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Phytochemical screening, Gas chromatography mass spectroscopy studies and antioxidant property of aqueous extract of Ogbono (*Irvingia gabonensis*)

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Abstract
This work presents Phytochemical Screening, Gas Chromatography Mass Spectroscopy Studies and Antioxidant Property of aqueous extract of Ogbono (*Irvingia gabonensis*). Standard chemical and biochemical chemicals and analytical techniques were used for this study. The phytochemical screening of the of the samples showed the presence of various secondary metabolites of which alkaloids, flavonoid, cardiac glycoside andalkaloids are moderately present while tannis, saponins, steroids, protein and reducing sugar were the most prominent as shown in the results. Antioxidant activities of Ogbono (*Irvingia gabonensis*) determined by the free radical scavenging activity (DPPH) assay method indicated a steady increase in the scavenging activity of free radicals in all extracts. It was observed that the ability of test materials to scavenge DPPH was assessed on the basis of their I_C50 values, defined above as the concentration of test material to reduce the absorbance at 515 nm of DPPH solution to half of its initial value. The high antioxidant potential, free radical scavenging activity and antioxidative enzymes of Ogbono (*Irvingia Gabonensis*) should be utilized to develop new drug contenders for antioxidant remedy.

Keywords: Antioxidant property, GC-MS, Ogbono, phytochemical screening

Introduction
Recently, the use of *Irvingia gabonensis* in most families in Nigeria is becoming a daily recipe for the preparation of soups. Researches have shown that *Irvingia gabonensis* has many biological potentials and some antioxidants properties which when properly used (Ejiofor, 2004) [8]. *Irvingia gabonensis* (Ogbono*)* fruit is a largely ellipsoid drupe, yellowish and having very juicy fibrous pulp when ripe. Its stony nut encases an oil rich dicotyledonous kernel wrapped inside a brown seed-coat. The average length, width and thickness of the nut are (43.3×30.62×22.11) mm respectively (Abreu *et al*., 2008) [1]. Cavin *et al*., (2006) [6] reported that the defatted flour of is potentially useful as raw material in food products development (Farasat *et al.*, 2014) [11]. The extraction, screening and identification of the medicinally active substances found in plants is referred to as phytochemical screening (Keay *et al*., 2013) [15]. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants and phenolic compounds (Abreu *et al.*, 2006) [1]. Though the information of how these substances provide medicinal value to humans reflects a comparatively recent scientific understanding, the use of plants and plant extracts to heal, relieve pain and promote good health dates back to before the beginnings of medical science (Gulcin *et al*., 2010) [12]. It is understood that there might be about 4,000 phytochemicals confined in plants that can be used in preventing, minimizing and also remedy for medical conditions such as metabolic syndrome, strokes, or cancer. From previous results obtained by scientific researchers have establish that the use of phytochemical supplements supports long-term healthy living as well as consuming the actual fruits, grains and vegetables from which they were taken (Farasat *et al*., 2014) [11]. Ogbono fruit is a full source of protein, ascorbic acid potassium, carbohydrate, dietary fibre, iron, vitamin C, water, energy, sodium, amino acids, calcium, phosphorus and magnesium (Anhwange *et al*., 2004) [3]. The seeds contain fatty acids such as stearic acid, oleic acid, palmitic acid, myristic acid and lauric acid. The first stage of the phytochemical screening of the aqueous leaf extract of Irvingia gabonensis indicates that it contains phenols, tannins, phlobatanins and saponins.

Ogbono is high in protein content, fiber and essential fats (Vaya *et al*., 2004) [25]. A 100 grams serving of ogbono seeds provides about 683 calories. It is rich in fatty acids such as oleic, palmitic, linoleic, linolenic, stearic, myristic, and lauric acids, which are essential for performing the various bodily functions, maintaining the health of the nervous system and for muscular development (Eka, 2000) [9]. Antioxidant is mostly used for two different groups of substances: industrial chemicals which are intensified to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects (Tiwari *et al*., 2009; Balogun and Fetuga, 2008) [23, 5]. The general aim of this research work is to determine the phytochemical properties of *Irvingia gabonensis* seed (ogbono) and to evaluate the antioxidant properties of *Irvingia gabonensis (ogbono)*.

Materials and Methods

Collection of sample

Fresh *Irvingia gabonensis* seed (Ogbono) were bought from Relieve market in Owerri, Imo State and were immediately transported to the biotechnology laboratory of the Imo State University for biochemical for proper authentication and identification by the senior lecturer in organic Chemistry Dr. Ikpa in comparison with voucher specimen present in herbarium.

