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GC-MS profile and antimicrobial activities of extracts from root of *Senna occidentalis* Linn.

Ngozi Francesca AMAKO*, Mary-Ann Nkoli MGBEMENA, and Sunday Peter ODO

Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Nigeria

Abstract. We considered the extraction of plant materials from *Senna occidentalis* root, and its fractionation monitored by bioassay towards isolating its bioactive principles. Pulverized root sample of *Senna occidentalis* (fam. leguminaceae) was extracted with methanol using a maceration method. The crude methanol extract (MSo) 6.06 g was partitioned into petroleum ether and ethyl acetate to yield their respective fractions viz: petroleum ether fraction (1.20 g), ethyl acetate fraction (1.86 g) and methanol fraction (2.92 g). The crude methanol extract was analyzed using phytochemical screening, infrared spectroscopy, and gas chromatography/mass spectrometry while the fractions were examined for antimicrobial properties. Phytochemical screening indicated the presence of tannins, phenolics, alkaloids, flavonoids, saponins, cardiac glycosides, phlobatannin, and absence of steroids. The infrared spectrum revealed a broad absorption band at 3437.26 cm^{-1} due to O-H symmetric stretch in polymeric alcohols, and absorption frequency of 1640.51cm^{-1} of moderate intensity due to C=O stretch. Enols are easily identified by the broad H-bonded O-H stretch absorption and low C=O stretch frequency as in β-keto enolic esters and phenol acetates, -CO-O-C=C-, (1690-1650 cm^{-1}). Gas chromatography/mass spectrometry identified the presence of ten compounds including *n*-hexadecanoic acid (23.76%), linoleic acid (1.64%), E-9-tetradecenoic acid (4.88%), octadecanoic acid, 2-(2 hydroxyethoxy) ethyl ester (6.24%) and E-2-octadecadecen-1-ol (13.74%). Similar broad spectrum antimicrobial activities were manifested by methanol and ethyl acetate fractions. *Escherichia coli* and *Bacillus cereus* were the most susceptible with the highest zone of inhibition of 30 mm and 28 mm respectively at minimum inhibition concentration of 1.35 $x10^3$ µg/ml. The methanol fraction has the highest potency against the tested pathogens whereas the petroleum ether fraction exhibited activity only on gram negative pathogens. The antimicrobial activities observed in these fractions suggest the presence of active chemical components in the crude methanol root extract of *Senna occidentalis* thus provides a potential source of novel antimicrobial agents. Further work is however, required to isolate and characterize these bioactive principles.

Keywords: *Senna occidentalis* Linn.; gas chromatography/mass spectrometry; bioactive principles; fatty acids; antimicrobial activity.

1. Introduction

Medicinal plants have long provided sources of healing drugs to local populations in Africa. Novel substances useful in enriching therapeutics can be sourced more easily and be selected based on their ethno-medicinal uses. *Senna occidentalis* Linn. common name, "negro coffee" or "coffee Senna" is a slender shrub, an annual woody and ramified plant about 0.5 - to 2.0 m in height, native to tropical and subtropical regions of the world. The plant can be found in open pastures and in fields cultivated with cereals such as soybeans, corn, sorghum, and others [1]. The seeds of *S. occidentalis* can be roasted and used as substitute for coffee and sometimes used as adulterant for coffee despite the reported poisoning of cattle and human by the plant [2].

In many African countries *Senna occidentalis* Linn. is useful for the treatment of skin diseases, sore throat, tetanus, wounds, worms, stomach ulcers, syphilis [3, 4]. The paste of the leaves is applied for the healing of wounds, sores, itch, cutaneous diseases, ringworm, skin diseases, throat infection, leprosy and an infusion of the plant bark is used in folklore for diabetes [5]. The juice squeezed out from the paste of the crushed root is effective in the treatment of ringworm (Personal communication). The plant has been reported to contain anthraquinones and the roots contain emodine [3, 4, 6], while the seeds contain chrysarobin (1,8-dihydroxy-3-methyl-9-anthrone) and N-methylmorpholine. Isolation of flavonoids and steroids from leaves, roots, and stems of *S. alata* was also documented [7, 8]. The presence of three new flavones glycosides viz: 5,7-dihydroxyflavone-5-O-d-xylopyranosyl-7-O-l-rhamno pyranosyl-(13)-O-l-arabinopyranoside, 3, 5, 7, 3', 4'-pentahydroxy flavone-3-O-l-rhamnopyranosyl-7-O-d-glucopyranosyl-(13)-O-d-xylopyranoside and 5, 7,3',4'-tetrahydroxy-6-methoxyflavone-5-larabino pyranosyl-(14)-O-l-rhamnopyranosyl-(13)-O-d-galactopyranoside was indicated in the seeds of *S. occidentalis* [4, 9]. The crude extracts of the various plant parts of *S. occidentalis*: leaves, seeds and pods have manifested antifungal activity against *Candida albicans*, *Aspergillus clavatus* and *A. niger* [9-11]. Chrysophanol which was isolated from methanol extract of *S. occidentalis* and its anthraquinone derivative upon application promoted wound healing activity in excision, incision, and dead space models in rats [12].

* Corresponding author. *E-mail address*: ngoziamako2011@gmail.com (Ngozi Francesca Amako)

Crude extracts from leaves, seeds and pod manifested excellent antifungal and antibacterial activities even better than the standard drugs such as nystatin and greseofulvin [9, 13]. The presence of antifungal anthraquinones including aloe, emodin, rhein, and chrysophanol and anthraquinone glycosides (sennosides) have been documented in literature [8, 14, 15].

The use of the juice squeezed from the crushed root of *S. occidentalis* for the treatment of ringworm motivated the present study. The major strategies employed in obtaining quality bioactive constituents involve selection of appropriate solvent, extraction methods, phytochemical screening procedures, fractionation methods, and identification techniques [16, 17]. The maceration process was employed in this study for the extraction of pulverized root sample of *Senna occidentalis* using methanol as solvent. This was followed by fractionation whereby the extract was partitioned into different solvents to give their soluble fractions; these were subjected to antimicrobial bioassay. Separation of the crude methanol extract was also achieved using gas chromatography and qualitative analyses of the constituents was determined via phytochemical screening, Fourier Transform Infrared spectroscopy (FT-IR) and mass spectrometry. The separation capabilities of gas chromatography were coupled with the capacity for qualitative and quantitative detection of mass spectrometry to provide accurate substance identification [18]. KBr pellet method was used as a carrier for the sample in the infrared spectrum because it is optically transparent with a transmittance of 100% in the range of IR measurement (4000-400 cm^{-1}) so that no interference in the absorbance would occur [19].

