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Research Article

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GC-MS Analysis of Medicinal Compounds and Reactive Oxygen Species Scavenging Properties of G*ongronema Latifolium* (Utazi) n-Hexane Extract

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Abstract *Gongronema latifolium* (Amaranth globe) is popularly known for its nutritional and health promoting values. This plant is widely found in the southern part of Nigeria and is used in local dishes and also for Medicinal purposes. In this project the essential Medicinal and nutritive compounds were detected using GC-MS analysis. The leaves of *Gongronema latifolium* were dried and extracted with n-hexane. The dry paste was subjected to GC-MS analysis. Various Medicinal and nutritive compounds were detected which included 1,5-diisopropyl-2,3-dimethylcyclohexane, 2,5-bis(1-methyl propyl)-phenol, 2,4,6-trmethyl propiophenone, Octadecamethylcyclononasiloxane, 2,2-dimethyl -2-[2,3,5,6-tetramethyl] ethanol, 5-flouro-2-triflouromethyl benzoic acid-4-tride, 4-t-butyl-2-(1-methyl -2-nitroethyl) cyclohexanone, are 1,2-dimethyl-2-peptene, Octamethylcyclotetrasiloxane, 1-methyl-2-(1-methylethyl)-benzene, Decamethylcyclopentasiloxane, Hexene-1-ylcyclohexane etc. The antioxidant and free radical scavenging activity of *Gongronema latifolium* were determined by several standard methods using spectrophotomer. Based on DPPH, total antioxidant, copper chelating and Nitric oxide radical scavenging activity, the extract of *Gongronema latifolium* showed strong scavenging activity when compared with standards. The total antioxidant capacity assay through the phosphomolybdenum method showed 0.74 ± 0.076 mg GAE/g dry extract. At 1mg/ml the percent of DPPH radicals scavenged by *Gongronema latifolium* and gallic acid were 31.74±1.79 % and 94.26±2.36 % respectively. *Gongronema latifolium* and quercetin used as reference showed higher NO scavenging of 54.11±2.68 % and 61.59±1.70 respectively at 0.2 mg/ml. The extract also has higher chelating potential as compared with standard EDTA. Conclusion n-hexane extract of *Gongronema latifolium* contain bio compounds important for the Medicinal and nutritive potentials. Hexane extract of *Gongronema latifolium* leaf is a potential source of natural antioxidants and serves as an effective free radical scavenger and/or inhibitor. Hence, *Gongronema latifolium* might be a good plant-based pharmaceutical product for several diseases caused by free radicals.

Keywords GC-MS Analysis, *Gongronema latifolium*, *n*-Hexane, Extract

1. Introduction

Gongronema latifolium (Utazi) is a medicinal plant that is important in preventing and treating certain diseases and ailments that are detrimental to human health. *Gongronema latifolium* leaf, can be chewed, infused or used for cooking is mainly used in the Western part of Africa for nutritional and medicinal reasons. *Gongronema latifolium* (Amaranth globe) is a tropical rainforest plant which belongs to the family Asclepiadaceae and genus Gongronema

(Nelson, 1965; Okafor, 1975). It is commonly found in West Africa and is locally called "Utasi" by the Ibibios, Quas and Efiks; "Utazi" by the Igbos in South East and "Arokeke" by the Yorubas in South Western part of Nigeria. The people of Ghana and Senegal called *Gongronema latifolium* as "Akan-Asante aborode" and "Sever gasule" respectively (Hutchinson, 1973, Morebise et al., 2005). The leaves can be eaten raw, dried and used as local powdery spice or as vegetable for food preparations such as unripe plantain porridge, white soup, sauces and salads (Morebise and Fafunso, 1998; Ezeonwu, 2013; Ezekwe et al., 2014). Many of the above underlining medicinal benefits of *Gongronema latifolium* are due to its antioxidant properties. Hence this present study is aimed to assess the antioxidant capacity of the N-hexane extract of *Gongronema latifolium* through measurement of activities in scavenging of different free radicals including nitric oxide, DPPH, total antioxidant activity with phosphomolybdenum, copper chelating and GC-MS analysis of bioactive compounds. In light of these nutritional and Medicinal properties of *Gongronema latifolium* there is the need to identify many of the phytomolecules responsible for these claim thus is why this research was sought.

2. Materials and Methods

2.1 Chemicals

n-hexane

Collection and Identification of Plant Sample

The plant, *Gongronema latifolium*, was collected from Niger Delta Botanical Garden, Wilberforce Island, Bayelsa State.

N-Hexane Extract of *Gongronema latifolium*

The plant Leaves were obtained in large quantity and left under shade at room temperature for two weeks to dry. Afterwards they were ground into a coarse powder. 243.6g of the powder was soaked in 800 ml of n-hexane and allowed to stand for 96 hours with intermittent stirring. The extracts were filtered with a filter paper and the filtrates were evaporated using a rotary evaporator at constant temperature of 40 ^{0}c. Percentage yield of n-hexane was 0.47%. The powdery residue was utilized for further experiments.

