

**Comparative Assessment of the Nutritional and Antioxidant Status Of *Euphorbia Heterophylla*
(*Euphorbiaceae*) And *Morinda Lucida* (*Rubiaceae*) Plants**

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ABSTRACT

This study comparatively and quantitatively ascertained the antioxidant, nutritional and phytochemical potentials of *Euphorbia heterophylla* and *Morinda lucida* plants by Standard methods. The results of moisture, crude protein, fibre and ash contents respectively obtained in proximate analysis were significantly higher ($p < 0.05$) in *Euphorbia heterophylla* than that of *Morinda lucida* except that of the total fat and carbohydrate contents. In mineral analysis, the relative amounts of potassium, calcium, magnesium, zinc and iron in the leaves of *Morinda lucida* were significantly higher ($p < 0.05$) as compared to *E. heterophylla*. With phytochemical analysis, there was a high significant difference ($p < 0.05$) of tannins, alkaloids and cyanogenic glycosides in both plants, and a higher significant difference ($p < 0.05$) in the amounts of phytate, oxalate and phenols in *Morinda lucida* than that of *Euphorbia heterophylla*. There was also, a significant difference ($p < 0.05$) for the antioxidant capacity of both plants with predominance of vitamin C, catalase, and superoxide dismutase activities in *Euphorbia heterophylla* than that of *Morinda lucida*. However, Glutathione peroxidase activity predominates in the latter plant. Therefore, these plants are rich in nutrients, phytochemicals and highly valuable sources of natural antioxidants, which may combat oxidative stress and inhibit degenerative disorders.

Keywords: *Euphorbia heterophylla*, *Morinda lucida*, Antioxidants, Nutrients

INTRODUCTION

Plants have formed the basis of traditional medicine system that has been the way of life for thousands of years. Mostly, herbs and spices contain polyphenols which are most powerful natural antioxidants and are highly valued for their antioxidant, anti-ageing and antimicrobial effects. Data from both scientific reports and laboratory studies showed that plants contain a large variety of bioactive substances that possess antioxidant activity (Goldie and Vijay, 2012). Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain good health and prevention of oxidative stress-mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids inhibit the mechanism that leads to degenerative diseases (Hamid *et al.*, 2010).

In Nigeria, a large percentage of the populace are dependent on herbal medicines because Orthodox medicines are becoming increasingly expensive and out of reach (Fasola and Egunyomi, 2005). One amongst such plants used for the management/treatment of various types of diseases and ailments is *Morinda lucida* and *Euphorbia heterophylla*.

The medicinal usefulness of the plant, *Euphorbia heterophylla* has been the object of numerous chemical and pharmacological studies. *Euphorbia heterophylla* is commonly called *Nono-kunchiya* in Hausa, *Egele* in Ibo and *Adimeru* in Yoruba, Nigeria. It is referred to as *Mexican fire plant*, *milk weed* and *Spurge weed* in English (Okeniyi *et al.*, 2012). The report by Omale and Emmanuel (2010), showed that the Ethanol extract and water free extract of *Euphorbia heterophylla* leaf contain some wound healing properties. Falodun and Agbakwuru, (2004) reported the isolation of a flavonoid, quercetin from crude extract of the leaves of this plant. The leaf is also known to possess antibacterial activity (Falodun *et al.*, 2003). In the Igbo community of Nigeria, *E. heterophylla* is used as a purgative (Erden *et al.*, 1999). It is also used for different purposes in herbal medicine practice as a purgative, antiasthmatic, anti-inflammatory, lactogenic and an abortifacient agent (Erden *et al.*, 1999; Falodun, *et al.*, 2006; Dokori, 1998).

Morinda lucida. (*Rubiaceae*) is a tropical West Africa rainforest tree also called *Brimestone* tree (Adeneye, and Agbaje, 2008). In Nigeria, the plant is used for the treatment of malaria, typhoid fever, jaundice and dressing of wounds to prevent infections (Akinmoladun *et al.*, 2010).

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The leaves are used as "oral teas", which are usually taken orally for the traditional medicine as a general febrifuge, analgesic, laxative and anti-infections (Raji *et al* 2005). The leaves also, have been reported to possess strong trypanocidal and aortic vasorelaxant activities (Asuzu and Chineme (1990); Ettar and Emeka (2004). Other parts of the plant (stem, bark and root) also have been shown to possess diverse therapeutic benefits. The major constituents of *Morinda lucida* extract are the various types of alkaloids, anthraquinones and anthraquinols (Adesogan, 1973). Further studies have shown that leaf and stem bark of *Morinda lucida* possess anticancer (Sowemimo *et al*, 2007), hepatoprotective (Oduola (2010), cytotoxic and genotoxic (Ajaiyeoba, *et al*, 2006; Akinboro and Bakare 2007) antispermatogenic, (Raji, *et al*, 2005) hypoglycemic and antidiabetic (Adeneye and Agbaje, 2008) activities respectively.