We hereby report the extraction of different phytochemicals, the FT-IR and gas chromatography/mass spectrometry analyses of constituents from the root of *S. occidentalis* and the *in vitro* antimicrobial properties of the extracts which may be attributed to the bioactive principles in the root extracts thus validating the use of this plant in folk medicine.

2. Experimental

2.1. Materials and reagents

The plant *Senna occidentalis* Linn. was collected on 21st of April 2015 from the pasture grazing field of Michael Okpara University of Agriculture, Umudike, Abia State Nigeria. The leaves, flowers and inflorescence of this plant were identified by comparison with voucher herbarium specimen and were assigned herbarium No. Brenam, 9170 by Mr. Ibe, K. Ndukwe of the Department of Forestry of the University. The roots of the plant were washed, air-dried pulverized and stored in polythene bags before use.

Freshly prepared reagents such as Dragendorff and Mayer's reagents for alkaloids, 3.5 % ferric chloride in 7.0 ml glacial acetic acid (cardiac glycosides), 10% sulfuric acid were used for identification of different groups of phytochemical constituents. Most chemicals used were analytical grade viz: petroleum ether (40 - 60 °C), ethyl acetate, methanol, these were redistilled before use.

2.2. Extraction and fractionation

The powdered *S. occidentalis* root sample (30.0 g) was soaked (maceration method) in methanol (250.0 cm^3) for seven days shaking occasionally to ensure saturation of the solvent. The solvent was renewed after occasional filtration steps to ensure exhaustive extraction of the plant materials. The pooled filtrate was concentrated *in vacuo* at 30 – 40 °C to give reddish brown solid mass of MSo (6.06 g). This was successively washed with the following solvents: petroleum ether (40-60 °C), ethyl acetate and methanol, these yielded 1.20 g, 1.86 g and 2.92 g solid mass fractions labelled: Pex, Eax and Mex respectively. The crude methanol extract was subjected to phytochemical screening, FT-IR, and GC/MS spectroscopic analyses while the fractionated extracts were screened for antimicrobial potency.

2.3. Characterization of the extracts

Phytochemical screening. Qualitative analysis of the various phytochemical constituents present in the crude methanol extract (MSo) was adapted from published methods [20, 21].

2.4. FT-IR spectroscopy

Infra-red spectrum of MSo was recorded neatly on a Shimadzu FT-IR 8400S KBr discs by using KBr pellet method [22, 23].

2.5. Separation and identification of constituents using gas chromatography /mass spectrometry (GCMS)

A Shimadzu GCMS- QP2010 PLUS with an AOC-20i autosampler and non-polar 60 M RTX- 5 MS gas chromatograph made of a capillary gas chromatographic column: 60 m x 0.25 mm id x 0.25 mm film thickness with 5% diphenyl 95% dimethylsiloxane as packing material was employed for the separation and analyses of the crude methanol root extract. Helium was used as carrier gas. 1.0 µL sample solution was injected in the sample port in a split less mode with an injection port dwell time of 0.3 sec. The injector was thermostated at 250 °C whereby the sample was volatilized. Column oven temperature ramping was initially set at 80 °C and held for 1.0 min, it was increased to 200 °C, and finally to 280 °C with holding time of 4.00, 5.00 min. respectively at a rate of 10 °C min^{-1}, total elution time was 28.0 min. As the separated components got into a mass selective detector a mass spectrum was obtained. Mass spectra were recorded over 40-600 m/z range with electron impact ionization energy of 70 eV. The mass spectra of separated compounds in the methanol root extract of *S. occidentalis* were identified by comparison with a library data search of mass spectra of authenticated compounds using standard reference National Bureau of Standards Library of Mass spectra (NIST) libraries [24]. Quantitative determinations were made by relating respective peak areas from GCMS to Total ion chromatogram (TIC) [25, 26].

2.6. Antimicrobial screening

Clinical isolates of *Staphylococcus aureus* (SA), *Staphylococcus epidermidis* (SE), *Bacillus cereus* (BC), *Streptococcus pyogenes* (SP), *Escherichia coli* (EC),

Klebsiella pneumonia (KP), *Pseudomonas aeruginosa* (PA), *Proteus vulgaris* (PV), *Aspergillus niger* (AN) and *Candida albicans* (CA) were obtained from Department of Medical Microbiology, Michael Okpara University of Agriculture, Umudike Medical Centre. All cultures were checked for purity and preserved in a slant of nutrient agar and Sabouraud Dextrose Agar for the bacteria and fungi respectively.

The initial sensitivity tests of the microorganisms: SA, SE, BS, SP, EC, KP, PA and PV on the extracts Pex, Eax and Mex were evaluated according to the standard recommended method [27, 28]. This was carried out by inoculating a small portion of each of the bacteria cultures separately into 3 ml sterile saline solutions in sterile bottles with 0.5 McFarland standard. Within 15 min after adjusting the turbidities of the inoculum's suspensions, sterile cotton swabs were dipped into the solution of the bacteria cultures and used to inoculate the Mueller–Hinton Agar (MH Agar) plates. The inoculated plates were allowed to dry. Solutions of the extracts were prepared at very low concentration (2.7 mg/ml). Each was dissolved in methanol which was also used as control.

0.1 ml aliquot of each extract was introduced on the MH Agar plates and incubated for 24 hours at 37 °C for the bacteria organisms and for the fungi the incubation was at a temperature of 22 °C for 48 h.

