

Phytochemical analysis and in-vitro antimicrobial screening of the leaf extract of *Senna occidentalis* (Fabaceae)

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Abstract. Tamasi AA, Shoge MO, Adegboyega TT, Chukwuma EC. 2021. Phytochemical analysis and in-vitro antimicrobial screening of the leaf extract of *Senna occidentalis* (Fabaceae). *Asian J Nat Prod Biochem* 19: 57-64. Due to the use of *Senna occidentalis* as an antimalarial, anti-inflammatory, antioxidant, hepatoprotective, and antibacterial agent traditionally, this study examined the phytochemical composition and antimicrobial activity of the isolated methanol, ethyl acetate, and hexane fractions of the leaves of *Senna occidentalis*. Qualitative and quantitative phytochemical analyses were carried out using standard procedures. Antimicrobial activity was performed by using standard procedures against known common clinical isolates. Qualitative phytochemical analysis showed the abundant presence of saponins, tannins, flavonoids, and terpenoids in the ethyl acetate fraction. Cardiac glycosides were not detected in all the tested isolated fractions while steroids were found to be present and abundant in ethyl acetate and hexane fractions respectively. Quantitative phytochemical analysis showed that the methanol fraction contained 4%w/w alkaloids, 0.51%w/w flavonoids, 9.5%w/w saponins, 1.3%w/w tannins 5.5%w/w terpenoids, and 0.6%w/w total phenol content. The antimicrobial susceptibility test shows a range of inhibitory zone of 10-16 mm. Hexane fraction has the highest zone of inhibition against *Candida albicans*. The lowest MIC values of 0.6mg/mL and 0.2mg/mL were observed in ethyl acetate fraction against *Candida albicans* and *Escherichia coli* respectively. The highest MIC was 20mg/mL was observed in the ethyl acetate fraction against *Staphylococcus aureus*. The MMC values were varied widely. The MMC value against *Bacillus subtilis*, *Candida albicans*, and *Trichophyton rubrum* was 5mg/mL while the MMC value against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* was 20mg/mL. The results from this study show that the leaves of *Senna occidentalis* can serve as a potential source of some phytochemicals and also have the potential to be developed as a source of antibiotics.

Keywords: Alternate medicine, antimicrobial, phytochemicals, *Senna occidentalis*

INTRODUCTION

Medicinal plants are traditionally used globally for the treatment of many diseases, including but not limited to; asthma, gastrointestinal problems, skin disorders, respiratory and urinary problems, hepatic and cardiovascular disease (Tian et al. 2014). Plants synthesize a wide range of biologically active compounds (Cushnie et al. 2014) that are important for them to survive and flourish in the natural environment, protecting them from abiotic stress, insect pests as well as disease infections (Vardhini and Anjum 2015). Since medicinal plants possess an effective source of antimicrobial natural products with proven potential of treating infectious diseases compared to the synthetic drug, there has been a gradual revival of interest in the use of medicinal plants in developed and developing countries (Akinyemi et al. 2006; Ajayi and Akintola 2010; Valarmathy et al. 2010). The natural products from medicinal plants are used in pharmaceutical preparations either as pure or as extracts (Gogtay et al. 2002).

Senna is a large genus of flowering plants in the legume family; Fabaceae (Leguminosae). This genus is distributed throughout the tropics comprising more than

300 species (Gebrelibanos et al. 2014). *Senna* species are commonly used as ornamentals, famine foods, and many species are used in both traditional and modern medicines (Haraguchi et al. 1998). *Senna occidentalis* (L.) Link. (Syn. *Cassia occidentalis* L.) is commonly known as coffee *Senna* (Odeja et al. 2014). It is found in open pastures and fields, cultivated with cereals such as soybean, corn, sorghum, and others (Barbosa-Ferreira et al. 2005). Various parts of *S. occidentalis* (seeds, roots, leaves, and stems) are traditionally claimed to be useful in treating different medical conditions and infections (Bekele and Reddy 2015). It has been used as a folklore medicine for hepatotoxicity treatment. The aqueous-ethanolic leaves extract was tested for hepatoprotective activity on liver damage in paracetamol-induced hepatotoxicity in rats shows that the paracetamol-induced hepatotoxic indications were to some degree reduced after 21 days of treatment with *Senna occidentalis* leaves extract in a dose-dependent manner (Uzzi and Grillo 2013). Aqueous extract of *Senna occidentalis* tested for antidiabetic activity showed a significant reduction in fasting blood glucose levels in the normal and alloxan-induced diabetic rats (Laxmi 2010).

