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Proximate and Phytochemical Composition of the Leaves of *Morinda lucida*

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Abstract
The present study investigated the proximate and phytochemical composition of the leaves of Morinda lucida. The results obtained from its quantitative phytochemical analyses indicated that M. lucida leaf contains 14.9 ± 0.23mg/100 g saponin, 82.2 ± 1.28 mg/100 g alkaloid, 32.3 ± 0.51 mg/100 g flavonoid, 12.4 ± 0.19 mg/100 g tannin, 137.2 ±2.13mg/100g phytate, 250.7 ± 3.90mg/100g oxalate, 10.2 ± 0.16mg/100g anthraquinones and 8.0 ± 0.12 mg/100 g cyanogenic glycosides. Furthermore, results of its proximate analysis revealed the presence of high levels of carbohydrate, and appreciable levels of protein, fibre, ash, moisture and lipid. Thus, the results obtained from this study revealed that M. lucida leaf is rich in phytochemicals and therefore will potentially serve very beneficial purposes for medicinal benefits if properly processed.

Keywords: *Morinda lucida*, Proximate, Phytochemical

Introduction
The use of medicinal plants as therapeutic agents or prophylaxis in disease conditions in developing and even in advanced countries is gaining momentum. Most inhabitants in the rural areas depend almost solely on traditional medicine obtained from medicinal plants to treat different array of diseases. The claims of the effectiveness of some of these medicinal plants have been documented scientifically. Serious concern is raised as to the crude methods of preparation of these extracts. This is largely due to illiteracy and penury. Much research is required to scientifically analyze the components of these herbs and ascertain their level of safety. So many bioactive substances that exhibit definite biochemical, physiological and pharmacological actions in the body are associated with medicinal plants. Some of these bioactive constituents include alkaloids, tannin, flavonoid, phenolic derivatives etc [1, 2]. Natural products derived from plants have gained much attention in later years due to the diverse pharmacological properties, which include antioxidant and antitumor activity [3]. In Southern Nigeria (Edo state to be precise), one of the frequently used medicinal plants for therapeutic purposes is *Morinda lucida*. The plant is about 15m tall and characterized with scaly grey bark, short crooked branches and shining foliage [4]. It is a tropical rain forest plant and it is commonly known as Brimstone tree [5]. It belongs to the Rubiaceae family. The leaf and stem bark possess anticancer [6], hepatoprotective, antispermatogenic [7], properties. Records have also shown that the leaves of *M. lucida* are used as "oral teas", which are usually taken for the traditional treatment of malaria, and as a general febrifuge, analgesic, laxative and anti-infections [8]. Igede People in the Middle Belt region of Nigeria, administer a decoction of *M. lucida* twice or thrice daily as anti – diarrhea and the leaves utilized for treatment of infertility in women [9,]. The plant has also been reported to contain a bio-friendly natural dye used for the staining of collagen fiber and muscle fiber [10]. *M. lucida* is regarded as an important medicinal plant in West Africa.
This study was undertaken to investigate the therapeutic bases for the use of *M. lucida* leaf. Specifically, the study investigated the phytochemical and proximate constituents of *Morinda lucida* leaves by quantifying them. The present study will likely stimulate interest on *M. lucida* due to the fact that there is an increasing demand for medicinal plants, its products and plant derivatives as alternatives to currently used imported drugs especially in developing countries.

Materials and Methods
Experimental Plant Material
Morinda lucida (*Rubiaceae*) leaves were obtained from a farmland at Ekpoma, Edo State, Nigeria, where it is locally called Ebogo. It was identified and authenticated at the Plant Biology and Biotechnology Department, University of Benin, Benin.
Preparation of Extracts: *M. lucida* leaves were rinsed with clean water to remove debris and dried under shade for 3 weeks and pulverized into coarse powdered form. The powder was stored in an air-tight container until used. 500g of the pulverized plant material was extracted with 3 liters of distilled water for 48 hours. The suspension was shaken occasionally and then filtered with muslin cloth, to yield the aqueous extract. The filtrate was concentrated in rotatory evaporator at 60^0c. The concentrate was then freeze-dried and kept in air-tight container until required. Also, freshly collected leaves were washed with clean water and its contents squeezed out, freeze dried and stored in air-tight container.

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Phytochemical Screening
Various phytochemical analyses were done using standard methods: flavonoids [11], saponins [12], tannins [13], anthraquinones [14], cyanogenic glycosides [15], Oxalate [16] and alkaloids [14]. All samples were analyzed in triplicates.
Proximate Analysis: The proximate composition of the samples was determined using AOAC methods [15].

Results and Discussion

Proximate composition of the dried and fresh extracts showed that they are rich in carbohydrate and crude proteins. However, the fresh extract had higher fibre content than the dried extract (Table 1).

Table 1: Proximate Composition of fresh and dried aqueous extracts of *Morinda lucida* Leaves

Parameters	Composition (%)	
	Fresh leaf	Dried leaf
Crude Protein	12.54 ± 0.01	8.13± 0.02
Lipid	2.75± 0.01	3.86± 0.01
Fibre	2.24± 0.01	0.98± 0.01
Ash	0.43± 0.01	0.62± 0.01
Carbohydrate	62.65± 0.01	60.49± 0.02

Each value represented the Mean ± sem of n=3 readings. The P<0.05 value was taken as statistically significant. The concentration of the phytochemicals was expressed as percentage.

The phytochemical constituents (quantitative) of the extracts showed that the dried leaf extract had higher content of flavonoids, saponins and tannins when compared with the fresh leaf extract. On the other hand, the fresh extract showed higher values for alkaloids and cardiac glycosides in contrast to the dried extract (Table 2).