The test microorganism which showed activity was used to determine the minimum inhibitory concentration (MIC). This was carried out using broth micro dilution methods. Each well of the microtiter plate was filled with 100.0 µg/l (0.1 ml) of MH broth except the first well which received 100.0 µl of the extracts to be tested. Serial dilutions of each extract in the broth were used to obtain concentrations: $2.7x10^5$, $1.35x10^5$, $6.75x10^4$, $3.33x10^4$, $1.69x10^4$, $8.40x10^3$, $4.2x10^3$ and $2.1x10^3$ µg/ml. The inoculum size for the microtiter plates procedure was $2.1x10^6$ cfu/ml. The plates were examined for changes in turbidity as an indicator of growth. The first well that appeared clear was taken to be MIC of the extracts. The minimum bactericidal /fungicidal concentration (MBC/MFC) were determined by sub-culturing the preparation that showed no growth in the MIC assay onto MH broth and further incubated at the temperature and time stated previously for bacteria and fungi respectively.

3. Results and discussion

Methanol extracted the largest amount of the plant material (9.73%), being a polar solvent, it extracted polar secondary metabolites [29]. The phytochemical analysis on the crude methanol extract from the root of *Senna occidentalis* Linn. revealed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, anthocyanins, cardiac glycosides and phlobatannins, and the absence of steroids (Table 1).

Table 1. Qualitative phytochemical screening of crude methanol root extract, MSo

Phytochemicals	Result
Tannin	+ +
Phenolics	+ +
Alkaloid	+
Flavonoid	+ +
Saponin	+
Anthocyanin	+ +
Cardiac glycoside	+
Phlobatannin	+
Steroid	-

Key: + = present in trace amount; ++ = present in reasonable amount; - = not present.

Certain literatures [30–32] indicated the presence of steroids in the leaves, seeds, and stem of *S. occidentalis*. It was also documented that different parts of *S. occidentalis* contain different classes of secondary metabolites [4]. The presence of high number of alkaloids, triterpenes, flavonoids, tannins, and little amount of anthraquinone glycosides were reported as phytochemical compounds in the methanol root extract of *S. occidentalis* [33]. Also, aqueous ethanol root extract of *S. occidentalis* were identified to contain unsaturated steroids, steroids, tannins, unsaturated sterols, flavonoids and saponins [34]. Flavonoids and steroids were isolated from leaves, stems, and root of *S. alata*, while anthraquinones and its derivatives were indicated in the methanol extract and emodine was reported in the root of *S. occidentalis* [4, 7, 8]. The absence of steroids in this study may be due to the use of different plant parts or geographical location. These phytochemicals contain a range of structurally diverse chemical compounds which can be isolated and characterized. Usually, these metabolites contain active components that can be of therapeutic benefits in treatment of diseases thus provide lead compounds for production of medications [35]. Tannins are astringent, plants containing polyphenolic compounds that bind and precipitate proteins. Naturally, tannins play a role in protection of plants against predators [36]. Flavonoids are important phytochemicals that contribute to the reduced mortality rates observed in people consuming high levels of plant-based foods. Flavonoids prevent cancer at several sites including skin, colon, breast, prostrate, oral cavity, and liver [37]. Saponins are responsible for numerous pharmacological properties of *Senna occidentalis*. Their presence protects the plant against microbes and fungi and serves as antifeedants [38]. Cardiac glycosides in this plant are used in the treatment of heart diseases e.g., congestive heart failure [39].

The infrared spectrum of MSo revealed a broad absorption band at 3437.26 cm^{-1} due to O-H symmetric stretch in polymeric alcohols [40, 41]. The observed band at 2939.61 cm^{-1} may be due to C-H stretch in methylesters, CH_2, C-H stretch and O-CH_3. This is in close comparison with 2915.64 cm^{-1}, 2921.69 cm^{-1}, FT-IR absorptions reported in the analysis of water / methanol extracts of whole plant and leaves respectively of *Senna occidentalis*. Similarly documented was 2929.70 cm^{-1} sharp band in FT-IR analysis of ethylacetate fraction of *Senna occidentalis* root. Also, an absorption band of moderate intensity observed at 1640.51 cm^{-1}could be due to C=O stretch in aldehydes, carboxylic acids, and esters. This can be compared with

1628.80 cm^{-1} reported in the FT-IR analysis of ethylacetate fraction of *Senna occidentalis* root [42, 43]. The slight variation in the FT-IR data with those of the earlier study may be due to the use of different solvents for extraction or different parts of the plant studied. Also in close comparison with vapor phase IR absorption band is 2930.00 cm^{-1} of linoleic acid, an omega 6 fatty acid [44]. Enols are easily identified by the broad H-bonded O-H stretch absorption and low C=O stretch frequency as in β-keto enolic esters and phenol acetates, -CO-O-C=C- (1690-1650 cm^{-1}). The narrow limits of C=O stretch frequencies for these compounds is noteworthy [40, 45], the absorption frequency of –C=C- stretch in alkenes is usually observed in this region. The absorption bands at 1267.27 cm^{-1} and 1055.10 cm^{-1} are due to asymmetric and symmetric stretch vibrations of –C-O-C- respectively, usually the asymmetric stretch is stronger than the symmetric stretch and α, β -unsaturated carboxylate esters show two –C-O stretch bands [31] which is like –C-O- stretch (1252 cm^{-1}) in esters of polyols such as hydroxyl- flavonoids [46].

The identity of chemical compounds present in the crude methanol extract was also ascertained using the gas chromatography-mass spectrometric technique. The Total Ion Chromatogram (TIC) of MSo presented the elution of ten prominent peaks as shown in Figure 1. On spectrum comparison of the peak areas with the database in the GC-MS NIST library, ten compounds were identified. Their mass spectrum is shown in Figure 2.

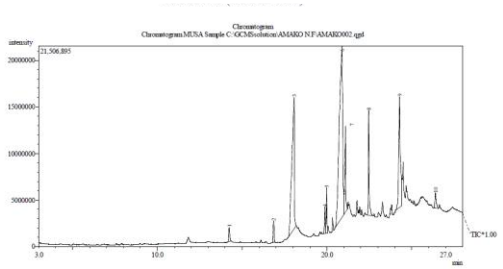

Figure 1. GC-chromatogram of methanol root extract (MSo) of *S. occidentalis*

The total percentage of the identified compounds of about 56.7% and some of the important features of these compounds are summarized in Table 2. Compounds were identified not only by comparing the R_t to a standard as in GC but also by its mass spectrum. An unknown compound in most cases can be identified based on its mass spectrum. Chromatographic peak areas are proportional to the total quantities of substances passing through the detector and are used for most quantitative chromatographic estimations [28, 29].