Previous studies on *S. occidentalis* extract in four different solvents (methanol, aqueous, benzene, petroleum

ether, and chloroform) showed that methanol extract had antimicrobial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. The aqueous extract was effective against *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*; while benzene and petroleum ether extracts were active against *Proteus mirabilis* and *Escherichia coli*; and chloroform extract was inactive against all tested strains (Arya et al. 2010). Further, a study by Daniyan et al. (2011) showed that flower extract of *S. occidentalis* had maximum inhibition against *Klebsiella pneumoniae* and no activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. Thus, the flower extract of *S. occidentalis* can be used to treat *Klebsiella*-associated ailments such as pneumonia, bronchitis, and other diseases caused by *Klebsiella pneumoniae*.

The antibacterial and antimalarial activities of leaves and root bark extracts of this plant have also been reported (Tona et al. 2001; Chukwujekwu et al. 2006). Studies on the ethanolic, dichloromethane and lyophilized aqueous extracts of *S. occidentalis* root bark were tested for antimalarial activity against *Plasmodium berghei*. The extracts produced significant chemo suppressions of parasitemia with 200 mg/kg dose when administered orally in experimental animals (mice). The species was found to be potential with 60% chemo suppression of the parasites.

Ibrahim et al. (2010) attempt to demonstrate the *in vitro* and *in vivo* activities of *S. occidentalis* against *Trypanosoma brucei*. Although the exact mechanism for the observed *in vivo* trypano-suppressive effect is not known, it is obvious that the extract contains some phytochemicals that could interfere with the survival of the parasites *in vivo*. The presence of alkaloids, flavonoids, tannins, and anthraquinones have been reported in this extract (Ogunkunle and Ladejobi 2006) and previous reports attributed the antitrypanosomal activity of several tropical plants to the flavonoids (azaanthraquinone), highly aromatic planar quaternary alkaloids, barbarine and harmaine (Nok 2001). The trypanosuppressive action of this extract could be due to the presence of one or more of these bioactive compounds. The present study examined the phytochemical compounds of *S. occidentalis* leaves which could be useful for the synthesis of orthodox drugs, and also its antimicrobial activity.

MATERIALS AND METHODS

Sample collection and preparation

The plant was collected at Unguwar Dosa, Kaduna, Nigeria and identified by Prof Gabriel Ajibade of Biological Sciences Department, Nigerian Defence Academy, Kaduna. It was authenticated at the Forest Herbarium Ibadan (FHI) (Holmgren et al. 1990). Upon collection, the leaves of the plant were rinsed with distilled water, air-dried at room temperature for two weeks, and then blended into fine powder. Active compounds from the

plant were extracted in a crude form through cold maceration extraction method using methanol for 72 hours, concentrated at 40°C using rotary evaporator and the crude extract fractionated with hexane, ethyl acetate, and methanol successively in a 500ml separating funnel.

Figure 1.0 illustrates the morphology of *S. occidentalis*, while Figure 2 shows the current distribution of the species in Nigeria.

Qualitative phytochemical screening

The hexane, ethyl acetate, and methanol fractions of *S. occidentalis* were assessed for the existence of some phytochemicals using the following standard methods:

Test for Alkaloids

(i) Dragendroff's reagent: 8 g of Bi (NO₃.5H₂O) was dissolved in 20 ml of nitric acid and 2.72g of potassium iodide in 50 ml of water, mixed and makeup to 100ml with distilled water.

Test: To 0.1g of extract, added 2.0 ml of HCl. To this acidic medium, 1 ml of reagent was added. An orange-red precipitate produced immediately indicates the presence of alkaloids.

(ii) Wagner's reagent: 1.0 g of iodine and 2.0 g of potassium iodide were dissolved in 5 ml sulphuric acid and the solution was diluted to 100 ml.

Test: 0.1g extract was acidified by adding 1.5% v/v HCl and a few drops of Wagner's reagent. The formation of yellow or brown precipitate confirmed the presence of alkaloids.

