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GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND FOURIER TRANSFORMED INFRA-RED ANALYSIS OF *SENNA OCCIDENTALIS* ROOT

[1] Balarabe, S.*, [2]Aliyu, B. S., [2]Muhammad, H., [3]Nafisa, M. A. and [2]Namadina, M. M.

[1]Department of Integrated Science, Federal College of Education, Kano, Nigeria

[2]Department of Plant Biology, Bayero University, Kano, Nigeria

[3]Department of Biology, Sa'adatu Rimi College of Education, Kumbotso, Kano, Nigeria

Correspondence: safiyabalarabe@gmail.com

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ABSTRACT

Senna occidentalis Linn is a member of the Fabaceae family (Leguminosae family). Different parts of the plant have been used in Hausa traditional medicine to treat various ailments ranging from microbial infections, jaundice and body weakness to fevers. The roots, leaves, flowers and seeds have been employed in herbal medicine around the world in various treatments. *Senna occidentalis* root was extracted using distilled water and methanol and was used to carry out phytochemical screening which revealed the presence of carbohydrates, monosaccharides, reducing sugars, combined reducing sugars, tannins, free anthraquinones, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids and alkaloids. Fractionation of the methanol extract was done with four different solvents. The ethyl acetate fraction was further used for thin layer chromatography (TLC) and column chromatography (CC) and the sub-fractions obtained were coded ARE-GRE. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on ARE sub-fraction where fifteen different phytochemical compounds were identified. Fourier Transformed-Infra Red (FT-IR) analysis also showed sixteen peaks of different shapes and wavelengths. The presence of these phytochemicals in the roots of the plant could justify its use in traditional medicine to treat antimicrobial infections and other diseases.

Keywords: *Senna occidentalis*, phytochemicals, column chromatography, thin layer chromatography, GC-MS, FT-IR analysis.

INTRODUCTION

Senna occidentalis Linn is a member of the Fabaceae family (Leguminosae family). Synonyms include *Cassia geminiflora*, *Cassia foetida*, *Cassia carolinian*, *Cassia ciliata*, *Cassia frutescens* and *Cassia linearis.* It is commonly known as the coffee senna, and is a large and economically important family of flowering plants. Plants of this family are found throughout the world, growing in many different environments and climates (Stevens, 2001). The plants range from giant trees to small annual herbs, with the majority being herbaceous perennials (Awomukwu *et al*., 2015). Reports have shown that *Senna occidentalis* in various parts of the world have been employed to treat ailments such as stomach-ache, dyspepsia, flatulence, constipation, hiccough, hepatitis, liver cirrhosis, whooping cough, diuresis, fortify liver, jaundice, fever, cough, asthma, sore throat, cold, flu, hypertension, ease of child delivery and female fertility (Awomukwu *et al*., 2015). The whole plant

*Author for correspondence

is useful as a purgative and as a tonic and the seeds and leaves are used as cure for cutaneous diseases (Yadaya and Satnami, 2011). The roasted seeds are used as a tonic for general weakness and illness (Usha *et al.,* 2007). The leaves are externally applied for wound-healing, itching, bone fracture, ringworm, skin diseases and throat infection. The plant has also been reported to cure leprosy. An infusion of the bark is used in folklore medicine for diabetes (Reeta *et al*., 2013). In addition, the leaf-sap is used in eye troubles in young and old as well as a febrifuge and laxative in the Gambia and Ijo area of Nigeria (Burkill, 1995). Gajalakshmi *et al*. (2012) also reported the plant to have considerable antimicrobial, antioxidant, hepatoprotective, antimalarial, larvicidal, anti-inflammatory, antidiabetic, antianxiety, antidepressant, analgesic, antipyretic and immunosuppressive activities. It has also been used as a laxative (National Research Council, (2008).

The leaves of *Senna occidentalis* have been used in local communities as food and its various parts as medicine to cure numerous ailments without adequate knowledge of its chemical compounds. This research aimed at investigating the phytochemicals present in *Senna occidentalis* roots with the view of verifying the ethnobotanicals use of the plant in the communities.