Preparation for extract

A substantial quantity of fresh *Irvingia gabonensis* seed was collected, thoroughly washed with clean water separately accordingly, based on how they were collected. They were oven dried until a constant weight was achieved then was spread out on laboratory bench for inspection. They were then grounded using electric blender to fine powder and passed through a 24 mesh sieve. 100g of the sample was weighed using a rough mechanical beam balance and allowed to air dry 24 hours at room temperature.

Extraction of plant material

The powdered sample (100g) of *Irvingia gabonensis* was successfully extracted with 500ml of distilled water, using magnetic stirrer and stirred for 3 hours. Then it was filtered using whatmann filter paper. Again, the residue was dissolved with 100ml of distilled water and stirred for 2 hours. The solvent containing the extract is dried under reduced pressure. The supernatant was boiled up to minimum volume. The extract obtained were kept in sterile sample tube and store (Sonawane *et al*., 2011) [21].

Methods of phytochemical screening

The freshly prepared crude extract was quantitatively tested for the presence of biochemical constituent.

Screening for Alkaloids

10ml of aqueous extract was added to 2ml of HCl. to this acidic medium, 1ml of wagners reagent was added. A reddish brown precipitate indicates the presence of alkaloids.

Screening for Glycosides

To a small amount of extract, 1ml of fehlings solution was added and heated, orange precipitate indicates the presence of glycoside.

Screening for Flavonoids

To 1ml of the extracts, a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Screening for Terpenoids (Lieberman-Burchad test)

To 1ml of extracts was treated with chloroform, acetic anhydride and drops of sulphuric acid was added, the formation of dark green colour indicates the presence of terpenoids.

Screening for Proteins (Ninihydrin test)

1ml of the extract was treated with aqueous ninihydrin and observed for the presence of blue colour, indicaton the presence of amino acid or purple colour indicationg the presence of protein.

Screening for Antharquinone (Bornthrager's test)

The powdered leaves (50mg) was heated with 10% ferric chloride solution and 1ml concentrated hydrochloric acid. The mixture was cooled filtered and the filtrate shaken with diethyl ether. The ether extract was further extracted with strong ammonia and observed for the formation of pink or deep red colouration of the aqueous layer (Ekpe *et al*., 2007; Middleton and Kandaswami, 2002) [10, 16].

Screening for Steroids

1ml of the extract was dilueted with chloroform, acetic anhydride and drops of sulphuric acid was added. Dark pink colouration indicates the presence of sterols.

Screening for Saponins

1ml of the extract was diluted with 20ml of distileed water and it was agitated in a graduated cyljnder for 15mins. The formation of 1cm layer of foam showed the presence of sapoins.

Screening for Phenolic compound

1ml of the extract was taken separately in water and tested for the presence of phenolic compound with dilute ferric chloride solution. Violet colour indicates the presence of phenolic components.

Screening for reducing sugar

To 1ml of the extract, 2ml of fehlings solution reagent and 3ml of water was added. Appearance of red orange indicates the presence of reducing sugar.

Screening for Tannins

1ml of the extract was treated with acetic acid solution and observed for the formation of red colour solution.

Screening for Phlobatannins

3ml of aqueous extract was added to 2ml of 1% HCl and the extract was boiled. Deposition of a red precipitate was taken as an evidence for the presence of Phylobatannins (Abreu *et al*., 2008; Aiyegroro and Okoh, 2010; Arulpriya and Lalitha, 2012; Periyanayagam *et al*., 2012) [1, 2, 4, 17].

Identification of components (GC-MS)

GC-MS analysis on the aqueous extract of *Irvingia gabonensis* was carried out using a Hewlett Packard gas chromatography (model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250 ^{0}C. The instrument was used employing the following conditions. column elite - fused silica capillary column (30nm x 0.25nm 1D x 1µm df, composed of 100% dimethyl polysiloxane operating in

electron impact mode at 70ev; helium (99.999%) was used as a carrier gas at a constant flow of 1ml/minute and an injection volume of 0.5µ was employed (split ration of 10:1) injection temperature 250^{0}C, ion – source temperature 280^{0}C. the oven temperature was programmed from 110^{0}C (isothermal for 2 minutes), was an increase of 10^{0}C / minutes, to 200^{0}C, then 5^{0}C / min to 280^{0}C, ending with a 9 minutes isothermal at 280^{0}C. mass spectra were taken at 70ev; a scan interval 0.5 seconds and fragment from 45 to 450Da. total GC running time is 36mins. the plant extract was dissolved I distilled water and filtered with polymeric acid phase extraction (SPE) column and analyzed in GC – MS for different component.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the NIST Database. The spectrums of the unknown components were compared with the spectrum of known component stored in the NIST library. The name, molecular weight and structure of the component of the test materials were ascertained.