(iii) Meyer's reagent: 1.36 g Mercuric chloride was dissolved in 60 ml of distilled water and 5g of potassium iodide in 10 ml of water. The two solutions were mixed and diluted to 100 ml with distilled water.

Test: To 0.1g ml of extract, few drops of reagent were added. The formation of white or pale precipitate showed the presence of alkaloids.



Figure 1. Morphology of *Senna occidentalis* (L.) Link.

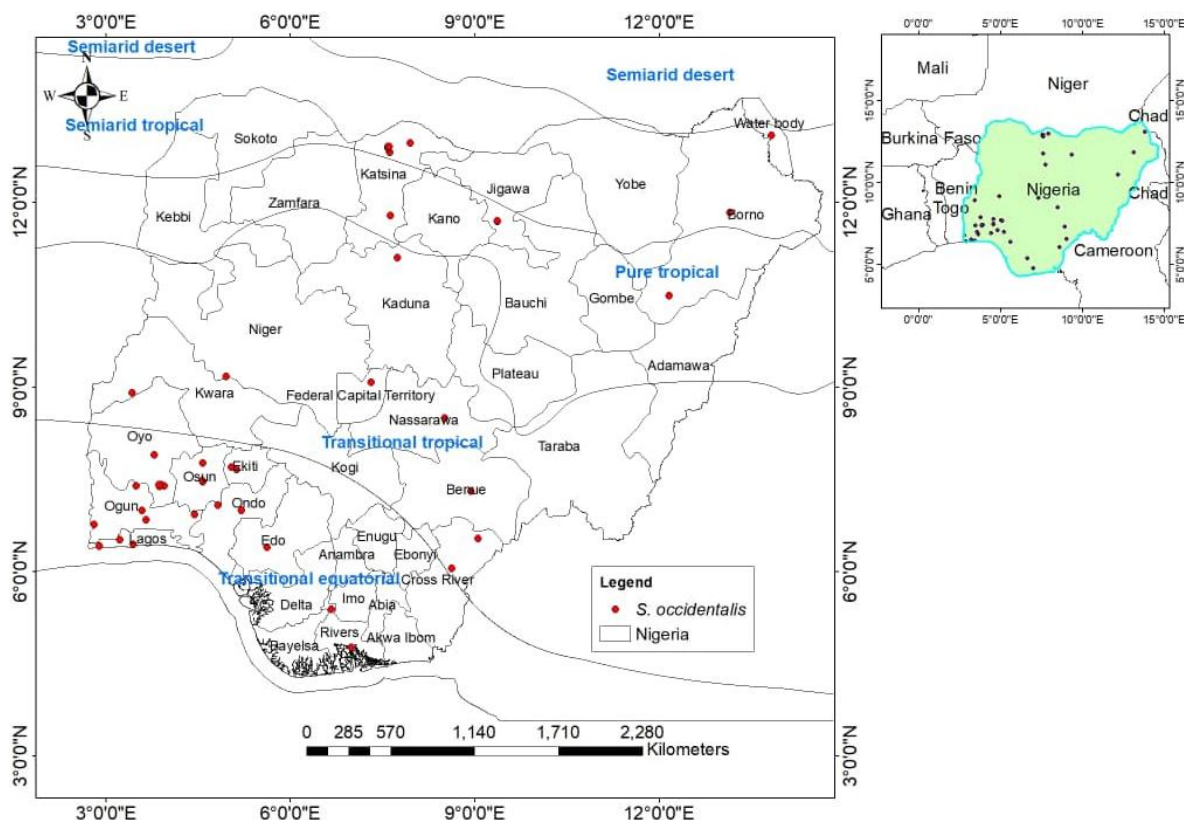


Figure 2. Distribution of *S. occidentalis* in Nigeria

Test for flavonoids: ammonia/H₂SO₄

The sample was added to 5 ml of diluted ammonia solution and concentrated H₂SO₄. The disappearance of yellow color on standing indicates the presence of flavonoids.

Test for saponins: Frothing Test

3 ml of the aqueous solution of the extract was mixed was stoppered in a test tube and shaken vigorously for about 30sec, then allowed to stand for 3min and observed for a persist honeycomb froth, which indicates the presence of saponins.

