Phenolics and bio compounds detected in *Senna alata* extract using HPLC and GC-MS analysis respectively

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Abstract*- Senna alata is a plant found in Africa and other tropical regions of the world. Senna alata has been used traditionally for the treatment of different ailments. That is the reason why we investigated the presence of phenolics and other bioactive compound in the methanolic extract of Senna alata. Standard GC-MS and HPLC procedures were carried out on the extract. The results showed the presence of Methyl-4-(3-oxocyclohexyl)-butanoate with a peak number 13, retention time 14.782 and % area of 8.13, another detected bioactive with higher % area of 5.05 and retention time 16.467 was n-hexadecanoic acid methyl ester with a peak number 21 and Quercetrin 155.39 mg/100g > cinnamic acid 107.04 mg/100g > naringin 82.26 mg/100g > apigenin 66.75 mg/100g > caffeic acid 45.05 mg/100g > quercetin amongst others. In conclusion Senna alata extract contained important phenolics and other bioactive compounds that are of importance in health and disease.

I. INTRODUCTION

Phenolics are a class of organic compounds that occurs in all plants as secondary metabolites in varying concentrations (Evans and Harbone, 1999). They are diverse group of biocompounds with simple chemical structures like C_6 – phenolics, C_6-C_n, C_6-C_n-C_6 among other complex structures (Evans and Harbone, 1999). Phenolic compounds from plants contribute to their quality, nutritional value, aroma and flavour. They are also known to provide the greatly to the beneficial health effects of many plants, such as anti-tumour, anti-ulcer and anti-inflammatory properties, (Konczak-Islam et al., 2003; Stintzing and Carle, 2004) especially due to their high medicinalactivity. Nowadays, efforts are made to find new sources of natural phenolic. (Ignat et al., 2011).

*Senna alata*is a medicinal/ therapeutic plant used throughout areas in which it is found. It is used widely in traditional medicine in the Niger Delta Region of Nigeria. *Sennaalata* leaf extract is used to treat constipation, inguinal hernia, intestinal parasitosis, syphilis, and diabetes (Dutta*et al*., 2012).

There are no or scarce research works that explore the quantification of phenolics in methanolic extract of *Senna alata* utilizing HPLC and also detecting other bioactive compounds with the aid of GC-MS. That is why this work was carried out.

II. MATERIALS AND METHODS

- PLANT MATERIAL

Fresh leaves of *Senna alata* were collected from Amassoma Community, Sothern Ijaw L. G .A Bayelsa State. The plant was identified by the Department of Botany, Niger Delta University, Bayelsa State. Plant was collected in November, 2021.

- PREPARATION OF METHANOIC EXTRACTS OF SENNA ALATA

Senna alata leaves were harvested and dried in the shade for 14 days. The grounded plant was marcerated in 1500ml methanol for three days (72 hours). The crude extract was then filtered with Whattman number 1 filter paper. The extract was evaporated to dryness in a rotary evaporator. The resulting paste was weighed and found to be 28.1g. It was then put in the refrigerator to be used later.

- DETECTION OF PHENOLICS IN *SENNA ALATA* METHANOL EXTRACT

HPLC MAKE	Agilent 1200 series
Detector	Agilent 1260
Wavelength	320 nm
Column	Chromspher 5, C18
Column temperature	40 ^{0}C

Dimension	5 mm, 3mm x 250 mm, with Hamilton microliter syringe
Injection volume	100 µL
Flow rate	0.7 ml/min
Pressure	180 x 10^5Pa
Isocratic elution	2% acetic acid in water-methanol mixture (82:18, v/v)

Gas chromatography- mass spectrophotometry analysis (GC-MS Analysis) was carried out according to manual guidelines and standard principles of operation.