The increasing rate of consumption of herbal medicines derived from the aforementioned plants and the belief that they can cure certain sicknesses, necessitated this research to focus on the two selected plants so as to ascertain the components, that is; the secondary metabolites, that aids in their curative abilities as well as establishing the distinctive efficacy of the two plants for therapeutic purposes. Therefore, this *in vitro* study was done to ascertain comparatively, the nutritional and antioxidant status of *Euphorbia heterophylla* and *Morinda lucida* plants.

MATERIALS AND METHODS

Collection, Identification and Extraction of Plant Samples

Fresh leaves of *Euphorbia heterophylla* were obtained from the environment of Western Delta University administrative building. *Morinda lucida* leaves were also obtained from Ida College, Benin city Edo state, Nigeria. Both plants were identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Edo State by Dr. H.A Akinnibosun where herbarium specimens were kept and voucher numbers UBHe0152 and UBHm155 were assigned to *Euphorbia heterophylla* and *Morinda lucida* respectively. The leaves of the plants were washed with distilled water and oven dried at 60°C to constant weight and ground to powder. The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 200 ml of distilled water for 12h. Thereafter, extracts were filtered using Whatman filter paper No. 42 (125 mm).

Proximate analysis

Moisture, ash and fibre content of the samples were determined by the method of the Association of Official and Analytical Chemists (A.O.A.C), 1980 while crude lipid content was by soxhlet method. Nitrogen and crude protein were determined by micro- 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 (Muller and Tobin, 1980).

Phytochemical Constituents

Total phenols in the samples were determined by spectrophotometric method (Singleton *et al*. 1999). The other phytochemical constituents of the plants were determined according to the following methods: alkaloid determination (Harborne, 1973), cyanogenic glycoside determination (AOAC, 2000), tannin determination (Van-Burden and Robinson, 1981), phytic acid determination (Lucas and Markakas 1975) and oxalate determination (Sanchez-Alonso and Lachica, 1987).

Elemental assay

Laboratory procedures for the preparation and determination of Na, K, Ca, Mg, Zn and Fe nutrients were used as outlined by Shah *et al*, (2009) for plant samples.

Antioxidant assays

Antioxidant assay of the plants were determined according to the following methods: catalase (Claiborne, 1985), Superoxide Dismutase (Misra and Fridovich 1972), ascorbic acid (Roe and Keuther, 1943), glutathione peroxidase (Tappel, 1978) and DPPH scavenging effect (Mensor *et al*, (2001)

Statistical Analysis

Triplicate data of various assay results were expressed as mean \pm standard error of mean (S.E.M). Statistical comparison was performed by one factor analysis of variance (ANOVA; LSD, DUNCAN and SNK tests), using the statistical package for social sciences version 20.0, (SPSS Inc, Chicago II, USA). $P < 0.05$ was considered significant.

RESULTS

Table 1 reveals proximate composition of *E. heterophylla* and *M. lucida*. *E. heterophylla* has higher composition ($p < 0.05$) of moisture, protein, ash and fibre contents than *M. lucida*. However, the Lipid and carbohydrate contents of *M. lucida* was higher ($p < 0.05$) than that of *E. heterophylla*.

Table 1: Proximate composition of *E. heterophylla* and *M. lucida* leaves

Parameters (%)	<i>Euphorbia heterophylla</i>	<i>Morinda lucida</i>
Moisture	16.40 ±0.34	5.52 ±0.12
Protein	5.50 ±0.11	0.84 ±0.15
Ash	0.15 ±0.01	0.11 ±0.00
Fibre	1.78 ±0.35	1.31 ±0.03
Lipid	1.42 ±0.32	2.61 ±0.57
Carbohydrate	73.11 ±1.49	87.63 ±1.79

*All mean results for *Euphorbia heterophylla* and *Morinda lucida* in the above table were considered significant different at $p < 0.05$

Table 2 exhibits the mineral composition of *E. heterophylla* and *M. lucida*. *M. lucida* has a higher composition ($p < 0.05$) of Potassium, Calcium,