Antioxidant assays

1, 1- Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Assay was evaluated as described by Blois, 1958. The total antioxidant capacity of *Gongronema latifolium* extract was evaluated by the method of Prieto et al., 1999. Nitric oxide radical scavenging assay quantified by the Griess reagent by Marcocci et al., 1994. Copper chelating ability was measured by the method as described by Torres-Fuentes et al.. 2011.

GC-MS analysis of *Gongronema latifolium* extract

The GC-MS analysis was carried out in line with standard operational procedures and according to manufacturer's instructions. Peaks where then probed with current libraries..

Table 1. % DPPH and Nitric Oxide Radical Scavenging activity of N-hexane extract of *Gongronema latifolium*

	Percent (%)	**Percent (%)**	**Percent (%)**	**Percent (%)**
Conc. (mg/ml)	*Gongronema latifolium*	*Gallic acid*	*Gongronema latifolium*	Quercetin
0.2	20.70 ± 2.79	36.96 ± 2.12	54.11 ± 2.68	61.59 ± 1.70
0.4	26.23 ± 3.52	43.1 ± 0.66	65.09 ± 2.11	79.68 ± 3.26
0.6	27.53 ± 1.29	69.18 ± 1.64	72.17 ± 1.24	85.92 ± 1.29
0.8	28.84 ± 0.68	83.58 ± 2.53	83.00 ± 0.69	88.92 ± 2.44
1.0	31.74 ± 1.79	94.26 ± 2.36	94.43 ± 2.26	91.54 ± 0.63

Values are mean±standard deviation of triplicate reading

Table 1 shows the free radical scavenging activity of extract of *Gongronema latifolium* and standard (Gallic acid). When compared, the extract of *Gongronema latifolium* possessed a high activity. At a concentration of 0.2 mg/ml, the scavenging activity of the extract was 20.70 ± 2.79, whereas at the same concentration, the standard Gallic acid was 36.96 ± 2.12 The Nitric oxide radical scavenging activity of the extract of *Gongronema latifolium* and that of the standard (quercetin) are shown to be moderately dose-dependent. At a concentration of 0.2 mg/ml, the

scavenging activity of the extract was 54.11 ± 2.68 %, whereas at the same concentration, standard (quercetin) was 61.59 ± 1.70 %.

Table 2 Total Antioxidant of N-hexane Extract of *Gongronema latifolium* using Phosphomolybdate method.

Extract	Total Antioxidant
Extract of *Gongronema latifolium*	0.74 ± 0.076 mgGAE/g dry extract

Values are mean±standard deviation of triplicate reading

Table 3 % Copper (Cu^{2+}) Chelating activity of N-hexane extract of *Gongronema latifolium*

	Gongronema latifolium	**EDTA**
Conc. (mg/ml)	Percent (%)	Percent (%)
0.2	17.78 ± 1.17	36.86 ± 0.28
0.4	20.14 ± 0.71	40.46 ± 1.60
0.6	22.14 ± 0.22	45.50 ± 1.69
0.8	24.15 ± 1.39	53.25 ± 0.56
1.0	26.5 ± 0.36	61.78 ± 3.71

Values are mean±standard deviation of triplicate reading

The copper (Cu^{2+}) chelating activity of n-hexane extract of *Gongronema latifolium* are shown in Table 4 The extract of *Gongronema latifolium* showed considerable chelating activity when compared with standard EDTA. The extract activity increases with increasing concentration also the standard chelating activity increase with increasing concentration.

