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Gc-MS Analysis of Bioactive Compounds on Aqueous and Ethanolic Extracts of *Azanza Garckeana* Fruit Pulp

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ABSTRACT

A novel Gas Chromatography-Mass Spectrometry (GCMS) analysis of aqueous and ethanolic extract of *Azanzagarckeana* fruit pulp was carried out to identify the composition and percentage abundance of various phytochemicals. GC-MS analysis revealed the presence of 22 compounds in aqueous extract and 35 compounds in its ethanol extract among which had few similarity in compounds such as 2-Furanmethanol and 5-hydroxymethylfurfural (HMF). Some fatty acids and volatile organic acids were identified.

Keywords: GC-MS; *Azanzagarckeana*; Compounds; Bioactive; Phytochemical

INTRODUCTION

There has been renewed interest in herbal remedies in several parts of the world with many of the herbal remedies being incorporated into orthodox medical practice. Some African countries: Egypt, Burkina Faso, Ghana, Nigeria, Zambia and South Africa have also made good advances in the area of the use of plants for production of new drugs [1]. In many tropical countries, rural dwellers traditionally harvest wide range of leafy vegetables, roots, tubers, and fruits from the wild as food supplements, spices, and sometimes for cultural uses. Labeled as famine or hunger food, some wild plants have been recognized to have the potentials to meet household food and income security [2]. The indigenous fruits collected from the wild plays a significant role in food and nutrient security of the poor and rural dwellers. Some wild fruits have been identified to have better nutritional value than cultivated fruits [3]. As a result, in recent years, a growing interest has emerged to evaluate various wild edible plants for their nutritional features [4].

Goron Tula (Hausa), having the botanical name, *A. garckeana* and known as African chewing gumis a valuable edible indigenous fruit tree. In northern Nigeria, the plant is found predominantly in Tula area of Kaltungo local government area of Gombe State, Kali hills of Zah district of Michika local government area of Adamawa State and available in most of the Northeast markets especially in rural areas. *A. garckeana* belong to a family malvaceae, which is the distrimotive family to which the hibiscus belongs. Most of the malvaceae in Nigeria are shrubs. *A. garckeana* is a semi-deciduous tree/shrub with a round medium crooked stem [5]. The tree can grow to a height of 3-15 meters depending on the environment in which it is grows [5], FAO (1982) reported that *Azanzagarckeana* grows naturally in semi-arid areas receiving lowest annual rain fall of 250 mm and height rain fall of 1270 mm. The mean annual rain fall is between 250 mm to 500 mm with attitude of 0 – 1900 m. The twigs are hairy when young but become smooth with age and branches have wooly hairs according to World Agroforestry (1992). The leaves are distinctively round with 8 to 12 cm long stalks. The leaves have 3 to 5 lobes, which are covered in brown star-shaped hairs, and have longitudinal fissures in the midrib (FAO, 1983). The flowers have many stamens and 5 petals, which are yellow or purplish in colour with dark purple or dark red Centre. The flowers are bisexual with all floral parts in fives. It flowers in wet season and fruits in dry season (April-August) (FAO, 1983) [6,7]. The fruits are hairy, spherical, hard and about 2.5 – 4 cm long in diameter, internally divided into 4 to 5 longitudinal sections, the fleshy gummy pulp which is generally eaten are a good source of proteins, minerals, fiber, vitamins, and contains five seeds inside with a seed in each segment. The seed is hemispherical in shape, up to 10mm long, 7mm thick with brownish and wooly floss [8]. They are yellowish to brownish green when mature (Figures 1,2).



Figure 1: Fresh *Azanzagarckeana* fruit pulp on its tree
Magnification: x40



Figure 2: Dried and peeled *Azanzagarckeana*
Magnification: x40

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients [9]. Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables [10]. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants [11]. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytochemicals for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [12]. Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants.

Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents. Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of such thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients.

