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 and the emergence of widespread drug resistance [3].

The active principle of many drugs found in plants are phytochemicals [4]. The medicinal value of these photochemicals are because of the presence of chemical substance that produces definite physiological action on the human bod. Some of the valuable ones include;

Alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, and body building [5]. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search for new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages. Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses [6].

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. More than 80 percent of African population is dependent on these cheap and effective traditional medicines, used against many diseases and infections [7].

Senna occidentalis is one of the Nigerian medicinal plant used by Yoruba tribe for the treatment many diseases. The leaves part are used for the treatment of yaws, scabies, itches and ringworm among the Yoruba tribe of south western Nigeria[8]. In addition to this, the leaves are also known to be effective against jaundice, headache and toothache. Infusion of *Senna occidentalis* leaves is used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria[9].

Different parts of this plant have been reported to possess anti-inflammatory and antiplasmodial activities [10].

Senna occidentalis is commonly known as Raidore in Hausa language and Coffee Senna in English language has been scientifically classified in Kingdom: plantae, Family: Caesalpiniaceae, Sub Family: Caesalpinioideae, Genus: Senna and Species: Occidentalis [11].

Experimental section

Sample Collection

The plant leaves of *Senna occidentalis* was collected from Gadau and their environs such as katsinawa and Malumawa. The plant was healthy and uninfected. And was identified by appropriate voucher in the Herbarium unit Botany Department at Bauchi State University Gadau, Bauchi State.

Preparation of Leaf Extract

The leaves were washed with distilled water to eliminate dusts and other foreign particles; the dried sample was grinded into powder using a well cleaned mortar and pestle. Then sieved to removed fibers' and large debris. The grounded powdered leaves were separately percolated in 500ml conical flask with 500ml of various solvents. Solvent extraction is the most frequently used technique for the isolation of bioactive compound from plants.

Phytochemical Screening

Test for Alkaloids

About 0.5g of each extract was mixed with 5ml of dilute HCL, and then 1ml of the mixture was treated with few drops of Meyer's reagent. Appearance of a yellow colour shows a positive test for alkaloids [12].

Test for Flavanoids

Sulfuric acid: The extract was dissolved into concentrated sulfuric acid giving a deep yellow solution, which indicates the presence of flavanone [13].

Test for Carbohydrate

Mohlisch reagent: this was prepared by dissolving α- naphthol into ethanol and was added to the extract in the test tube followed by the addition of 2ml of concentrated H_2SO_4, the appearance of violet ring colour indicates the presence of carbohydrate.

Test for Saponins

The extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins [14].

Test for Steroids

1. Liebermann-Buchard test: To 0.2g of each portion, 2ml of acetic acid was added, the solution was cooled well in ice followed by the addition of concentrated H2SO4 carefully, colour development from violet to blue or bluish-green indicate the presence of a steroids [13].
2. Salkowski's test : 0.2 g of the extracts was dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicated the presence of steroids [15].

Test for other Polyphenols

Extracts were treated with few drops of ferric chloride solution formation of bluish black colour indicates the presence of other polyphenols [16].

Test organisms

Two gram positive organisms (*Staphylococcus aureus* and *Streptococcus pneumonia*) and two gram negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*) were clinically isolated. These micro-organisms were obtained from Federal Medical Centre Microbiology department, Azare.

Preparation of Sensitivity Discs

Discs of about 6mm in diameter were made from Whatmans No.1 filter paper using about 50 discs was transferred into each Bijour bottle (16 Bijour bottle) and sterilized at 121C^{o} for 15 minutes[17].

Preparation of Culture Media

30g of nutrient agar was dissolved into 750ml of distilled water then sterilized by Autoclaving at 121^{o}C for 15 minutes and then cooled at room temperature. The nutrient agar solution then was poured into petri dishes and allowed to cool and gel.

Standardization of Inoculums

The standardization of inoculums were carried out using inoculation wire loop. Enough material from an over nigh culture of the test organisms were transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5Mc farland standards as described by national community for clinical laboratory standards [17].

Bioassay Procedure

Standard inoculate of the isolates were swabbed on the surface prepared and solidified nutrient agar in a separate petri dishes. The discs of the extracts (3000µg/ml, 2000µg/ml 1000µg/ml, 100µg/ml) and the standard antibiotic discs (Erythromycin) will be place on the surface of the inoculated media at intervals. The plates will be incubated at 37^{o}C for 24 hours before observation and measurements of zones of inhibition in millimeters.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the extract and fractions were prepared by serial doubling dilution using distilled water to obtain concentrations of 2000µg/ml, 1000µg/ml and 100µg/ml. Equal volume (2ml) of extractsan+d Muller-Hinton broth were mixed. Specifically 0.1ml of standardized inocula (3.3×10^{6} CFU/ml) was

added to each of the test tube above. The tubes were incubated aerobically at 35ºC for 24 hours. Tubes containing broth and leaf extract without inocula which served as positive control while tubes containing broth and inocula served as negative control. The tubes were observed after 24 hours incubation to determine minimum inhibitory concentration. That is the lowest concentration shows no evidence of growth.

Determination of Minimum Bacterial concentration growth (MBC)

Sterile Muller-Hinton agar plates were separately inoculated with ample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35ºC for 24 hours and observed. The highest dilution that yielded no bacterial growth was regarded as MB

Result and Discussion

Results

The extract of Acetone, Ethanol and Distilled water were subjected to phytochemical screening and the result obtained was summarized in Table 1.

