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GC-MS Analysis and Antifungal Activity of *Senna alata* Linn

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Abstract: This study evaluated the antifungal activity, chemical and the phytochemical constituents of *Senna alata* Linn using standard agar well diffusion and broth dilution techniques, GC-MS and standard recommended method of the Association of Analytical Chemists respectively. Results obtained revealed that all the different extracts showed reasonable zone of inhibitions but to varying degree of efficacies. Of the different extracts tested, ethanolic extract displayed the highest activity as reflected in their mean zone of inhibition ranging from 73.6mm to 167.4mm. This was followed by the activity of the chloroform extract that ranges from average zone of inhibition of 38-91mm. The aqueous extract showed the least mean zones of inhibition that ranges from 33-57mm. This observation was also corroborated by the MIC and the MBC values.The mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis of ethanolic extract led to identification of 78compounds including xylene, alcohol, aldehydes, alkanes, alkenes, fatty alcohol, acetic acid, ketones and ester. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Also, *Senna alata* Linn was also found containing saponins, alkaloids, tannins, phlobatannins, anthraquinones, cardenolides, steroidal ring and flavonoids. It can thus be inferred that *Senna alata* Linn posseses good antifungal activity and such activities might be ascribed to the presence of the phytochemicals and some of the chemical constituents.

Key words: GC-MS · Antifungal · *Senna alata* Linn · Phytochemicals

INTRODUCTION

Herbal medicine is an alternative form of therapy and has become the mainstream throughout the world due to the growing resistance of pathogens to conventional antimicrobials [1]. The development of herbal products is dependent on local botanical flora. Medicinal plants are distributed worldwide and many abound in tropical countries. Nigeria has a rich variety of medicinal plants distributed in the different geoecological regions of the country. The genus *Senna* Mill, originates from the Arabic name "Sana" and belongs to the subtribe Cassiinae tribe Cassieae, sub-family Caesalpinioideae, family Leguminosae and order Fabales.

Senna alata L. Roxb. is an important ethno medicinal plant known as *ringworm senna*, as the leaves of the plant are directly used for curing skin infections like ring worm. The plant is commonly known as candle brush tree or empress candle. The leaves of the plant are known to possess antimicrobial [2], anti-tumor [3,4], antioxidant [5], antimutagenic [6] and analgesic [7] activities. The leaves of the plant are also known to possess potent antifungal properties [8, 9]. A 10 year study on human proved that the leaf extracts can be readily used as a herbal medicine for treatment of Pityriasis versicolor, a fungal infection without any side effects [10].

The ointment made from the ethanolic extracts of the leaves is used as topical treatments on acute lesions of dermatophytosis in bovine and prevented its reoccurrence [11]. Apart from the above mentioned properties, the plant is known to possess hepatoprotective [12], antihyperglycemic [13] activities

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and is also used in the treatment of opportunistic infections in AIDS patients. Recently, the extracts of the plant have been used in cosmetics and dermatological skin care products [14]. This research was aimed at determining the antifungal activity, chemical and phytochemical constituents of *Senna alata* Linn

MATERIAL AND METHODS

Collection of Plant Material and Plant Authentication: *Sennaalata* Linn leaves were collected around Sagamuenviron, identified and vouchered by a senior plant taxonomist (Mr T.K. Odewo) at the Dept. of Botany, University of Lagos, Akoka, Lagos, Nigeria.

Preparation of Plant Materials and Extracts: The procured plant materials were air dried and processed by method described by Ijeh *et al.* (2005) The resulting filtrates were then concentrated by evaporation on a rotary Evaporator.

Phytochemistry Analysis: This was carried out on the aqueous extract following the procedure described by Sofowora [15].

Antimicrobial Activity: The antimicrobial activity of the tested plants was carried out using both the broth dilution and Agar diffusion method. Both techniques were carried as described by Parekh and Chanda (2007).