METHODS

Collection and extraction of Plant Samples

Fruit pulps were gotten from Tula, Kaltingo L.G.A of Gombe State, Northern Nigeria by August 2019 by hand plucking from the tree. The fruit was air dried for a month after opening and then oven dried in the laboratory at 40°C. It was subsequently pulverized using hammer type milling machine (Mecan, CM/L-1364548, India). The powdered material was dissolved in ethanol of analytical grade for 72 h and in water for 48 h. It was filtered with a muslin cloth and the ethanolic extract concentrated using a rotary evaporator (RE300). Both extracts were then placed in water bath set at 40°C [13]. The resultant product is a Jelly like substance which was placed in a freeze dryer to make it hard from its jelly state.

GC-MS Analysis

GC-MS analysis was carried out by standard procedures using GC-MS QP2010SE spectrometer. Gas chromatography mass spectrometry (GC/MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified such as essential oils, hydrocarbons and solvents. An electron ionization system with ionization energy of 70eV was used. Helium gas was used as carrier gas and a mass transfer line temperature set at 200°C and 240°C respectively. The Oven temperature was programmed from 70 to 220°C at 10°C/min, held isothermal for 1min and then raised to 300°C. At 10°C, 2ml of the samples were manually injected in vials in the split less mode. Total running time was 35minutes. Data constituents were expressed as compound name, molecular formula, molecular weight, peak area, and height. Interpretation of mass spectrum of GC-MS was done using database of National Institute Standard and Technology (NIST).

RESULTS

GC-MS analysis on Aqueous and Ethanolic extract of Azanzagarckeana

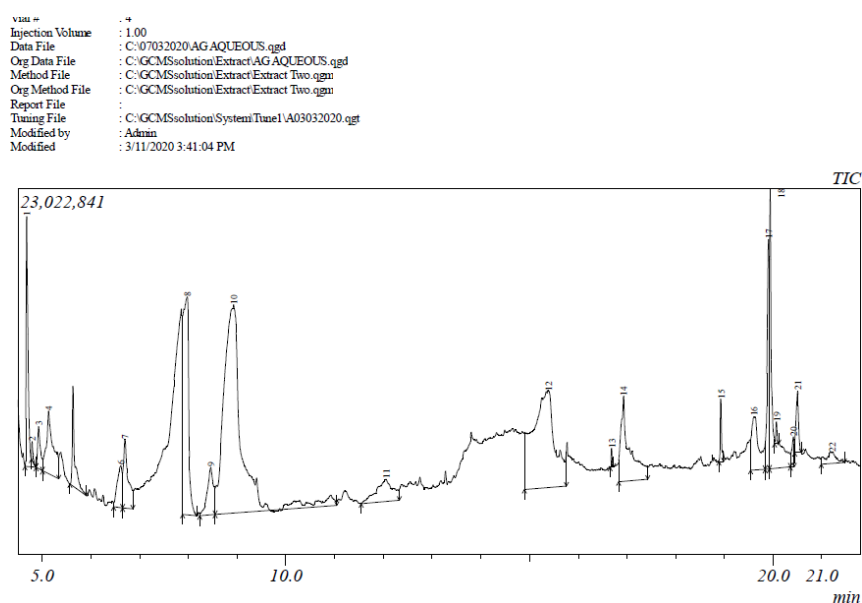


Figure 3: GC-MS Chromatogram of Aqueous extract of Azanzagarckeana

Table 1: GC-MS Phytocomponents identified in Aqueous Sample of Azanzagarckeana

S/N	R/T	Name of Compound	Mol. formular	Mol. Weight	Peak Area	Area %	Height %
1	4.684	2-Furanmethanol	C ₅ H ₆ O ₂	98	40800226	3.69	12.30
2	4.789	Acetic acid, 4-oxocyclohexyl ester	C ₈ H ₁₂ O ₃	156	2176195	0.20	0.93
3	4.925	Methoxyacetic acid, cyclobutyl ester	C ₇ H ₁₂ O ₃	144	10445328	0.95	2.07
4	5.128	2-Nonanol	C ₉ H ₂₀ O	144	38796925	3.51	3.08
5	5.635	2,4-Dihydroxy-2,5-dimethyl-3(2H)- furan-3-one	C ₆ H ₈ O ₄	144	21732682	1.97	4.94
6	6.615	2,5-Dimethyl-4-hydroxy-3(2H)- furanone	C ₆ H ₈ O ₃ C ₇ H ₁₂ O ₂	128	19111821	1.73	2.08