III. RESULTS

Fig 1 chromatogram of phenolics in methanolic extract of *Senna alata*

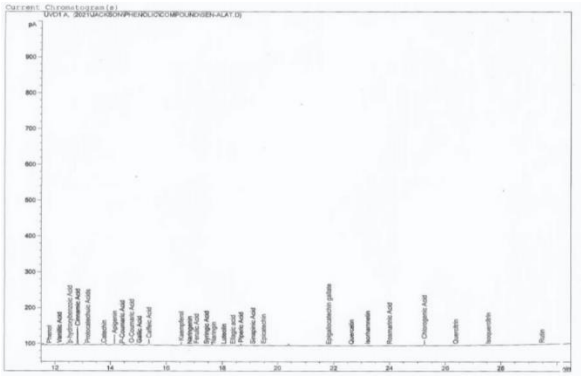

Table 1 showing the different quantities of phenolic in *Sennaalata*

Peak number	Retention time (min)	Area	Amount (mg/100g)	Name of compound
1	11.797	10.077	7.24 x 10-4	Phenol
2	12.155	11.92	1.43 x 10-2	Vanillic acid
3	12.520	11.74	23.32	p-hydroxybenzoic acid
4	12.826	92.83	107.04	Cinnamic acid
5	13.188	5.17	1.5 x 10-4	Protocatechuic acid
6	13.772	9.08	14.51	Catechin
7	14.154	57.14	66.75	Apigenin
8	14.421	5.60	3.43 x 10-3	p-coumaric acid
9	14.762	15.54	4.70 x 10-4	o-coumaric acid
10	15.029	8.66	3.79 x 10-3	Gallic acid
11	15.384	41.62	45.05	Caffeic acid
12	16.541	18.18	37.01	Kaempferol
13	16.838	1.57	1.89 x 10-5	Naringenin
14	17.080	6.28	7.40 x 10-1	Ferulic acid
15	17.437	1.30	2.36 x 10-4	Syringic acid
16	17.692	7.11	82.26	Naringin
17	18.056	0.89	10.75	Luteolin
18	18.383	2.55	7.78 x 10-4	Ellagic acid
19	18.673	10.24	1.00 x 10-5	Piperic acid
20	19.094	1.25	4.4 x 10-6	Sinapinic acid
21	19.479	2.72	1.92 x 10-2	Epicatechin
22	21.831	2.41	1.97 x 10-2	Epigallocatechingallate
23	22.632	3.12	36.08	Quercetin
24	23.215	5.43	6.37 x 10-5	Isorhambetin
25	24.009	4.37	8.85 x 10-3	Rosmarinic acid
26	25.250	13.70	155.39	Chlorogenic acid
27	26.368	4.94	5.94 x 10-5	Quercitrin
28	27.549	3.10	1.43 x 10-2	Isoquercetrin

29	29.465	6.26	3.83 x 10-4	Rutin

Fig 2 showing the chromatogram of biocompounds in methanolic extract of *Senna alata*

The phenolics detected in *Senna alata* methanolic extract via HPLC are displayed in table 1. Quercetrin 155.39 mg/100g > cinnamid acid 107.04 mg/100g > naringin 82.26 mg/100g > apigenin 66.75 mg/100g > caffeic acid 45.05 mg/100g > quercetin 36.08 mg/100g > kaempferol 37.01 mg/100g > p-hydroxybenzoic acid 23.32 mg/100g > catechin 14.51 mg/100g. These phenolic compounds and others on table 1 contribute greatly to the health benefits of *Senna alata.* Phenols detected in large and minute quantity as shown in table 1 have several mechanisms of action, like protection of membrane layers, metal coordination, free radical quenching, modulation of oxidative and antioxidative enzymes the potency of these abilities relies on the number and position of free OH groups and the stereochemistry of the phenol ring (Panche et al., 2016; Benjakul et al., 2014).

