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# Evaluation of Nutritional and Anti-nutritional Compositions of Leaves of *Gingko biloba* (Maiden Hair) Tree Found in Nigeria

**[*]Nwosu OK[1,2], Ubaoji KI[1] and Okaka ANC[1]**

[1] Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[2] National Biosafety Management Agency, Abuja, Nigeria.

***Author for Correspondence:** nwosuonyeka6@gmail.com

**ABSTRACT**

*Ginkgo biloba* (GB) leaves and extracts have been recognized worldwide for its renowned nutritional and medicinal properties. GB tree is highly grown and used in Asian and South American countries unlike Nigeria. In this study, we examined the nutritional and anti-nutritional compositions of GB leaves (whole dried, and aqueous and ethanol extracts) grown in Nigeria. Association of Official Analytical Chemists (AOAC) method was used to determine the proximate and vitamin compositions while Atomic Absorption Spectrophotometer (AAS) was used to analyze the minerals. Anti-nutrients analysis was done using gas chromatography. The results of analyses on whole dried leaves showed that the nutritional composition was high in carbohydrate (59.70±1.02mg/100g) and energy value (287.00±2.59Kcal/g) and low in protein (6.65±0.38mg/100g), lipid (2.40±0.14mg/100g), fibre (2.50±0.21mg/100g) and moisture (15.65±0.38mg/100g). High concentrations was also observed for vitamins A (79.75±9.05), C (79.20±2.56) and E (59.31±2.84) while vitamins $B_1$, $B_2$, $B_3$, $B_6$ and $B_{12}$ (1.53±0.04, 2.98±0.62, 2.44±0.25, 3.57±0.24 and 0.28±0.01 mg/100g) respectively, were found in lower concentrations. The mineral analysis also showed high concentrations in macro minerals especially calcium and magnesium (24.620±0.410 and 18.450±0.28mg/100g) followed by phosphorous (4.896±0.003mg/100g), potassium (4.332±0.000mg/100g), and sodium (2.340±0.001mg/100g). The concentrations in micro minerals were iron (6.667±0.003mg/100g), zinc (1.851±0.001mg/100g), manganese (0.626±0.025mg/100g), copper (0.640±0.000mg/100g) and selenium (0.391±0.003mg/100g). However, the absence of molybedenum (Mo) in the leaves was observed. The calcium/phosphorous and sodium/potassium ratios were 5.029±0.087 and 0.540±0.000 mg/100g respectively. The anti-nutrients analysis of the aqueous and ethanol extracts of the leaf showed low concentrations of phytate, oxalate and tannin. The low concentrations observed are considered to be non-toxic to man. These investigations have revealed the nutritional potentials of *Ginkgo biloba* leaves grown in Nigeria. The findings therefore, will be useful for nutritional and medical practice in Nigeria and beyond for maintenance of good health of individuals.

**Keywords:** *Ginkgo biloba*, Nutritional compositions, Anti-nutritional factors, Gas chromatography, Atomic absorption spectrophotometer.

## INTRODUCTION

Green leafy medicinal plants are occupying an important role among the food and medicinal crops as they provide adequate amount of many nutritional substances for human (Achikanu et al. 2013). They constitute an indispensable constituent of nutraceuticals in Africa and West Africa in particular. It is worthwhile to note that consumption of such green leafy medicinal plants as nutraceuticals could be beneficial to nutritionally and health marginal population especially in developing countries where poverty and climate is causing havoc to the rural populace.

The nutritional properties of plant leaves are attributed to macro molecular (carbohydrate, lipid and protein) contents and other micro-molecules like vitamins, minerals etc. present in it. These chemicals are said to be nutritional as they are adequately required from our food consumptions in order to maintain healthy condition. The development of such medicinal plants into drugs or food for the utilization of such chemicals is now recognized globally, and such a drug, food or supplement is regarded as nutraceuticals (Bieselkl, 2001). In recent years, there has been an explosion of interest regarding plants, their medicinal and nutritional properties, and Africa has not been left out. Indeed, the African herbal medicine constitutes an important source for ethnopharmacological research (Uraku et al. 2015).