7	6.696	2-Amino-4,5-dimethylthiazole	C ₅ H ₈ N ₂ S	128	28342920	2.56	3.37
8	7.974	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₆ O ₄ C ₆ H ₈ O ₄	144	101658730	9.20	10.74
9	8.457	5-Hydroxy-2-methylthiopyrimidine	C ₅ H ₆ N ₂ OS	142	26672788	2.41	2.34
10	8.926	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	330510408	29.91	10.18
11	12.056	9,9-Dimethoxybicyclo[3.3.1]nona- 2,4-dione	C ₁₁ H ₁₆ O ₄	212	33718905	3.05	1.09
12	15.389	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194	194599845	17.61	4.78
13	16.687	7-Hexadecenoic acid, methyl ester, (Z)-	C ₁₇ H ₃₂ O ₂	268	1700818	0.15	0.94
14	16.930	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	65708734	5.95	4.20
15	18.928	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	C ₃₅ H ₆₈ O ₅	568	8264695	0.75	3.09
16	19.607	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	35305470	3.19	2.64
17	19.907	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl	C ₂₁ H ₃₈ O ₄	354	46052353	4.17	11.30
18	19.940	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl	C ₂₁ H ₃₈ O ₄	354	66090473	5.98	13.78
19	20.069	7-Hexadecenoic acid, methyl ester, (Z)-	C ₁₇ H ₃₂ O ₂	268	2265054	0.20	1.07
20	20.416	2-Myristinoyl-glycinamide	C ₁₆ H ₂₈ N ₂ O ₂	280	6137925	0.56	1.48
21	20.500	7-(2-Hydroxy-1-methylethyl)-1,4a- dimethyldecahydronaphthalen	C ₁₅ H ₂₈ O ₂	240	14714783	1.33	3.04
22	21,206	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	10349479	0.94	0.56

Injection Volume : 1.00
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 Org Data File : C:\GCMSsolution\Extract\AG ETHANOLIC.qgd
 Method File : C:\GCMSsolution\Extract\Extract Two.qgm
 Org Method File : C:\GCMSsolution\Extract\Extract Two.qgm
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 Tuning File : C:\GCMSsolution\System1\Tune1\A03032020.qgt
 Modified by : Admin
 Modified : 3/11/2020 4:10:57 PM