Test for tannins: Ferric chloride method

2 ml of the aqueous extract was added to 3 drops of 10% Ferric chloride solution. The occurrence of blue-black color showed the presence of tannins

Anthraquinone test: Borntrager's test

0.1g of the dried extract was placed in a dry test tube and 5 ml of chloroform was added and was heated in a steam bath for 1 min. The extract was filtered while hot and allowed to cool. A 10% ammonia solution was added to the filtrate and then shaken. The upper aqueous layer was observed for bright pink indicates the presence of anthraquinones.

Terpenoids test: Salkowski test

0.1g of the sample was mixed with 2ml of CHCl₃ in a test tube 3ml of concentrated H₂SO₄ was carefully added to the mixture to form a layer. An interface with a reddish-brown coloration was formed if terpenoids constituent is present.

Cardiac glycosides Test: Keller-Killiani test

A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl₃ was mixed with 10 ml aqueous plant extract and 1 ml concentrated H₂SO₄. A brown ring formed between the layers showed the presence of cardiac glycosides.

Phenol test: Ferric chloride test

To 1 ml aliquot of each of the fractions, was added with 3-4 drops of neutral 5% ferric chloride solution. The formation of dark green color indicated the presence of phenols.

Steroids test: Liebermann-Burchard test

The extracts were added 5ml of chloroform, shake and filtered. and few drops of acetic anhydride along with a few drops of concentrated sulphuric acid were added through the side of the tube. The appearance of green color indicates the presence of sterols in the extracts.

Quantitative phytochemical screening

Quantitative determination of alkaloids

Quantitative determination of alkaloids was performed according to the phytochemical methods described by Harborne (1973). Exactly 200 cm³ of 10% acetic acid in ethanol was added to each fraction (1g) in a 250 cm³ beaker and allowed to stand for 4 hours. The fractions were concentrated on a water bath to ¼ of its original volume and 10ml of concentrated ammonium hydroxide was added until the precipitation was complete and filtered after 3 hours with a pre-weighed filter paper. The residues were dried in an oven and the percentage of alkaloid for each fraction is calculated mathematically as follows:

$$\% \text{ Alkaloids} = \frac{\text{Weight of alkaloid} \times 100}{\text{Weight of samples}}$$

Quantitative determination of terpenoids

Dried plant extracts (1g) of each fraction (Wi) were soaked in 20 ml of ethanol for 24 hours (Indumathi et al. 2014). After filtration, the extracts were extracted with 10 ml of petroleum ether using a separating funnel. The ether extract was separated into pre-weighed glass vials and kept completely dry (Wf). The yield (%) of total terpenoids contents was measured by the formula:

$$\% \text{ Terpenoids} = \frac{W_i - W_f \times 100}{W_i}$$

Quantitative determination of saponins

Quantitative determination of saponins was carried out using the method by Ejikeme et al. (2014). 100 cm³ of 20% aqueous ethanol was added to 1g of each fraction in 250 cm³ conical flasks. The mixtures were heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixtures was re-extracted with another 100 cm³ of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The extracts were evaporated to 40 cm³ over a water bath at 90°C. 20 cm³ of diethyl ether was added to the concentrate in a 250 cm³ separating funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 40 cm³ of n-butanol was added to the aqueous portion, shook, allowed to partition and the butanol layer collected after 10min. this process was also repeated twice and the combined butanol layer was washed with 20 cm³ of 5% sodium chloride and heated in a pre-weighed crucible. The saponin content was calculated as follows:

$$\% \text{ Saponins} = \frac{\text{Weight of saponins} \times 100}{\text{Weight of samples}}$$

Quantitative determination of tannin (using the spectrophotometric method)

Tannins contents were quantitatively determined by the Folin-Ciocalteu method for ethyl acetate and methanol fractions. 0.1ml of each fraction were added to volumetric flasks (10 ml) both containing 7.5 ml of distilled water and

0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na₂CO₃ solution was diluted to 10 ml with distilled water. The mixtures were shaken well and kept at room temperature for 30 minutes. Reference standard solutions of gallic acid (32, 63, 125, 250, 500, and 1000 µg/mL) were prepared. Absorbance for test and standard solutions were measured against the blank at 760 nm with a UV/Visible spectrophotometer (Miean and Mohamed 2001; Ribarova and Atanassova 2005).