Magnesium, Zinc and Iron than *E. heterophylla*. However, the Sodium content of *E. heterophylla* was higher ($p < 0.05$) than that of *M. lucida*

Table 2: Mineral composition of *E. heterophylla* and *M. lucida* leaves

Mineral parameters (mg/kg)	<i>E. heterophylla</i>	<i>M. lucida</i>
Sodium	136.43 ± 2.79	43.10 ± 0.88
Potassium	67.94 ± 1.39	175.63 ± 3.59
Calcium	21.26 ± 0.44	36.04 ± 0.74
Magnesium	12.51 ± 0.26	21.20 ± 0.44
Zinc	5.64 ± 0.12	17.58 ± 0.36
Iron	65.04 ± 1.33	130.07 ± 2.66

*All mean results for *Euphorbia heterophylla* and *Morinda lucida* in the table above were considered significant different at $p < 0.05$

Table 3 elucidates the quantitative phytochemical composition of *E. heterophylla* and *M. lucida*. *M. lucida* contains larger amount of the phytochemicals ($p < 0.05$), Phytate, Oxalate and

Phenols than *E. heterophylla*. However, the Tannin, Alkaloids and Cyanogenic glycosides content of *E. heterophylla* was higher ($p < 0.05$) than that of *M. lucida*

Table 3: Phytochemical composition of *E. heterophylla* and *M. lucida* leaves

Phytochemical parameter (mg/100g)	<i>E. heterophylla</i>	<i>M. lucida</i>
Phytate	4.09 ± 0.09	7.51 ± 0.16
Oxalate	5.67 ± 0.12	11.57 ± 0.24
Tannins	4.87 ± 0.10	1.01 ± 0.02
Phenols	5.75 ± 0.12	11.87 ± 0.24
Alkaloids	4.87 ± 0.10	1.01 ± 0.02
Cyanogenic glycosides	0.31 ± 0.01	0.16 ± 0.01

*All mean results for *Euphorbia heterophylla* and *Morinda lucida* showed significant different at $p < 0.05$

Table 4 reveals the antioxidant activities of Catalase, Glutathione peroxidase, Superoxide dismutase and Vitamin C in *E. heterophylla* and *M. lucida*. The overall results showed that *E. heterophylla* leaves exhibited a higher antioxidant

activity ($p < 0.05$) in comparison with *M. lucida* as exemplified in its Catalase, Glutathione peroxidase and Vitamin C antioxidant activities. However, the Superoxide dismutase activity of *M. lucida* was higher ($p < 0.05$) than that of *E. heterophylla*.

Table 4: Antioxidant activities of *E. heterophylla* and *M. lucida* Leaves

Antioxidant parameters (IU/L)	<i>E. heterophylla</i>	<i>M. lucida</i>
Catalase	0.43 ± 0.01	0.31 ± 0.01
Glutathione peroxidases	0.37 ± 0.01	0.11 ± 0.00
Superoxide dismutase	0.14 ± 0.01	0.17 ± 0.01
Vitamin C	0.08 ± 0.00	0.05 ± 0.00

*All mean results for *Euphorbia heterophylla* and *Morinda lucida* were considered significant different at $p < 0.05$

The examination of antioxidant activities of plant extracts from *E. heterophylla* and *M. lucida* depicted by Table 5, showed different values. It can be clearly seen that the scavenging activity of *Euphorbia heterophylla* and *Morinda lucida* extracts were concentration-dependent. With the increase of the concentration of the samples, the

scavenging activity of the extracts increased accordingly. The obtained values varied from 3.3% to 10.6%. *M. lucida* manifested a stronger capacity for neutralization of DPPH radicals at 50%, 23%, 12.5% and 6.12% aqueous Stock concentrations compared to that of *E. heterophylla* respectively.

Table 5: % Inhibition of DPPH –Free –Radical Scavenging Activity of Leaf Extracts

Aq. Extract conc.	<i>E. heterophylla</i>	<i>M. lucida</i>
Stock	10.6	7.2
50%	6.7	3.8
25%	4.7	0.6
12.25%	8.6	3.3
6.12 %	7.0	3.9

DISCUSSION

Plant-derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and relatively less expensive. In respect of the healing power of plants and a return to natural remedies, it is an absolute requirement of our time (Sen *et al.*, 2009; Ramchoun *et al.*, 2009).