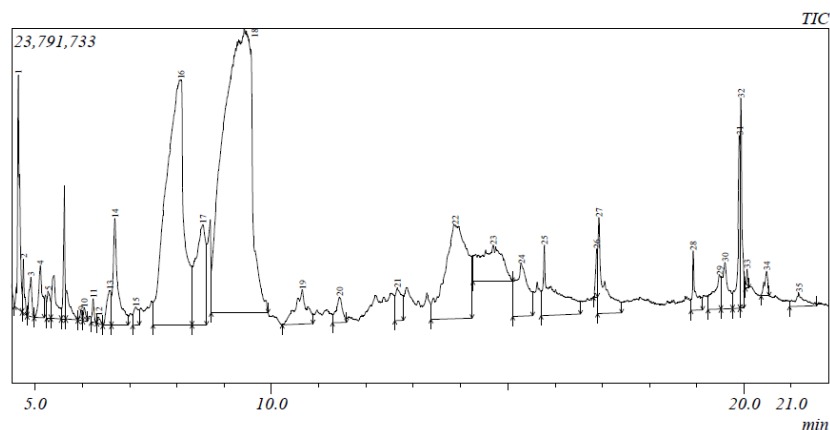


Figure 4: GC-MS Chromatogram of Ethanolic extract of *Azanzagarckeana*

Table 2: GC-MS Phytocomponents identified in Ethanol Sample of *Azanzagarckeana*

S/N	R/T	Name of Compound	Mol. formular	Mol. Weight	Peak Area	Area %	Height %
1	4.639	2-Furanmethanol	C ₅ H ₆ O ₂	98	49667960	2.20	9.36
2	4.750	1,4-Dioxaspiro[5.5]undecan-2-one	C ₉ H ₁₄ O ₃	170	7709328	0.34	2.15
3	4.908	3-[N-Aziridyl]propionylhydrazide	C ₅ H ₁₁ N ₃ O	129	9710069	0.43	1.58
4	5.106	1H-Pyrrole, 2,5-dihydro-1-nitroso-	C ₄ H ₆ N ₂ O	98	18112503	0.80	2.08
5	5.269	2-Octene, 1-(methoxymethoxy)-, (E)-	C ₁₀ H ₂₀ O ₂	172	8144171	0.36	1.08
6	5.397	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	C ₇ H ₁₀ O ₆	190	18271625	0.81	1.72
7	5.617	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144	22403844	0.99	5.32
8	5.667	Octanoic acid, 6-hydroxy-8-methoxy-, .epsilon.-lactone	C ₉ H ₁₆ O ₃	172	10856556	0.48	1.16
9	5.971	S-[2-Aminoethyl]-dl-cysteine	C ₅ H ₁₂ N ₂ O ₂ S	164	1787252	0.08	0.37
10	6.038	Methoxyacetic acid, decyl ester	C ₁₃ H ₂₆ O ₃	230	2716924	0.12	0.51
11	6.224	2-Heptanol, 5-ethyl-	C ₉ H ₂₀ O	144	3963526	0.18	0.94
12	6.339	Stevioside	C ₃₈ H ₆₀ O ₁₈	804	1655945	0.07	0.26
13	6.580	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C ₆ H ₈ O ₃	128	15873267	0.70	1.39
14	6.685	Tridemorph	C ₁₉ H ₃₉ NO	297	49183439	2.18	4.26
15	7.131	IsosorbideDinitrate	C ₆ H ₈ N ₂ O ₈	236	8274858	0.37	0.72

16	8.087	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	144	427482140	18.90	9.80
17	8.550	2-Butanone, 4-hydroxy-3-methyl-	C ₅ H ₁₀ O ₂	102	99004721	4.38	4.00
18	9.421	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	789838593	34.93	11.35
19	10.656	.alpha.-D-Glucopyranoside, O-.alpha.-D-glucopyranosyl	C ₁₈ H ₃₂ O ₁₆	504	38428259	1.70	1.36
20	11.444	Cytidine, 5-methyl	C ₁₀ H ₁₅ N ₃ O ₅	257	15990944	0.71	1.03
21	12.664	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	C ₁₁ H ₁₆ O ₄	212	20592331	0.91	1.29
22	13.885	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene	C ₈ H ₁₅ N ₃ O	169	205468818	9.09	3.73
23	14.691	3-Deoxy-d-mannonic lactone	C ₆ H ₁₀ O ₅	162	79042270	3.50	1.44
24	15.292	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl	C ₂₁ H ₃₈ O ₂	322	61226659	2.71	2.10
25	15.776	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312	73259152	3.24	2.56
26	16.886	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	8896349	0.39	1.94
27	16.929	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	50788950	2.25	3.84
28	18.925	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl	C ₁₉ H ₃₈ O ₄	330	18520745	0.82	1.96
29	19.483	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	22505693	1.00	1.34
30	19.602	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	27234822	1.20	1.82
31	19.902	Cyclopropaneoctanoic acid, 2-[[2-(2-ethylcyclopropyl	C ₂₂ H ₃₈ O ₂	334	38688967	1.71	6.90
32	19.934	Oleoyl chloride	C ₁₈ H ₃₃ Cl	300	32763202	1.45	8.40
33	20.067	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	2157567	0.10	0.74
34	20.473	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	7909444	0.35	0.98
35	21.161	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	13108337	0.58	0.51

