**Partial Oil Characterization and Antioxidant property evaluation of *Irvingia Gabonensis* Seed**

**ABSTRACT**

**Background:** African mango (*Irvingia gabonensis*) commonly known as Bush Mango, is a tree belonging to the genus *Irvingia*. The fruits of these trees are valued for their fats- and protein- rich nuts. The nutritional value of African mango seed (*Irvingia gabonensis*) is estimated to contribute to human health and food consumption safety.

**Aim:** This research work is aimed to highlighting the Antioxidant activity and partial oil characterization of African mango seed (*Irvingia gabonensis*).

**Materials and Method:** The study was carried out at the Biochemistry Department, Biochemistry Department Research Laboratory, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria from March to May 2021. The antioxidant activity and partial oil characterization were analyzed using standard laboratory methods.

**Results:** The antioxidant results obtained showed that the sample had its highest antioxidant activity as follows: Ferric reducing antioxidant power activity at 4.8%, nitric acid scavenging activity at 3.6%, superoxide scavenging activity at 60%, hydroxyl scavenging activity at 73% & ABTS scavenging at 67%. The partial oil characterization result shows as follows: Free Fatty acid (%) (2.092$\pm $0.181), Acid value (%) (4.183$\pm $0.36), Saponification (mgKOH/kg) (323.51$\pm $14.118), Peroxide (mleq/kg) (10.2$\pm $0.2), Iodine value (104.860$\pm $0.381), Refractive index (1.421$\pm $0.001), Specific gravity (0.9301$\pm $5.7753E-05), Viscosity (164.3$\pm $2.157), Cloud point (12.4$\pm $0.2), Flash point (246$\pm $1.732), Melting point (6.3$\pm $0.115), Boiling point (322$\pm $2).

**Conclusion:** This study showed that African mango seed exhibits effective antioxidant properties and contain adequate oil components thus making it fit and edible for consumption.

**Keywords:** African Mango Seed, Antioxidants, Partial oil characterization, insulin sensitivity

**INTRODUCTION**

“Ogbono seed (*Irvingia gabonensis*), is a species of African tree in the genus Irvingia, sometimes known by the common names, African mango or bush mango. These trees are valued for their fats- and protein-rich nuts. Traditionally the leaves of the plant are used for the treatment of diabetes while the seeds are used for ‘African soup’ and is believed to have antidiabetic effects. Based on available experimental evidence, it is proven that the leaves and stem extracts of *Irvingia* regulate blood glucose and lipid profile” (Atanu et al., 2022). “The Bush Mango trees are found in the humid forest zone from the northern tip of Angola including Congo, DR Congo, Nigeria, Ivory Coast and south-western Uganda. They belongs to the family Plantae and the natural order Malpighiales. They are also known as African mango seed (English), ‘Oroto’ (Yoruba), ‘Goronor’ (Hausa) And ‘Ogbono’ (Igbo), bobo in Sierra Leone, andok in Cameroon, boboruor wanini in Ivory Coast and meba or mueba in Zaire” (Olayiwola *et al.,* 2013). “The fruits are consumed by humans and other mammals such as monkeys, gorillas, elephants and many more”. tion (Ekpe *et al.,* 2019).

*“Irvingia gabonensis* has gained attention for its potential role in weight management. Some studies suggest that it may help reduce body weight, and waist circumference, and improve markers of metabolic health. It is believed to work by suppressing appetite, increasing fat breakdown, and reducing fat cell growth” (Oben *et al.,* 2008). “Irvingia gabonensis has been reported to have a positive impact on cholesterol levels and lipid profiles. It may help lower total cholesterol, LDL cholesterol (often referred to as "bad" cholesterol), triglycerides, and increase HDL cholesterol (often referred to as "good" cholesterol). These effects may contribute to improved cardiovascular health” (Ngondi *et al*., 2009). “Preliminary studies suggest that Irvingia gabonensis may have a positive impact on blood sugar control. It may help regulate blood glucose levels and improve insulin sensitivity. This potential benefit could be helpful for individuals with or at risk of developing type 2 diabetes” (Oben *et al.,* 2008). “Irvingia gabonensis possesses antioxidant compounds that can help neutralize harmful free radicals in the body. Antioxidants are important for protecting cells from oxidative stress and reducing the risk of chronic diseases” (Njamen *et al.,* 2005).