Antioxidant activity

The antioxidant capacity of extracts of ogbono was evaluated against ascorbic acid as percent inhibition of ABTS free radicals. ABTS radical is a blue chromophore produced by the reaction between ABTS and potassium persulfate. The intensity of the color is related to the amount of antioxidant reductants in the samples.

Results

Table 1: Phytochemical analysis of the extracts

S/N	Test	Result
1.	Tannins	++
2.	Saponins	++
3.	Flavonids	+
4.	Steroids	++
5.	Terpenoids	-
6.	Cardiac Glycoside	+
7.	Phylobactanins	+
8.	Phenolic Compounds	-
9.	Proteins	++
10.	Reducing Sugars	++
11.	Anthraquinnones	-
12.	Alkaloids	+

Key: (++) = Present, (-) = Indicates Absence, (+) = moderately present

Aqueous extract of seed of *Irvingia gabonensis* showed the presence of the various phytochemicals; saponin, flavonoids, steroid, protiens, anthraquinnones, tannins, protein, reducing sugar, cardiac glycoside, terpenopid, phenolic compounds and alkaloids. The presence of flavonoid, cardiac glycoside andalkaloids are moderately present while tannis, saponins, steroids, protein and reducing sugar were heavily present. These bioactive components are naturally occurring in *Irvingia gabonensis* and known to possess interesting biological activities. Several studies have shown that a diet rich in fruit and vegetables has an important role in reducing the incidence of diseases. Some of these preventive actions have been related to the presence of bioactive substances such as polyphenols. Flavonoids are characterized by a common benzopyrene ring structure (Havsteen, 2002) [13]. The biological purposes of flavonoids, apart from its antioxidant properties include defense against allergies, free radicals, hepatoxins, platelet aggregation, inflammation, microbes, ulcers, viruses and tumors. Flavonoids reduced cancers by interfering with the enzymes that produce estrogen (Williams *et al*., 2005) [26]. The phytochemical screening of the extract of the seed of *Irvingia gabonensis* showed the presence of numerous secondary metabolites of which flavonoid, cardiac glycoside and alkaloids are moderately present while tannis, saponins, steroids, protein and reducing sugar were heavily present as shown in Table 1. Similarly, this result was in line with a research work done on the phytochemical screening of seeds of *Irvingia gabonensis***,** which revealed the presence of alkaloids, flavonoids, tannins, terpenoids and anthraquinones in the extract (Srivastava *et al.*, 2011) [22]. Presence of tannis, saponins, steriods, alkaloids and Flavonoids are usually found more in seed oil and it is reported to be an effective antioxidant and radical scavenging activity (Tiwari *et al*., 2009) [23]. Several reports have shown a close relationship between total phenolic content and high antioxidant activity (Havsteen, 2002) [13]. Some researchers that has worked on seed plants have examined the relationship between antioxidant activity and polyphenol content. Polyphenol compounds are reported to be a good source of natural antioxidants (Abreu *et al.*, 2006) [1]. Plant phenols represent one of the major groups of compounds acting as primary antioxidants or free radical terminators. Thus, it was reasonable to determine their total amount in the selected plant extracts (Pin *et al.*, 2000) [18].

