



Full Length Research Paper

## Proximate Analysis And Phytochemical Composition Of Fresh And Dried Fruit Of *Morinda Lucida*

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### ABSTRACT

The numerous ethno-medicinal properties of *Morinda lucida*, an evergreen rainforest medium-sized tree also known as Brimstone tree, have called for a serious research investigation of all the various parts, including the fruits, in order to promote maximum utilization of the plant. In this present study therefore, the nutritional (proximate) and phytochemical evaluation of the fresh and dried fruit of *Morinda lucida* were carried out using standard methods such as AOAC methods, Macro kjedahl's method etc, in order to determine the nutritional potential of the plant. The result of the proximate analysis showed that the dried fruit of *Morinda lucida* contains in percentage (%), 3.23±0.04 moisture, 4.25±0.35 ash, 4.98±0.05 crude protein, 27.80±0.14 crude fiber, 16.95±0.21 total lipids and 42.79±0.11 total carbohydrate while the fresh fruit contains in percentage (%), 70.15±2.62 moisture, 2.55±0.07 ash, 3.88±0.05 crude protein, 4.61±0.57 crude fiber, 2.27±0.09 total lipids and 15.58±0.46 total carbohydrate. The phytochemical analysis showed that the dried fruit of *Morinda lucida* contains phytate, alkaloids, oxalate, saponins and cardiac glycosides with mg/100g values of 140±0.02, 3310±0.47, 1336±0.91, 4450±0.78 and 3910±0.13 respectively while the fresh fruit contains 60±0.00 mg/100g phytate, 470±0.02mg/100g alkaloid, 488±0.17mg/100g oxalate, 1710±0.13mg/100g saponin and 1950±0.78mg/100g cardiac glycoside. The tannin content of the dried fruit was 1496±0.36 mg/100g. However, the fresh fruit did not have a detectable amount of tannin. The observation that equal grams of the dried fruit had higher concentrations of the nutrients and Phytochemicals compared to the fresh fruit may be attributed to the fact that drying of fruits concentrates the fiber and phytochemicals in them. These results have shown that the fruits of *Morinda lucida* have pharmacologically active compounds and is also nutritionally potent and as such, could be used in ethno-medicine as well as an alternative source of feed for animals.

Key Words: proximate analysis, anti-nutrients, *Morinda lucida*

## INTRODUCTION

Nutrition is one key to developing and maintaining a state of health that is optimal to an individual. A poor diet coupled with a sedentary lifestyle are known to be risk factors for life threatening chronic diseases and death (Wardlaw and Kessel, 2002). Food provides both the energy and the nutrients needed to build and maintain all body cells. The various classes of nutrients found in food are: carbohydrate, lipids (fats and oils), proteins, vitamins, minerals and water. The ultimate source of these important nutrients is plants. Plants play significant roles in the lives of both humans and animals; from providing basic needs such as food, shelter and clothing to being used as herbal medications (Addy *et al.*, 2013).

The increasing population of many tropical countries has led to the awareness of the importance of some plants as sources of essential nutrients owing to their high vitamins, minerals, essential fatty acids, crude protein and fiber content. Although conventional food plants have the capacities of providing most of the nutrients needed for energy, body building, maintenance and regulation of body processes, the need to explore some seemingly unappealing sources of nutrients have become imperative. This is due to the serious threat to growth, development and survival posed by increasing human population, food scarcity, insecurity and economic crisis in most developing countries like Nigeria. Therefore, the search for wild edible food plants for human consumption has been identified to maintain a balance between population

growth and agricultural productivity (Enemchukwuet *al.*, 2015).

In order to face the problem of food scarcity, fruits can serve as a good source of nutrients and food supplements. Fruits are commonly well known for the excellent source of nutrients such as minerals and vitamins; and also contain carbohydrates in form of soluble sugars, cellulose and starch. Fruits are very vital portion of an adequate diet and they serve as food supplement, and an appetizer (Desai and Gaikwad, 2010).

