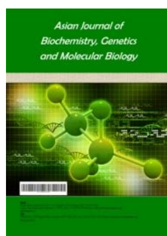

Asian Journal of Biochemistry, Genetics and Molecular Biology

7(4): 25-34, 2021; Article no.AJBGMB.63380
ISSN: 2582-3698

Phytochemistry and Antioxidant Activity of *Irvingia gabonensis* (Bush mango) Seed Sample

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EAS and NCN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EAS, WGP and GBO managed the analyses of the study. Authors EAS, NCN and GBO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2021/v7i430181
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal Centre for Advanced Research (MCAR), India.
Reviewers:
(1) Hattab Sabrine, Sousse University, Tunisia.
(2) Niranjan M.H, Davangere University, India.
(3) R. Deveswaran, M.S.Ramaiah University of Applied Sciences, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63380

Original Research Article

Received 05 October 2020
Accepted 17 December 2020
Published 08 May 2021

ABSTRACT

Phytochemistry and antioxidant activity of *Irvingia gabonensis* (Bush mango) seed sample were evaluated. Dried milled seeds of *I.gabonensis* popularly known as "ogbono" passed through phytochemical screening, Gas chromatography-mass spectrometry (GC-MS) analysis and antioxidant studies. Tannins, saponins, flavonoids, alkaloids, steroids, and reducing sugars were among the phytochemicals found present after screening. About 20 constituents were revealed by the GC-MS analysis out of which 1,3-O-Benzylidine glyceryl-2-myristate had the highest molecular weight, 2-Undecanone had the highest peak area of 45.39% whereas 3-Dibenzofuranamine had the highest retention time. Some of these constituents could have one or two applications in medicinal, food or cosmetic industries. *I.gabonensis* seeds showed better antioxidant activity against the

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control at concentrations considered in this study. Some of these constituents as well could be behind the observed antioxidant activity. This study has evaluated the phytochemistry and antioxidant activity of *I.gabonensis* (Bush mango) seed sample.

Keywords: Antioxidants activity; phytochemistry; medicinal plants; Irvingia gabonensis.

1. INTRODUCTION

Plants have contributed immense to humans and humanity [1-6]. The benefits of plants to humans cannot be quantified and are as old as mankind on earth [7-8]. The use of plant to salvage some of the problems of humans could date to creation. Apart from food production [9-19], plants are put to different uses by man [7, 20-27]. The medicinal aspect of plants took a dimension after the Alma-Ata declaration in 1978, where the use of plant in primary healthcare was publicly accepted [7, 28]. Since then, efforts are being made to discover plants and plant products with medicinal potentials. Studies began to see edible portion of plants beyond serving as food only. Presently, thousands of studies have revealed the medicinal potentials of different plants as well as different parts of plants [29-33]. Further studies have also linked the constituents of both edible and non-edible portion of plants to phytoactivity, of which many are bioactive in nature [31-39]. With the phyto and bioactivity of constituents found in plants, they are able to show potency against diseases and disease causing pathogens [40-51]. Different authors have reported the physiological activity of most of the constituents found in plants against disease causing pathogens [52-56].

The potency of plants has also been extended to their ability to quench some biomolecules that are associated with stress in living organisms [57-60]. Oxidative stress as found in living organisms has been described as a situation that rises when there is excessive production of reactive oxygen species (ROS) against the defense mechanisms. The known defense mechanisms include group of complex antioxidant systems. Antioxidants counter the activities of reactive oxygen species. They include the major cellular redox buffers ascorbate (AsA) and glutathione (γ-glutamyl-cysteinyl-glycine, GSH) as well as tocopherol, carotenoids, and phenolic compounds; and enzymatic components such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [61-66].

Irvingia gabonensis, a species of African tress could be among the plants with phyto and bioactivity of constituents that can as well quench some biomolecules associated with stress in living organisms. *I.gabonensis* is commonly known as bush mango, African mango, or wild mango [67]. It bears edible fruit that looks like mango fruit. The fruits also bear seeds popularly known as “ogbono” in Southern Nigeria. “ogbono” is referenced because of its usefulness as a thickener in the preparation a soup known as “ogbono soup”, a particular type of draw soup. *I.gabonensis* is a deep soil plant, which survives in sandy loam soil of acidic pH [68]. It could be propagated by grafting, budding, air layering, cutting, marcoting or seeds. The tree can grow up to 40 m in height and 1 m in diameter. The leaves bear slightly obovate, dark green coloration and elliptic shape and can measure up to 5 to 15 x 2.5 to 6 cm. The Igbos of Southeastern Nigeria call the fruit of *I.gabonensis* “ugiri” and the seed “ogbono” [67-70]. The fruit is also known as “ogwi” by the Benin people; “biri” or “goronor” by the Hausa people; and “oro”, “apon” or “aapon” by the Yoruba people. The high glucose lowering potency [71-72], weight reducing ability [71], analgesic properties [73], serum cholesterol level regulation [71], antidysentery potency, antibacterial and antifungal properties [74] as well as anti-constipation, anti-indigestion, astringent [75] and the industrial uses of *I. gabonensis* have been reported by different authors. The stem has also been implicated in dental care as chewing stick for cleaning of teeth [67].