Determination of total flavonoid content

Total flavonoids content was determined from the calibration curve of quercetin and expressed as a microgram of quercetin equivalent per milligram of extract. Total flavonoid content was determined according to the procedure of (Chang et al. 2002), validated by (Nugroho et al. 2013) with some modifications. 1mg/1 ml standard solution was prepared, and series concentrations of quercetin were then obtained. The sample solution (0.5ml) was added with 1.5 ml methanol, 0.1 ml of 10% AlCl₃, 0.1 ml Potassium acetate 1M, and 2.8 ml of distilled water, and then incubated for 30 minutes. Absorbance was measured at 415 nm. Distilled water was used as blank. Total flavonoid content was expressed in mg quercetin per 1g of plant extract.

Total phenolic content

The total phenolic content of ethyl acetate and methanol extracts (200-1000 µg/mL) were quantified using the Folin-Ciocalteu's phenol reagent (Singleton and Rossi 1965) with gallic acid (50-200µg/mL) as standard. Each extract (1 ml) was added to 1 ml Folin-Ciocalteu 's reagent (diluted tenfold in distilled water) in separate test tubes. The content of each test tube was mixed and allowed to stand for five minutes at 25°C in an incubator. 1 ml of 2 % sodium carbonate solution (Na₂CO₃) was added to the mixture. This was allowed to stand for 2 hours at 25°C in an incubator and centrifuged at 1000 g for 10 minutes to get a clear solution. The absorbance of the supernatant was then determined at 760 nm using a UV spectrophotometer. Distilled water (1 ml) was added to 1 ml Folin-Ciocalteu's reagent (diluted ten-fold in distilled water) processed in the same way as done for the test samples and used as blank. All measurements were done in triplicates. The total phenolics were expressed as micrograms per milliliter of gallic acid equivalents through the calibration curve with gallic acid.

Antimicrobial susceptibility test of *Senna occidentalis*

Determination of antimicrobial activity by standard procedures

Isolates of micro-organisms were obtained from the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. The antimicrobial activity of methanol, ethyl acetate, and hexane fractions of *S. occidentalis* was determined against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Trichophyton rubrum*. A suspension of micro-organism pure culture by disk diffusion was spread evenly over the face of a sterile agar plate using a sterile swab. For all the fractions, 100 mg/mL and 50

mg/mL each were applied to the center of the various agar plates (in a fashion such that the antimicrobial doesn't spread out from the center). Gentamicin, 10µg/mL and 1% Ketoconazole were inoculated as controls. The agar plate was incubated for 24 hours at a temperature of 37°C. The zone of inhibition appears when the plant extract fractions exert a growth-inhibiting effect. The diameter of the inhibitory zone was measured in millimeters and it is related to the level of antimicrobial activity present in the fraction; the larger the inhibitory zone usually means the better the antimicrobial potency.

Determination of Minimum Inhibitory Concentration (MIC) & Minimum Microbicidal Concentration (MMC)
(Pramila et al. 2012)

The minimal inhibitory concentration (MIC) is defined as the lowest concentration of the compounds which inhibit the growth of the microorganisms. Minimal bactericidal concentration (MBC) is defined as the lowest concentration of the compounds that kill and show no growth of the microorganisms on an agar plate. The test microbes were grown in nutrient broth and potato dextrose broth for bacteria and fungus respectively as previously described and the cultures were adjusted to 0.5 McFarland standard turbidity. MIC and MBC values of the plant extract against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Trichophyton rubrum* were determined based on a micro-well dilution method. The 24-well microtiter plates were prepared by dispensing 2 ml of MH broth into each well and 3 µl of the bacterial and fungal inoculum. The plant extract was dissolved in the fractions to obtain the concentration of 1 mg/mL and was serially diluted and mixed thoroughly. Then the plates were incubated at 37°C for 24h

RESULTS AND DISCUSSION

The screening of plant parts for medicinal properties starts with crude extract preparation using a range of solvents with different polarities to dissolve different phytochemicals compounds in the plant parts. This is a vital step in drug development from the plant. Phytochemical constituents vary quantitatively and qualitatively in different plant species and even in plant parts of the same species depending upon various atmospheric conditions and storage conditions (Jahan et al. 2008). Three solvents (hexane, ethyl acetate, and methanol) were used for the extract preparation. The qualitative phytochemical screening of the three fractions is shown in Table 1.

