GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Antioxidant and gas chromatography-mass spectrometry characterization of methanol extract of *Alchornea cordifolia*

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GSC Biological and Pharmaceutical Sciences, 2022, 21(03), 071–076

Publication history: Received on 19 October 2022; revised on 01 December 2022; accepted on 04 December 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.3.0449

Abstract

Antioxidant, phytochemical and GC-MS analyses of methanol extract of *Alchornea cordifolia* were determined by various methods to quantify each of the phytochemical constituent present in the sample. The quantitative phytochemical tests conducted include; alkaloids, saponins, phenols, flavonoids, flavonol and proanthocyanidin content estimation. The extract was subjected to antioxidant assay; total antioxidant, nitric oxide, DPPH radical scavenging and copper chelating properties of *Alchornea cordifolia*. Results: The quantitative phytochemical analysis revealed the different percentages of phytoconstituents in the extract. The results showed that the saponin content in extract was found to be 19±2.5% is present in high concentration as compared to the total alkaloid content (2.5±0.5%). The phenol, flavonoid, flavonol and proanthocyanidin contents were found to be 1.5±1.0 mgGAE/g, 16±0.2 mgQE/g, 8.6± 0.3mgRE/g and 10 ± 0.6mg TAE/g respectively. The NO, DPPH scavenging and copper chelating of *Alchornea cordifolia* at 1 mg/ml are 52.25±4.44%, 55.7±5.62% and 33.5±0.36% respectively. GC-MS results revealed the presence of many polar and nonpolar compounds in order of decreasing abundance Vitamin E > 1,2-dimethyl-cyclooctane >campesterol>stigmasterol> 1, 4-dimethoxy-2-methyl cyclohexane >Bicycle[4.4.0]dec-2-ene-4-ol > Pregn-4-en-17,21-diol-3,20-diene-9,11-epoxy > 2, 4,6-trimethyl-octane. *Alchornea cordifolia* is a medicinal plant found in Africa with many polar and nonpolar compounds that contributed to its traditional uses medically. In conclusion *Alchornea cordifolia* is endowed with phytochemicals of antioxidant and antiradical potentials.

Keywords: Phytochemicals; *Alchornea cordifolia*; Nitric oxide; Flavonoids, Free radicals; Vitamin E

1. Introduction

Plants are therapeutic agents for treatment of diseases being that they are important sources of curative aids. Medicinal plants have a commanding role in health all over the world [1]. *Alchornea cordifolia* is an African medicinal plant which is widely used in traditional medicine and found along the coastal areas of West Africa. *Alchornea cordifolia* leaves or leafy stems can be chewed fresh for sedative, antispasmodic and respiratory problems such as; sore throat, cough and bronchitis. Genital-urinary problems, Intestinal disorders which include; gastric ulcers, diarrhoea and worms could be treated with *Alchornea cordifolia*. *Alchornea cordifolia,* also taken to treat anaemia and epilepsy [2]. The decoction of the leaves of *Alchornea cordifolia* is used a stimulant, also increases the sexual activity in males, used in rheumatic pains, fever and wounds [3-5]. The rural people of Southern Nigeria used decoction of *the* leaves in the management of convulsion, analgesics, anti-inflammatory, and antimicrobial [3, 5-6].

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The aim of the study was to characterized phytochemical and antioxidant activity of methanolic extract of *Alchornea cordifolia* in order to corroborate its used in folkloric medicine.

2. Material and methods

2.1. Chemicals

Acetic acid, ethanol, ammonium hydroxide, diethyl ether, n-butanol, sodium chloride, folin reagent, sodium carbonate, gallic acid, methanol, sodium nitrite, aluminum chloride, sodium hydroxide, quercetin, rutin, sodium acetate, acetone, n-hexane, vanillin, tannic acid.

2.2. Plant collection

The plant *Alchornea cordifolia* fresh leaves were collected from Niger Delta University Agricultural farm, Wilberforce Island, Bayelsa State. The plant was identified and authenticated in the Department of Botany, Niger Delta University, Bayelsa State. April, 2021 and voucher specimen was deposited in Department of Botany Herbarium, Faculty of Science, Niger Delta University.