Table 2. Compounds identified in GC/MS analysis of MSo

GC peak #	Molecular – ion (m/z)	R_T (min)	Compound identified	MS fragmentation pattern (m/z)	Peak area (%)
1	228	14.231	Tetradecanoic acid, $C_{14}H_{28}O_2$ (Mol.wt. 228)	228, 185, 129, 115, 87, 73, 60, 41, 27	1.12
2	228	16.858	Tridecanoic acid methyl ester, $C_{14}H_{28}O_2$ (Mol. wt. 228)	228, 143, 87, 74, 57,41, 39	1.38
3	256	18.067	*n*-Hexadecanoic acid, $C_{16}H_{32}O_2$ (Mol. wt. 256)	256, 129, 115, 85, 73, 60, 43, 41.	23.76
4	163	19.892	9,12-Octadecadienoic acid methyl ester (E, E), $C_{19}H_{34}O_2$ (Mol. wt. 294)	163, 109, 95,81, 67, 55, 41, 39	1.64
	150	19.892	Linolelaidic acid methyl ester, 11,14-Eicosadienoic acid methyl ester (E, E), $C_{21}H_{38}O_2$, linoleic acid (Mol. wt. 322)	150, 123, 109, 93, 81, 67, 55, 41, 39	
5	194	19.892	7-Hexadecenoic acid methyl ester (Z-11), $C_{17}H_{32}O_2$ (Mol. wt. 268)	194, 152, 137, 123, 98, 87, 74, 69, 55, 41	2.48
6	236	20.900	Hexadecenoic acid (Z-11), $C_{16}H_{30}O$ (Mol.wt. 254)	236, 192, 152, 138, 123, 97, 83, 69, 55, 41	4.88
	226	20.900	E-9-Tetradecenoicacid, $C_{16}H_{30}O_2$ (Mol. wt. 226)	226, 208, 166, 124, 98, 83, 69, 55, 41	
7	284	21.100	Octadecanoic acid 2-(2-hydroxyethoxy) ethyl ester, $C_{22}H_{44}O_4$ (Mol. wt. 372)	284, 241, 213, 185, 129, 85, 73, 60, 43, 41	6.24
8	237	24.292	E-2-Octadecadecen-1-ol, $C_{18}H_{36}O$ (Mol. wt. 268)	237, 137, 109, 96, 82, 69, 55, 41	13.74
9	180	26.408	E-2-Octadecadecen-1-ol, $C_{18}H_{36}O$ (Mol. wt. 198)	180, 111, 96, 82, 57, 55, 43, 41	1.46

Volatile saturated and unsaturated fatty acids (FAs), their methyl esters and alcohols were the major compounds viz: saturated *n*-hexadecanoic acid predominates $C_{16}H_{32}O_2$ (23.76%). Other major compounds identified were: hexadecenoic acid (Z-11), $C_{16}H_{30}O_2$ and E-9-tetradecenoic acid $C_{16}H_{30}O_2$ (4.88%), octadecanoic acid 2-(2-hydroxyethoxy)ethylester (aquacera) $C_{22}H_{44}O_4$ (6.4%) and E-2-octadecadecen-1-ol, $C_{18}H_{36}O$ (13.74%). The presence of these compounds is consistent with the observed functional groups in the FT-IR spectrum of the methanol extract. Arshad Javaid et al. [47] reported the presence of 9,12- octadecadienoic acid methyl ester (E, E) in the GCMS analysis of *Senna occidentalis* root extract which might be the cause of its antifungal activity against *Macrophomia phaseolina* a destructive soil fungus and 11-octadecanoic acid has been indicated as the major constituent in both root and leaf extracts of *Senna occidentalis* [48]. Hexadecenoic

acid (Z-11), E-9-tetradecenoic acid, and 9,12-octadecadienoic acid have been identified in *Gracilaria* spp [49] and have been isolated from seed extracts of *Buchhoolzia cariacea* Engler [50]. *n*-Hexadecanoic and 11,14-eicosadienoic acid methylester (E, E), $C_{21}H_{38}O_2$ linoleic acid were identified in leaves extract of different Senna species [51]. There is no previous report of the presence of tetradecanoic acid, E-9-tetradecenoic acid, linolelaidic acid methyl ester and E-2-octadecadecen-1-ol in any Senna spp. Conjugated FAs is still undergoing research, some examples such as linoleic acid an essential FA needed for healthy body growth for mammals cannot be synthesized by them and as such must be added in their diets. They have antitumor activity and are beneficial in the fight against hypertension, obesity, and diabetes [52]. Antioxidant and anti-atherosclerosis properties of *n*-hexadecenoic acid have been reported [53, 54]. Octadecanoic acid 2-(2-hydroxyethoxy)ethylester is used as plasticizer, lubricant, binding, and thickening agent in cosmetic and textile industries [55]. Several therapies such as antieczemic, antihistaminic and anti-arthritic have been documented in literature with respect to the activities of 9,12-octadecadienoic acid [56].

The antimicrobial potency of the root extracts was evaluated against different human and animal pathogens. The results of sensitivity test/zones of inhibition as well as minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) / minimum fungicidal concentration (MFC) are summarized in Tables 3 and 4. Mex and Eax root extracts showed similar broad spectrum *in vitro* antimicrobial activity on all the tested microorganisms. Both gram-positive and gram-negative bacteria as well as different species of fungi were inhibited.

Table 3. Susceptibility test / diameter of zones of inhibition (mm) of the root extracts of *Senna occidentalis* on tested microorganisms.

