**ANTIOXIDANT CONTENT AND PARTIAL OIL CHARACTERIZATION OF AFRICAN MANGO (*Irvingia gabonensis*) SEED**

**ABSTRACT**

**Background and Objective:** African mango seed (*Irvingia gabonensis*) is a species of African trees in the genus Irvingia, sometimes known by the common names, african mango or bush mango. They bear mango-like fruits and are especially 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. This research work is aimed to highlighting the Antioxidant activity and partial oil characterization of African mango seed (*Irvingia gabonensis*). **Materials and Methods:** 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 exhibit a good antioxidant property and also contain good oil component which is fit and edible for consumption.

**KEYWORDS**

African Mango Seed, Antioxidants, Partial oil characterization.

**INTRODUCTION**

Ogbono seed (*Irvingia gabonensis*); is a species of African trees in the genus Irvingia, sometimes known by the common names, african mango or bush mango. They are especially valued for their fats- and protein- rich nuts. They are grown and consumed mostly 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 fruit are often eaten fresh by humans and other mammals such as monkeys, gorillas, elephant and many more. Irvingia gabonensis is another traditionally essential fruit known as oro or oba in Nigeria,. Applications of its fleshy mesocarp can be used in the treatment of gastrointestinal disorder and infection (Ekpe *et al.,* 2019). Methanolic extract of the fruit has been used in the treatment of bacterial and fungal infections (Arogba and Omede, 2012). In recent years, novel Irvingia gabonensis herbal weight loss dietary supplement appeared in the market (Sun and Chen, 2012). Irvingia gabonensis seed extract was significant in inhibiting adipogenesis in adipocytes – an enzyme mediated process of managing obesity (Oben *et al.,* 2008). Indeed, studies have been done on the nutritional, health benefits and overall food potential of the seed. This informs the objective of this study, which was to characterize the seed regarding their quality parameters, and *in vitro* antioxidant capacities. This study is an extension of the existing information; that could aid commercial production and eventual domestication of these essential fruits.

Understanding the composition and characteristics of African Mango seed is essential for exploring its various applications in the fields of food, pharmaceuticals, cosmetics, and biofuels. The oil is primarily extracted from the seed, which contain approximately 34-62% oil content. The composition of the oil is known to vary depending on factors such as the geographical location of the trees, extraction methods, and processing techniques employed.

Irvingia gabonensis has gained attention for its potential role in weight management. Some studies suggest that it may help reduce body weight, 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).

In this study the antioxidant activity and partial oil characterization of *Irvingia gabonensis* are investigated.

**MATERIALS AND METHODS**

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

**Materials:** Beaker, Spatula, Conical flask, Filter paper, Test tubes, Refrigerator, Distilled water , Measuring cylinder, Test tube rack, Stop watch, Centrifuges, Pipette, Manual grinder, Water bath, Spectrophotometer, Weighing balance.

**Sample Collection, Preparation and Extraction:** The sample was collected from Eke Awka market, Awka. It was grounded into fine powder using electric blender. The ground samples were stored in an air-tight container for the analyses.

**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% naphthylethylenediaminedihydrochloride), 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**

**Principle:** The principle of the assay is the quantification of ferric degradation product, by its condensation with the extract.

**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 will be added and centrifuge at 2000rpm for 10 minutes, 1ml of the supernant will be mixed with 1ml of distilled water and 0.2ml of ferric chloride and 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 decolourisation assay according to the method of Shirwaikar et al. (2006).

**Reagent:** ABTS Solution (7mM with 2.45mM ammonium per sulfate)

**Procedure:** 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 in a spectrophotometer (Genesys 10-S, USA) and the per cent 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.

**Reagents:**

1. EDTA (0.1M containing 1.5mg of NaCN)
2. Nitrobluetetrazolium (NBT – 1.5mM)17
3. 3.Riboflavin (0.12mM)
4. Phosphate buffer (0.067M, pH 7.6)

**Procedure:** Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf samples (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml o phosphate buffer. The control tubes were also set up where DMSO was added instead of the sample samples. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). 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 indicative of superoxide anion scavenging activity.

**Nitric Oxide Scavenging activity**

The extent of inhibition of nitric oxide radical generation *in vitro* was followed as per the method reported by Green et al. (1982).

**Principle:** Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546nm.

**Reagents:**

1. Sodium nitroprusside (100mM)

2. Phosphate buffered saline (pH 7.4)

3. Griess reagent (1% sulphanilamide, 2% H3PO4 and 0.1% naphthylethylene

diaminedihydrochloride)

**Procedure:** The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of leaf samples (50mg) and incubated 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, in a spectrophotometer (Genesys 10-S, USA).

**Hydroxyl Radical Scavenging Activity**

The extent of hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao (1990).

**Principle:** The principle of the assay is the quantification of 2'-deoxyribose degradation product, malondialdehyde, by its condensation with thiobarbituric acid.

**Reagents**

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%)

**Procedure:** 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 per cent TBARS production for 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:** Twenty five milliliters (25ml) of diethyl ether solution was mixed 25ml alcohol and 1ml phenolphthalein (1%) and carefully neutralize with 0.1M NaOH.Exactly 1-10g of the oil was dissolved in the mixed neutral solvent and titrate with aqueous 0.1M NaOH shaking constantly until pink colour which persists for 15 seconds is obtained.

Calculation:

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, add 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 g of the oil to be taken can be calculated by dividing 20 by the highest expected iodine value. Ten milliliters (10ml) of carbon tetrachloride was added to the oil or melted fat and dissolve. Twenty milliliters (20ml) of wijis’ solution was added, the stopper was inserted (previously moistened with potassium iodine solution) and allow to stand in the dark for 30 minutes. Fifteen milliliters (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:**

Eight grams (8g) of iodine trichloride was dissolved in 200ml glacial acetic acid. Nine grams (9g) of iodine was dissolved in 300ml carbon tetrachloride. The two solutions were mixed and diluted to 1000ml with glacial acetic acid.

**Peroxide Value**

**Procedure:** One gram (1g) of oil was weighed into a clean dry boiling tube and 1g of powdered potassium iodide and 20ml of 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 was poured quickly into a flask containing 20ml of potassium iodide solution (5%), and the tube was washed twice with 25ml water and tirate 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 was weighed into a conical flask and added 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 milliliters (1ml) of phenolphthalein (1%) solution was added and the excess alkali was titrated with 0.5M hydrochloric acid (titration = aml).A blank was carried out at the same time ( titration  = bml).

Calculation:

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

**Specific gravity**

A 50ml pycometer bottle 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 form of a dark background. The telescope tubes was moved using the fine adjustment until the lack shadow appears central in the cross wire indicator. The refractive index was read off.

**Smoke, flash and fire point.**

Ten milliliters (10ml) volume of the oil was poured into an evaporating dish. A thermometer was suspended at the centre of the dish ensuring that the bulb just dips inside the oil without touching the bottom of the dish. The temperature of oil was gradually raised using 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 the 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: Result 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 the lower activity in nitric oxide antioxidant activity compared to the standard garlic acid with higher activity in nitric oxide antioxidant activity.

**Figure 2: Result 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: Result 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 the 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: Result 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: Result 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.

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| --- |
|  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 a similar research (Efosa *et al* 2016) on antioxidant activity of irvingia gabonensis using DPPH activity, Reducing power (RP) and total phenolic compound assay and it was observed that that African mango seed has strong antioxidant activity in both researches 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 a 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 exhibit a good 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 contain a good oil component which is edible for consumption.

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