2.3. Preparation of Methanolic Extract of *Alchornea cordifolia*

Alchornea cordifolia leaves were shade dried for three weeks, grounded using warring blender. About 150 g of the pulverized plant was extracted using cold maceration with 1.5 L of methanol and kept for 72 hours, filtered using whatman No.4 filter paper, concentrated *in vacuo* using Rotary evaporator, weighed and stored in the refrigerator for further use.

2.4. Biocompound Estimation

Total alkaloid content in *Alchornea cordifolia* was estimated by Unuofin *et al*., 2017 [7]. The total saponin content was by Onyesife *et al.,* 2014 [8], total phenol in extract of *Alchornea cordifolia* was estimated by the method of Singleton *et al*., 1999 and Demiray *et al*., 2009 [9-10], total flavonoid content of the extract was determined by Zhishen *et al.*, 1999 [11], total flavonol in *Alchornea cordifolia* was estimated as rutin equivalent expressed as mg of rutin per gram of extract by the method of Miliauskas *et al.,* 2004 [12], total proanthocyanidin content in *Alchornea cordifolia* was determined using the procedure reported by Caceres-Mella *et al*., 2013 with slight modifications [13].

2.5. Antiradical Assays

Alchornea cordifolia extract against DPPH (1, 1-diphenyl–2 picrylhydrazyl) was "appraised as described by Blois, 1958" [14]. Nitric oxide radical scavenging was quantified by the method of "Chidambaram *et al*., 2013" [15]. The antioxidant capacity of *Alchornea cordifolia* extract and standard ascorbic acid was evaluated by the "method of Prieto and colleagues 1999" [16]. Copper chelating ability activity was measured by the methods as described by "Torres-Fuentes *et al*., 2011" [17].

2.6. Gas chromatography- mass spectrophotometry analysis (GC-MS Analysis)

The *Alchornea cordifolia* extract was subjected to GC-MS (GC-MS QP2010SE Shimadzu Japan) analysis and spectra were compared with NIST library to detect and identify the phytoconstituents in the sample.

3. Results

Percentage yield of plant= 20.59%

The result of quantitative phytochemicals analysis (Table 1) of the extract showed percentage of chemical composition of the sample. The percentage of the total alkaloid content was 2.5 ± 0.5% in *Alchornea cordifolia* which is relatively low and safe for human consumption. The percentage of the saponin content was found to be 19±2.5%. This result showed that saponin is present in high quantity. The phenol content of the *Alchornea cordifolia* extract was 15 ± 1.0 mgGAE/g which shows that *Alchornea cordifolia* extract act as an antioxidant. The flavonoid content was found to be (16 ± 0.2mgQE/g). The flavonol content was found to be (8.6 ± 0.3mgRE/g) which is about half of the total flavonoid content. The proanthocyanidin content was found to be (10±0.6mgTAE/g). Total antioxidant capacity of the extract of *Alchornea cordifolia* expressed as the number of milligram equivalents of quercetin, is shown in Table 1 (0.0586 ± 0.0629 mgQE).

Table 1 Results of quantitative phytochemicals and total antioxidant in *Alchornea cordifolia*

Phytochemicals	Quantitative values
Alkaloids	2.5±0.5%
Saponins	19±2.5%
Phenols	15±1.0 (mgGAE/g)
Flavonoids	16±0.2 (mgQE/g)
Flavonol	8.6±0.3 (mgRE/g)
Proanthocyanidin	10±0.6 (mgTAE/g)
Total antioxidant	0.0586±0.0629 mgQE

Each value is a mean ± SD of triplicate samples. GAE = Gallic acid equivalent, QE = Quercetin equivalent, RE = Rutin equivalent and TAE = Tannic acid equivalent

Table 2 Nitric oxide, DPPH radical scavenging and copper chelating properties of *Alchornea cordifolia*