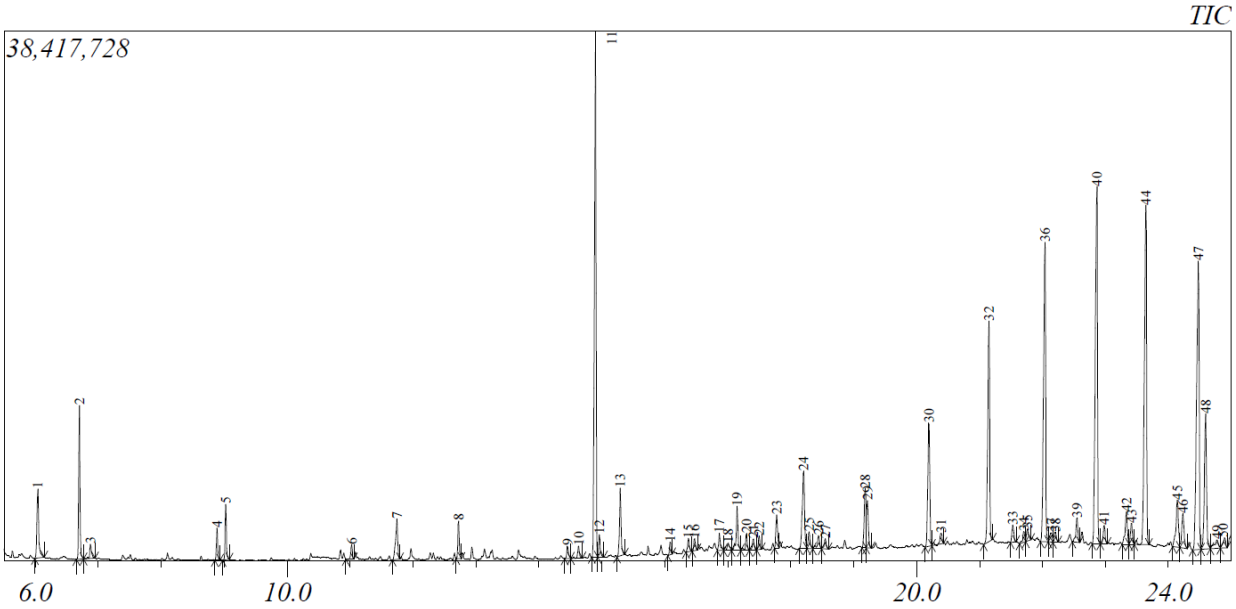

Figure 1: GC–MS chromatogram for phytomolecules in hexane extract of Gongronema latifolium

Table 4 Phytomolecules detected in GC-MS analysis of hexane extracts of *Gongronema latifolium*

S/N	COMPOUND NAME	RT	% area
1	1,2-dimethyl-2-pentene	6.03	1.75
2	Octamethylcyclotetrasiloxane	6.69	2.85
3	1-methyl-2-(1-methylethyl)-benzene	6.87	0.39
4	Decamethylcyclopentasiloxane	6.88	0.60
5	Hexene-1-ylcyclohexane	9.02	1.01
6	Dodecamethylcyclohexasiloxane	11.02	0.30
7	Cholistan-2-one oxime	11.74	1.23
8	Butylatedhydroxytoluene	12.72	0.82

9	3-methyl-2-propionyl benzoic acid	14.45	0.32
10	Hexadecamethylcycloctasiloxane	14.63	0.33
11	1,5-diisopropyl-2,3-dimethylcyclohexane	14.89	12.21
12	2,5-bis(1-methyl propyl)-phenol	14.96	0.50
13	2,4,6-trmethyl propiophenone	15.29	1.54
14	Octadecamethylcyclononasiloxane	16.08	0.27
15	2,2-dimethyl -2-[2,3,5,6-tetramethyl] ethanol	16.37	0.34
16	5-flouro-2-triflouromethyl benzoic acid-4-tride	16.47	0.29
17	4-t-butyl-2-(1-methyl -2-nitroethyl) cyclohexanone	16.86	0.43
18	I,3-bis(1,1-dimethyl ethyl) benzene	17.00	0.33
19	4-(1,1- dimethyl ethyl)benzeneacetic acid methyl ester	17.14	1.17
20	Octadecane	17.29	0.44
21	Dodecamethylcyclohexasiloxane	17.40	0.33
22	2,4-bis (1,1-dimethyl propyl) phenol	17.49	0.31
23	4-Methoxy-2(IH)-quinolone	17.77	0.85
24	Trans Phytol	18.20	2.59
25	6-tert-butyl-2,4-dimethyl Phenol trifluoroacetate ester	18.29	0.47
26	Diethylheptyloxy(3-methylbutoxy)-silane	18.44	0.45
27	Dimethylhexyloxyheptyloxy-silane	18.54	0.28
28	Heptadecane	19.18	1.67
29	Citronellol acetate	19.21	1.11
30	10-Methyl eicosane	20.19	3.50
31	Tetracosamethylcyclododecasiloxane	20.39	0.39
32	n-octane	21.14	6.19
33	Megestrol acetate	21.52	0.49
34	7-Hexyl eicosane	21.70	0.33
35	2,6 bis[(trimethylsilyl)oxy]-benzoic acid	21.76	0.37
36	Hentriacontane	22.03	9.06
37	12-hydroxy-9-octadecenethioic acid- s-t-butyl ester	22.13	0.49
38	[6-cyclopentyl 3-(3-cyclopentylpropyl)hexyl]-cyclohexane	22.20	0.33
39	11-decyl-tetracosane	22.54	0.71
40	Tetratetracontane	22.86	11.20
41	2,6-bis[(trimethylsilyl)oxy]-benzoic acid trimethylsilyl ester	22.98	0.53
42	2-methyl octacosane	23.33	1.05
43	5-ethyl-5-methyl – tetracosane	23.41	0.57
44	Hentriacontane	23.64	11.12
45	Erythro-9,10-dibromopentacosane	24.14	1.71
46	2-phenyl-m-dioxan-5-yl ester cis-stearic acid	24.23	1.16
47	Octacosane	24.47	10.50
48	Squalene	24.59	4.33
49	3-iodo-cholestane	24.77	0.51
50	3,3,13,13-tetraethylpentadecane	24.89	0.40