Gas Chromatography (GC)–Mass Spectrometer (MS) Analysis

Instruments and Chromatographic Conditions: GC-MS analysis was carried out on GC-MS-QP2010 Shimadzu system comprising a gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column VF-5MS fussed silica capillary column (30.0m x 0.25mm x 0.25ìm, composed of 5% phenyl/95% dimethylpolysiloxane), operating in electron impact mode at 70ev; helium (99.999%) was used as carrier gas at a constant flow of 1. ml/min and an injection volume of 0.5ìl was employed (Split ratio of 10:1) injector temperature 240°C ion-source temperature 200°C. The oven temperature was programmed from 70°C (Isothermal for 3 min), with an increase of 10°C/min, to 240°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 40 to 440Da. Total GC running time was 40min.

Identification of Compounds: Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

The antifungal activity of *Senna alata* Linn is depicted in the table below. As shown in the Table 1, all the different extracts shows reasonable zone of inhibition but to varying degree of efficacy. Of the different extracts tested, ethanolic extract displays the highest activity as reflected in their mean zone of inhibition ranging from 73.6mm to 167.4mm. This was followed by the activity of the chloroform extract that ranges from average zone of inhibition of 38-91mm. The aqueous extract shows the least mean zones of inhibition that ranges from 33-57mm. This observation was also corroborated by the MIC and the MBC values.

Table 1: *In vitro* Antibacterial Activities of Crude Ethanolic Extracts of Medicinal Plants

	Concentration of extracts/ Zones of inhibition (mg/ml)													
	Aqueous extract			Ethanolic extract			Chloroform extract			n-Hexane extract				
													Flucona zole	
Org	50	75	150	50	75	150	50	75	150	50	75	150	250 mg/ml	50% ethanol/Chloroform/n-Hexane
AN	0	0	0	10	13	26	12	15	20	15	21	25	19.2	0.00/0.00/0.00
AC	0	0	0	13	18	19	0	14	15	0	10	15	17.4	0.00/0.00/0.00
AF	10	16	18	12	22.8	33.2	0	12	20	0	0	10	16.0	0.00/0.00/0.00
PV	15	18	20	15.6	26.4	36.8	13	16	18	5	8	10	28.0	0.00/0.00/0.00
FS	0	0	6	13	16	20	0	0	0	6	8	14	17.0	0.00/0.00/0.00
TA	8	7	13	10	16.3	32.4	13	16	18	0	10	15	22.4	0.00/0.00/0.00

AN = *Aspergillus niger*, AC= *Aspergillus carbonarius*, AF= *Aspergillus flavus*, PV= *Penicillium verrucosum*, FS= *Fusarium solani*, TA= *Trichoderma atrovidae*, AE= Aqueous extract, ET= Ethanolic extract, CE= Chloroform extract, NHE = normal hexane extrac

Table 2: Minimum inhibitory and fungicidal concentration of *Senna alata* Linn

	MIC (mg/ml)				MFC (mg/ml)			
ORG	AE	ET	CE	NHE	AE	ET	CE	NHE
AN	310	38.8	77.5	155	>620	155	310	620
AC	310	19.4	77.5	77.5	620	77.5	620	620
AF	155	4.8	38.8	77.5	620	38.8	310	310
PV	19.4	2.4	19.4	38.8	77.5	9.7	77.5	310
FS	310	9.7	19.4	19.4	>620	38.8	155	310
TA	155	19.4	77.5	155	620	77.5	310	620

Library Search Report

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Acq On : 15 Aug 2014 23:45
Operator : MEJIDA/ACHEM
Sample : KOLAMOLE PLANT
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: C:\Database\NIST08.L Minimum Quality: 90
C:\Database\NIST11.L Minimum Quality: 90