Medicinal plants are of great importance to the health of individuals and communities (Enemchukwu *et al.*, 2015). The tropic is enriched with many useful plant resources. *Morinda lucida Benth(Rubiaceae)*, an evergreen rainforest medium-sized tree also known as Brimstone tree is one of the most popular medicinal plants widely distributed in Africa. The different parts of *Morinda lucida* are used across Africa for various purposes such as dye, flavorings, timber, charcoal, cleaning agent, etc. The fruits are also edible. The most extensive use of *Morinda lucida* is as a medicinal plant. It is used among traditional healers to treat fever, dysentery, abdominal colic and intestinal worm infestation, diabetes, hypertension and gonorrhoea. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth (Adejo *et al.*, 2015).

Phytochemical studies have shown that *Morinda lucida* is a natural resource rich in anthraquinones like oruwacin, oruwal, 3-

hydroxyanthraquinone-2-carboxyaldehyde, 1,3-dihydroxy-2-methylanthraquinone, 1,3-dihydroxyanthraquinone-2-carboxyaldehyde and many others. *Morinda lucida* has also been shown to contain in addition to nutrients, certain phytochemicals known as anti-nutrients or anti-nutritional factors. Anti-nutritional factors are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods and they play a vital role in determining the use of plants for humans (Gemede and Ratta, 2014). These anti-nutrients include tannins, phytate, oxalate, alkaloids, saponins and cardiac glycosides. Though the anti-nutrients present in plants reduce the optimum nutrition obtained from the plant by several mechanism during absorption, they also exert medicinal effects. Thus, quantification of the anti-nutritional content of plants is very important.

However, most research works that has been carried out on *Morinda lucida* has been done using the leaves, stem, bark and roots. There is paucity of literature on the proximate, phytochemical and anti-nutrient compositions of the fruit of *Morinda lucida*. The provision of empirical data on nutritional values of the fruit of *Morinda lucida* is desirable as it would go a long way in creating awareness and improve the consumption rate of this fruit. Therefore, the aim of this study was to evaluate the proximate and phytochemical composition of the fresh and dried fruit of *Morinda lucida* to determine its utility as an edible and medicinal part of the plant.

## MATERIALS AND METHODS

### *Collection, Identification and Preparation of fruit samples*

Fresh fruits of *Morinda lucida* were obtained from the environment of Department of Botany Laboratory building, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The fruit was identified and authenticated at the Department of Botany, Nnamdi Azikiwe University, Awka by Mr Tooohukwu Egboka, a taxonomist. For the dried sample, the fruits were sliced, air dried, ground to powder using a manual grinding machine, sieved and stored in an airtight jar to be used for analysis. For the fresh sample, the fruits were sliced into bits and used as such.

### *Proximate analysis*

Moisture, ash and fibre content of the samples were determined by the methods of the Association of Official and Analytical Chemists (A.O.A.C), 2005 while crude lipid content was carried out by Soxhlet method. Nitrogen and Crude protein were determined by Macro- Kjeldahl method. The total carbohydrate was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100 (Usunobun *et al.*, 2015)

### *Phytochemical Constituents*

The tannin content of the samples were determined by spectrophotometric method (AOAC, 1995) in which One gram of the sample was extracted with 10ml of 70%

ethanol and centrifuged at 2500rpm for 5 minutes to remove the residue. The supernatant (0.5ml) was diluted with 4.5ml of distilled water. This was followed by the addition of 0.5ml of 0.1M FeCl<sub>3</sub> and 0.3ml of 0.1M potassium ferrocyanate. Distilled water (6ml) was added to dilute the whole mixture and the absorbance was read at 720nm using Spectrophotometer. The other phytochemical constituents of the plants were determined according to the following methods: Saponins determination was carried out using the AOAC method of 1995 in which two different solvents were used. The first solvent, acetone, was used to extract crude lipid from the samples while the second solvent (methanol) was used for the extraction of the saponin proper. Cardiac glycoside determination was carried out using a method described by Osagie (1998) in which One millilitre (1ml) of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate is observed) and the boiled sample was filtered. The weight of the filter paper was taken before filtration. The filter paper with the absorbed residue was dried in an oven at 50°C till dryness and weight of the filter paper with residue was noted. Phytic acid determination was carried out using Young and Greaves method (1940) in which the sample, (0.2g) of each of the fresh and dried fruit was weighed into different 250ml conical flasks. Each sample was soaked in 100ml of 2% concentrated HCL for 3h. The

samples were then filtered. 50ml of each filtrate was placed in 250ml beaker and 100ml distilled water added to each sample. 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per ml. Alkaloid determination was carried out using a method described by Harbone (1973) while oxalate determination was by a method described by Osagie (1998) which involves three stages namely: Digestion, Oxalate precipitation and permanganate titration.