Fig 2 showing the GC-MS chromatogram of methanolic extract of *Senna alata*

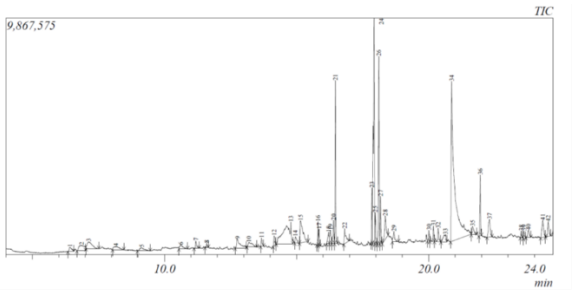

Table 2 GC-MS biocompounds detected from methanolic extract of *Senna alata*

Peak no	Compound Name	RT	% Area
1	2-ehtyl hexanal	6.426	0.60
2	Phenyl carbamate	6.865	1.57
3	Xylitol	7.131	1.78
4	Succinamic acid	8.160	0.93
5	Cyclohexene-3,5-diol	9.136	0.69
6	2-methoxy-4-vinyl phenol	10.616	0.66
7	2,3-bis-(4-methoxy phenyl)-1,4,5-trimethyl piperizine	11.185	0.43
8	Tridecane	11.598	0.17
9	2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde	12.764	1.80
10	2,5-dihydroxyl-4-isopropyl-2,4,5-cycloheptatrien-1-one	13.191	1.02
11	1-chloro-octadecane	13.670	0.44
12	2-tridecynyl-3-cyclopentylpropionate	14.148	0.59
13	Methyl-4-(3-oxocyclohexyl)-butanoate	14.782	8.13
14	Trispiro[4,2,4,2,4,2] heneicosane	14.950	0.45
15	n-butyl benzylsulfonamide	15.154	3.31
16	6,10-dimethyl-2-undecanone	15.808	0.71
17	4-dodecene-1-ol	15.850	0.55
18	3,7,11,15-tetramethyl-2-hexadecene-1-ol	16.207	0.80
19	Methyl cis-9-octadecenoate	16.277	0.88
20	Methyl palmitoleate	16.396	1.14
21	n-hexadecanoic acid methyl ester	16.467	5.05
22	Palmitic acid	16.816	1.28
23	Methyl cis,cis-9,12-octadecadienoate	17.850	1.91
24	Methyl cis-9-octadecenoate	17.930	12.22
25	Trnas-13-octadecenoic acid methyl ester	17.974	1.06
26	Trans -1,3-trans-1,4-menthol	18.107	7.11
27	Methyl stearate	18.174	1.75
28	Decylmercaptan	18.355	2.05

29	Methyl linolelaidate	18.680	0.87
30	2,3,4;4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)-naphthalene	20.002	0.60
31	Eicosanoic acid methyl ester	20.174	0.87
32	4,8,12,16-tetramethylheptadecan-4-olide	20.355	0.96
33	Gamma tocopherol	20.631	1.09
34	(5-methyl-3-benzoburyl)-phenyl methanone	20.860	27.67
35	1,4-di-O-acetyl-2,3,5-tri-O-methyl ribitol	21.658	0.78
36	Bis (2-ethylhexyl) pthalate	21.945	2.32
37	Alpha tocopherol	22.297	1.78
38	Tetracosanoic acid methyl ester	23.498	0.27
39	Dibenzo [c1]chrysene-8-carboxylic acid methyl ester	23.599	0.40
40	Campesterol	23.766	0.66
41	Stigmasterol	24.309	1.62
42	Heptadecaflourononanoic acid decyl ester	24.516	1.02

RT = retention time

Methyl-4-(3-oxocyclohexyl)-butanoate is one of the biocompounds detected in the methanolic extract of senna alata with a peak number 13, retention time 14.782 and % area of 8.13, another detected bioactive with higher % area of 5.05 and retention time 16.467 was n-hexadecanoic acid methyl ester with a peak number 21. Methyl cis-9-octadecenoate was also found in higher concentration because it showed % area of 12.22, RT of 17.930 and a peak number of 24. Trans -1,3-trans-1,4-menthol and (5-methyl-3-benzoburyl)-phenyl methanone have peak number 26 and 34, they are eluted at RT of 18.107 and 20.860 with % area of 7.11 and 27.67 respectively.

In general, HPLC and GC-MS data showed that the leaves of *Senna alata* methanolic extract under study contained high amounts of bioactive compounds and high amounts of phenol is a valuable source of health-promoting compounds.

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