Saponins and terpenoids were found to be present in all three extracts of *S. occidentalis*. Tannins, flavonoids, alkaloids, and phenols were found to be present in ethyl acetate and methanol extracts only. Steroids and anthraquinones were positive in hexane and ethyl acetate extracts, while cardiac glycoside was completely negative in all three extracts.

The occurrence of this wide range of phytochemicals shows the medicinal potentials of *S. occidentalis*. Alkaloids, saponins, tannins, flavonoids, and steroids have been known to be biologically active thus partially responsible for the antimicrobial activities of plants (Nethathe and Ndip 2011). Flavonoids are known to be synthesized by plants in response to microbial infection (Cowan 1999), antimicrobial properties of saponins are due to the leakage of proteins and certain enzymes from the cell (Zablotowicz et al. 1996) while tannins bind to proline-rich proteins and interfere with the protein synthesis (Shimada 2006).

As revealed in Table 2, the three fractions had a range of terpenoids from 5.5% to 7.5%w/w. Malik et al. (2017) also reported a wide range of terpenoid content, i.e. *Cassia absus* contains 40%, and *Carissa carandas* 80%, in which these two plants were used as medicinal plants in Punjab. Terpenoids are chemically interesting groups of secondary metabolites (Ajaib et al. 2016) of important therapeutic use as antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, antioxidants, and antiparasitic (Brahmkshatriya and Brahmkshatriya 2013). Jaeger and Cuny (2016) reported that terpenoids are naturally formed in a stereospecific manner and can be obtained in enantiopure form by extraction from natural sources. Thus, the pure enantiomeric form is needed for the production of highly active medical compounds of high pharmaceutical value.

Saponins have a wider range of 1.5% to 9.5% w/w across the tested fractions. A previous study by Ezeonu and Ejikeme (2016) also reported a wide range of saponin content between *Protea elliottii* (1.6%) to *Anogeissus leiocarpus* (12.5%). The extraction of saponins from these plants serves as a good source of saponins since saponins protect plants against microbial attacks such as yeast and fungal infections (Sheikh et al. 2013). Oyekunle et al. (2006) reported that saponins show antimicrobial activity by inhibiting the growth of Gram-positive and Gram-negative microorganisms. However, some saponins are not effective against Gram-negative microorganisms because saponins are not able to penetrate the cell membranes of Gram-negative bacteria.

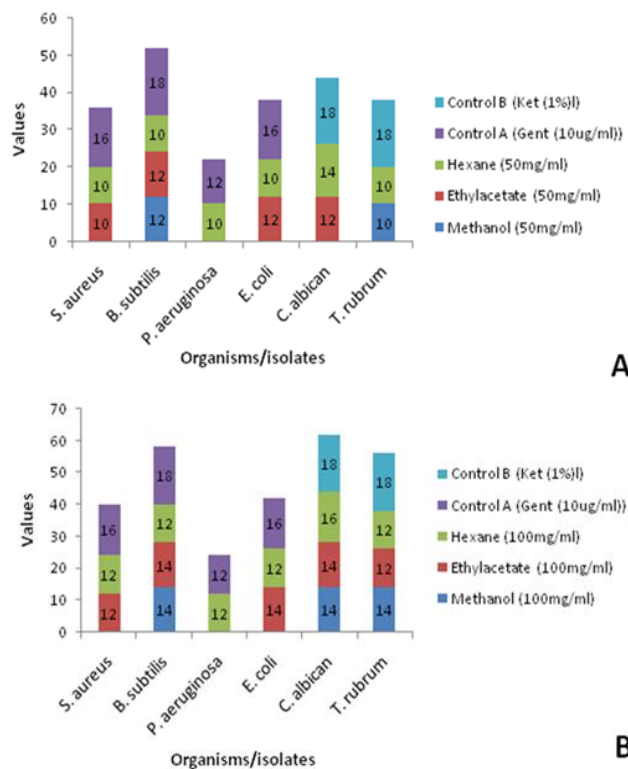
Table 1. Phytochemicals compounds of three fractions of *S. occidentalis* leaves

Test	Hexane Fraction	Ethyl acetate Fraction	Methanol Fraction
Saponins	+ve	++ve	++ve
Tannins	-ve	++ve	+ve
Flavonoids	-ve	++ve	+ve
Cardiac glycosides	-ve	-ve	-ve
Anthraquinones	+ve	+ve	-ve
Terpenoids	+ve	++ve	+ve
Steroids	++ve	+ve	-ve
Alkaloids	-ve	+ve	+ve
Phenol	-ve	+ve	+ve