## RESULTS

The Proximate composition of the dried and fresh fruit of *Morinda lucida* is shown in Table 1. Percentage compositions were expressed as mean  $\pm$  standard deviation of triplicate determination. The percentage ash, crude protein, crude fiber, total lipids and carbohydrate content of the dried fruits are with values 4.25 $\pm$ 0.35, 4.98 $\pm$ 0.05, 27.80 $\pm$ 0.14, 16.95 $\pm$ 0.21 and 42.79 $\pm$ 0.11 respectively which were higher when compared to that of the fresh fruit which has 2.55 $\pm$ 0.07% ash, 3.88 $\pm$ 0.05% crude protein, 4.61 $\pm$ 0.57% crude fiber, 2.27 $\pm$ 0.09% total lipids and 15.58 $\pm$ 0.46% carbohydrate. Moisture content was higher in the fresh fruit (70.15 $\pm$ 2.62%) compared to that of the dried fruit (3.23 $\pm$ 0.04%).

**Table 1: Proximate Composition of dried and fresh fruit of *Morinda lucida*** (Values were expressed as mean  $\pm$  standard deviation of triplicate determinations.)

Parameters	% Composition	% Composition
	<b>Dried fruit</b>	<b>Fresh fruit</b>
Moisture (%)	3.23 $\pm$ 0.04	70.15 $\pm$ 2.62
Ash (%)	4.25 $\pm$ 0.35	2.55 $\pm$ 0.07
Crude protein (%)	4.98 $\pm$ 0.05	3.88 $\pm$ 0.05
Crude fiber (%)	27.80 $\pm$ 0.14	4.61 $\pm$ 0.57
Total lipids (%)	16.95 $\pm$ 0.21	2.27 $\pm$ 0.09
Total Carbohydrate (%)	42.79 $\pm$ 0.11	15.58 $\pm$ 0.46

Phytochemical composition of the dried and fresh fruit of *Morinda lucida* is shown in Table 2. The values were expressed as mean  $\pm$  standard deviation of triplicate determination. The phytate, alkaloid, oxalate, saponin and cardiac glycoside content of the dried fruit, with mg/100g values of 140 $\pm$ 0.02, 3310 $\pm$ 0.47, 1336 $\pm$ 0.91,

4450 $\pm$ 0.78 and 3910 $\pm$ 0.13 respectively were higher when compared to that in the fresh fruit, which has 60 $\pm$ 0.00 phytate, 470 $\pm$ 0.02 alkaloid, 488 $\pm$ 0.17 oxalate, 1710 $\pm$ 0.13 saponin and 1950 $\pm$ 0.78 cardiac glycoside. The tannin content of the dried fruit was 1496 $\pm$ 0.36 while that of the fresh fruit was not detected.

**Table 2: Phytochemical Composition of the dried and fresh fruit of *Morinda lucida*** (Values were expressed as mean  $\pm$  standard deviation of triplicate determinations)

Phytochemical	Concentration (mg/100g)	Concentration (mg/100g)
	<b>Dried fruit</b>	<b>Fresh fruit</b>
Phytate	140 $\pm$ 0.02	60 $\pm$ 0.00
Alkaloid	3310 $\pm$ 0.47	470 $\pm$ 0.02
Oxalate	1336 $\pm$ 0.91	488 $\pm$ 0.17
Saponin	4450 $\pm$ 0.78	1710 $\pm$ 0.13
Cardiac glycoside	3910 $\pm$ 0.13	1950 $\pm$ 0.78
Tannin	1496 $\pm$ 0.36	Not detected