“The methanolic extract of the fruit has been used in the treatment of bacterial and fungal infections” (Arogba and Omede, 2012). “In recent years, a novel *Irvingia gabonensis* herbal weight loss dietary supplement appeared in the market” (Sun and Chen, 2012). “*Irvingia gabonensis* seed extract was found to significantly inhibit adipogenesis in adipocyte tissues” (Oben *et al.,* 2008). Furthermore, studies concerning the nutritional, health benefits and overall food potential of the seed have been conducted in recent years.

The objective of this study, which was to characterize the seed regarding their quality parameters, and *in vitro* antioxidant capability. This study aims to validate and provide additional support for the existing information; that could aid commercial production and eventual domestication of these essential fruits.

**MATERIALS AND METHODS**

**Study area:** The study was carried out at the Biochemistry Department Research Laboratory, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria from March to May 2021.

**Sample Collection, Preparation and Extraction:** The sample was collected from Eke Awka market, Awka. The seeds/leaf were ground into fine powder using an electric blender. The ground samples were then stored in an air-tight container for further analysis.

**Reagents:** ABTS Solution (7mM with 2.45mM ammonium persulfate), EDTA (0.1M containing 1.5mg of NaCN), Nitrobluetetrazolium (NBT – 1.5mM), Riboflavin (0.12mM), Phosphate buffer (0.067M, pH 7.6), Sodium nitroprusside (100mM), Phosphate buffered saline (pH 7.4), Griess reagent (1% sulphanilamide, 2% H3PO4 and 0.1% naphthyl ethylenediamine dihydrochloride), Deoxyribose (2.8mM), Ferric chloride (0.1mM), EDTA (0.1mM), H2O2 (1mM), Ascorbate (0.1mM), KH2PO4-KOH buffer (20mM, pH 7.4), Thiobarbituric acid (1%), diethyl ether, Alcohol, aqueous 0.1M NaOH, wijis’ solution, potassium iodine solution, alcoholic potassium hydroxide solution, potassium iodide solution, Ethanol and other materials for analytic purposes

**ANTIOXIDANT**

**Ferric reducing antioxidant power activity**

**Procedure:** 0.25ml of the extracts was mixed with 0.25 of 200nm Sodium phosphate buffer pH 6.6 and 0.25ml of 1% potassium ferrocyanide. The mixture was incubated at 50$℃$ for 20 minutes, thereafter 0.25ml of 10% trichoroacetic acid was added and centrifuged at 2000rpm for 10 minutes. 1ml of the supernant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride. The absorbance was measured at 700nm.

**ABTS Scavenging activity**

The antioxidant effect of the leaf samples was studied using ABTS (2,2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay following the method described by Shirwaikar et al. (2006).

ABTS radical cations (ABTS+) were produced by reacting ABTS solution (7mM) with 2.45mM ammonium per sulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the different samples were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance was read at 745nm using a Spectrophotometer (Genesys 10-S, USA) and the percent inhibition was calculated using the formula

Inhibition (%) =$\frac{(Control – test) }{Control}× 100$

**Superoxide Scavenging activity**

The superoxide scavenging ability of the samples was assessed by the method of Winterbourn et al. (1975).

**Principle:** This assay is based on the inhibition of the production of nitrobluetetrazoliumformazon of the superoxide ion by the sample samples and is measured spectrophotometrically at 560nm.

Superoxide anions were generated in samples that contained 0.02ml of the leaf extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin, in 2.64ml o-phosphate buffer. The control tubes were also set up using DMSO instead of the sample samples. The tubes were vortexed and the initial optical density was measured at 560nm using a spectrophotometer (Genesys, 10-S, USA). Post-measurement, the tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was measured for the superoxide anion scavenging activity.

**Nitric Oxide Scavenging activity**

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of leaf samples (50mg) and incubating at 25$°$C for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546nm against the reagent blank, using a spectrophotometer (Genesys 10-S, USA).

**Hydroxyl Radical Scavenging Activity**

Elizabeth and Rao (1990).

The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of FeCl3, 0.1ml of EDTA, 0.1ml of H2O2, 0.1ml of ascorbate, 0.1ml of KH2PO4-KOH buffer and 20ml of sample samples in a final volume of 1.0ml. The mixture was incubated at 37$°$C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95$°$C for 20 minutes to develop the colour. After cooling, the TBARS formation was measured spectrophotometrically (Genesys 10-S, USA) at 532nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the -absorbance of the control with that of the samples. The percent TBARS production for the positive control (H2O2) was fixed at 100% and the relative per cent TBARS was calculated for the sample treated groups.

