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Phytochemical Screening and Antimicrobial Activities of *Senna occidentalis* Stem Extracts

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ABSTRACT

This research work investigated the phytochemical constituents and the antimicrobial activities of stem extract of *Senna occidentalis*. The powdered stem was extracted with ethyl acetate, ethanol and methanol using sequential extraction method and the preliminary analysis was carried out using standard methods. The antimicrobial assay was investigated using Agar-diffusion method against some human pathogens. The phytochemical analysis revealed the presence of alkaloids, tannins, saponins, steroids, terpenoid, cardiac glycoside, glycosides, phenols, anthraquinone, phlobatannins, and absence of flavonoid in ethanol and methanol extracts. The antimicrobial activities showed that ethyl acetate extract was most effective in inhibiting *Klebsiella spp* at 18 mm zone of inhibition at 100 mg/mL concentration. The methanol extract showed the highest zone of inhibition of 17 mm against *Candida albican*. Ethyl acetate showed the highest zone of inhibition of 13 mm against *Moulds* at 100 mg/mL. The three extracts effectively inhibited the growth of all the human pathogens tested at 100 mg/mL with the exception of ethanol extract which did not inhibit *Rhizopus stolonifera*. Only ethyl acetate actively inhibited *Rhizopus stolonifera* at 100 mg/mL with zone of inhibition of 12 mm. The results obtained showed that important bioactive compounds are present in *Senna occidentalis* stem extract and these constituents may be responsible for the antimicrobial activities.

Keywords: Phytochemical, *Senna occidentalis*, antimicrobial, ethyl acetate, ethanol and methanol extracts

INTRODUCTION

Medicinal plants are important to human for the treatment of several ailments. They are utilized by the pharmaceutical industry for research and for new drug discovery. Plants have been a vital source of drugs for traditional medicine [1,2]. Medicinal plants contribute significantly to rural livelihoods. Apart from the traditional healers practicing herbal medicine, many people are involved in collecting and trading medicinal plants. This has resulted to increased demand in

both local and international markets as well as bio-prospecting activities for sources of new drugs [3]. Plants contain a wide range of chemical constituents that used to treat chronic and infectious diseases [4]. Plants are commonly used in the form of crude extract through decoction, infusion, or tincture for the treatment of common infections and chronic diseases in Nigeria and in other parts of the world [1, 2, 5].

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics is continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and on-going epidemics of human immunodeficiency virus (HIV) infections. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections [6]. This shift to herbal drugs has been endearing them duue to factors like the low cost of herbal drugs, the 'green' movement in the developed world that campaigns on the inherent safety and desirability of natural products and the individualistic philosophy of western society that encourages self-medication [3, 7].

Plant sources play an essential role in the development of drugs. This is due to the promising biological activity of plants materials in treating infectious diseases successfully with little or no side effects [8]. Natural products research has gained a lot of attention as an effective and alternative route for new product development via which novel bioactive agents from plants sources has been discovered [9]. The potential activity of these herbs in prevention and treatment of diseases depend on their bioactive compounds or phytochemicals. The active principles of many drugs found in plants are secondary metabolites and are widely used in traditional medicine to treat various ailments [10]. The presence of secondary metabolites indicates a great potential for plants as a useful source of phytomedicine [10, 11]. These are responsible for many biological or pharmacological activities [12].

Senna occidentalis is one of the most widely used herbal plants among people of tropical and subtropical regions of the world [13]. It is used for various therapeutic purposes in traditional medicine [10]. *Senna occidentalis* Linn (formerly *Cassia occidentalis*) [3, 5] is a short-lived slender erect herbaceous plant or small shrub of about 3 metres high [1, 2] with a yellow flower and a green foliage and has a characteristic foetid smell [11, 14]. It belongs to the Fabaceae (pea) family and subfamily Caesalpiniaceae [5, 9, 15, 16]. It is commonly known as coffee senna in English [5, 11, 17]. In Nigeria, *Senna occidentalis* is locally called Sanga sanga or Rai ndori by