Test organism	Sensitivity test				Diameter of zones of inhibition (mm)		
	Mex	EAcx	PEx	Control	Mex	EAcx	Pex
Escherichia coli	S	S	R	R	30	20	ND
Klebsiella pneumoniae	S	S	R	R	15	12	ND
Pseudomonas aeruginosa	S	S	R	R	14	11	ND
Proteus vulgaris	S	S	R	R	16	14	ND
Staphylococcus aureus	S	S	S	R	25	20	15
Staphylococcus epidermidis	S	S	S	R	20	18	12
Bacillus cereus	S	S	S	R	28	25	20
Streptococcus pyogenes	S	S	S	R	25	22	15
Aspergillus niger	S	S	R	R	21	16	ND
Candida albicans	S	S	R	R	15	13	ND

Key: S = Susceptible; R = Resistant; ND = Not determined.

These extracts must contain similar chemical components. *E. coli* and *B. cereus* were the most susceptible with the highest zone of inhibition of 30 mm and 28 mm respectively at a minimum concentration of 1.35 x10^3 µg/ml as shown by the methanol extract. The lowest zones of inhibition of 14 mm (MIC 1.35 x10^3 µg/ml) and 11 mm (MIC 0.68 x 10^3 µg/ml) were exhibited by Mex and Eax extracts respectively against *P. aeruginosa*. Each extract was used at very low concentration (2.7 mg/ml). *E. coli, K. pneumonia, P. aeruginosa, P. vulgaris, A. niger* and *C. albicans* were resistant to Pex. All the extracts manifested activity against *S. aureus, S. epidermidis, B. cereus* and *S. pyogenes* with significant zones of inhibition being exhibited by *B. cerus*. Petroleum ether did not extract compounds which could inhibit the growth of *E. coli, K. pneumonia, P. aeruginosa, P. vulgaris, A. niger* and *C. albicans*. The lowest MIC and MBC / MFC value of 0.68 µg/ml exhibited by Mex and Eax is noteworthy. It corresponds to the most active extracts. MBC / MIC (≤ 4) is the minimum amount of plant extract needed to kill the bacteria spp. or fungi [57].

Table 4. The MIC and MBC / MFC of the *Senna occidentalis* root extracts against selected pathogens (x10^3 µg/ml)

Test organism	Mex		EAcx		PEx	
	MIC	MBC/ MFC	MIC	MBC/ MFC	MIC	MBC/ MFC
Escherichia coli	1.35	2.7	0.68	1.36	ND	ND
Klebsiella pneumoniae	1.35	2.7	0.34	0.68	ND	ND
Pseudomonas aeruginosa	1.35	2.7	0.68	1.36	ND	ND
Proteus vulgaris	1.35	2.7	1.35	2.7	ND	ND
Staphylococcus aureus	1.35	2.7	1.35	2.7	1.35	2.7
Staphylococcus epidermidis	1.35	2.7	0.68	1.36	1.35	2.7
Bacillus cereus	1.35	2.7	1.35	2.7	0.68	1.36
Streptococcus pyogenes.	1.35	2.7	1.35	2.7	0.34	0.68
Aspergillus niger	0.68	2.7	1.35	1.36	ND	ND
Candida albicans	0.68	2.7	1.35	1.36	ND	ND

Key: ND: Not determined.

MBC values were twice their MIC values while the MFC values were about four times the MIC values for Mex and about the same value for the Eax. This suggests that twice the concentration of the Mex and Eax extracts were needed to completely kill the bacteria rather than to merely inhibit their growth whereas four times the concentration of Mex and about the same concentration of Eax is required to kill the fungi (Table 4). Emodin isolated from ethanol root extract of *S. occidentalis* has been reported to manifest activity against *Bacillus subtilis* and *Staphylococcus aureus* with MIC value 7.8 x 10^{-3} and 3.9 x 10^{-3} mg/ml respectively and was not active against *K. pneumonia* and *E. coli* gram negative bacteria at the tested concentration wide range of infections including wound sepsis, burns and skin abscesses. *E. coli* is indicated in gastroenteritis and urinary tract infections while *S. pyogenes* causes tonsillitis, wound sepsis, and scarlet fever. *B. cereus*, a widely distributed environmental pathogen, is a common cause of gastrointestinal tract infections e.g., diarrhea, nausea and vomiting associated with food poisoning as well as other non-gastrointestinal tract infections such as severe eye infections, respiratory tract, wounds, and urinary tract infections also in anthrax-like pneumonia. *B. cereus* has been reported to show resistance to β-lactam antibiotics such as penicillin. However, antimicrobials that could be used for effective management of *B. cereus* infections include ciprofloxacin and vancomycin [59]. The result agrees with the reported activities demonstrated by ethanol leaves extract of *C. occidentalis* [30, 60]. A few

literature attributed the presence of flavonoids and terpenoids as defensive agents against any pathogen [57]. Crude extracts of various parts: leaves, seeds, and pods manifested antifungal activity against *Candida albicans*, *Aspergillus clavatus* and *A. niger* [10]. The significant activities manifested by these extracts may be attributed to the presence of secondary metabolites in the crude methanol extract (Table 1).

In folk medicine the paste of the leaves of *C. occidentalis* is externally applied to wounds, sores, itch, cutaneous diseases, ringworm, and skin diseases. The paste of the leaves together with calcium hydroxide is applied to abscesses for quick rupture and clearing of pus. An infusion of the plant bark is used in folklore for diabetes, throat infection [5, 31]. In Cameroon *S. alata* is used for treatment of gastro-intestinal disorders, gonorrhea, and skin diseases. Previous studies reported the isolation of different anthraquinones such as rhein, emodin and chrysophanol which exhibited antifungal properties and anthraquinone glycosides with laxative properties [57, 59]. Different parts of *S. occidentalis* viz: leaves, seeds, stems have been investigated. There are very few reports on the chemical profile and therapeutic effects of extracts from *S. occidentalis* roots.