**PARTIAL OIL CHARACTERIZATION**

**Determination of Fatty Acid**

**Acid value**

**Procedure:** 25ml of diethyl ether solution was mixed 25ml alcohol and 1ml phenolphthalein (1%) and carefully neutralize with 0.1M NaOH.Additionally, 1-10g of the oil was dissolved in the mixed neutral solvent and titrate with aqueous 0.1M NaOH shaking constantly until pink color persists for 15 seconds.

The acid value of fatty acid was determined using the formula given below:

Acid value = $\frac{titre (ml) x 5.61}{weight of sample used}$

The FFA figure is usually calculated as oleic acid (1ml 0.1M sodium hydroxide = 0.0282g oleic acid), in which case the acid value = 2 x FFA.

For most oils, acidity begins to be noticeable to the palate when the FFA calculated as oleic acid is about 0.5- 1.5 %

For palm oil as palmic (1ml 0.M NaOH = 0.0256g).

For palm kernel, coconut and similar lauric acid (1ml 0.M NaOH =0.0200g).

**Iodine Value**

**Determination of iodine value:**

The oil was poured into a small beaker, added a small rod and weigh out a suitable quantity of the sample by difference into a dry glass-stoppered bottle of about 250ml capacity. The approximate weight in grams of the oil to be taken can be calculated by dividing 20 by the highest expected iodine value. (10ml) of carbon tetrachloride was added to the oil or melted fat and dissolved. (20ml) of wijis’ solution was added, the stopper was inserted (previously moistened with potassium iodine solution) and allowed to stand in the dark for 30 minutes. 15ml of potassium iodine solution (10%) was added to 100ml water, it was mixed and titrated with 0.1M thiosulphate solution using starch as indicator just before the end-point (titration = aml). A blank was carried out at the same time commencing with 10ml of carbon tetrachloride (titration = bml).

Iodine value = $\frac{(b –a) x 1.269}{   wt. (g) of sample}$

**Note:** if (b-a) is greater than b/2 the test must be repeated using a smaller amount of the sample.

It should be noted also that the less unsaturated fats with low iodine values are solid at room temperature, or conversely, oils that are more highly unsaturated are liquid (showing there is a relationship between melting points and the iodine value).

**Preparation of wijis’ solution:**

8gms of iodine trichloride was dissolved in 200ml glacial acetic acid. 9gms of iodine was dissolved in 300ml carbon tetrachloride. The two solutions were mixed and diluted to 1000ml with glacial acetic acid.

**Peroxide Value**

One gram (1g) of oil was weighed into a clean dry boiling tube and 1g of powdered potassium iodide and 20ml of the solvent mixture was added (2 vol glacial acetic acid + 1 vol chloroform). The tube was placed in boiling water and allowed to boil vigorously for not more than 30 seconds. The contents were poured quickly into a flask containing 20ml of potassium iodide solution (5%), and the tube was washed twice with 25ml water and titrate with 0.002M sodium thiosulphate solution using starch. A blank was performed at the same time.

(i) = 7.8D

**Saponification Value**

**Procedure:** Two grams (2g) of the oil or fat were weighed into a conical flask and exactly 25ml of the alcoholic potassium hydroxide solution.A reflux condenser was attached, the flask was heated in boiling water for 1hr, and was also shaked frequently.One milliliter (1ml) of phenolphthalein (1%) solution was added and the excess alkali was titrated with 0.5M hydrochloric acid (titration = a-ml).A blank was carried out at the same time (Titration  = b-ml).

Calculation:

Saponification value = $\frac{(b-a) x 28.05 }{wt. (g) of sample}$

**Specific gravity**

A 50ml pycometer bottle was washed thoroughly with detergent, water and petroleum ether, dried and weighed. The bottle was filled with water and weighed. After drying the bottle, it was filled with the oil sample and weighed.

**Calculation**

Specific gravity = $\frac{weight of Xml oil}{Weight of Xml water}$

**Refractive index**

The Abbe refractometer was reset with a light compensator. The oil sample was seared on the lower prism of the instrument and close. A light was passed by means of the bangled mirror; the reflected light appears in the form of a dark background. The telescope tubes were moved using the fine adjustment until the lack of shadow appears central in the cross-wire indicator. The refractive index was read off.