*Gingko biloba* also known as "Maiden hair tree" has been described as a living fossil, being last remaining living member of the *Ginkgoaceae* family. *Gingko biloba* tree is indigenous to Asia (Korea, China, India and Japan) and South America. It is popular for lining the streets and parks, although its seed are reported as known to give off a foul odour when ripe (Defeudis, 1998). There are several claims made about the plant beneficial effects to human. Scientific evidence has been used to support some of these claims whereas others currently remain only but legend. The standardized extract of the leaves popularly known as EGb 761, has been shown to exert numerous therapeutic effects for human health and many papers have cited studies utilizing this standardized extract. Among the therapeutic effects as reported by Koch (2005) are the prevention and treatment of thrombosis, illness of blood vessel of heart and brain, asthma, bronchitis and allergic reactions. Generally, clinical studies indicate use of the *Gingko* extract in the treatment of poor circulation, impotence, heart diseases, eye diseases, chronic cerebral insufficiency, short term memory loss (like Alzheimar's diseases), depression, dementia (Kwon et al. 2004). It as well acts as an aphrodisiac (Malviya et al. 2011).

This paper was designed to quantify the proximate, vitamins, minerals and anti-nutrient factors of *Gingko biloba* grown in Enugu State, South-East Nigeria.

## MATERIALS AND METHOD

### Collection and Preparation of the Plant Leaves

Healthy fresh leaves of *Gingko biloba* were collected from *Gingko biloba* tree at Park Avenue, GRA, Enugu, Enugu State, Nigeria in a large quantity. The plant was identified and authenticated by Prof. Okeke, C.U., a plant taxonomist in the Botany Department of Nnamdi Azikiwe University, Awka. The voucher specimen was deposited in the Hebarium of the Department. Healthy leaves of the plant were removed from plant stalk, and were dried at room temperature (20 to 23$^0$C) for 3weeks. The dried plant leaves were ground to fine powder with local steel grinder, packaged in air tight container and stored at 4$^0$C prior to nutritional analysis. For the anti-nutritional analysis, aqueous and ethanol extracts was obtained. To obtain an aqueous extract, 15g of the ground leaves was mixed with 150ml of distilled water. Fifteen (15g) of the ground leaves was also mixed with 80% (v/v) ethanol in order to obtain an ethanol extract. Both mixture was refluxed in a water bath at 65$^0$C for 1 hour and filtered using Whatman filter paper No 1 followed by the evaporation of the filtrate using a rotary evaporator.

### Proximate Analysis

The proximate analysis to determine the moisture content, protein, fibre and fat in the *Gingko biloba* leaves was conducted using AOAC (2005) method. Carbohydrate content was determined by difference method (calculated by subtracting the sum of crude protein, crude fat, crude fiber and ash from total dry matter content). The energy value of *Gingko biloba* leaves was determined by multiplying the carbohydrate, protein and fat by the At water conversion factors of 4, 4 and 9 respectively (Food and Agriculture Organization, 1998). The values of the multiplication are sum up to give the energy value of the sample.

### Vitamin Analysis

Vitamin analysis to determine the content of provitamin A carotenes, Ascorbic acid (Vitamin C), Tocopherol (Vitamin E), thiamine (Vitamin $B_1$), riboflavin (Vitamin $B_2$), niacin (Vitamin $B_{3)}$, pyridoxine (Vitamin $B_6$) and cobalamine (Vitamin $B_{12}$) in the *Gingko biloba* leaves was conducted using AOAC (2005) method.