MATERIALS AND METHODS

Collection and Extraction of the Plant Materials

Senna occidentalis roots were collected from Zawachiki, Kumbotso Local Government Area of Kano State, air-dried at room temperature and crushed with mortar and pestle. Powdered sample (100 g) was weighed and extracted exhau-stively with 1,000 ml methanol and 1,000 ml of distilled water for two (2) weeks using maceration technique. The extracts obtained were transferred into clean sterile airtight containers, weighed and kept in desiccators until required for use (Sofowora, 1993; Trease and Evans, 2002). Percentage yield of the extracts was calculated from the weight
of the extracts and other physical parameters such as colour, texture and odour of the extracts were recorded.

Phytochemical Tests

Phytochemical screening was carried out on the methanol and aqueous extracts of *Senna occidentalis* root following standard methods described by Trease and Evans (2002) and Yadav and Munin (2011).

Partitioning of Methanol Extracts of the Plant

Crude (methanol) extracts of *Senna occidentalis* root were subjected to fractionation by solubiliszation in distilled water followed by partitioning with n-hexane (Jamil *et al.,* 2012) repeatedly for complete separation of n-hexane soluble materials. The remaining extracts were then partitioned with ethyl acetate and n-butanol following the same process to leave just the aqueous portion. All the fractions obtained were collected in beakers and evaporated to dryness (Juvaid *et al.,* 2017).

Column Chromatographic (CC) Separation of the Plant Fractions

A glass tube 60 cm high and 3 cm wide was used for the column chromatography. The adsorbent, silica gel (100, 60-120 µm) was carefully packed using wet slurry method to about 25 cm in the glass tube. Ethyl acetate fraction of *Senna occidentalis* was selected and used for the column chromatography. Two (2) g of the extract fraction was loaded onto the packed adsorbent and allowed to stabilise for about 3 hours before elution started.

The column was eluted with solvents of increasing polarity in the following stepwise gradients (Mudi and Dauda, 2013). The ethyl acetate fraction of *Senna occidentalis* was eluted using hexane (100%), hexane: ethyl acetate (2:1), hexane: ethyl acetate (1:1), hexane: ethyl acetate (1:2), ethyl acetate (100%), ethyl acetate: methanol (2:1), ethyl acetate: methanol (1:1), ethyl acetate: methanol (1:2) and methanol (100%). Volumes of 50 ml and 100 ml were collected at a time until elution was completed and allowed to evaporate to dryness at room temperature. All the eluates were labeled accordingly. The fractions collected from the plant were monitored on TLC plates and those with similar TLC profiles were pooled together and coded ARE - GRE accordingly (Tomer *et al.,* 2009; Patra *et al.,* 2012).

Thin-Layer Chromatography (TLC)

Thin-layer Chromatography (TLC) was performed on pre-coated 20 cm x 20 cm and 0.25 mm thick aluminum plate. The plate was cut into sizes depending on the number of spots to be marked. The positions of the spotting origin were marked in a straight line with a soft pencil. One hundred (100) mg/ml of each of the extracts was dissolved in equal volume (1 ml) of solvent in different tubes. These were spotted on the TLC plates by using glass capillary tubes and the distance of the spotting points of the extracts on the plates was measured to be 2 cm from the base. This was followed by running the chromatogram with suitable solvent system of hexane: ethyl acetate (7:3) in an air-tight chromatographic tank at room temperature. This was run to a certain level depending on the size of the plate used, then the solvent front was marked with a pencil and the plates were removed to evaporate the solvent.

The different spots of bioactive compounds were visualised under an ultraviolet lamp at 365 nm (Sun *et al.,* 2014) before they were sprayed using Vanillin Sulphuric acid spray (vanillin 0.5 g, H_2SO_4 10 ml, alcohol 90 ml) followed by heating in an oven at 110^oC for 2 minutes.

Gas Chromatography-Mass Spectrometry (GC-MS)

Ethyl acetate fraction (ARE) of *Senna occidentalis* root was selected and used to perform Gas Chromatography- Mass Spectrometry (GC-MS) on agilent 7890 instrument with column 30 m long, 250 µm wide and 0.25 µm film thickness. The oven temperature was at 50^oC to 325^oC at 3°C min^{-1} for 15 min. Injection temperature was set at 50^oC using a split mode. Helium (99.9%) was the carrier gas fixed with flow rate of 3 ml min^{-1} and split ratio of 5:1. The components of the test samples were evaporated in the injection part of the GC equipment and segregated in the column with suitable temperature controlled by software. The ionization mass spectroscopic analysis was done at 70 eV. The total running time was 99 minutes and the peak areas were compared with the database in the GC-MS library, that is, those stored in the National Institute of Standards and Technology (NIST) Library, and Fatty Acid Methyl Esters Library (FAME Library). The name, molecular formula, molecular weight and structure of the compounds were recorded.