Table 2: GCMS data of importance bioactive compound

S/N	RT	Name of compound	Molecular formula	M/W	Peak Area %
1.	3.965	Glycerin		92	7.51
2.	4.427	2-Undecanone	$C_{13}H_{22}O$	170	45.39
3.	4.457	Glycerin	$C_3H_8O_3$	92	5.03
4.	5.000	3,4-Furandiol, tetrahydro-,trans	$C_4H_8O_3$	104	0.93
5.	5.090	3,4-Furandiol, tetrahydro-,trans	$C_4H_8O_3$	104	1.1
6.	5.562	2-Tridecanone	$C_{13}H_{26}O$	198	0.99
7.	5.705	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	2.03
8.	5.922	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.02
9.	6.713	Tetradecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	2.97
10.	6.927	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.48
11.	11.089	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.26
12.	8.374	Dodecanoyl chloride	$C_{12}H_{23}ClO$	219	9.93
13.	9.2.44	1,3-O-Benzylidine glyceryl-2-myric	$C_{24}H_{38}O_3$	374	8.11
14.	9.510	2-Nonene, (E)-	C_9H_{18}	126	1.25
15.	10.133	Hexadecanoic acid, 2-hydroxyl-1-(h	$C_{19}H_{38}O_4$	330	1.19
16.	10.346	1-Dodecanol, 2-methyl-,(5)-	$C_{13}H_{28}O$	700	1.12
17.	11.035	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	2.07
18.	14.424	4-Dibenzofuranamine	$C_{12}H_9NO$	183	1.25
19.	16.227	2(3H)-Benzothiazolethione, 6-ethc	C_9H9NOS_2	211	1.21
20.	16.325	3-Dibenzofuranmine	$C_{12}H_9NO$	183	1.17

The following in the table 2, shows all the GCMS bioactive compounds, their molecular formula and their peak area. GCMS analysis showed the presence of 2-Undecanone as a main compound in the extraction. The aqueous extraction showed 45.39%. And the least compound showed 3,4-Furandiol, tetrahydro-, trans and the aqueous extraction is 0.93%. The presence of various bioactive compounds from *Irvingia gabonensis* justified the use of various treatments by local traditional practitioners. tetradecanoic acid is a food additive permitted for direct addition to food for human consumption as synthetic flavouring substances, (Williams *et al*., 2005) [26]. Hexadecanoic acid or palmitic acid is the most common fatty acid (Saturated) found in animals plants and microorganisms. It is used to produce soaps, cosmetics, and release agents. Methyl esters are commonly used as fragrance and found in essential oils and phenomones, (Cook *et al.,* 2003) [7]. Hexadecanoic acid are used as adhesives and sealant chemicals, agricultural chemicals, (non-pesticidal), filters, finishing agents and lubricants, (Sanchez *et al.,* 2010) [20]. Octadecadienal is used in the production of detergents, soaps and cosmetics such as shampoos and shaving cream products. It is also used as lubricants, softening ad release agents and Niche uses. Stearic acid is one of the most common saturated fatty acids found in nature. 9-Oct 9 dececanoic acid (z)-, methyl ester is used as agricultural products, ink toner and colorant products it is also used as lubricants, grasses and water lubricants products. Sterculic acid (9, 12, octadececanoic acid, methyl ester) is an organic compound found in some tropical vegetable oils, it helps in the treatment of neuro generative diseases, (Roussel *et al*., 2003; Vandana and Shalini, 2014) [19, 24].

Table 3: Concentration and Absorbance of ascorbic acid

Oxidant: Dpph					
Concentration (Mg/L)					
Sample	0	10	20	30	40
Absorbance @ 517nM					
Ascorbic Acid	0.089	0.088	0.075	0.068	0.053
1	0.089	0.071	0.064	0.058	0.042
2	0.089	0.073	0.060	0.053	0.046
3	0.089	0.069	0.061	0.055	0.038

The frequency distribution of vitamin C concentration of the extract is shown in Table. 3. Vitamin C contents recorded in this study are generally lower than the USDA database, but similar to that reported by Kabasakalis (2000) [14], in which ascorbic acid content of ofor (*Detarium microcarpum*) ranged from 0.04 to 0.143 mg/100 ml of *Detarium microcarpum*.

Conclusion

This study has critically studied the phytochemical screening of *Irvingia gabonensis* and the anti-oxidant nature. The high antioxidant potential, free radical scavenging activity and antioxidative enzymes of *Irvingia gabonensis* should be utilized to develop new drug candidates for antioxidant therapy. Due to its many uses, *Irvingia gabonensis* has great potential for poverty or hunger stricken areas where growing conditions are poor especially. Attempts should be put forth to reassure its use and domestication. Research in the following areas could aid in furthering its benefits: genetic variation associated with drought tolerance; causes underlying variation in tree growth and fruit production; more information on its medicinal, nutritional and wood-energy properties; effective population sizes in semi-natural farmland populations and minimum viable populations for conservation and long-term sustainable use. Additionally, regulation is needed for exploitation of wood, controlling fires, reducing fuel-wood demand and encouraging re-forestation. Rural communities require aid to develop sustainable use and conservation practices for the species; this must be done using local knowledge.

Conflict of interest

The authors declared no conflict of interest

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