Unknown Spectrum: Apex
Integration Events: ChemStation Integrator - events.e

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			2-Indanol	15273	004254-29-9	25
			Benzene, 1,2-diethyl-	14837	000135-01-3	22
			6-Octenal, 3,7-dimethyl-, (R)-	26751	002385-77-5	15
2	5.539	3.90	C:\Database\NIST11.L			
			Benzene, 1-methyl-3-propyl-	14856	001074-43-7	46
			Benzene, 1-methyl-3-propyl-	14858	001074-43-7	42
			Benzene, 1-methyl-3-propyl-	14853	001074-43-7	42
3	5.613	4.72	C:\Database\NIST11.L			
			Benzene, 1,2-diethyl-	14842	000135-01-3	87
			Benzene, 1,2-diethyl-	14837	000135-01-3	60
			Benzene, 1,3-diethyl-	14839	000141-93-5	55
4	5.653	18.64	C:\Database\NIST08.L			
			Benzene, 1,2-diethyl-	14664	000135-01-3	90
			Benzene, 1,2-diethyl-	14659	000135-01-3	89
			Benzene, 1,4-diethyl-	14662	000105-05-5	78
5	6.020	6.14	C:\Database\NIST08.L			
			Benzene, 2-ethyl-1,4-dimethyl-	14714	001758-88-9	93
			Benzene, 1-methyl-2-(1-methylethyl)-	14737	000527-84-4	92
			Benzene, 1-ethyl-2,3-dimethyl-	14702	000933-98-2	91
6	6.082	1.99	C:\Database\NIST11.L			
			Benzene, 4-ethyl-1,2-dimethyl-	14888	000934-80-5	87
			o-Cymene	14811	000527-84-4	86
			Benzene, 2-ethyl-1,4-dimethyl-	14876	001758-88-9	60
7	6.128	7.48	C:\Database\NIST08.L			
			Benzene, 2-ethyl-1,4-dimethyl-	14712	001758-88-9	91
			Benzene, 4-ethyl-1,2-dimethyl-	14710	000934-80-5	91
			Benzene, 1-methyl-2-(1-methylethyl)-	14737	000527-84-4	91
8	6.266	22.63	C:\Database\NIST08.L			
			Undecane	27916	001120-21-4	93
			Undecane	27913	001120-21-4	92
			Undecane	27915	001120-21-4	70
9	6.678	2.62	C:\Database\NIST08.L			
			Benzene, 1-ethyl-3,5-dimethyl-	14705	000934-74-7	92
			Benzene, 1-methyl-2-(1-methylethyl)-	14737	000527-84-4	91
			Benzene, 1-ethyl-2,3-dimethyl-	14708	000933-98-2	86
10	6.763	2.54	C:\Database\NIST08.L			
			Benzene, 1,2,4,5-tetramethyl-	14694	000095-93-2	94