### Mineral Analysis

Macro and micro mineral analyses to determine the content of calcium, magnesium, sodium, potassium, copper, iron, zinc, molybdenum, manganese and selenium were analyzed using Varian AA240 Atomic Absorption Spectrophotometer (AAS) according to the method of APHA (2005)

**Atomic Absorption Spectrophotometer (AAS) Working Principle*:*** Atomic absorption spectrophotometer working principle is based d

on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiation interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

**Preparation of the Reference Solution:** A series of standard metal solutions in the optimum concentration range was prepared, the reference solutions were prepared daily by diluting the single stock element solution with water containing 1.5ml concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

**Phosphorous Determination:** Phosphate was measured using standard method as explained by Pradhan and Pokhrel (2013).

**Anti-nutrients Analysis**

Anti-nutrients (phytate, oxalates and tannin) were quantified in aqueous and ethanol extracts of *Gingko biloba* leaves. The quantification was done using Gas Chromatography fitted with Flame Ionization Detector (GC-FID) employing Martin and Synge (1941) method and modified by Ujowundu et al. (2015).

**Conditions for the Quantification:**

The quantification of the anti-nutrients was performed on a BUCK M910 Gas Chromatograph equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15 x 250 x 0.15µm) was used. The injector temperature was 2800$^0$C with splitless injection of 2µl of the sample and a linear velocity of 30cms-1. Helium 5.0pa.s was the carrier gas with a flow rate of 40mlmin-1. The oven operated initially at 2000$^0$C, it was heated to 3300$^0$C at a rate of 30C min-1 and was kept at this temperature for 5 minutes. The detector operated at a temperature of 320$^0$C. The anti-nutrients were then determined by the ratio between the area and mass of internal standards and the area of the identified anti-nutrients. The concentration of the different anti-nutrients was expressed in µg/ml.

## RESULTS

The data obtained in this study were evaluated using Statistical Package for Social Sciences (SPSS). The values were expressed as a means of triplicate determination ± standard deviation.

**Table 1: Proximate Composition of *Gingko biloba* Leaves**

| Constituents | Composition (mg/100g) |
|---|---|
| Moisture | 15.65 ± 0.28 |
| Ash | 13.10 ± 0.00 |
| Crude Fibre | 2.50 ± 0.21 |
| Total Lipid | 2.40 ± 0.14 |
| Crude Protein | 6.65 ± 0.38 |
| Total Carbohydrate | 59.70 ± 1.02 |
| Energy (Kcal/g) | 287.00 ± 2.59 |

*Values are means ± standard deviation of triplicate determination*

**Table 2: Vitamin Composition of *Gingko biloba* leaves**

| Vitamins | Concentration (mg/100g) |
|---|---|
| Provitamin A Carotenes | 79.75 ± 9.05 |
| Ascorbic acid | 79.20 ± 2.56 |
| Tocopherol | 59.31 ± 2.84 |
| Thiamine | 1.53 ± 0.04 |
| Riboflavin | 2.98 ± 0.62 |
| Niacin | 2.44 ± 0.25 |
| Pyrodoxine | 3.57 ± 0.24 |
| Cobalamine | 0.28 ± 0.01 |

*Values are means ± standard deviation of triplicate determination.*

**Table 3: Mineral Composition of *Ginkgo biloba* leaves**

| | Minerals | Concentration (mg/100g) |
|---|---|---|
| Macro Minerals | Calcium (Ca) | 24.620 ± 0.410 |
| | Magnesium (Mg) | 18.450 ± 0.283 |
| | Potassium (K) | 4.332 ± 0.000 |
| | Sodium (Na) | 2.340 ± 0.001 |
| | Phosphorous (P) | 4.896 ± 0.003 |
| Micro Minerals | Iron (Fe) | 6.667 ± 0.003 |
| | Zinc (Zn) | 1.851 ± 0.001 |
| | Manganese (Mn) | 0.626 ± 0.025 |
| | Molybedum (Mo) | - -- - |
| | Copper (Cu) | 0.640 ± 0.000 |
| | Selenium (Se) | 0.391 ± 0.003 |
| | Ca/P | 5.029 ± 0.087 |
| | Na/ K | 0.540 ± 0.000 |