4. Discussion

The compounds detected in the chromatogram of hexane extract of *Gongronema latifolium* from peak 1 to 10 are 1,2-dimethyl-2-peptene, Octamethylcyclotetrasiloxane, 1-methyl-2-(1-methylethyl)-benzene, Decamethylcyclopentasiloxane, Hexene-1-ylcyclohexane, Dodecamethylcyclohexasiloxane, Cholistan-2-one oxime, Butylatedhydroxytoluene, 3-methyl-2-propionyl benzoic acid, Hexadecamethylcycloctasiloxane with very low %

area of 0.32 to 2.85. Despite their minute concentrations they also contribute greatly to the medicinal efficacy of *Gongronema latifolium*. These results are similar to the reports of Eboh and Robert 2022.

1,5-diisopropyl-2,3-dimethylcyclohexane, 2,5-bis(1-methyl propyl)-phenol, 2,4,6-trmethyl propiophenone, Octadecamethylcyclononasiloxane, 2,2-dimethyl -2-[2,3,5,6-tetramethyl] ethanol, 5-flouro-2-triflouromethyl benzoic acid-4-tride, 4-t-butyl-2-(1-methyl -2-nitroethyl) cyclohexanone, I,3-bis(1,1-dimethyl ethyl) benzene, 4-(1,1- dimethyl ethyl)benzeneacetic acid methyl ester and Octadecane are also contributing to the numerous properties of *Gongronema latifolium*. They are detected from peak number 11 to 20 few among these also have benzene or phenol rings that could confer some antioxidant properties (Eboh, 2014). There are some with very high concentrations as indicated by their % area in the chromatogram and the table. These are 1,5-diisopropyl-2,3-dimethylcyclohexane and 2,5-bis(1-methyl propyl)-phenol, 2,4,6-trmethyl propiophenone.

The total antioxidant of N-hexane extracts of *Gongronema latifolium* was evaluated and the result showed in table 2 as 0.7373± 0.0762 mg Gallic acid/g dry extract these results are in line with the works of Ahmed et al., 2015 who showed the total antioxidant property of *Adiantum caudatum* extract.

The results of DPPH radical scavenged revealed that the N-hecane extract of *Gongronema latifolium* leaves had a similar free radical scavenging activity when compared with standard Gallic acid (table 1). At concentration of 0.2 mg/ml the percentage of DPPH radical scavenging by extract of *Gongronema latifolium* and gallic acid were 20.70 ± 2.79 and 36.96 ± 2.12 respectively. The scavenging ability of *Gongronema latifolium* may be due to bioactive compounds (phenols) that can decolorize DPPH from violet to yellow. The result was shown in table 1 at 0.2 mg/ml *Gongronema latifolium* and quercetin scavenge NO at a percent of 54.11± 2.68 and 61.59 ± 1.70 respectively. This could be as a result of the presence of bioactive compounds in *Gongronema latifolium* revealed in GC-MS analysis.

The percentage chelation in extracts of *Gongronema latifolium* against copper ions were compared to the standard chelating agent EDTA at concentrations of 0.2-1mg/ml *Gongronema latifolium* chelated copper in a concentrated dependent manner (17.78 ± 1.17, 20.14 ± 0.71, 22.14 ± 0.22, 24.15 ± 1.39, 26.5 ± 0.36) respectively as showed in table 3 above. These results are in agreement with the works of Sharma and Singh 2012 who also showed the chelating potential of *Nardostachys jatamansi DC extract.*

These biocompounds detected from *Gongronema latifolium* are from peak numbers 21 to 50 with very high % area of 2 to 12 they included Trans Phytol, 10-Methyl eicosane, n-octane, Hentriacontane, Tetratetracontane, Hentriacontane, Squalene, Octacosane. Trans phytol is a precursor of chlorophyll and other terpenes (Nelson and Cox, 2000) which also contribute to the spicy nature of *Gongronema latifolium* and its medicinal potentials. Squalene is a precursor of tocopherol which acts as antioxidant, other compounds found in larger quantity also contribute to the medicinal and nutritive value of *Gongronema latifolium*. In conclusion n-hexane extract of *Gongronema latifolium* contain Medicinal and nutritive compounds. The results obtained in the present study indicate that *Gongronema latifolium* exhibit free radical scavenging, $NO^{\cdot}$, total antioxidant and metal chelating activity due to many bioactive compounds in GC-MS analysis.

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