Results obtained from the Proximate composition (moisture, ash, fat, protein, crude fibre and carbohydrate) of *Euphorbia heterophylla* and *Morinda lucida* revealed that; there was significant difference at $p < 0.05$ for the two plants. All the data obtained in this part of the study were from wet basis and expressed as percentage (%). Although literature regarding the comparative investigation of these plants is lacking, *E. heterophylla* has higher composition ($p < 0.05$) of moisture, protein, ash and fibre contents than *M. lucida*. However, the Lipid and carbohydrate contents of *M. lucida* were higher ($p < 0.05$) than that of *E. heterophylla*. Therefore, *E. heterophylla* is a better source of hydration than *M. lucida* for the body as well as possessing the ability to quench thirst (Ogbonna *et al.*, 2013). The results obtained from the comparative assessment of the two plants are consistent with the reports given by Omale and Emmanuel (2010), and Lawal *et al.* (2012).

Proteins are essential components of diets needed for survival of animals and humans, their basic function in nutrition is to supply adequate amounts of required amino acids in nutrition (Pugalenthal *et al.*, 2004).

Protein deficiency causes growth retardation, muscle wasting, edema, abnormal swelling of the belly and collection of fluids in the body (Perkins – Veazie *et al.*, 2005). In the present study, therefore, *E. heterophylla* could be considered as a better source of dietary protein than *M. Lucida*.

The amount of ash present can be translated to be the quantity of minerals present in the plant samples (Coimbra and Jorge, 2011). The percentage ash in the samples gave an idea about the inorganic content of the samples from where the mineral content could be obtained. Therefore, *E. heterophylla* with high percentage of ash content than *M. Lucida* is expected to have high concentrations of various mineral elements, which are required to speed up metabolic processes in order to improve growth and development (Bello *et al.* 2008; Ekpete and Edori (2013).

The results of the mineral contents of the two plants are in consonance with the ash content of the plants. As for the fibre content of the plants, Hassan *et al.* (2009) reported that foods rich in dietary fibre contributes to the prevention of various diseases such as constipation, hemorrhoids, colon cancer, excess cholesterol, diabetes and diverticulitis. In view of this comparative assessment, *E. heterophylla* may be a better potent source of dietary fibre than *M. lucida* in the management of these diseases.

The results obtained for carbohydrate contents in the plants demonstrated that *M. lucida* may be more advantageous than *E. heterophylla* to grazing animals, as source of energy as well as index of increased rate of digestibility.

The results also indicated an appreciable amount of macro and micro nutrients (i.e. Na, K, Ca, Mg, Zn and Fe respectively) in *E. heterophylla* and *M. Lucida* with significant predominance of all minerals except sodium in *E. heterophylla*. There has also been an increasing concern in the amount of minerals in food as human's fundamental minerals (Arslan and Özcan, 2008). Potassium plays a crucial role in energy metabolism and membrane transport. A major function of

potassium is membrane polarization (Omale and Emmanuel, 2010). In contrast to the deleterious effects of high sodium intakes that frequently increase blood pressure, ingestion of more potassium may influence blood pressure favorably by lowering any elevations (Gough *et al.*, 1979). The beneficial effects of potassium (even magnesium and calcium) work, at least in part, through an effect on sodium balance. Potassium is more effective in salt sensitive individuals. Consequently, the high level of potassium in *E. heterophylla* relative to *M. lucida* can be considered advantageous as it could be a potential agent for blood pressure lowering in high blood pressure individuals. Moreso, adequate calcium intake can potentiate the advantage of physical activity (Specker, 1996), estrogen (Nieves *et al.*, 1998) and on bone formation. The primary strategies for reducing the risk of osteoporosis are to maximize development of peak bone mass during growth and to reduce bone loss later in life. Adequate calcium intakes are important for both of these aims. The high calcium level in *E. heterophylla* is of immense advantage medically as it can be used to prevent osteoporosis when compared to *M. lucida*.

Zinc serves catalytic, structural and regulatory functions in biological systems. The catalytic role of zinc is required for the biological functions of about 300 enzymes in all six classes of enzymes and from different species of all phyla (McCall *et al.*, 2000; Omale and Emmanuel, 2010). Another major function of zinc in metalloenzyme is the structural role whereby the zinc stabilizes the tertiary structure of the enzymes (Vallee *et al.*, 1991). There is no doubt that the presence of Zn in both *E. heterophylla* and *M. lucida* could be contributory to their medicinal and nutritional significance claimed by traditional users. It was also observed that *M. lucida* had a lower concentration of iron (Fe) than *E. heterophylla*. The observed higher iron concentration in *E. heterophylla* could justify its use in the management of iron deficiency conditions like anemia, and could be used as supplement during pregnancy.