## DISCUSSION

Proximate and phytochemical analyses are very useful in the evaluation of the nutritional and medicinal value of fruits, seeds, leaves and other parts of plants. The present study assessed the proximate as well as the phytochemical composition of the fresh and dried fruit of *Morinda lucida*. The results of the proximate analysis indicated that the fruit of *Morinda lucida* has appreciable amounts of nutrients. The ash, crude protein, crude fiber, total lipids and carbohydrate content of the dried fruit, with percentage values  $4.25 \pm 0.35$ ,  $4.98 \pm 0.05$ ,  $27.80 \pm 0.14$ ,  $16.95 \pm 0.21$  and  $42.79 \pm 0.11$  respectively were higher compared to that of the fresh fruit which has  $2.55 \pm 0.07$  ash,  $3.88 \pm 0.05$  crude protein,  $4.61 \pm 0.57$  crude fiber,  $2.27 \pm 0.09$  total lipids and  $15.58 \pm 0.46$  carbohydrate. The observation that equal grams of the dried fruit had higher concentrations of the nutrients compared to the fresh fruit can be explained by the fact that drying of fruits concentrates the fiber and phytochemicals in them (McMordie, 2017). A food product is said to be a source of protein when at least 12% of the energy value of the food is provided by protein, high in fiber if it contains at least 6g of fiber per 100g, low in sugar if the product contains no more than 5g of sugar per 100g for solids and low in fat if it contains no more than 3g of fat per 100g for solids (European Commission, 2019). The crude protein content of the dried fruit ( $4.98 \pm 0.05\%$ ) was far lower than that of the dried leaf (29.02 %) as reported by Aborisade *et al.*, 2017, but higher than that of

the dried root (2.28%) as analyzed by Gbadamosiet *et al.*, 2011. However, it is close to that of the dried stem bark (5.70%) as reported by Olanipekun *et al.*, 2016 and to that of the dried fruit of *Gardenia erubescens* ( $5.68 \pm 0.01\%$ ), a lesser known Nigerian fruit belonging to the same family as *Morinda lucida* (Bello *et al.*, 2008). The carbohydrate content of the dried fruit ( $42.79 \pm 0.11\%$ ) is similar to that of the dried leaf (42.18%) as analyzed by Olanipekun *et al.*, 2016 but lower than that of *Gardenia erubescens* ( $70.69 \pm 0.22\%$ ) as reported by Bello *et al.*, 2008. The fruit can thus be said to be a fair source of protein and a good source of carbohydrate. The total lipid content of the dried fruit ( $16.95 \pm 0.21$ ) was higher than the  $1.54 \pm 0.20\%$  reported for *Gardenia erubescens* by Bello *et al.*, 2008, but lipid content of the fresh fruit was similar to that of the fresh fruit of two related species, *Morinda citrifolia* (3.2%) and *Morinda pubescens* (2.1%) as analyzed by Desai and Gaikwad, (2010). The appreciable amount of lipid in the dried and fresh fruit indicate that the fruit of *Morinda lucida* may be palatable because dietary fats function to increase food palatability by absorbing and retaining flavors. The crude fiber content of the dried fruit is close to the 22.01% of the dried leaf reported by Olanipekun *et al.*, (2016). The high amount of crude fiber ( $27.80 \pm 0.14\%$ ) in the dried fruit and ( $4.61 \pm 0.57\%$ ) in the fresh fruit indicates *Morinda lucida* fruits is a good source of roughages. It has the ability to lower blood cholesterol concentration, a risk factor of coronary heart disease. Thus, it is an ideal food for the prevention of atherosclerosis. The fiber content of the

dried fruit ( $27.80 \pm 0.14$ ) is higher than that of *Gardenia erubescens* ( $14.57 \pm 1.10$ ) as reported by Bello *et al.*, 2008. The ash content is the lowest for both the dried fruit and fresh fruit having the values,  $4.25 \pm 0.35\%$  and  $2.55 \pm 0.07\%$  respectively. These values are lower than that of the leaf ( $12.25\%$ ) as analyzed by Aborisade *et al.*, 2017. The result of the proximate analysis showed a very high moisture content ( $70.15 \pm 2.62\%$ ) for the fresh fruit, while for the dried fruit, it was ( $3.2 \pm 0.04\%$ ). The moisture content of the fruit aids in adequate metabolism, improves digestion of food and body hydration, and improves freshness of food substances. However, high moisture content subject foods to microbial spoilage and deterioration and short shelf life (Olanipekun *et al.*, 2016). Thus, the fresh fruit is more prone to perishability than the dried fruit.