Table 1: Phytochemical contents of the leaf extracts of *Senna occidentalis*

Phytochemical	Acetone Extract	Ethanol Extract	Distilled water
Saponins	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Sterol Method1	+	+	+
Sterol Method2	+	+	+
Carbohydrates	+	–	–
Other polyphenols	–	–	–
Tannins	+	+	+

\+ = Presence of secondary metabolites - = Absence of secondary metabolites

The results of the phytochemical tests carried out revealed the presence of Alkaloids, Saponins, Flavonoids, steroids and Tannins in all the extracts. Carbohydrate is only evident in acetone while other polyphenols are absent in all the three extracts

Table 2: Results of the antimicrobial screening of Acetone Extract Fraction.

Test organisms	Zones of Inhibition (mm)			
	3000 µg/disc	2000 µg/disc	1000 µg/disc	100 µg/disc
Staphylococcus aureus	27	25	20	15
Escherichia coli	30	23	21	19
Streptococcus pneumonia	26	24	19	17
Pseudomonas aeruginosa	28	24	21	16

Table 3: Results of the antimicrobial screening of Ethanolic Extract Fraction

Test organisms	Zones of Inhibition (mm)			
	3000 µg/disc	2000 µg/disc	1000 µg/disc	100 µg/disc
Staphylococcus aureus	17	15	14	11
Escherichia coli	35	27	24	18
Streptococcus pneumonia	24	21	19	15
Pseudomonas aeruginosa	28	27	23	19

Table 4: Results of the antimicrobial screening of Distilled Water Extract Fraction

Test organisms	Zones of Inhibition (mm)			
	3000 µg /disc	2000 µg/disc	1000 µg/disc	100 µg/disc
Staphylococcus aureus	19	14	13	11
Escherichia coli	28	20	17	15
Streptococcus pneumonia	24	27	25	22
Pseudomonas aeruginosa	19	17	15	11

Table 2, 3 and 4 show the antimicrobial activity of *Senna occidentalis* extracts on the test organisms. The result reveals that Ethanol extract has the highest zone of inhibition (35) on *E. coli at* 3000 µg, followed by acetone extract (30) then distilled water extract (28) at the same concentration (3000µg). The effectiveness of extracts against the microorganisms under consideration decrease with decrease in concentration as shown above.

Table 5: MIC and MBC of *Senna occidentalis* leaf extracts

Isolates	Ethanol extract		Acetone extract		Distilled water extract	
	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	2000	***	2000	***	1000	2000
Escherichia coli	1000	1000	***	***	1000	***
Streptococcus pneumonia	1000	***	***	***	100	1000
Pseudomonas aeruginosa	100	***	2000	***	***	***

KEY: MIC means minimum inhibitory concentration, MBC means minimum bacteriacidal concentration***- MIC OR MBC value greater than 2000µgm/ml.

Antimicrobial susceptibility of the plant extract (*Senna occidentalis*) of the isolate identified were indicated by observation and measurement of inhibitory zones formed around a prepared disc. The larger the zone of inhibition the effectiveness of the phyto-extract against the isolated organism as shown in table 2 to 4.

Table 6: Mineral composition of *Senna occidentalis* leaves (mg/100g dry weight)

Minerals	Concentration in (mg/100)
Phosphorus	3.35 ± 0.02
Iron	2.44 ± 0.01
Zinc	1.75 ± 0.02
Sodium	1.71 ± 0.01
Calcium	0.77 ± 0.01
Potassium	0.66± 0.01

Results are mean of duplicate determinations on a dry weight basis ± standard deviation

Discussion

The therapeutic value of medicinal plants lies in the various chemical constituent in it. The bioactivity of this plant extract is attributed to phytochemical constituents. The leaf extract contain tannins (1), which have antibacterial potential due to their character that allow them to react with proteins to form stable water soluble compounds there by killing the bacteria by destructing the cell membrane causing cell disequilibrium and death[18].

The presence of Saponins (Table 1), supports the facts that *Senna occidentalis* leaf has cytotoxic effects such as permialization of the intestine as saponins are cytototoxic, it also gives the leaves bitter taste [19].

Alkaloids isolated from plant are commonly found to have antimicrobial properties and are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives are used as medicinal agent because of their analgesic, antispasmodic and antibacterial properties. They show marked physiological effects when administered to animals [20].

The antimicrobial screening of the ethanol fraction, Acetone fraction and distilled Water fraction showed that the ethanol extract of the isolate *Escherichia coli* has more inhibitory effects than that of the acetone whereas that of distilled water has the least effect against *E. coli at* 3000µg. this research indicated that higher dose of the plant leaf extract require to be administered to treat a disease caused by *Escherichia coli.* The presence elements such as Phosphorus, Iron, Zinc. Sodium, Calcium and Potassium suggests that, the plant leaf is a source mineral elements.

Conclusion and Recommendation

Conclusion

Variations of phytochemical present in medicinal plant depend on solvents used for extraction and the extraction procedure. It is concluded that *Senna occidentalis* leaf possess some vital phytochemical components that can be used medicinally. The study thus provides further evidence on the traditional usage of this plant in treating diseases.

Recommendation

Further research needs to be carried out on this plant to determine the antimicrobial activity of the plant against a wider group of pathogens including fungi and parasites.

Finally, the traditional medicine practitioners, herb users, herb sellers, and health institutions should be using such research work in order to understand the health and economic importance of *Senna occidentalis* leaves

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