**Smoke, flash and fire point.**

Ten milliliters volume of the oil was poured into an evaporating dish. A thermometer was suspended at the center of the dish ensuring that the bulb just dips inside the oil without touching the bottom of the dish. The temperature of the oil was gradually raised using a hot plate. The temperature at which the oil sample gives off a thin bluish smoke continuously is noted as the smoke point. Similarly, the temperature at which the oil started flashing without supporting combustion is equally noted as the flash point. The temperature at which the oil starts supporting combustion is recorded as the fire point.

**RESULTS**

**Ferric Reducing Antioxidant Power Activity Result**

The result shows that the African mango seed has a lower activity in ferric-reducing power antioxidant activity compared to the standard garlic acid with higher activity in ferric-reducing power antioxidant activity.

**Figure 1: Graph showing the ferric reducing power antioxidant activity of African mango seed and garlic acid standard.**

**Nitric oxide Scavenging Activity Result**

The result shows that the African mango seed has a lower activity in nitric oxide antioxidant activity compared to the standard garlic acid with higher activity in nitric oxide antioxidant activity.

**Figure 2: Graph showing the nitric oxide scavenging antioxidant activity of African mango seed and garlic acid standard.**

**Superoxide Scavenging Activity Result**

The result shows that the African mango seed has the lower activity in superoxide antioxidant activity compared to the standard garlic acid with higher activity in superoxide antioxidant activity.

**Figure 3: Graph showing the superoxide scavenging antioxidant activity of African mango seed and garlic acid standard.**

**Hydroxyl Radical Antioxidant Activity Result**

The result shows that the African mango seed has a lower activity in hydroxyl radical antioxidant activity compared to the standard garlic acid with higher activity in hydroxyl radical antioxidant activity. From the result, the % scavenging increase at the concentration of 5mg/ml and then there is an increase up to 100mg/ml.

**Figure 4: Graph showing the hydroxyl radical antioxidant activity of African mango seed and garlic acid standard.**

**ABTS Radical Scavenging Activity Result**

The result shows that the African mango seed has the lower activity in ABTS radical scavenging activity compared to the standard garlic acid with higher activity in ABTS radical scavenging activity.

**Figure 5: Graph showing the ABTS radical scavenging antioxidant activity of African mango seed and garlic acid standard.**

**PARTIAL OIL CHARACTERIZATION**

From the Sample, %Free fatty acid, %Acid value, saponification value, peroxide value, the iodine value, the refractive index, the Specific gravity, the viscosity, cloud point, flash point, melting point, boiling point was recorded.

Table 1: PARTIAL OIL CHARACTERIZATION

|  |
| --- |
|  PARAMETERS CONCENTRATION |
| Free Fatty acid (%) 2.092$\pm $0.181Acid value (%) 4.183$\pm $0.363Saponification (mgKOH/kg) 323.51$\pm $14.118Peroxide (mleq/kg) 10.2$\pm $0.2Iodine value 104.860$\pm $0.381Refractive index 1.421$\pm $0.001Specific gravity 0.9301$\pm $5.7753E-05Viscosity 164.3$\pm $2.157Cloud point 12.4$\pm $0.2Flash point 246$\pm $1.732Melting point 6.3$\pm $0.115Boiling point 322$\pm $2 |

**DISCUSSION**

“In this study, the antioxidant activity and partial oil characterization of *Irvingia gabonensis* were studied and evaluated. The investigation of the antioxidant activity was carried out using ferric reducing antioxidant power, nitric oxide scavenging, superoxide scavenging, ABTS scavenging assay. And the result gotten was compared to similar research” (Efosa et al 2016) on the antioxidant activity of *Irvingia gabonensis* using DPPH activity, Reducing power (RP) and total phenolic compound assay and it was observed that African mango seed has strong antioxidant activity in both types of research and is effective in reducing oxidative stress.

“However, further investigation was carried out on the oil extract of African mango seed and the oil was proved to be edible and could be a potential source of vegetable oil. The research was carried out to study the chemical and physical characteristics of the oil extract and the result revealed that African mango seed contained higher Free Fatty acid (%), higher Acid value (%), lower Peroxide value, higher Saponification value, lower Iodine value, lower cloud point, lower flash point, lower melting/ freezing point, in comparison with similar research” (Okonkwo et al, 2014) on the physical and chemical characteristics of African mango seed oil extract.

**CONCLUSION**

This research showed that *Irvingia gabonensis* exhibits effective antioxidant properties and can be recommended as an alternative source of potential antioxidants that can provide an effective means to combat the deleterious effects of reactive oxygen species due to its antioxidant potential and it also contains a good oil component which is edible for consumption.

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Details of the AI usage are given below:

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