RESULTS

Table 1 shows the percentage yield of *Senna occidentalis* root extracts which was higher in the aqueous (8.0%) than in the methanol (7.2%).

Table 1: Percentage yield (%) and physical characteristics of *Senna occidentalis* root extract using methanol and distilled water as solvents

Solvent used	Methanol	Aqueous
Solvent volume (mls)	100	100
Mass of powder (g)	100	100
Extract mass (g)	7.2	8.0
Percentage yield (%)	7.2	8.0
Appearance	Solid	Solid
Colour	Deep brown	Brown
Odour	Woody	Woody
Texture	Hard	Hard

The methanol extract of *S. occidentalis* root showed the presence of carbohydrates, monosaccharides, reducing sugars, combined reducing sugars, tannins, free anthraquinones, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids and phenols but alkaloids were absent while the aqueous extract revealed the presence of carbohydrates, reducing sugars, combined reducing sugars, free anthraquinones, cardiac glycosides, saponins, flavonoids but monosaccharides, tannins, glycosides, terpenoids, alkaloids and phenols were absent.

Table 2: Phytochemical constituents of *S. occidentalis* plant extracts

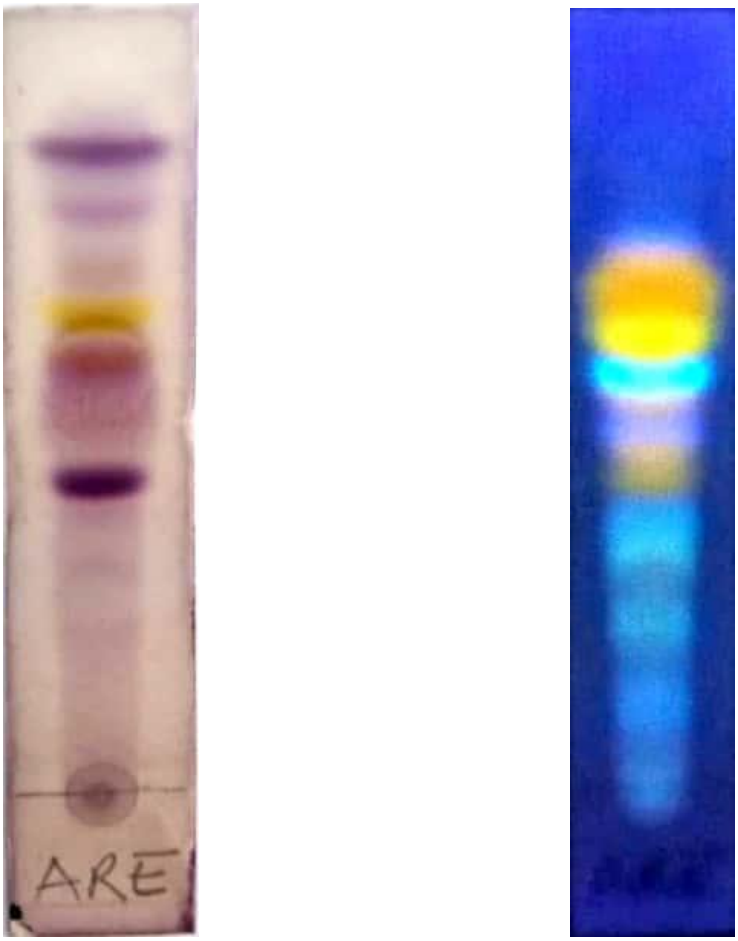
Phytochemical	Extract	
	Methanol	Aqueous
Carbohydrates	+	+
Monosaccharide	+	-
Reducing sugar	+	+
Combined reducing sugar	+	+
Tannins	+	-
Free anthraquinones	+	+
Cardiac glycosides	+	+
Glycosides	+	-
Terpenoids	+	-
Saponins	+	+
Flavonoids	+	+
Alkaloids	-	-

KEY: + = present - = absent

Table 3 shows that eluates of 100 ml each were collected from the column chromatography of *S. occidentalis* root partitioned, fractioned and analysed on TLC using a suitable solvent system. Fractions with similar profiles were pooled together and coded accordingly.