			Benzene, 1,2,4,5-tetramethyl-	14688	000095-93-2 94
			Benzene, 1,2,4,5-tetramethyl-	14693	000095-93-2 93
11	6.912	0.71	C:\Database\NIST11.L		
			Naphthalene, decahydro-2-methyl-	25447	002958-76-1 60
			Cyclodecene, 1-methyl-	25420	066633-38-3 55
			Naphthalene, decahydro-2-methyl-	25445	002958-76-1 50
12	7.170	2.14	C:\Database\NIST08.L		
			Benzene, 1,3-diethyl-5-methyl-	22456	002050-24-0 90
			Benzene, 1-methyl-2-(2-propenyl)-	13931	001587-04-8 62
			Benzene, 1-methyl-4-(1-methylpropyl)-	22480	001595-16-0 42
13	7.381	4.54	C:\Database\NIST08.L		
			Benzene, 1-methyl-4-(2-propenyl)-	13929	003333-13-9 90
			Benzene, (1-methyl-1-propenyl)-, (	13934	000767-99-7 60
			Benzene, 1-methyl-2-(2-propenyl)-	13931	001587-04-8 60
14	8.177	5.79	C:\Database\NIST08.L		
			Naphthalene	11815	000091-20-3 95
			Azulene	11808	000275-51-4 94
			1H-Indene, 1-methylene-	11817	002471-84-3 93
15	8.371	0.27	C:\Database\NIST11.L		
			Azulene	11935	000275-51-4 35
			Naphthalene	11941	000091-20-3 35
			Benzene, 1,3-dimethyl-5-(1-methylethyl)-	22830	004706-90-5 30
16	8.411	1.21	C:\Database\NIST11.L		
			1H-Indene, 2,3-dihydro-1,2-dimethyl-	21646	017057-82-8 42
			Benzene, 1-ethyl-4-(1-methylethyl)	22810	004218-48-8 38
			Benzene, (3-methyl-2-butenyl)-	21631	004489-84-3 38
17	9.338	0.59	C:\Database\NIST11.L		
			2-Ethyl-2,3-dihydro-1H-indene	21617	056147-63-8 58
			Bicyclo[4.2.1]nona-2,4,7-triene, 7-ethyl-	21640	1000184-42-6 41
			1H-Imidazole, 4,5-dihydro-2-phenyl	22235	000936-49-2 35
18	10.946	1.67	C:\Database\NIST08.L		
			Naphthalene, 1-methyl-	18987	000090-12-0 94
			Naphthalene, 1-methyl-	18989	000090-12-0 91
			Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	18998	002443-46-1 91
19	11.398	0.42	C:\Database\NIST08.L		
			Naphthalene, 2-methyl-	18992	000091-57-6 91
			Naphthalene, 1-methyl-	18989	000090-12-0 86
			Benzocycloheptatriene	18985	000264-09-5 86
20	13.252	0.78	C:\Database\NIST08.L		
			5-Octadecene, (E)-	99558	007206-21-5 91
			9-Octadecene, (E)-	99559	007206-25-9 87
			1-Tridecene	46098	002437-56-1 68
21	16.657	2.04	C:\Database\NIST08.L		
			Phenol, 2,5-bis(1,1-dimethylethyl)	63983	005875-45-6 95
			Phenol, 2,4-bis(1,1-dimethylethyl)	63980	000096-76-4 94
			Phenol, 2,4-bis(1,1-dimethylethyl)	63985	000096-76-4 90
22	18.408	1.33	C:\Database\NIST08.L		
			1-Hexadecene	78199	000629-73-2 98
			1-Hexadecene	78198	000629-73-2 96
			1-Heptadecene	88761	006765-39-5 95

23	18.585	0.33	C:\Database\NIST08.L			
			Hexadecane	79882	000544-76-3	95
			Hexadecane	79880	000544-76-3	91
			Heptacosane	187748	000593-49-7	87
24	23.105	1.35	C:\Database\NIST08.L			
			1-Octadecene	99556	000112-88-9	95
			Trifluoroacetoxy hexadecane	163912	006222-03-3	94
			1-Nonadecene	110437	018435-45-5	94
25	24.147	0.72	C:\Database\NIST11.L			
			Phytol	141396	000150-86-7	68
			Bicyclo[3.1.1]heptane, 2,6,6-trime thyl-, (1.alpha.,2.beta.,5.alpha.)	17013	006876-13-7	60
			(-)-trans-Pinane	16859	033626-25-4	60
26	26.865	0.59	C:\Database\NIST11.L			
			n-Hexadecanoic acid	107547	000057-10-3	47
			1-Decanol, 2-hexyl-	95994	002425-77-6	38
			Octatriacontyl pentafluoropropiona te	242842	1000351-89-1	38
27	27.357	1.11	C:\Database\NIST08.L			
			Trifluoroacetoxy hexadecane	163912	006222-03-3	95
			n-Tetracosanol-1	174227	000506-51-4	94
			1-Heptacosanol	194343	002004-39-9	94
28	27.717	0.65	C:\Database\NIST11.L			
			1-Ethanone, 1-[4-acetyl-2,5-dimeth yl-1-(8-quinolinyl)-1H-pyrrol-3-yl]-	149782	1000350-21-8	47
			Propanoic acid, 3-(3,4-dihydro-6,7 -dimethoxy-3,3-dimethyl-1-isoquino linylamino)-	149352	299923-04-9	43
			Indazol-4-one, 3,6,6-trimethyl-1-p hthalazin-1-yl-1,5,6,7-tetrahydro-	149516	1000310-86-5	43
29	29.445	0.08	C:\Database\NIST11.L			
			Nonahexacontanoic acid	243830	040710-32-5	81
			Sulfurous acid, octadecyl 2-propyl ester	200449	1000309-12-7	72
			1-Dodecanol, 2-octyl-	143260	005333-42-6	53
30	30.349	0.24	C:\Database\NIST11.L			
			Tricosyl trifluoroacetate	223732	1000351-75-1	46
			Dotriacontyl heptafluorobutyrate	242399	1000351-84-2	46
			Tricosyl heptafluorobutyrate	238204	1000351-83-4	46
31	31.219	0.77	C:\Database\NIST08.L			
			Dotriacontyl pentafluoropropionate	218181	1000351-81-4	93
			Octacosyl trifluoroacetate	213793	1000351-74-9	91
			Dotriacontyl trifluoroacetate	216734	1000351-75-4	91