*Values are mean ± standard deviation of triplicate determination*

**Table 4: Anti-nutrients content of *Ginkgo biloba* aqueous and ethanol extracts**

| | Anti nutrients Concentration (µg/ml) | | |
|---|---|---|---|
| | Phytate | Oxalates | Tannin |
| Aqueous Extract | 0.586 ± 0.03 | - | 7.986 ± 0.04 |
| Ethanol Extract | 0.555 ± 0.01 | 0.650 ± 0.03 | 7.430 ± 0.02 |

*Values are means ± standard deviation of triplicate determination*

## DISCUSSION

Table 1 showed the macro nutrient analysis of *Gingko biloba* leaf sample. The analysis was carried out with the intention of providing a better understanding on the base nutrient constituents in *Gingko biloba* leaves grown in Enugu, Nigeria. It can be observed from the table that carbohydrate have the highest amount (59.70±1.02mg/100g) and the total fat or lipid with the lowest amount (2.40±0.14mg/100g). The high carbohydrate content of Nigerian grown *Gingko biloba* leaves suggest that it can be considered as a potential source of energy. The lower amount of fat in the leaves confers a more healthy character to the medicinal plant such that it may

be useful for individuals on weight reducing diet. The content of the protein recorded in this study may suggest that the leaves can be a potential source of plant protein and if used as nutraceutical, can be a protein supplement. The energy value of the sample was high and quite appreciable for a nutraceutical. Andzouana and Mombouli (2012) reported that high energy content plant leaves can serve as supplements which assures an adequate food security for the locality. The result is in consonance to the reports of Pereira et al. (2013) who indicated a low amount of total fat in *Gingko biloba* leaves obtained in Portugal. The ash content was also similar to the work of Salem et al. (2015) who reported the ash content of *Gingko biloba* leaves from Egypt to be 16.44%. They also reported high content of carbohydrate and minimal amount of crude protein (3.93%) and total lipid (4.86%). These results are also similar to the results of this study.

The results of the vitamin compositions of *Gingko biloba* leaf as shown in Table 2 indicated appreciable vitamins A, C and E contents in the plant leaves. Vitamin C and E are potent natural antioxidants that scavenge free radicals and ameliorate their deleterious effects. Vitamin C apart from being a potent antioxidant, facilitates the transport and uptake from non-heme iron at the mucosa, the reduction of folic acid intermediates and the synthesis of cortisol. Its deficiency is fragility to blood capillaries, scurvy and gum decay (Bender, 2009). Vitamin E also plays a vital role in the formation and normal functioning of red blood cells and muscles (Lusaki, 2004). Certainly, the high content of Vitamin C and E in *Gingko biloba* leaves contributed to a greater percentage in the highly affirmed antioxidant properties of *Gingko biloba* leaf extracts. Vitamin A from plant source is known as Provitamin A Carotenoids or Carotenes that must be converted by the body into Vitamin A (Retinol) before they can be used. Of all the various carotenes, beta-carotene is one that is most easily converted to Vitamin A (Lusaki, 2004). Vitamin A is important for normal vision and its high content in the sample supports the potential effect of *Gingko biloba* extract in the treatment of cataract as studied by Salem et al. (2015). Among the many other acclaimed t. The benefits of *Gingko iloba* is that it significantly improves long distance vision and may reverse damage of the retinol of the eye. Vitamin A content in this study supports such claim. The vitamin B-complex in *Gingko biloba* leaves is also significant. The vitamin B-complex is highly beneficial to growth, digestion, stimulation of appetite and control of nerve disturbances.