Table 3: Eluates obtained from ethyl acetate fractions of *S. occidentalis* root

Solvent	Eluate obtained	Pooled fraction code		Weight (g)
Ethyl acetate Fractions (2.0 g)				
Hexane 100%	1-5	1-8	ARE	0.18
Hexane: Ethyl acetate 2:1	6-10	9-10	BRE	0.10
Hexane Ethyl acetate 1:1	11-15	11-12	CRE	0.12
Hexane Ethyl acetate 1:2	16-20	13-14	DRE	0.14
Ethyl acetate 100%	21-31	15-18	ERE	0.16
Ethyl acetate: methanol 2:1	32-34	19-24	FRE	0.64
Ethyl acetate: methanol 1:1	35-37	25-37	GRE	0.24



I A I B

Plate I: TLC profile of ethyl acetate fraction of *S. occidentalis* root (ARE)

The chromatogram of *S. occidentalis* root (ARE) ethyl acetate fraction (Plate I A) shows eleven spots with the following Rf values: 0.23, 0.32, 0.40, 0.44, 0.53, 0.61, 0.68, 0.71, 0.76, 0.84 and 0.93. Plate I B shows the spots as viewed under UV before spraying.

Total Ion Chromatogram (TIC) of ethyl acetate fraction (ARE) of *Senna occidentalis* root revealed fifteen phytochemical compounds as shown in Table 3. D-Allose and octanoic acid, silver(1+) salt (17.50%), Thiophene, tetrahydro- (1.02%), 2-Fluoropropene (1.18%), Cyclohexanemethanol (22.70%), 1,2:4,5:9,10-Triepoxydecane (5.39%), o-Allylhydroxylamine (1.07%), 3-Hydroxy-3-phenyl-2-fluoropropene (4.66%), Cyclohexanebutanoic acid (2.53%), 7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl- (1.87%), 9-Decen-1-ol, pentafluoropropionat (2.03%), Polygalitol (2.99%), 7-Octenoic acid (3.02%), 1,3-Hexadiene, 4-diethylboryl-3-trimethylsilyl- (1.05%), .beta.-d-Lyxofuranoside, methyl (1.70%) and 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, glucose (1.20%).

Table 3: Phytochemical compounds identified from ethyl acetate fraction (ARE) of *Senna occidentalis* root

Peak	Retention time (min)	% Area	Compound	Molecular weight	Molecular formula
1	50.603	17.50	D-Allose,	180.16	$C_6H_{12}O_6$
			octanoic acid, silver(1+) salt	251.07	$C_8H_{15}AgO_6$
2	51.043	1.02	Thiophene, tetrahydro-	88.17	C_4H_8S
3	54.267	1.18	2-Fluoropropene	60.07	C_3H_5F
4	56.355	22.70	Cyclohexanemethanol	114.19	$C_7H_{14}O$
5	56.868	5.39	1,2:4,5:9,10-Triepoxydecane	184.23	$C_{10}H_{16}O_3$
6	57.491	1.07	o-Allylhydroxylamine	73.09	C_3H_7NO
7	62.253	4.66	3-Hydroxy-3-phenyl-2-fluoropropene	152.17	C_9H_9FO
8	67.309	2.53	Cyclohexanebutanoic acid	170.25	$C_{10}H_{18}O_2$
9	78.409	1.87	7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl-	140.18	$C_8H_{12}O_2$
10	79.252	2.03	9-Decen-1-ol,	156.27	$C_{10}H_{20}O$
			Pentafluoropropionat	613.02	$C_3F_5O_2$
11	80.571	2.99	Polygalitol	164.16	$C_6H_{12}O_5$
12	84.198	3.02	7-Octenoic acid	142.20	$C_8H_{14}O_2$
13	91.195	1.05	1,3-Hexadiene, 4-diethylboryl-3-trimethylsilyl-	222.25	$C_{13}H_{27}BSi$
14	93.027	1.70	beta-d-Lyxofuranoside, methyl	164.16	$C_6H_{12}O_5$
15	98.009	1.20	5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, glucose	426.44	$C_{17}H_{22}N_4O_7S$