There is need to ascertain the constituents that could be linked to the aforementioned medicinal properties of *I.gabonensis*. The present study looked into this area and evaluated the Phytochemistry and antioxidant activity of *I.gabonensis* (Bush mango) with its seeds.

2.1 MATERIALS AND METHODS

2.1 Sample Collection and Identification

The *I.gabonensis* fruits used were plucked from the tree found in a farm in Umunchi village, Isiala Mbano LGA of Imo State. The fruits were properly identified by a Botanist in the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. The fruits were peeled to obtain the nuts. The nuts were forcefully cracked open to expose the seeds, which were harvested from the nuts. The harvested seeds were dried in the sun for few days and were milled into powder for further studies.

2.2 Preparation of Aqueous Extract

Ten gram of the milled seeds was extracted by maceration in 50 ml of water for 3 days with frequent agitation at a speed of 280 rpm at 28°C in dark. Between extractions, the sample was centrifuged for 10 min with 2000 rpm. The combined supernatants were collected, filtered through Whatman No. 1 filter paper and concentrated in vacuum. It was kept in a vacuum desiccator for complete removal of solvent. The yield extract was thus used for some of phytochemical screening, GCMS analysis and assessment of antioxidant activity.

2.3 Qualitative Phytochemical Determinations

2.3.1 Test for tannins

To 1 mL of the extract, equal volume of bromine water was added. The formation of a greenish to red precipitate was taken as the presence of tannins,

2.3.2 Test for saponins

One mL of the extract was boiled with 5 mL of distilled water for 5 min. and decanted while hot. 4 mL of distilled water was added to 1 mL of the filtrate before it was shaken vigorously for observation of stable froth on standing.

2.3.3 Test for flavonoids

0.5 g of the extract was added, in a test tube and 10 ml of distilled water, 5 mL of dilute ammonia solution were added to a portion of the aqueous filtrate of the extract followed by addition of 1 mL concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoid in each extract.

2.3.4 Test for alkaloids

One (1) mL each of the extract was shaken with 5 mL of 2% HCl on a steam bath and then filtered. To I mL of the filtrate, Wagner`s reagent (iodine in potassium –iodide solution) was added and reddish brown precipitates was observed for positive result.

2.3.5 Test for steroids

Half (0.5 g) gram of the extract was dissolved in 10 mL anhydrous chloroform and filtered. The filtrate was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one mL of acetic anhydride followed by the addition of 1 mL of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration as indicative of steroids.

2.3.6 Test for terpernoids

One gram of seed sample was shaken in a test tube with 10 mL of methanol, and then filtered. 5 mL extract was then mixed with 2 mL of chloroform and 3 mL of sulphuric acid was added. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

2.3.7 Cardiac glycosides

One mL of the seed extract was dissolved in 2 mL of chloroform in a test tube. 1 mL conc. H_2SO_4 was carefully added to the test tubes through the side and was observed for a red or reddish brown colouration at the interphase, which indicates positive result.

2.3.8 Test for phlobatannins

One percent aqueous hydrochloric acid was added to the seed extract in a test tube (about 2 mL), and then boiled with the help of Hot plate stirrer. Formation of red coloured precipitate confirmed a positive result.

2.3.9 Test for phenolic compounds

To 2 mL of the seed extract, 1% $FeCl_3$ was added and observation was made for blue, violet, purple, green or red-brown colour.

2.3.10 Test for proteins

Five drops of 1% hydrated copper sulphate was added to 2 mL the seed extract in a test tubes. Two mL of 40% NaOH was also added, and the test tube was shaken vigorously to mix the content and presence of purple colouration indicated the presence of proteins.

2.3.11 Test for reducing sugars

One mL of ethanol was mixed with 2 mL each of the plant extract, after which 1 mL each of Fehling solution A and B were added to the test tubes. The test tubes were heated to boiling while observation was made for presence of reddish brown colouration which indicates positive results.

2.3.12 Test for anthroquinones

One gram of the seed extract was placed in a dry test tube and 20 mL of chloroform was added. This was heated in steam bath for 5 min. The extract was filtered while hot and allowed to cool. To the filtrate was added with an equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration, which for the presence of anthraquinones. This was repeated with all the plant samples.