Note: ++ve: Abundant, +ve: Present, -ve: Absent

Table 2. Percentage chemical composition of *S. occidentalis* leaf fractions

Sample	% Alkaloids	% Flavonoid	% Saponin	% Tannin	% Terpenoid	% Total Phenol
Hexane fraction	0	0	1.5%w/w	0	6%w/w	0
Ethylacetate fraction	8%w/w	0.2%w/w	2.5%w/w	1%w/w	7.5%w/w	0.73%w/w
Methanol fraction	4%w/w	0.51%w/w	9.5%w/w	1.3%w/w	5.5%w/w	0.6%w/w

**Figure 3.** Diameter of inhibitory zone of *S. occidentalis* leaf fractions against several microbes. A. Fraction concentrations of 50 mg/mL; B. Fraction concentrations of 100 mg/mL

Flavonoids and phenols content in ethyl acetate and methanol were low, i.e. 0.2%w/w, 0.7%w/w, and 0.51%w/w, 0.6%w/w respectively. These results are also supported by previous studies on the flavonoid content of *Erythrina velutina* (0.2%) and *Mimosa tenuiflora* (0.21%) both of the plants that belong to the Fabaceae family with reported antimicrobial activity (Siqueira et al. 2012). Also, the low phenol content is similar to the report on stem bark (0.75%) and leaves (0.09%) of *Mangifera indica* (Okwu and Ezenagu 2008). Phenols protect plants from oxidative damage and do the same for humans (Okwu 2005).

Tannins content in ethyl acetate and methanol fractions were 1%w/w and 1.3%w/w respectively. Alkaloids were present in ethyl acetate fraction at 8%w/w and methanol fraction at 4%w/w. These values are much higher than reported values of alkaloids in *Cucumis sativus* (cucumber) and *Momordica charantia* (bitter melon) leaves with 1.23%w/w where bitter melon ethanolic leaf extract showed 70% percent inhibition against *E. coli*, *S. dysenteriae* 1, and *S. pneumoniae* while *Cucumis sativus* leaf extract was

found to have less antibacterial activity (Debnath et al. 2015). The differences in methanol and ethyl acetate differences could be due to their inherent polarity and hence influences their performance.

The antimicrobial activities of *S. occidentalis* against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Trichophyton rubrum* were examined in this study and their potency was qualitatively and quantitatively assessed by the presence or absence of inhibitory zones, minimum inhibitory concentration (MIC), and minimum microbicidal concentrations (MMC).

The zones of inhibition of the tested fractions against the selected bacterial and fungal strains are shown in Figure 3. in a concentration-dependent manner. The result showed that methanol and ethyl acetate fractions at 100 mg/mL and 50 mg/mL did not inhibit the growth of *Pseudomonas aeruginosa*. However, an inhibitory zone of 12 mm was observed for both hexane fraction and the tested control (Gentamicin) at 100 mg/mL and 10 µg/mL respectively. The zone produced by the standard antibiotic was similar to that produced by the hexane fraction of *S. occidentalis*. Similar to the present work, studies on the antibacterial activity of methanolic leaf extract of *Plukenetia conophora* against *Pseudomonas aeruginosa* isolated from urinary tract infection showed a concentration-dependent activity with *Pseudomonas aeruginosa* having zones of inhibition of 10, 12, 14, and 18 mm at 50, 100, 150 and 200 mg/mL respectively (Enitan et al. 2014).

The methanol fraction did not show any inhibition zone against *Staphylococcus aureus* and *Escherichia coli* at the concentrations of 100mg/mL and 50mg/mL. The diameter of inhibition of the ethyl acetate and hexane fractions at the concentration of 100mg/mL and 50mg/mL against *Staphylococcus aureus* was the same, i.e., 12mm and 10mm, respectively. These values were lower than positive control Gentamicin (16 mm). A study by Kumar et al. (2010) showed that the inhibitory zone of cardamom extract against *S. aureus* was 6 mm to 9 mm. The inhibitory zone of hexane, ethyl acetate, and dichloromethane extract against *Escherichia coli* was 12 mm.