the Hausas [18]; Akidi agbara in Igbo and Abo rere by the Yoruba tribe [10, 19]. *Senna occidentalis* is native to the tropical and subtropical regions of America and naturalized in the tropical world like Australia, eastern Africa, southern and eastern United State of America (USA) [1, 2, 5 10]. It grows in moist, disturbed environments in and around abandoned fields, waste areas, open woodlands, dumping sites, pasture, and roadsides [20]. It can be found in open pastures and are cultivated with cereals such as soybean, corn, sorghum, and others; thus, during the harvest it is almost impossible to prevent this plant from mixing with the cultivated crops [2,5]. The various parts of *Senna occidentalis* plant (such as roots, leaves, flowers, stems, and seeds) are traditionally used in medication [13, 21]. In traditional medicine, the roots of *Senna occidentalis* are used to treat diabetics and other ailments in spite that its adequate validation as antidiabetic and hepatoprotective effects has not been established. The plants have been used in different parts of the world by traditional healers in treating different forms of diseases [5, 10]. The leaves of this plant are boiled alongside *Magnifera indica* and *Carica papaya* prior to drinking or bathing the decoction for the treatment of malaria across West Africa [9]. The infusion of the leaves of *Senna occidentalis* have been reported to be effective in the treatment of typhoid fever in northern part of Nigeria [16]. The leaves of *Senna occidentalis* are made into a paste and applied externally on healing wounds, sores, itches, cutaneous diseases, bone fracture, fever, ringworm, skin diseases and throat infection [16].

Previous studies on *Senna occidentalis* consisting of the various parts of the plant have been reported to possess valuable phytochemical constituents such as anthraquinone, glycosides, cardiac glycosides, steroids, flavonoids, saponins, [22], alkaloids, tannins, reducing sugar, phenols [11, 21] and terpenoid [18]. The presence of this wide range of phytochemicals indicates the medicinal potentials of *Senna occidentalis*. Research on the stem part of *Senna occidentalis* has been scantly reported and it is important to further screen for other phytochemicals that could be present using different solvents system different from the ones used in earlier studies. The antimicrobial activities will help to ascertain the efficacy of *Senna occidentalis* stem in inhibiting some human pathogens-causing diseases. This will further provide evidence on the usefulness of this plant in phytomedicine in the treatment of ailments. Hence, this study aims to examine the phytochemical constituents of *Senna occidentalis* stems and their antimicrobial activities since limited research have been done on the stem part of the plant.

MATERIALS AND METHODS

Sample collection

Senna occidentalis stem bark was collected from Amike-Aba in Ebonyi Local Government Area, Ebonyi State, Nigeria. The plant collected was identified and authenticated at the Department of Applied Biology, Ebonyi State University, Abakaliki by a Taxonomist using a standard chart. The sample was chopped into small sizes and air-dried for 2 weeks. The dried sample was ground into powder using electric blender and stored in a tight container for further analysis.

Plate 1: *Senna occidentalis* plant

Preparation of plant extract

About 1000 g of the ground powdered sample were extracted successively with 3000 mL of ethyl acetate, ethanol and methanol respectively using sequential extraction method. The solvent was left in contact with the powered plant sample for 72 hours (3 days) in each case with intermittent shaking. The extract was decanted and allowed to stand, after which the marc was filtered through a Whatman No. 42 filter paper. The extracts was concentrated using water bath at 40 ºC until a paste form of the extract was obtained, weighed, and stored in a refrigerator at 4 °C until when required. The percentage yield of extraction was calculated using equation 1:

$$\text{Percentage yield} = \frac{\text{Weight of the dry concentrated crude extract}}{\text{Weight of the dried crushed plant sample used}} \times 100\% \qquad (1)$$

Qualitative phytochemical screening

The ethyl acetate, ethanol, and methanol crude extracts of the stem of *Senna occidentalis* were analyzed qualitatively for the presence of phytochemicals using the following standard methods [12, 23, 24].

Test for alkaloids

i. Dragendorff's reagent: One milliliter (mL) of each extract was shaken with 1% hydrochloric acid (HCl) for two minutes respectively. The mixture was filtered and three drops of Dragendorff's reagent was added. Formation of an orange-red precipitate indicated the presence of alkaloids.

ii. Wagner's reagent: One milliliter (mL) of each solvent fraction (extract) was acidified by adding 1.5% v/v hydrochloric acid and a three drops of Wagner's reagent were added. The formation of yellow or brown precipitates confirmed the presence of alkaloids

Test for flavonoids

i Alkaline reagent test

One milliliter (mL) of each extract was taken and placed into a test tube. Then one milliliter of sodium hydroxide (NaOH) solution was added and shaken. Appearance of intense yellow color that turned to colorless after adding one milliliter dilute hydrochloric acid implied the existence of flavonoids.

ii Lead acetate test

One milliliter (mL) of each extract was taken and placed into a test tube. Then two drops of lead acetate was added and shaken. Formation of yellow precipitate signified the presence of flavonoids.