	% NO scavenging		% DPPH scavenging		% Copper chelating	
(mg/ml)	A.C	Quercetin	A.C	Gallic acid	A.C	EDTA
0.2	12.43±3.90	45.48±0.15	11.62±1.01	26.83±4.39	13.18± 1.13	30.81±0.18
0.4	25.33±1.09	57.32±2.54	29.68±1.49	31.76±0.92	20.84 ±0.71	37.09±1.60
0.6	36.95±2.43	68.4±6.87	34.09±3.30	44.61±3.99	25.14 ± 0.82	43.50±1.69
0.8	43.21±0.67	71.01±1.3	41.87±4.61	50.47±7.99	29.17 ± 1.239	51.05±0.16
1	52.25±4.44	85.85±3.79	55.70±5.62	65.23±0.76	33.5 ±0.36	59.78±3.1

Values are mean ±SD (n=3), A.C = *Alchornea cordifolia*

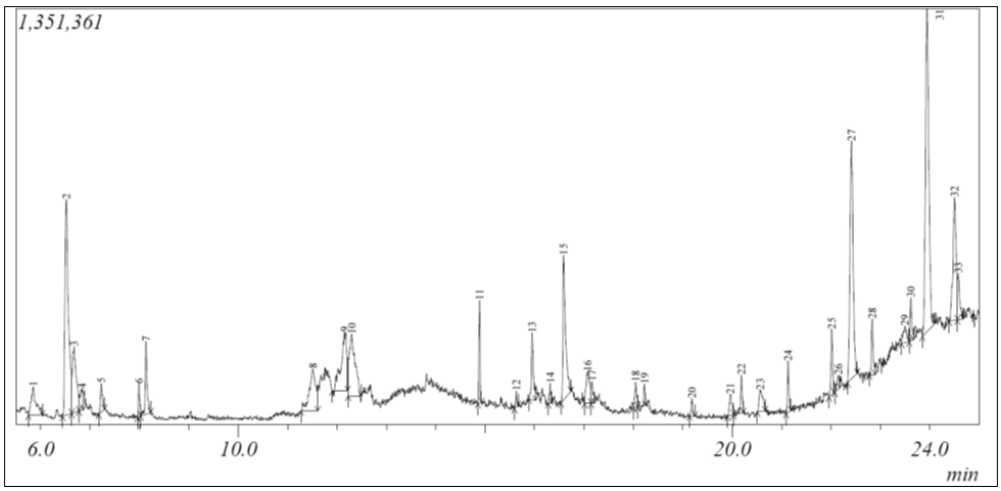

Figure 1 GC–MS chromatogram for phytocompounds in methanolic extract of *Alchornea cordifolia*

Table 3 Major phytocompounds detected in GC-MS analysis of Methanolic Extracts of *Alchornea cordifolia* extract

Peak no	Name of compound	Retention time	Area %
1	2-amino-4-chloromethyl-thiazole	5.849	2.16
2	1,2-dimethyl-cyclooctane	6.520	11.83
3	2,4,6-trimethyl-octane	6.676	4.15
4	Cis-3-decene	6.841	1.07
5	2-trimethylsilyloxy-1,3-butadiene	7.227	0.93
6	8,8-dimethoxy-2-octanol	7.989	0.70
7	Trimethylsilyl-2-butynoate	8.131	2.14
8	5-benzofuranacetic acid	11.509	4.36
9	Pregn-4-en-17,21-diol-3,20-diene-9,11-epoxy	12.149	5.36
10	Bicycle[4.4.0]dec-2-ene-4-ol	12.297	5.50
11	1,5-diisopropyl-2,3-dimethyl cyclohexane	14.883	1.67
12	2,4-dimethylhexane	15.626	0.35
13	Tetrahydrosmilagenin	15.951	1.98
14	1-octadecyne	16.314	0.43
15	1,4-dimethoxy-2-methyl cyclohexane	16.586	6.05
16	3-methoxy-3-methyl-tetrahydropyran-2-one	17.067	1.87
17	2,6-bis(1,1-dimethylethyl)-phenol	17.158	0.62
18	7-hexadecenoic acid	18.044	0.74
19	1-methyl-4-(1-methylethyl)-cyclohexanol	18.223	0.46
20	Heptadecane	19.183	0.37
21	Pentadecyl Ester trifluoroacetic acid	19.962	0.84
22	Gamma tocopherol	20.185	1.02
23	Gamma tocopherol	20.566	1.27
24	2-methyloctacosane	21.130	1.28
25	Nonadecane	22.012	1.79
26	Benzyldimethylsilyl Ester nonadecanoic acid	22.149	0.55
27	Vitamin E	22.413	11.99
28	Tetratetracontane	22.834	1.48
29	Ergosterol	23.492	0.95
30	2-bromotetradecane	23.615	1.15
31	Campesterol	23.941	17.05
32	Stigmasterol	24.499	6.39
33	Oxirane	24.575	1.48