Table 3 showed the composition of minerals present in *Gingko biloba* leaf grown in Nigeria. Among the macro minerals, calcium has the highest amount whereas sodium has the lowest amount. Calcium plays a great amount of vital roles in human as it functions as compositions of bones and teeth, activates large number of enzymes like ATPase, lipase etc (Soetan et al. 2010) and together with potassium regulates the nerve and muscle function thereby may have contributed a lot towards the mental functioning activity of *Gingko biloba* leaf extracts. Sodium regulates plasma volume and acid balance, thereby maintaining membrane potential, transmission of nerve impulse (Aremu and Ibrahim, 2014). The presence of sodium in the leaves may have contributed towards the benefits of *Gingko biloba* leaf extracts to some diseases like Parkinson disease, Alzheimer and mental function. Magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis and maintenance of electrical potential of the nervous tissues and cell membranes (Mohammed and Sharrif, 2011). Phosphorous is highly concerned with many metabolic processes including those involving buffers in the body fluid. It is virtually located in every cell in the body. Table 3 also showed the concentration of micro mineral present in *Gingko biloba* leaf of Nigerian origin. Their amount in the sample is in the order of Fe> Zn> Cu> Mn> Se. However, the analysis showed the absence of molybdenum. Iron is part of haemoglobin, myoglobin and cytochromes and Selenium is an antioxidant. Therefore, this study may have suggested *Gingko biloba* ability to aid in blood synthesis and helps prevent oxidative stress and inflammation (Arinola, 2008). High phosphorous content in diet may promote the loss of calcium in urine (Arinola,

2008). If Ca/P ratio is low (i.e. low calcium and high phosphorous intake), more than normal amount of calcium may lose in the urine thereby decreasing the functionalities of calcium in the body. As reported by Aremu et al. (2013), food with Ca/P ratio above 1 is "good" while if the ratio is less than 0.5, it is poor. The results of this study as shown in Table 3, conforms to this rule. The sodium to potassium (Na/K) ratio is recommended for the prevention of high blood pressure if it is less than 1 (Nieman et al. 1992). Hence *Gingko biloba* leaves would probably reduce high blood pressure disease because Na/K ratio less than 1 (0.540 ± 0.000).

Anti-nutritional factors reduce the maximum utilization of nutrients like vitamins, minerals and proteins, thus reducing the optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Fekadu et al. 2013). The anti-nutritional composition in aqueous and ethanol leaf extracts as shown in Table 4 indicated that Tannin was highest in both extracts, however it was higher in aqueous extract (7.986 ± 0.04) than in ethanol extract (7.430 ± 0.02). Adeparusi (2001) reported that tannin affects the bioavailability of non-heme iron and protein and carbohydrate digestibility thereby leading to poor iron and calcium absorption, inadequate supply of protein and reduced energy value of a diet containing tannin. However, the tannin observed in this study may not be significant and toxic since the acceptable tannic acid daily intake for man is 56gμg/g or ml as reported by Fekadu et al. (2013). Phytate and oxalates were found in very low concentrations in both extracts, however oxalate was absolutely not observed in the aqueous extract. Ethanol may have been a better solvent for extraction of oxalate in plant leaves. Phytate and oxalates causes low absorption of minerals by binding to some essential mineral nutrients especially calcium (Bello et al. 2008). Oxalates when bind with calcium can lead to formation of kidney stones (Fekadu et al. 2013) as majority of urinary stones formed in humans are calcium oxalate stones (Gemade and Ratta, 2014). Massey et al. (2001) and Elinge et al. (2012) advised individuals to limit their intake of foods with total intake of oxalate not exceeding 5-6μg/g or ml and phytate not exceeding 1-6μg/g or ml respectively. Phillipy et al. (2004) on a positive approach indicated the evidence that dietary phytate at low level may have beneficial role as an antioxidant and in controlling atherosclerosis. The very little concentrations of phytate and oxalate in this study may have proved that *Gingko biloba* leaves grown in Nigeria is nutritionally acceptable.

## CONCLUSION

In conclusion, this study has revealed that leaves of *Gingko biloba* Tree grown in Nigeria are potential sources of macro and micro nutrients highly needed by every individual, as it also does not contain in toxic amount, the anti-nutritive factors that impairs nutrient absorption and metabolism. The importance of these nutrients cannot be over emphasized for effective maintenance of individual's health.

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