The result of the phytochemical screening revealed the presence of phytate, alkaloid, saponins, oxalate, cardiac glycosides and tannin. These findings agreed with an earlier report by Olanipekun *et al.*, (2016) who recorded similar results for the dried leaf and dried bark of *Morinda lucida*. The invaluable pharmacological properties of *Morinda lucida* may be attributed to the presence of some of these phytochemicals. Tannin concentration was ( $14.96 \pm 0.36$ ) mg/100g for the dried fruit. However, it didn't exist in appreciable amounts to be detected in the fresh fruit. This significant amount of Tannins may form insoluble complexes with proteins thus reducing protein digestibility. From medicinal point of view, polyphenol to which tannin belongs

has been reported to act as antioxidant by preventing oxidative stress that causes diseases such as coronary heart disease, some types of cancer and inflammation (Bello *et al.*, 2008). This shows that *Morinda lucida* fruit is likely to have antioxidant activity. The fruit also showed a high concentration of oxalate., ( $1336 \pm 0.9$ )mg/100g and ( $488 \pm 0.17$ )mg/100g in the dried fruit and fresh fruit respectively. Oxalate is a concern because of its negative effect on mineral availability. High oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones (Bello *et al.*, 2008). The concentration of phytate was ( $140 \pm 0.02$ ) mg/100g in the dried fruit and ( $60 \pm 0.00$ ) mg/100g in the fresh fruit. The problem with phytic acid in foods is that it can bind some essential minerals/nutrients in the digestive tract and can result in mineral deficiency. Phytic acids also have a negative effect on amino acid digestibility, thereby posing problem to non-ruminant animals due to insufficient amount of intrinsic phytase necessary to hydrolyze the phytic acid complex, but the presence is also beneficial because it may have a positive nutritional role as an anti-oxidant and anti-cancer agent (Bello *et al.*, 2008). However, the level of phytate in *Morinda lucida* fruits is low and might not pose any health hazard. The alkaloid content was ( $3310 \pm 0.47$ ) mg/100g in the dried fruit and ( $470 \pm 0.02$ ) mg/100g in the fresh fruit. The presence of alkaloid may partly be responsible for the bitter taste of the fruit, and if it is consumed in high quantity, it might have adverse effect on the central nervous system. The fruit contained a significant amount of saponin ( $4450 \pm 0.78$

mg/100g) in the dried fruit and (1710±0.13mg/100g) in the fresh fruit which contribute further to its bitter taste. The cardiac glycoside content of the dried fruit was 3910±0.13mg/100g while that of the fresh fruit was 1950±0.78mg/100. Phytochemicals present in plant foods are responsible for the beneficial health effects derived from consuming such plants. For example, phytate, tannins and saponins have been shown to reduce the blood glucose and insulin responses to starchy foods and/or the plasma cholesterol and triglycerides. In addition, phytate, tannins, saponins and oxalates have been implicated in reduced cancer risks (Gemede and Ratta, 2014). This implies that the fruits of *Morinda lucida* can play a very useful role in ethno-medicine.

## CONCLUSION

The results of this study show that the fruit of *Morinda lucida* is nutritionally potent. Therefore, it could serve as an alternative source of feed for animals. The presence of phytochemicals such as alkaloids, saponins, tannins, phytate and oxalate show that the fruit is also pharmacologically potent just like the leaves, root, stem and bark and can as well be used for medicinal preparations. It is however recommended that the dried fruits be used in medicinal preparations as they contain higher levels of the phytochemicals.