4. Conclusions

The findings made in the present study have enabled the detection of useful constituents viz: alkaloids, flavonoids, tannins, phenolic, saponins, anthocyanin, cardiac glycosides and phlobatannins, and absence of steroids as determined by phytochemical screening. The absence of steroids may be peculiar to the part of the plant studied or geographical location of the plant. Major compounds identified by GC/MS were volatile saturated and unsaturated fatty acids, their methyl esters, and alcohols. *n*-Hexadecanoic acid $C_{16}H_{32}O_2$ predominates, also identified was octadecanoic acid 2-(2-hydroxyethoxy) ethylester, $C_{22}H_{44}O_4$. The presence of tetradecanoic acid $C_{14}H_{28}O_2$, E-9-tetradecenoic acid $C_{16}H_{30}O_2$; linolelaidic acid methyl ester and E-2-octadecadecen-1-ol. $C_{18}H_{36}O$ in Senna species is hereby reported for the first time. The significant broad spectrum antimicrobial potency of the extracts of root of *S. occidentalis* can be attributed to the presence of these identified bioactive principles. This justifies the use of the extracts of the root of *S. occidentalis* in ethnomedicine. The identified compounds if isolated, purified and characterized shall provide useful candidates for drug discovery studies.

Conflict of interest

The authors have no competing interests.

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References

[1]. J. Valez-Gavilan, *Senna occidentalis* (Coffee Senna), Invasive species compendium, CABI Compendium. CABI Internatıonal (2016). Doi: 10.1079/cabicompendium.11450

[2]. R.B. Bhat, E.O. Etejere, V.T. Oladipo, Ethnobotanical studies from central Nigeria, Economic Botany 44 (1990) 382-390. Doi: 10.1007/bf03183923

[3]. J.P. Yadav, V. Arya, S. Yadav, M. Panghal, S. Kumar, S. Dhankhar, *Cassia occidentalis* L. A review on its ethnobotany, phytochemical, and pharmacological profile, Fitoterapia 81 (2010) 223-230. Doi: 10.1016/j.fitote.2009.09.008

[4]. M.M. Alshehri, C. Quispe, J. Harrera- Bravo, J. Sharifi-Rad, M. Butnariu, M. Kumar, D. Calina, W.C. Cho, A review of recent studies on the antioxidant and anti-infectious properties of Senna plants, Oxidative and Medicines and Cellular Longevity (2022). Doi: 10.1155/2022/6025900

[5]. R.S. Reeta, K. Patel, R.K. Sukumaran, C. Larroche, A. Pandey, Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production, Bioresource Technology 127 (2013) 500-550. Doi: 10.1016/j.biortech.2012.09.012

[6]. V.V. Sing, J. Jain, A.K. Mishra, Pharmacological and phytochemical profile of *Cassia occidentalis* L: A review, Journal of Drug Delivery and Therapeutics 6 (2016). Doi: 10.22270/jddt.v6i5.1284

[7]. H.L. Kim, B.J. Camp, R.D. Grigsby, Isolation of N-methyl morpholine from the seeds of *Cassia occidentalis* (Coffee senna), Journal of Agricultural and Food Chemistry 19 (1971) 198-199. Doi: 10.1021/jf60173a026

[8]. T. Hennebelle, B. Weniger, H. Joseph, S. Sahpaz, F. Bailleul, *Senna alata*, Fitoterapia 80 (2009) 385-393. Doi: 10.1016/j.fitote.2009.05.008

[9]. Z. Zhang, S.W. Ni, X. Xu, W.Z. Huang, S.S. Wang, H. Zhu, X.M. Gao, Chemical constituents from *Cassia occidentalis*, Zhongguo Zhong Yao Za Zhi 15 (2021) 3873-3876 (in Chinese). Doi: 10.19540/j.cnki.cjcmm.20210427.201

[10]. P. Jeruto, P.F. Arama, B. Anyango, T. Akenga, R. Nyunja, D. Khasabuli, *in vitro* antifungal activity of methanolic extracts of *Senna didymobotrya* (Fresen.). H.S. Irwin & Barneby Plant Parts, African Journal of Traditional, Complementary and Alternative Medicine 13 (2016) 168-174. Doi: 10.21010/ajtcam.v13i6.24

[11]. M.C. Mahanthesh, A.S. Manjappa, M. Shinde, J.I. Disouza, Biological activities of *Cassia occidentalis* Linn. A systematic review, World Journal of Pharmaceutical Research 8 (2019) 400-417. Doi: 10.20959/wjpr20199-15430

[12]. M.B. Delmut, L.M. Parente, J.R. Paula, E.C. Conceicao, A.S. Santos, I.A.H. Pfrimer, *Cassia occidentalis*: Effect on healing skin wounds induced by *Bothrops moojeni* in mice, Journal of Pharmaceutical Technology and Drug Research 2 (2013) 10. Doi: 10.7243/2050-120X-2-10

[13]. A.A. Tamasi, M.O. Shoge, T.T. Adegboyega, E.C. Chukwuma, Phytochemical analysis and *in-vitro* antimicrobial screening of the leaf extract of Senna *occidentalis* (Fabaceae), Asian Journal of Natural Product and Biochemistry 19 (2021) 58-65. Doi: 10.13057/biofar/f190203

[14]. S.K. Agarwal, S.S. Singh, S. Verma, S. Kumar, Antifungal activity of anthraquinone derivatives from *Rheum emodi*, Journal of Ethnopharmacology 72 (2000) 43-46. Doi: 10.1016/S0378-8741(00)00195-1

[15]. N.T. Manojlovic, S. Solujic, S. Sukdolak, Antimicrobial activity of an extract and anthraquinones from *Caloplaca schaereri*, Lichenologist 34 (2002) 83-85. Doi: 10.1006/lich.2001.0365

[16]. R.A. Abdullahi, H. Mainul, Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes, Journal of Pharmacy and Bio-allied Sciences 12 (2020) 1-10. Doi: 10.4103/jpbs.jpbs_175_19

[17]. A. Altemimi, N. Lakhssassi, A. Baharlouei, D. G. Watson, D.A. Lightfoot, Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts, Plants. (Basel) 6 (2017) 42. Doi: 10.3390/plants6040042

[18]. K.K. Aathira, B. Kariyil, D. Dhanusa, J.S. Haima, Qualitative and quantitative analysis (GC-MS) of methanol extract of *Crataeva nurvala* stem bark, Journal of Veterinary and Animal Sciences 52 (2021) 135-141.
Doi: 10.51966/jvas.2021.52.2.135-141