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Title :

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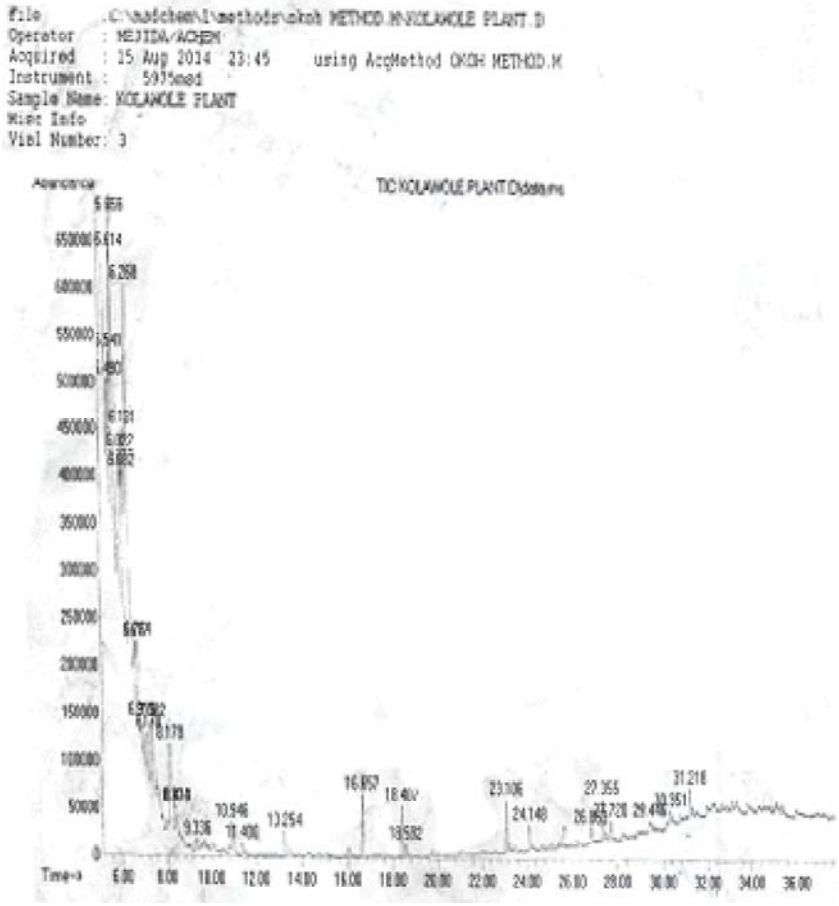
2	5.541	73	79	85	VV 3	140932	5140027	17.23%	3.899%
3	5.614	85	92	94	VV 3	268878	6218672	20.84%	4.717%
4	5.656	94	99	144	VV 6	345319	24566680	82.35%	18.635%
5	6.022	144	163	170	PV 3	147603	8099039	27.15%	6.144%
6	6.082	170	174	176	VV 4	139599	2620850	8.78%	1.988%
7	6.131	176	182	195	VV 3	191789	9866464	33.07%	7.484%
8	6.268	195	206	264	VV 2	369825	29833625	100.00%	22.631%
9	6.677	264	278	286	VV 7	62203	3449597	11.56%	2.617%
10	6.764	286	293	311	VV 2	78461	3344384	11.21%	2.537%
11	6.915	311	319	334	PV 2	21065	936203	3.14%	0.710%
12	7.170	348	364	385	PV 9	42365	2816487	9.44%	2.136%
13	7.382	385	401	436	PV 9	68889	5988489	20.07%	4.543%
14	8.178	525	540	571	PV 4	86350	7638798	25.60%	5.794%
15	8.374	571	574	576	VV 3	25679	361861	1.21%	0.274%
16	8.413	576	581	609	VV 7	27379	1592670	5.34%	1.208%
17	9.336	717	743	764	BV 7	12126	771362	2.59%	0.585%
18	10.946	1007	1024	1067	VV 2	34506	2207160	7.40%	1.674%
19	11.400	1086	1103	1121	BV 3	12440	555978	1.86%	0.422%
20	13.254	1412	1427	1448	BV 4	26343	1033245	3.46%	0.784%
21	16.657	2000	2022	2053	BV 2	66915	2683192	8.99%	2.035%
22	18.407	2305	2328	2344	BV 3	53810	1757975	5.89%	1.334%
23	18.582	2344	2359	2373	VV 5	13323	431106	1.45%	0.327%
24	23.106	3130	3149	3165	PV 3	55598	1779057	5.96%	1.350%
25	24.148	3293	3331	3347	BV 7	25395	946312	3.17%	0.718%
26	26.863	3791	3806	3823	VV 7	17292	780074	2.61%	0.592%
27	27.355	3879	3892	3903	VV 5	44937	1458176	4.89%	1.106%
28	27.720	3930	3955	3968	BV 6	20228	856613	2.87%	0.650%
29	29.446	4242	4257	4261	PV 6	5277	103025	0.35%	0.078%
30	30.351	4393	4415	4422	PV 6	9815	318250	1.07%	0.241%
31	31.218	4551	4567	4577	PV 5	31034	1009858	3.38%	0.766%