Test for tannins

Ferric chloride, $FeCl_3$ method: One milliliter (mL) of each extracts was stirred with 5 mL distilled water in a test tube and filtered. 3 drops of 10% ferric chloride was added to the filtrate. A blue-black precipitate indicated the presence of tannins.

Test for saponin

Frothing test: One milliliter (mL) of each extracts was shaken with 5 mL of distilled water in a test tube. Frothing which persisted on warming evidenced the presence of saponins.

Test for steroid

Salkowski's test: Two milliliter (mL) of each extracts was dissolved in 2 mL of chloroform, $CHCl_3$ and 2 mL of concentrated H_2SO_4 was carefully added to form a lower layer. A reddish-brown colour at the interphase indicated the deoxy sugar characteristics of cardenolides.

Test for terpenoid

Salkowski's test: Two milliliters (mL) of chloroform was added to 1 mL solution of each extract. The solution was shaken and filtered. Then, three drops of concentrated sulphuric acid (H_2SO_4) was added to the filtrate, shaken, and allowed to stand. Development of golden-yellow precipitate indicated the presence of triterpenes.

Test for cardiac-active glycoside

Keller-killani test: One milliliter (mL) of each extract's solution was dissolved in 2 mL of glacial acetic acid (CH_3COOH) containing one drop of 10% ferric chloride solution, followed by the addition of 1 mL of concentrated sulphuric acid (H_2SO_4). A brown ring at the interface confirmed the presence of cardiac glycoside.

Test for anthraquinone

Borntrager's test: Exactly 0.5 gram of the dried extract was placed in a test tube and 5 mL of chloroform was added and heated in a steam bath for 1 minute. The extract was filtered while hot and allowed to cool. A 10% ammonia solution (NH_4OH) was added to the filtrate and then shaken. The appearance of bright pink at the upper aqueous layer indicated the presence of anthraquinones.

Test for phenol

One milliliter (mL) of each extract's solution was dissolved in 2 mL 10% ferric chloride solution. A dirty green precipitate indicated the presence of phenolic compound.

Test for glycoside

One milliliter (mL) of each extract's solution was hydrolyzed with 1% hydrochloric acid (HCl) solution and neutralized with sodium hydroxide (NaOH) solution. Then, two drops of Fehling's solution A and B was added. Formation of a red precipitate indicated the presence of glycosides.

Test for phlobatannins

Exactly 0.5 gram of each extract was dissolved in 2 mL of distilled water and filtered. The filtrate was boiled with 2 mL of 2% HCl solution. Emergence of a red precipitate showed the presence of phlobatannins.

Antimicrobial assay of *Senna occidentalis*

Determination of antimicrobial activity by standard procedures.

Isolates of microorganisms were obtained from the laboratory of Microbiology Department, Ebonyi State University, Abakaliki, Nigeria. The antimicrobial activity of ethyl acetate, ethanol and methanol fractions of *Senna occidentalis* stem were determined against five bacteria, *Staphyloccus spp, Bacillius spp, Pseudomonas spp, Esherichia coli,* and *Klebsiella spp* and five fungi, *Candida albicans, Pencillin notatum, Moulds, Aspergillus spp,* and *Rhizopus stolonifera.* A suspension of pure cultured micro-organisms by disk diffusion were spread evenly over the face of a sterile Muller Hinton agar plate using a sterile swab stick and allowed for 5 minutes to dry with their lids in place. Sterile cork borer was used to punch wells (6 mm in diameter) on the cultured plates. For all the fractions 100 mg/ml, 50 mg/ml and 25 mg/ml each were applied to the holes of the various agar plates. Each well and the plates was labelled appropriately with the concentration of the extracts with sterile micropipette. Exactly, 30 mL of each concentration of the extracts was introduced into independent wells while 30 µg disc of ciprofloxacin and fluconazole was inoculated as controls. The agar plate was incubated for 24 hours at a temperature of 37^{o}C. The zone of inhibition appeared when the plant extract fractions exert a growing inhibiting effect. The diameter of the inhibitory zone was measured in millimeter and it was related to the level of antimicrobial activity present in the fraction; the larger the inhibitory zone, the better the antimicrobial potency [21].

RESULTS AND DISCUSSION

The results of yield determination are presented in Table 1

Table 1: The colour and percentage yield of ethyl acetate, ethanol, and methanol stem extract of *Senna occidentalis*

Extract	Colour of Extract	Weight of sample (g)	Weight of crude extract (g)	Percentage yield (%)
Ethyl acetate	dark green	1000	8.75	0.86
Ethanol	dark green	1000	12.85	1.29
Methanol	brown	1000	12.45	1.25

The phytochemical analysis of the three extract is presented in Table 2.