[19]. M. Pakkirisamy, S.K. Kalakandan, K. Ravichandran, Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (Black Tumeric), Pharmacognosy Journal 9 (2017) 952-956. Doi: 10.5530/pj.2017.6.149

[20]. J.R. Shaikh, M.K. Patil, Qualitative tests for preliminary phytochemical screening: An overview, International Journal of Chemical Studies 8 (2020) 8834. Doi: 10.22271/chemi.2020.V8.I2I.8834

[21]. C.S. Ezeonu, C.M. Ejikeme, Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods, New Journal of Science 2016 (2016) 5601327. Doi: 10.1155/2016/ 5601327

[22]. A. Finnegan, R.C. Susserott Gouramanis, A simple sample preparation method to significantly improve Fourier Transform Infrared (FT-IR) spectra of micro plastics, Applied Spectroscopy 76 (2022) 783-792.
Doi: 10.1177/00037028221075065

[23]. J. van der Weerd, R.M.A. Heeren, J.J. Boon, Preparation methods and accessories for Infrared spectroscopic analysis of multi-layer paint films, Studies in Conservation 49 (2004) 193-210. Doi: 10.2307/25487692

[24]. G.Y. Nyerges, J. Matyasi, J. Balla, Investigation and comparison of 5% dipheny l-95 % dimethylpolysiloxane capillary columns, Periodica Polytechnica Chemical Engineering 64 (2020) 430-436. Doi: 10.3311/ppch.15289

[25]. S.H. Hansen, Quantitative and qualitative chromatographic analysis, In Bioanalysis of pharmaceuticals: Sample preparation, separation techniques and mass spectrometry (2015). Doi: 10.1002/9781118716830

[26]. Y. Zuo, C. Wang, J. Zhan, Separation, characterization, and quantitation of benzoic and phenolic antioxidants in American cranberry fruit by GC-MS, Journal of Agricultural and Food Chemistry 50 (2020) 3789-3794. Doi: 10.1021/jf020055f

[27]. T.V. Benjamin, A. Lamikanra, Investigation of *Cassia alata*, a plant used in Nigeria in the treatment of skin disease, Pharmaceutical Biology 19 (2008) 93-96. Doi: 10.3109/13880208109070583

[28]. K. Das, R.K. Tiwari, D.K. Shrivastava, Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends, Journal of Medicinal Plant Research 4 (2010) 104-111. Doi: 10.5897/jmpr09.030

[29]. H.I. Aletan, H.A. Kwazo, Qualitative and quantitative phytochemical analysis of *Maerua crassifolia* leaves using various solvents, Nigerian Journal of Pure & Applied Science 32 (2019) 3315-3323. Doi: 10.7910/dvn/6kblbh

[30]. A.S. Saganuwan, M.L. Gulumbe, Evaluation of *in-vitro* antimicrobial activities of phytochemical constituents of *Cassia occidentalis*, Animal Research International 3 (2006) 556-569. Doi: 10.4314/ari.v3i3.40793

[31]. S.Y. Fatmawati, A.S. Purnomo, M.F. AbuBakar, Chemical constituents, usage and pharmacological activity of *Cassia alata*, Heliyon 6 (2020) e04396. Doi: 10.1016/j.heliyon.2020.e04396

[32]. A.M. Ibrahim, B. Lawal, N.A. Tsado, A. Awwal, Phytochemical screening, and GC-MS determination of bioactive constituents from methanol leaf extract of *S. occidentalis*, Journal of Coastal Life Medicine 3 (2015) 992-995. Doi: 10.12980/jclm.3.2015j5-135.

[33]. T.O. Issa, A.I. Mohammed Ahmed, Y.S. Mohamed, S. Yagi, A.M. Makhawi, T.O. Khider Tarig, Physiochemical, insecticidal and antidiabetic activities of *Senna occidentalis* Linn root, Biochemistry Research International 2020 (2020) 8810744. Doi: 10.1155/2020/8810744

[34]. S. Shehu, I. Saleh, S.U. Otokpa, E.V. Madaki, Z.A. Sambi, Phytochemical and antiemetic studies on aqueous ethanol extract of root of *Senna occidentalis* (L.) Link, Bayero Journal of Pure and Applied Sciences 11 (2018) 94-98. Doi: 10.4314/bajopas.v11i2.11

[35]. R.A. Husein, A.A. El-Anssary, Plants secondary metabolites: The key drivers of pharmacological actions of medicinal plants, in: Herbal Medicine, IntechOpen (2018).
Doi: 10.5772/intechopen.76139

[36]. A.P. Singh, S. Kumar, Applications of tannins in Industry, In: Tannins-structural properties,