The result showed a similar antibacterial activity of methanol and ethyl acetate fractions at the concentration of 100 mg/mL against *Bacillus subtilis* was 14mm, while hexane fraction has the inhibitory zone of 12mm. These values were lower compared to that of Gentamicin (18mm at 10ug/mL). The inhibitory zone of hexane fraction against *C. albicans* at the concentrations of 100 mg/mL and 50 mg/mL were 16mm and 14mm.

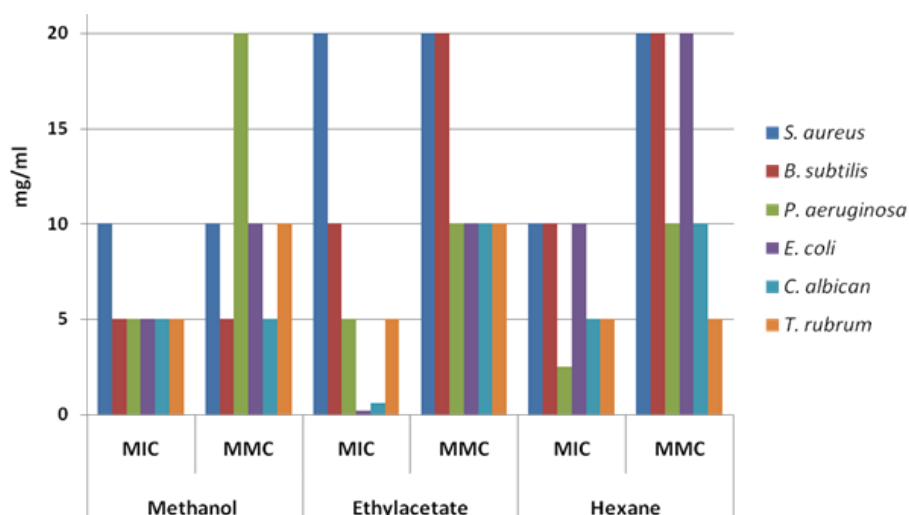


Figure 4. The Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) of *S. occidentalis* leaf fractions

This fraction has a similar zone of inhibition for all tested bacteria at the concentrations of 100 mg/mL and 50 mg/mL, i.e. 12 mm and 10mm respectively. However, these values are much lower than the positive control Ket 1% (18 mm). Methanol extract of *S. occidentalis* showed a zone of inhibition of 14mm at 100mg/mL against *Trichophyton rubrum* and 12 mm for ethyl acetate and hexane fractions respectively, which are lower than that of positive control Ket 1% (18mm).

The MIC and MMC results of the tested fractions are depicted in Figure 4. Ethyl acetate fraction shows the lowest MIC value of 0.2mg/mL and 10 mg/mL MMC against *Escherichia coli*. This same fraction also has a MIC value of 0.6mg/mL and 10 mg/mL MMC against *Candida albicans*. The MIC and MMC values of hexane fractions were 2.5mg/mL and 10mg/mL against *Pseudomonas aeruginosa*. The MIC value (5 mg/mL) of methanol fraction was similar against all tested microbial strains except for *Staphylococcus aureus* with the MIC and MMC value of 10 mg/mL. The highest MIC value (20mg/mL) was recorded for ethyl acetate fraction against *Staphylococcus aureus*. Ethyl acetate fraction could be described as having a better antimicrobial activity than other fractions. The results of this study have added to the existing information on *S. occidentalis*, it is also important to consider its sustainability. On the distribution of *S. occidentalis*, the species seem to be more abundant around the transitional equatorial states in Nigeria (Oyo, Ogun, Lagos, Kwara, Osun, Edo, Ekiti, Ondo, Rivers, etc) and less in the Northern regions. Hence, its natural habitats need some attention, to ensure its sustainable collection and use.

In conclusion, the phytochemical analysis of the leaves of *Senna occidentalis* shows the presence of flavonoids, tannins, and phenols and the abundance of alkaloids, saponins, and terpenoids. Antimicrobial activities of the *S. occidentalis* fractions were more potent against the tested fungi; *Candida albicans* and *Trichophyton rubrum* than Gram-positive and Gram-negative bacteria. These results

could be used as scientific evidence of the use of the plant as a source of remedy for various ailments traditionally. Further pharmacognostic studies are suggested to ascertain its potency in the treatment of diseases.

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