Table 2: Phytochemical screening of ethyl acetate, ethanol and methanol stem extract of *Senna occidentalis* stem

Phytochemicals	Tests	EtAc	EtOH	MeOH
Alkaloids	Dragendorff's reagent	+	+	+
	Wagner's reagent	+	+	+
Flavonoid	Alkaline reagent	-	-	-
	Lead acetate	-	-	-
Tannin	5% FeCl solution	+	+	+
Saponin	Frothing test	+	+	+
Steroid	Salkowski's test	+	+	+
Terpenoid	Salkowski's test	+	+	+
Cardiac glycoside	Keller-Killanite	+	+	+
Glycoside		+	+	+
Phenol		+	+	+
Anthraquinone	Borntrager's test	-	+	+
Phlobatannins		-	+	+

EtAc = Ethyl acetate EtOH = Ethanol MeOH = Methanol

+ = present - = absent

The antibacterial activities of ethyl acetate, ethanol and methanol extracts are presented in Table 3

Table 3: Effect of ethyl acetate, methanol, and ethanol extracts of *Senna occidentalis* stem at various concentration (mg/mL) against bacteria strains

	Zone of Inhibition (mm)									
Bacteria strains	EtAc			EtOH			MeOH			
Ciprofloxacin										
	100	50	25	100	50	25	100	50	25	
Staphylococcus spp	10	03	-	12	8	-	14	05	03	28
Escherichia coli	13	06	-	16	10	04	13	04	-	22
Klebsiella spp	18	10	04	16	11	05	13	05	-	23
Pseudomonas spp	10	5	-	12	07	-	14	07	03	20
Bacillus spp	13	07	03	14	09	05	12	06	-	25

The antifungal activities of ethyl acetate, ethanol and methanol extracts are presented in Table 4

Table 4: Effect of ethyl acetate, methanol, and ethanol extracts of *Senna occidentalis* stem at various concentration (mg/mL) against fungi strains

	Zone of inhibition (mm)									
Fungi strains	EtAc			EtOH			MeOH			Fluconazole
	100	50	25	100	50	25	100	50	25	
Candida albican	12	08	-	16	14	11	17	15	11	24
Pencillin notatum	10	-	-	10	-	-	10	08	-	20
Moulds	13	09	-	11	09	-	11	07	-	21
Aspergillus spp	09	07	-	13	08	-	12	09	07	22
Rhizopus stolonifera	12	08	-	-	-	-	08	-	-	20

In this research work, the solvent system used were selected based on the degree of polarities to dissolve different phytochemical components in the plant [21]. Ethyl acetate, ethanol, and methanol were selected to enable the extraction and separation of a wide range of bioactive component that are present in the plant sample. The powdered plant sample was extracted using sequential extraction method. The percentage yields of the crude extract were summarized in Table 1. The ethanol extract had the highest percentage yield of 1.29% followed by the methanol extract, 1.25% and ethyl acetate showed the least percentage yield of 0.86%. The colour obtained from the crude extract of methanol was brown while ethyl acetate and ethanol were dark green.

The preliminary qualitative phytochemical analysis of the three extracts revealed the presence of alkaloids, tannins, saponins, steroids, terpenoid, cardiac glycoside, glycosides, phenols, anthraquinone, and phlobatannins with the exception of flavonoid which was absent in all the three extracts as presented in Table 2. The absence of flavonoid was confirmed by early studies carried out by Terver *et al* [2]. The methanol and ethanol extracts of *Senna occidentalis* stem showed the presence of alkaloids, tannins, saponins, steroids, terpenoids, cardiac glycoside, glycosides, phenol, anthraquinone, and phlobatannins while flavonoids were absent. The ethyl acetate showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycoside, glycosides, and phenols while anthraquinone, and phlobatannins were absent. Alkaloids, tannins, saponins, steroids, cardiac glycosides, and glycosides were present in all the three solvents extracts. The presence of these phytochemicals in the stem part of *Senna occidentalis* indicates that if properly screened, it could yield a drug of pharmaceutical significance [22].