## REFERENCES

Aborisade, A. B., Adetutu, A. and Owoade, A. O. (2017). Phytochemical and

proximate analysis is Some Medicinal Leaves. *Clinical Medicine Research*. **6**(6):209-214.

Addy, B. S., Owodo, H. T., Gyapong, R. N, Umeji, C. O. and Mintah, D. N. (2013). Phytochemical Screening and Antimicrobial Study on the leaves of *Morinda lucida*. *Journal of Natural Science Research*. **3**(14):131-135.

Adejo, G. O., Akintayo, C. O. and Obinna, M. (2015). *Morinda lucida* leaf and fruit extract may Attenuate diarrhoea arising from Ebola. *Basic Research Journal of Medicine and Clinical Sciences*. **4** (3):95-100

AOAC. (2005). Methods for Proximate Analysis. Association of Official and Analytical Chemists. Pp 2217-2280.

AOAC. (1995). Official Method of Analysis. Association of Official and Analytical Chemists. 18<sup>th</sup> ed., Washington DC. Journal of Nutrition. Pp 1147-1161

Bello, M. O., Folade, O. S., Adewusi, S. R. and Olawore, M. O. (2008). Studies on the Chemical Compositions and Anti-nutrients of some lesser Known Nigeria fruits. *African Journal of Biotechnology*. **7**(21):3972-3979.

Desai, N. and Gaikwad, D. K. (2010). Proximate Composition and some Phytochemical Properties of *Morinda* pulp. *International Journal of Applied Biology and*

- Pharmaceutical Technology*. **1**(2):679-682).
- Enemchukwu, B. N., Uchenna, N., Udedi, S. C., Ubaoji, K. I. and Akalonu, X. C. (2015). Nutritional and Anti-nutritional Evaluation and Phytochemical Composition of Aqueous leaf extract of *Mucunapruriens*. *The Bioscientist*. **3**(1):93-100.
- European Commission (2019). [https://ec.europa.eu/food/safety/labelling\\_nutrition/claims/nutrition\\_claims\\_en](https://ec.europa.eu/food/safety/labelling_nutrition/claims/nutrition_claims_en), accessed 25/5/19.
- Gbadamosi, I. T., Moody, J. O. and Lawal, A. M. (2011). Phytochemical Screening and Proximate Analysis of Eight Ethnobotanicals used as Anti-malarial Remedies in Ibadan, Nigeria. *Journal of Applied Biosciences*. **44**:2967-2971.
- Gemedede, H. F. and Ratta, N. (2014). Anti-nutritional Factors in Plant Foods: Potential Health benefits and adverse effects. *International Journal of Nutrition and Metabolism*. **3**(6):72-76.
- Harbone, J. B. (1973). *Phytochemical methods*, Chapman and Hall Limited, London. Pp. 49-188.
- McMordie, K. (2017). Dried fruits: Underconsumed but oh so nutritious. *Today's Dietitian* **19**(6):11
- Olanipekun, M. K., Adewuyi, D. and Adedeji, D. (2016). Ethnobotanical Implications and Phytochemical Analyses of Some Selected Medicinal Plants used in Ado-Ekiti LGA, Ekiti State, Nigeria. *Journal of Herbal Medicine Research*. **1**(3):0007-0016.
- Osagie, A.U. (1998). Anti-nutritional Factors. In: *Nutritional Quality of Plant Foods*. Ambik Press Ltd, Benin City, Nigeria. Pp. 1-40; 221-244
- Usunobun, U. I., Okolie, N. P., Anyanwu, O. G., Adegbeji, A. J. and Egharerba, M. E. (2015). Phytochemical Screening and Proximate Composition of *Anonamoricata*. *European Journal of Botany, Plant Science and Phytology*. **2**(1):18-28.
- Wardlaw, G. M. and Kessel, M. W. (2002). *What Nourishes You. Perspectives in Nutrition*, Mc Graw Hill, New York, Ed. 5:p.4
- Young and Greaves. (1940). *Laboratory Handbook of Methods of Food Analysis*, Leonard Hill, London. Pp. 217-273.