4. Discussion

Alchornea cordifolia inhibits NO radicals generated via sodium nitroprusside as shown in the table above. At a concentration of 0.2 mg/ml 12.43±3.90% NO was inhibited as compared to Quercetin at same concentration inhibited 45.48±0.15%. At the highest concentration of 1 mg/ml *Alchornea cordifolia* scavenged 52.25±4.44% NO, where as Quercetin at 1 mg/ml inhibited 85.85±3.79% of NO.

DPPH a non-biological radical is also inhibited by *Alchornea cordifolia* as shown in Table 2. At a concentration 0.2 mg/ml *Alchornea cordifolia* scavenged 11.02±1.01% of DPPH, whereas gallic acid a standard antioxidant scavenged 26.83 ± 4.39. However, at the maximum concentration of 1 mg/ml *Alchornea cordifolia* inhibits DPPH with 55.70±5.62 while gallic acid at the same concentration scavenged 65.23 ± 0.76 of DPPH.

Percentage of copper chelation was concentration dependent; at 0.2 mg/ml to 1 mg/ml ranges from 13.18 ± 1.13 to 35.51 ± 0.36. EDTA which is a standard also chelated copper at a little higher percentage than *Alchornea cordifolia*.

The most abundant polar and non-polar compounds found in *Alchornea cordifolia* methanolic extract are Vitamin E > 1,2-dimethyl-cyclooctane >campsterol>stigmasterol> 1, 4-dimethoxy-2-methyl cyclohexane > Bicycle[4.4.0]dec-2-ene-4-ol > Pregn-4-en-17,21-diol-3,20-diene-9,11-epoxy > 2, 4,6-trimethyl-octane. Vitamin E has been shown as a wonder molecule, Vitamin E is particularly important for the protection of lipoproteins, membranes, carbohydrates, nucleic acid and other lipophilic environments. The quenching properties of vitamin E in the presence of reactive oxygen species lead to tocopheryl radicals, thus vitamin E can be regenerated by the antioxidant properties of ascorbic acid and glutathione [18-19]. The ergosterol showed antioxidant activity as revealed by the GC-MS analysis. This might be due to endophytic fungi presence in the leaves. Stigmasterol and campestrol have anti-inflammatory, antioxidant effect and inhibit proinflammatory factors, campesterol is known as cholesterol lowering and anticarcinogenic agents [20-22]. These polar and non polar compounds as well as others found in the chromatogram and table 1 contribute to the overall medicinal properties of *Alchornea cordifolia* extract; 2-amino-4-chloromethyl-thiazole, 2-trimethylsilyloxy-1,3-butadiene, 5-benzofuranacetic acid, Tetrahydrosmilagenin, 2,6-bis(1,1-dimethylethyl)-phenol, Gamma tocopherol, Benzyldimethylsilyl ester nonadecanoic acid.

5. Conclusion

The methanolic extract of *Alchornea cordifolia* contains a good number of phytochemicals which are present in different concentration and showed good antioxidant effect in DPPH and Nitric oxide models when compared to other models.

Compliance with ethical standards

Acknowledgments

We are grateful to contribution made by the staff of Department of Biochemistry and Botany, Niger Delta University.

Disclosure of conflict of interest

There is no conflict of interest.

Author Contribution

EAS conceived the study, collected the plant and performed the experiments. ADCO analyzed and interpret data. The manuscript was written by EAS and ADCO. ROF supervised the experiments and edited the manuscript.

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