biological properties and current knowledge, Intechopen 2020.
Doi: 10.5772/intechopen.85984
[37]. A. Ullah, S. Munir, S. LalBadshah, N. Khan, L. Ghani, B.G. Paulson, A.H. Emwas, J. Jaremko, Important flavonoids and their role as a therapeutic agent, Molecules 25 (2020) 5243. Doi: 10.3390/molecules25225243
[38]. A.K. Estrada, G.S. Laarveid, B. Bari, Isolation and evaluation of immunological adjuvant activities of saponins from *Polygala Senegal* L., Comparative Immunology: Microbial Infectious Diseases 23 (2000) 27-43. Doi: 10.1016/S0147-9571(99)00020-X
[39]. H. Sun, X. Young, Y. Ye, Advances in saponin-based adjuvants, Vaccine 27 (2009) 1787-1796. Doi: 10.1016/J.VACCIN.2009. 01. 091
[40]. A.B.D. Nandiyanto, R. Oktiani, R. Ragadhita, How to read and interpret FTIR spectroscopy of organic material, Indonesian Journal of Science & Technology 4 (2019) 15806. Doi: 10.17509/IJOST.V4I1.15806
[41]. S. Sharaf, A. Higazy, A. Hebeish, Propolis induced antibacterial activity and other technical properties of cotton textiles, International Journal of Biological Macromolecules 59 (2013) 408-416. Doi: 10.1016/j.ijbiomac.2013.04.030
[42]. K. Kavipriya, M. Chandran, FTIR and GCMS analysis of bioactive, phytocompounds in methanolic leaf extract of *Cassia alata*, Biomedical and Pharmacology Journal 11 (2018) 18736. Doi: 10.13005/bpj/1355
[43]. P. Priyadharsini, D. Dhanasekaran, B. Kanimozhi, Isolation, structural identification, and herbicidal activity of N-phenylpropanamide from Streptomyces spp. KA_1-3. Archive of Phytopathology and Plant Protection 46 (2013) 364-373. Doi: 10.1080/03235408.2012.758418
[44]. M. Kachel, A. Matwijczuk, A. Przywara, A. Kraszkiewicz, M. Koszel, Profile of fatty acids and spectroscopic characteristics of selected vegetable oils extracted by cold maceration, Agricultural Engineering 22 (2018) 61-71. Doi: 10.1515/agriceng-2018-0006
[45]. M. Matwijczuk, G. Zajac, R. Kowalski, M. Kachel-Jekubowska, M. Gagos, Spectroscopic studies of the quality of fatty acid methyl esters (FAME) derived from waste cooking oil, Polish Journal of Environmental Studies 26 (2017) 2643-2650. Doi: 10.15244/PJOES/70431
[46]. A.J. Silver, S. De Souza, Membranes from latex with propolis for biomedical applications, Materials Letters 116 (2014) 235-238. Doi: 10.1016/J.MATLET.2013.11.045
[47]. A. Javaid, H. Qudsia, I.H. Khan, A. Anwar, M.F. Ferdosi, Antifungal activity of *Senna occidentalis* root extract against *Macrophomia phaseolina* and its GC-MS analysis, Pakistan Journal of Weed Science Research 28 (2022) 115-122. Doi: 10.28941/PJWSR.V28I1.1033
[48]. A.V. Audipudi, R. Badri, C.V.S. Bhaskar, GC-MS and in silico molecular docking analysis of secondary metabolites present in leaf extract of *Cassia occidentalis* Linn., in: S.M. Malik, C. Long, K. Tamasiri, H. Lutken (Eds.) Medicinal Plants: Biodiversity, Sustainable Utilization and Conservation, pp. 501-508, Springer Nature Singapore Pte Ltd; 2020. Doi: 10.1007/978-981-15-1636-8_27
[49]. A.C. de Andrade Tomaz, G.E. C. de Mirande, M.F.V. de Souza, E.V.L. da Cunha, Analysis and characterization of methyl esters of fatty acids of some *Gracilaria spp*., Biochemical Systematics and Ecology 44 (2012) 303-306. Doi: 10.1016/j.bse.2012.02.006
[50]. M. Chukwuonye Ojinnaka, K.I. Nwachukwu, M.N. Ezediokpu, The chemical constituents and bioactivity of seed (fruit) extracts of *Buchholzia coriacea* Engler (Capparaceae), Journal of Applied Sciences and Environmental Management 19 (2015) 795-801. Doi: 10.4314/jasem.v19i4.29
[51]. G.A. Juliana Silva, A. Alexander Silva, D. Isabel Coutinho, P.O. Claudia, J.C. Alberto, G. V. Maria Silva, Chemical profile, and cytotoxic activity of leaf extracts from *Senna spp*. from Northeast of Brazil, Journal of the Brazilian Chemical Society 27 (2016) 1872-1880. Doi: 10.5935/0103-5053.20160073
[52]. M. Cholewski, M. Tomczykowa, M. Tomczy, A comprehensive review of chemistry, sources, and bioavailability of omega-3 fatty acids, Nutrients 10 (2018) 1662. Doi: 10.3390/NU10111662
[53]. E. Fattore, R. Farnelli, Palm oil and palmitic acid: A review on cardiovascular effects and carcinogenicity, International Journal of Food Science Nutrition 64 (2013) 648-659. Doi: 10.3109/09637486.2013.768213
[54]. G.O. Igile, U.L. Okoli, I.A. Iwara, M.U. Etang, Volatile constituents of two fractions of leaves of *Ficus vogelli*, Natural Product Chemistry & Research 6 (2018) 344. Doi: 10.4172/2329-6836.1000344
[55]. M.A. Andrade, R.R. Santos, A. Sanches-Silva, Essential oils from plants industrial application and biotechnological productions, In: S. Malik (Eds.), Exploring Plant Cells for The Production of Compounds of Interest pp. 145-170, Springer (2021). Doi: 10.1007/978-3-030-58271-5_6
[56]. B. Sharmeen Jugreet, S. Suroowan, R.R. Kannan Rengasamy, M.F. Mahomoodally, Chemistry, bioactivities, mode of action and industrial applications of essential oils, Trends in Food Science & Technology 101 (2020) 89-105. Doi: 10.1016/j.tifs.2020.04.025
[57]. S. Pattanaik, S. Chandra Si, A. Pal, J. Panda, S.S. Nayak, Wound healing activity of methanolic extract of the leaves of *Crataeva magna* and *Euphorbia nerifolia* in rats, Journal of Applied Pharmaceutical Science 4 (2014) 046-049. Doi: 10.7324/japs.2014.40310
[58]. J.C. Chukwujekwu, P.H. Coombes, D.A. Mulholland, J. Van Staden, Emodin, an antibacterial anthraquinone from the roots of *Cassia occidentalis*, South African Journal of Botany 72 (2006) 295-297. Doi: 10.1016/J.sajb.2005.08.003

[59]. N. Rajalingam, J. Jung, S. Seo, H. Jin, B. Kim, M. Jeong, D. Kim, J. Ryu, K. Ryu, K. Kyo Oh, Prevalence, distribution, enterotoxin profiles, antimicrobial resistance, and genetic diversity of *Bacillus cereus* group isolates from lettuce farms in Korea, Frontiers in Microbiology 13 (2022) 906040. Doi: 10.3389/fmicb.2022.906040

[60]. F.O. Taiwo, D.A. Akinkpelu, O.A. Aiyegoro, S. Olabiyi, M.F. Adegboye, The biocidal and phytochemical properties of the leaf extract of *Cassia occidentalis* Linn., African Journal of Microbiology Research 7 (2013) 3435-3441. Doi: 10.5897/AJMR 2013.5673

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