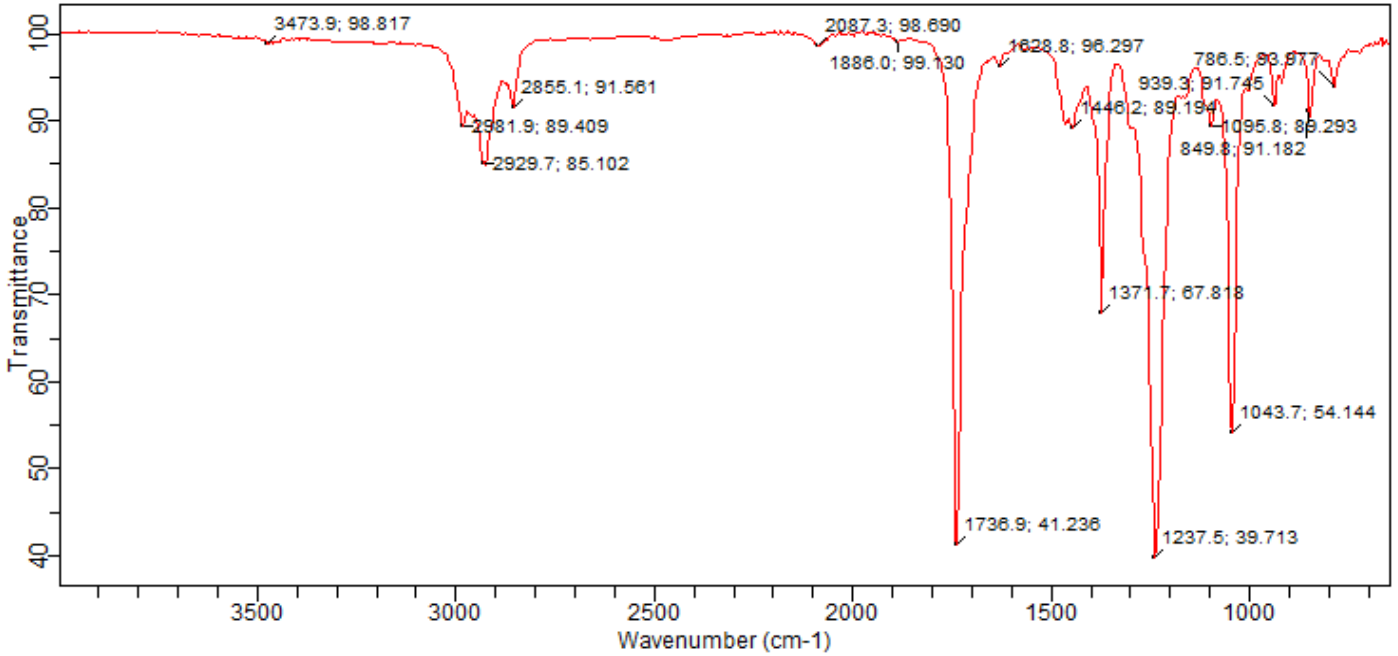


Figure 1: FT-IR spectrum of ethyl acetate fraction (ARE) of *Senna occidentalis* root

Sixteen peaks of different shapes and wavelengths were seen from the FT-IR spectrum of ethyl acetate fraction (ARE) of *Senna occidentalis* root (Figure 1). Detail of the spectrum is shown in Table 4. Table 4 shows various functional groups, chemical bonds and nature of identified compounds from ethyl acetate fraction of *Senna occidentalis* root by FT-IR analysis. The table shows different characteristic absorptions of the spectrum ranging from 3473.9-786.6 cm^{-1}