Sum of corrected areas: 131828761

okoh METHOD.M Sat Aug 16 20:52:07 2014



DISCUSSION AND CONCLUSION

In recent years, the search for phytochemicals possessing antimicrobial have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Phytochemical screening of *Senna alata* reveals the presence of alkaloids, saponins, tannins, phlobatannins, anthraquinones, steroidal nucleus, cardenolides, steroidal ring and flavonoids. These phytochemicals have been shown to possess several biological activities including antimicrobial activity [16]. The flavonoids are mostly recognized for their antioxidant activity while their role in modifying the body reaction to allergens, viruses and carcinogens has also been reported [17,18]. According to Jiksika *et al*. [18], alkaloids are organic compounds that contain nitrogen having sedative and analgesic properties. In another studies, the toxigenic effect of this phytochemical was reported [19]. The fact that ethanolic extract of *Senna alata* Linn was more efficacious than other tested extract is not unexpected as Obi and Onuohia [19] have earlier reported ethanol as the solvent of choice when extracting plant active ingredients. Their findings however negate that which documented normal hexane as the best for extracting active ingredients of plant [20, 21]. These two studies buttressed that, solubilization of required active ingredients in solvent may probably be the major factors influencing the selection of the most appropriate solvent of choice [22-23]. The MIC and MFC results showed that the extracts exhibited definite fungistatic and fungicidal activity. On comparison of the mass spectra of the constituents with the NIST library, the 78phytoconstituents were characterized and identified, which are listed with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the scanned material above. According to the peak area, the major phyto constituents present in *Senna alata* were xylene, alcohol, aldehydes, alkanes, alkenes, fatty alcohol, acetic acid, ketones and ester. It can be inferred that *Senna alata* Linn posseses good antifungal activity and such activities might be ascribed to the presence of the phytochemicals and some of the chemical constituents.

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