Phytochemicals are chemical compounds that are naturally found in plants. They are responsible for the colour and organoleptic properties of the plant (Liu, 2004; Ugwu *et al.*, 2013). The present study carried out on *E. heterophylla* and *M. lucida* revealed quantitatively the presence of medicinally active phytochemicals. The results obtained in the comparative study separately and qualitatively agreed with earlier reports (Omale and Emmanuel, 2010; Balogun and Akinloye, 2012; Abbasi *et al.*,

2013). Phytate, oxalate and phenolic contents significantly predominate in *M. lucida* while tannins, alkaloids and cyanogenic glycosides significantly predominate in *E. heterophylla*. First, the knowledge of phytate level in foods is necessary because high concentration can cause complicated effect in human system including indigestion of food and flatulence (Nwoko and Bragg, 2000). Phytic acid intake of 4.00–9.00mg/g, reduces iron absorption by 4–5 folds in humans (Hurrell, 2003). But phytate in moderate levels has an antioxidant effect and also prevents colon cancers by reducing oxidative stress in the lumen of intestinal tracts. Consequently, the moderate concentrations in both plants explain why the plants may be able to prevent or ameliorate colon cancer with utmost potency in *E. heterophylla* via intestinal reduction of oxidative stress.

WHO and FAO (2003) reported that a daily intake of 450mg of oxalic acid has been reported to interfere with various metabolic processes. Therefore, the values obtained for phytate and oxalate are lower than the lethal dosage reported in other studies while the toxic effect of these anti-nutrients may not occur when these plants are consumed because their levels are not enough to elicit toxicity. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. They are one of the major groups of non-essential inhibition of atherosclerosis that have been associated with the inhibition of atherosclerosis and cancer, as well as for age-related degenerative brain disorders (Cheung *et al.* 2003). Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun *et al.* 2009). Numerous investigations of the antioxidant activity of plant extracts have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity (Borneo *et al.*, 2008; Katalinić *et al.* 2004). It is therefore, imperative to comparatively establish that *M. lucida* may likely have a greater antioxidant activity than *E. heterophylla*.

Most alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al.*, 2010). Therefore; the comparative study clearly indicated that *E. heterophylla* may likely elicit higher pharmacological property as an anesthetic and CNS stimulant than *M. Lucida*. Plants that have tannins as their main components are astringent in nature and are used for treating of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001). Therefore, *E. heterophylla* may be more

potent in the treatment of intestinal disorders than *M. Lucida*.

Cyanogenic glycosides are considered to have an important role in plant defense against herbivores due to the bitter taste and release of toxic hydrogen cyanide upon tissue disruption (Haque and Bradbury, 2002). The level of cyanogenic glycoside in both plants is tolerable and significantly low. This result corroborates some previous studies on the phytochemical screening of *Morinda lucida* which also revealed the presence of tannins, alkaloids, flavonoids and glycosides components (Ajayeoba *et al.* 2006), although flavonoid was not analyzed in this study.

The results of the enzymatic and non-enzymatic antioxidant status of *E. heterophylla* and *M. lucida* significantly ($p < 0.05$) revealed the presence of catalase, glutathione peroxidase, superoxide dismutase and vitamin C respectively. *E. heterophylla* showed higher antioxidant activity both enzymatically and non-enzymatically than *M. lucida* with the exception of glutathione peroxidase activity, which was slightly higher in *M. lucida*. Several antioxidants of plant origin are experimentally proved and used as effective protective agents against oxidative stress by quenching various free radicals (Reddy, 2012).

IC_{50} is defined as the concentration of the extracts to quench 50% of DPPH in the solution under the chosen experimental conditions. IC_{50} value is inversely related to the activity as it is the measure of inhibitory concentration and a lower value would reflect greater antioxidant activity of the fraction (Muhammad *et al.*, 2013). Therefore, *M. lucida* exhibit greater proton-donating ability to DPPH molecule, and could serve as a better free radical inhibitor or scavenger than *E. heterophylla*.

In conclusion, findings from this research reveal that both plants are natural sources of nutrients and phytochemicals, thereby lending further credence to their ethno-botanical potentials. Both plants exhibited some antioxidant properties. However it is not very clear which of them possess more antioxidant properties since *Morinda lucida* exhibited higher antioxidant activity in scavenging free radical formation but lower enzymatic and non-enzymatic antioxidant levels. Therefore, further study is required on this account.

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