Table 4: FT-IR Analysis of ethyl acetate fraction of *Senna occidentalis* root

Peak No.	Wavelength (cm^{-1})	Peak shape	Functional group	Type of bond
1	3473.9	Weak	Amines	N-H stretch
2	2981.9	Sharp	Amines	N-H stretch
3	2929.7	Sharp	Alkanes, ketones, carboxylic acid	C-H stretch
4	2855.1	Sharp	Alkenes, carboxylic acids, alcohol	C-H stretch
5	2087.3	Narrow	Isothiocyanate	N=C=S stretch
6	1886.0	Weak	Aromatic compound	C-H bendig
7	1736.9	Very sharp	Esters	C=O stretch
8	1628.8	Weak	Alkene	C=C stretch
9	1446.2	Sharp	Alkane	C-Hbend
10	1371.7	Sharp	Sulfonate	S=O
11	1237.5	Very sharp	Alkyl aryl ether	C-O stretch
12	1095.8	Sharp	Aliphatic amines	C-N stretch
13	1043.7	Sharp	Aliphatic amines	C-N stretch
14	939.3	Sharp	Amines	C-N stretch
15	849.8	Sharp	Halo compound	C-Cl stretch
16	786.6	Sharp	Alkenes	C-H bend

DISCUSSION

The probable chemical compounds present in the plant fractions were identified using Gas Chromatography-Mass Spectrometry analysis. The methanol extract of *S. Occidentalis* root showed the presence of carbohydrates, monosaccharides, reducing sugars, combined reducing sugars, tannins, free anthraquinones, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids and phenols but alkaloids were absent while the aqueous extract revealed the presence of carbohydrates, reducing sugars, combined reducing sugars, free anthraquinones, cardiac glycosides, saponins, flavonoids, but monosaccharides, tannins, glycosides, terpenoids, alkaloids and phenols were absent. Chinnala *et al.* (2010) also reported phytochemical analysis of methanol extract of *Senna occidentalis* root, which showed the presence of tannins, saponins, flavonoids, triterpenoids, sterols, cardiac glycosides and carbohydrate. Phytochemical analysis showed that the different parts of *S. occidentalis* contained different chemical groups including alkaloids, steroids, tannins, flavonoids, anthraquinones, saponins, terpenes, carbohydrates, sugars and cardiac glycosides (Ajagbonna *et al.,* 2000; Saganuwan and Gulumbe, 2006; Daniyan *et al.,* 2011; Veerachari and Bopaiah, 2012). In another study by Garba *et al.* (2015), phytochemical screening of methanol root extract of *C. occidentalis* revealed the presence of a high amount of sterols, flavonoids, tannins, phenols, terpenes and anthraquinones with moderate amount of cardiac glycoside and saponin while the test for alkaloid showed negative result. Similarly, carbohydrates, saponins, terpenes, sterols, flavonoids, alkaloids, cardiac glycosides and anthraquinones were found in the root (Sadiq *et al.,* 2012). Usha *et al*. (2007) reported the presence of alkaloids in the aqueous root extract of the same plant.

The TLC chromatogram of ethyl acetate fraction of *S. occidentalis* root (ARE) in Hexane: Ethyl acetate (7:3) solvent system showed a good separation with eleven bands. The different bioactive spots observed from the TLC result showed that these phytochemicals are present in the plant fraction with high purity and are responsible for the activity of the extract. This result agreed with Ifeoma *et al.* (2015) who also reported TLC result with high purity of crude fractions and extracts that were effective against some test organisms.

The GC-MS spectrum of *Senna occidentalis* fraction showed the presence of various chemical components with different retention times and percentage area, indicating that the ethyl acetate fraction of the plant contained various bioactive compounds of pharmacological importance, which were responsible for the reported activity of the plant. This finding is in line with previous reports indicating that medicinal plants contain active ingredients that are responsible for their therapeutic effects (Yin *et al.,* 2009; Gomathi *et al.,* 2015; Kohoude *et al.,* 2016; Baha'uddeen *et al.,* 2017).

CONCLUSION

The results of this study showed that the methanol and aqueous root extracts of *Senna occidentalis* contain numerous phytochemicals used for the treatment of various infectious diseases in traditional medicine in different regions of the world. The Thin Layer Chromatography (TLC), Gas Chromatography-Mass Spectrometry (GCMS) as well as Fourier Transformed Infra-red (FTIR) analyses of the plant fraction revealed the presence of numerous chemical components in the plant with various chemical structures and physiological action in the body. Result of this study justified the use of *Senna occidentalis* for the treatment of various diseases by indigenous people in traditional medicine.

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