DISCUSSION

GC-MS Chromatogram showed the phytochemicals of ethanol extract of *A. garckeana* with different peaks based on their concentration. Several compounds were found using GC-MS in large and little quantities. The Aqueous extract identified a total of 22 compounds while the ethanol extract identified 35 compounds all with peaks. Of 35 compounds found in the ethanol extract, those with high peaks include: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-Hydroxymethylfurfural, 2-Furanmethanol, 9, 12-Octadecadienoic acid (Z,Z)-, Oleoyl chloride and Cyclopropaneoctanoic acid. GC-MS Chromatogram showing the phytochemicals of aqueous extract of *A. garckeana* with different peaks based on their concentration. Of 22 compounds found in the aqueous extract, those with high peaks include: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-Hydroxymethylfurfural, 2-Furanmethanol, 9, 12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl and Oleoyl chloride. Those with high peaks are discussed below:

2-furanmethanol was detected having 3.69% in aqueous extract and 2.20% in ethanolic extract. It is used as a flavoring agent in some food

industries. It has a slightly bitter taste. When ingested in high quantities it can lead to breast cancer which can be detected in urine. Other foods that possess this chemical are dry foods like coffee. Nuts and popcorn [14,15]. 2-furanmethanol aids the breakdown of carbohydrates and amino acids in the presence/absence of reducing sugars.

5-Hydroxymethylfurfural (HMF) was detected having an area percentage of 34.93% in ethanolic extract and it's the highest compound detected in this extract while in aqueous form, it had a 29.29% area which also happens to be the highest amount detected in the extract. Therefore 5-HMF is the most abundant compound found in *Azanzagarckeana*. HMF is absent in fresh food but it is naturally generated in sugar containing food during heat treatments like drying or cooking. It is found in foods such as coffee and honey. It serves as a good preservative especially is sweet wines like madeira. HMF bind intracellular sickle haemoglobin. Preliminary in vivo studies using transgenic sickle mice showed that orally administered 5-HMF inhibits formation of sickled cells in the blood; thereby it has been considered for treatment of sickle cell disease [16]. 5-HMF rapidly inhibits high glucose-induced activation of IL-8 which is a glucose activator and is therefore useful pharmacologically to reduce blood sugar levels. A study by Shaplaet *et al.*, (2018) reports HMF as antioxidant, treats hypoxic injury, anti-allergen and anti-sickling agent.

9,12-Octadecadienoic acid (Z,Z)- was detected having an area of 4.17% in aqueous extract and 2.64% in ethanolic extract. This is also known as linoleic acid. It is a polysaturated omega-6-fatty acid and is one of two essential fatty acids for humans who must obtain it through diet [18]. Industrially, it is used for making quick drying oils that are used in beauty oils on the skin and also in producing hair shampoo and conditioner. It has been investigated as a carrier for delivering medications through the skin or mucous membranes particularly the lungs [19]. Research points to it as anti-inflammatory, acne reductive, antiarthritic, antidiabetic, hypocholesterolemic, skin lightening and moisture retentive when applied to the skin [19]. It can be found in sunflower oil, corn oil and soybean oil.

Tridemorph was found only in ethanolic extract but in low quantity having an area percentage of 2.18%. This is a fungicide used to control the fungus *Erysiphegraminis*. In large doses it can be harmful to the eyes and the World Health Organization (WHO) categorized it as class II moderately hazardous pesticide.

Cyclopropaneoctanoic acid was detected having a small percentage area of 1.71%. It is largely found in Adipose tissue which is the main depot of energy stored as triacylglycerols (TAG) in human body.

CONCLUSION

Plants are natural reservoirs of several phytonutrients and compounds which are inevitable and essential to life in general. The phytochemicals found in both extracts of *Azanzagarckeana* revealed their bioactive components by GC-MS analysis and depicts its importance in folklore, phytopharmaceuticals, nutraceuticals, cosmetics and food industries.

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