The antibacterial activities of methanol, ethanol, and ethyl acetate stem extracts of *Senna occidentalis* were investigated against five bacteria strains viz *Staphylococcus spp*, *Bacillus spp*, *Escherichia coli*, *Klebsiella spp*, and *Pseudomonas spp* as presented in Table 3. The methanol extract showed the highest zone of inhibition of 14 mm against *Staphylococcus spp* and *Pseudomonas spp* at 100 mg/mL. All the bacteria isolates were resistant at 25 mg/mL and 50 mg/mL concentration with the exception of *Pseudomonas spp* which was slightly inhibited at 50 mg/mL as shown in Table 3. Ethanol extract showed the highest zone of inhibition of 16 mm against *Escherichia spp* and *Klebsiella spp* at 100 mg/mL. All the bacteria organisms were resistant against the ethanol extract at 25 mg/mL. The least inhibited pathogen was *Pseudomonas spp* with the zone of inhibition of 07 mm at 50 mg/mL. The ethanol extract actively inhibited the

growth of the bacteria organism at 50 mg/mL and 100 mg/mL among the three extracts used. The result obtained for *Escherchia coli* in this study is similar to that reported by Ishaku *et al* [9]. They reported that the bacteria isolates were inhibited by ethanol extract at a concentration between 250 mg/mL to 1000 mg/mL and *Escherichia coli* was mostly inhibited. In contrast to this finding, Olatunji *et al* [5] who worked on in vitro antibacterial and antitubercular activities of leaf extract of *Senna occidentalis* observed that all the named bacteria pathogens were resistant in the ethanol extract at the various concentration of 10 mg/mL to 80 mg/mL. Similarly, *Staphylococcus aureus* was resistant across all the concentrations of 30 mg/mL to 120 mg/mL according to the work done by Sadiq *et al* [17] which was also contrary to the result obtained in this work. Ethyl acetate extract showed the highest zone of inhibition of 18 mm against *Klebsiella spp*. All the bacteria organism were effectively inhibited by ethylacetate extract at 100 mg/mL concentration while they were all resistant at a concentration of 25 mg/mL. *Staphyloccus spp*, *Escherichia coli*, and *Pseudomonas spp* were resistant in the ethyl acetate extract at 50 mg/mL.

The antifungal activities of *Senna occidentalis* stem was also examined using five fungi strains such as *Candida albican, Penicillin notatum, moulds, Aspergillus spp,* and *Rhizopus stolonifera* as presented in Table 4. The methanol and ethanol extracts showed the highest zones of inhibition of 17 mm and 16 mm against *Candida albican*, at 100 mg/mL concentration respectively. Fluconazole was used as the standard control with the inhibitory zone of 24 mm as presented in Table 4. Ethyl acetate showed the highest inhibitory zone of 13 mm against *Moulds* at 100 mg/mL concentration while the *Moulds* were resistant at 25 mg/mL. Methanol, ethanol, and ethyl acetate extracts effectively inhibited the growth of all the fungi isolates at 100 mg/mL (Table 4) with the exception of ethanol extract which did not inhibit *Rhizopus stolonifera* at the various concentrations. Methanol and ethanol showed the same zone of inhibition of 11 mm against *Candida albican* at 25 mg/mL while the five fungi isolates were resistant in the ethyl acetate extract at the same 25 mg/mL concentration as shown in Table 4. Ethanol extract only showed zone of inhibition against *Candida albican* at 25 mg/mL while the methanol extract inhibited only *Candida albican* and *Aspergillus spp* at 25 mg/mL. Only ethyl acetate actively inhibited *Rhizopus stolonifera* at 100 mg/mL with inhibitory zone of 12 mm against the 20 mm showed by Fluconazole (control).

From this study, it was observed that the ethyl acetate, ethanol and methanol extracts of the stem of *Senna occidentalis* inhibited the growth of various species of bacteria and fungi strains mainly at 100 mg/mL and 50 mg/mL. The lack of activities at the dose of 25 mg/mL of these extracts could be as a result of low concentration of the phytochemicals. It was also observed that the antimicrobial activities of these three fractions of *Senna occidentalis* stem extracts used in this work increased with increase in concentration. This result was similar to the findings made by Odeja *et al* [1] where it was observed that all the hexane, ethyl acetate and methanol extract were effective antibacterial and antifungal agent.

CONCLUSION

In this study, the phytochemical analysis of the *Senna occidentalis* stem extracts showed the presence of alkaloids, tannins, saponins, steroids, terpenoid, cardiac glycoside, glycosides, phenols, anthraquinone, phlobatannins, and reducing sugar with the exception of flavonoid which was absent. The ethyl acetate, ethanol and methanol extracts were highly effective in inhibiting microbial growth. The ability of the extracts to inhibit the microbial organism tested could be due to phytochemical constituents present in the stem part of *Senna occidentalis*. This could also help to justify the claim of the use of the plant in folk medicine.

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