2.4 GC-MS Analysis of the Extracts

GC-MS analysis of the aqueous extracts was carried out using AOC-20i auto sampler and gas chromatograph interface to a mass spectrometer (GC-MS) instrument. Employing the following conditions; column Elite-1 fused silica capillary column (30 mm×0.25 mm ID×1Mm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 Ev; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/ min, and an injection volume of 0.5µl, Split ratio of 10:1), with injector temperature 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 mins isothermal at 280°C. Mass spectra were taken at 70 Ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da. Total GC running time was 36 mins. The plant extract was dissolved in aqueous and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test were ascertained.

2.5 Determination of Antioxidant Activity

2.5.1 DPPH (1, 1-Dipheny l-2-picrylhydrazyl) radical scavenging assay

The free radical scavenging activity was measured by DPPH assay method. Four mg of DPPH (0.1 Mm) was dissolved in 100 Ml of distill water to obtain working solution. One Ml of each extract was mixed separately with 2.0 Ml of 0.1 Mm DPPH followed by 30 min incubation in dark. The reduction of the DPPH free radical was measured by taking the absorbance at 517 nm [76]. Colour of DPPH was reduced from purple to yellow. The antioxidant activity of each extracts was evaluated by calculating the inhibition % of free radical formation using the formula:

% inhibition = [(A-A1)/A] x 100; A=absorbance of the blank (DPPH); A1=absorbance of the extract (DPPH+ extract).

3. RESULTS AND DISCUSSION

Phytochemical screening of *I.gabonensis* as represented in Table 1 showed the presence of tannins, saponins, flavonoids, alkaloids, steroids, cardiac glycosides, phlobatannins, proteins, and reducing sugars. These phytochemicals become important when their effects are considered in living organisms. The astringency of tannins [35,41], foaming and bitter tastes of saponins [44-45], antibacterial and anti-inflammatory property of flavonoids [46-47, 53-54], stimulant and analgesic property of alkaloids [77], hormonal nucleus role of steroids [41], the beneficial nature of cardiac glycosides to the heart at low concentration[ ], the anti-inflammatory, analgesic and wound healing properties of phlobatannins [54, 59], tissues replacement ability of proteins and energy production potency of sugars [41] have all been reported. Tannins, saponins, steroids, and proteins were present in high concentrations. Flavonoids, alkaloids, cardiac glycosides, phlobatannins, and reducing sugars were

present in moderate concentrations whereas terpernoids, phenolic compounds and anthroquinones were absent in *I.gabonensis* milled seed.

Table 1. Phytochemical screening of *I.gabonensis*

Phytochemical	*I.gabonensis*
Tannins	++
Saponins	++
Flavonoids	+
Alkaloids	+
Steroids	++
Terpernoids	-
Cardiac glycosides	+
Phlobatannins	+
Phenolic compounds	-
Proteins	++
Reducing sugars	+
Anthroquinones	-

++: present in high concentration; +: moderate concentration; -: absent

Result of GC-MS analysis of *I.gabonensis* showing retention time, molecular formula, molecular weight and peak area as presented in Table 2, revealed a total of 20 constituents which include glycerin, 2-Undecanone, 3,4-Furandiol, tetrahydro-, trans-,2-Tridecanone, Dodecanoic acid, methyl ester, Dodecanoic acid, Tetradecanoic acid, methyl ester, Tetradecanoic acid, Hexadecanoic acid, methyl ester, Dodecanoyl chloride, 1,3-O-Benzylidine glyceryl-2-myristate, 2-Nonene, (E)-, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 1-Dodecanol, 2-methyl-, (S)-, 9,17-Octadecadienal, (Z)-, 4-Dibenzofuranamine, 2(3H)-Benzothiazolethione, 6-ethoxy- and 3-Dibenzofuranamine. 1,3-O-Benzylidine glyceryl-2-myristate had the highest molecular weight, 2-Undecanone had the highest peak area of 45.39% whereas 3-Dibenzofuranamine had the highest retention time. Some of these constituents could have one or two applications in medicinal, food or cosmetic industries. Glycerine is used as a hyperosmotic, osmotic diuretic, and ophthalmic agent. Glycerin has many other uses in the agricultural, food and pharmaceutical industry. 2-Undecanone is an insect and tick repellant. 3,4-Furandiol, tetrahydro-, trans- is an excellent manufacturer of organic compound. 2-Tridecanone is a nonalkaloid and has insecticidal potency. Dodecanoic acid, methyl ester, is a fatty acid of methyl and dodecanoate esters, which has a metabolite role. Dodecanoic acid, a considered lauric acid, can be applied in soaps, cosmetics, resin and wetting agents. Tetradecanoic acid,

Table 2. Result of GC-MS analysis of *I.gabonensis* showing retention time, molecular formula, molecular weight and peak area

SN	RT	Component	Formula	MW	%
1	3.965	Glycerin	$C_3H_8O_3$	92	7.51
2	4.427	2-Undecanone	$C_{11}H_{22}O$	170	45.39
3	4.457	Glycerin	$C_3H_8O_3$	92	5.03
4	5.000	3,4-Furandiol, tetrahydro-, trans-	C4H8O3	104	0.91
5	5.090	3,4-Furandiol, tetrahydro-, trans-	C4H8O3	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	7.620	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.26
12	8.374	Dodecanoyl chloride	C12H23ClO	219	9.93
13	9.244	1,3-O-Benzylidine glyceryl-2-myristate	$C_{24}H_{38}O_3$	374	8.11
14	9.510	2-Nonene, (E)-	C9H18	126	1.25
15	10.113	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	1.19
16	10.346	1-Dodecanol, 2-methyl-, (S)-	$C_{13}H_{28}O$	200	1.12
17	11.035	9,17-Octadecadienal, (Z)-	C18H32O	264	2.07
18	14.424	4-Dibenzofuranamine	$C_{12}H_9NO$	183	1.25
19	16.227	2(3H)-Benzothiazolethione, 6-ethoxy-	C9H9NOS2	211	1.21
20	16.325	3-Dibenzofuranamine	C12H9NO	183	1.17

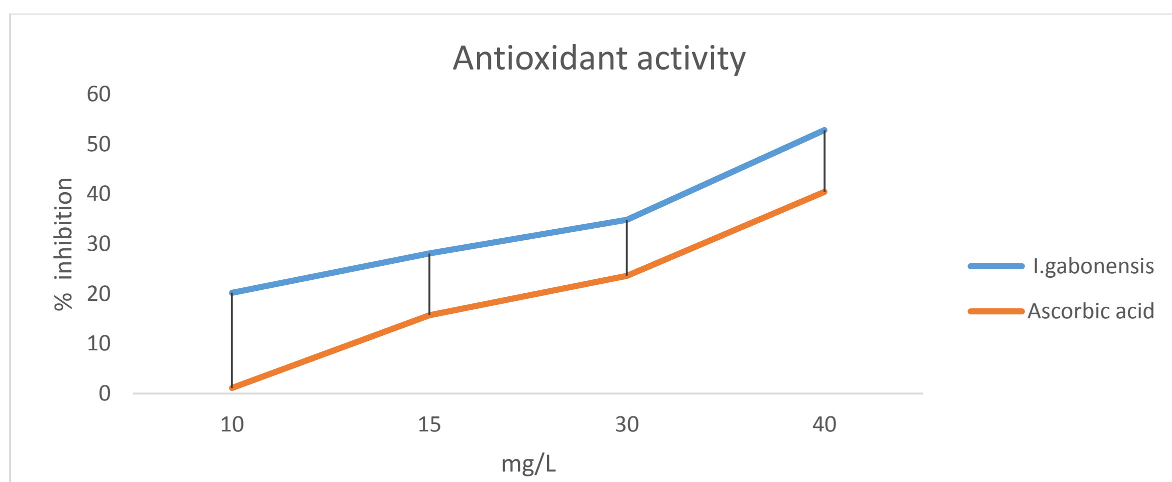

Fig. 1. The result of antioxidant activity of *I.gabonensis*

methyl ester has metabolite role in plants and could be used as a fragrance or a flavouring agents. Extracts of Hexadecanoic acid, methyl ester, from leaves, stems, and fruits exhibited antimicrobial activity against *Staphylococcus aureus*. Dodecanoyl chloride could be applied in surface modification of cellulose.

Antioxidant activity as presented in Fig. 1, showed percentage inhibitions of *I.gabonensis* taken at the 10, 15, 30 and 40 mg/L concentrations as 20.22%, 28.09%, 34.83%, and 52.81%. These inhibitions were against 1.12%, 15.73%, 23.60% and 40.45% observed for the control. In generality, *I.gabonensis* showed higher antioxidant activity than the control in the present study. Plants with antioxidant activity have been reported by different authors [58-66].

4. CONCLUSION

Tannins, saponins, flavonoids, alkaloids, steroids, cardiac glycosides, phlobatannins, proteins, and reducing sugars were found in *I.gabonensis.* The GC-MS analysis revealed a total of 20 constituents of either medicinal, food or cosmetic importance. Some of these constituents could also be behind the antioxidant activity of the plant. This study has revealed the Phytochemistry and antioxidant activity of *Irvingia gabonensis* (Bush mango) seed sample.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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