Table 2: levels of Phytochemicals of *M. lucida*

Parameters	Composition (mg/100g)	
	Fresh leaf	Dried leaf
Tannin	2.79 ± 0.01	4.63± 0.01
Saponin	1.88 ± 0.01	3.42± 0.01
Alkaloid	3.21 ± 0.01	0.56± 0.01
Flavonoid	2.06 ± 0.01	4.29± 0.01
cardiac glycoside	4.05 ± 0.01	2.95 ± 0.01
Anthraquinones	3.11 ± 0.02	1.57 ± 0.01

Each value represented the Mean ± sem of n=3 readings. The P<0.05 value was taken as statistically significant. The levels of the phytochemicals were expressed as mg/100g.

The proximate composition of *M. lucida* leaf as given in Table 1 showed that it contains 2.9 ± 0.04% of moisture. The low content of moisture associated with the extract points to the fact that the plant is less susceptible to infections by microorganisms. This increases the shelf- life considering the fact that the moisture content of food substances can be explored as a measure of its keeping quality. *M. lucida* leaves contain proteins (15.6 ± 0.24%). The plant may serve as a rich source of protein supplementation.
It also contains appreciable levels of crude lipid (4.2 ± 0.07%), crude fibre (5.8 ± 0.07%) and ash (4.7 ± 0.05%). Fibre is known to affect the gastrointestinal tract through variation in faecal bulkiness and water, transit time and elimination of bile acids which tend to lower the body cholesterol. Reduction in the incidence of coronary disorder and breast cancer by crude fibre has been reported [17].
The leaf contains high carbohydrate content (65.2 ± 1.01%). This shows that *M. lucida* leaf could be used as a ready source of carbohydrate. In the body, energy is provided to cells by carbohydrates. The brain particularly depends on only carbohydrate for energy derivation [17].
The quantitative phytochemical composition of leaf as shown in Table 2 revealed that it contains saponin, alkaloid, flavonoid, tannin, phytate, oxalate, anthraquinones and cyanogenic glycosides. Phytochemicals are

chemical substances whose precursors are products of primary metabolism in plants. Both pharmacological and biochemical effects of various degree are known to be exhibited on living organisms by these phytochemicals. [13]. Therefore, the presence of these secondary metabolites is attributable to its pharmacological properties.

Alkaloids and flavonoids showed very high presence in *M. lucida*. It therefore suggests that *M. lucida* is a potent medicinal plant. This is because many medicinal plants that contain alkaloids and flavonoids exhibit diuretic, antispasmodic, anti-inflammatory and analgesic effects [18]. In the same vain, flavonoid has been reported to possess similar properties including antiviral and anti-carcinogenic activities [19 , 20] . Moreover, modification of the production of cyclo-oxygenase 1 and 2 and lipooxygenase have been attributed to the extracts of plants that contain flavonoids [21, 22, 23], thereby inhibiting prostaglandin production, a known agent that stimulate intestinal motility and secretion. Flavonoids have also been documented to exhibit antioxidative properties and therefore the observed inhibitory action on several enzymes. Of particular interest are those enzymes involved in the arachidonic acid metabolism [24]. Alkaloids are known to affect glucagons and thyroid stimulating hormones [25]. Tannins in medicinal plants are well known to alter the three dimensional structure of proteins and this leads to a decrease in intestinal mucosa hyper-secretion in diarrhea [26, 27]. These may support its use in the management/treatment of diarrhea. Researchers have reported of the anti-diarrheal properties exhibited by tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars [28].

The concentration of saponins (14.9 ± 0.23mg/100) in *M. lucida* leaf suggests that it is capable of reducing blood cholesterol. Saponins have been reported to decrease blood cholesterol. This is achieved by preventing cholesterol reabsorption, and that the non-sugar component of saponins has a proportional antioxidant activity which can reduce risk of cancer and cardiovascular diseases. [29].

Results from this study further showed that *M. lucida* leaf contains 8.0 ± 0.12mg/100g of cyanogenic glycosides, which is lower than the permissible limits of 10 – 20mg/100g [30]. It has been reported that hydrogen cyanide (a toxic derivative of cyanogenic glycosides) can be substantially reduced by boiling, heating and soaking [31].

The presence of significant levels of anthraquinones is an indication that *M. lucida* leaf possesses anti-malarial property since it inhibits the growth of *Plasmodium falciparum* [32]. The inhibition is probably due to the presence of aldehyde group at C-2 and a phenolic hydroxyl group at C-3. The levels of phytate present in the leaf of M. lucida as shown in table 2 is within the acceptable range as it has also been reported that the acceptable upper limit of phytate in the body is from 250 - 500mg/100g [33]. Although phytate is known to inhibit the absorption and utilization of important mineral elements, [34] reported that new evidence indicates that phytate confer several positive effects on human health. Analysis on *M. lucida* leaf from table 2 showed that the oxalate content of the leaf is within the acceptable range. The toxic range of oxalates is between 3-5 g for man [35]. The levels of phytate and oxalate show that *M. lucida* leaf is relatively safe.

Phytochemical screenings are extensively explored to search for bioactive agents. Plants have provided agents which serves as starting materials for the partial synthesis of some useful drugs. An example is the steroidal sapogenins produced by *Dioscorea species* (or Mexican yams) and also by the Balanites and Trigonellai species. The 'Solanum alkaloids' from Solanum species have been used reportedly in the partial synthesis of drugs. Steroidal drugs such as corticosteroids, the sex hormones, and oral contraceptives are synthesized using plant steroidal sapogenins as precusor [36]. Results from this study therefore, revealed that the leaves of *M. lucida* possess important bioactive compounds which could be screened for several medicinal purposes. These phytoconstituents could also act in a synergistic manner to produce pharmacological effects.

Conclusion

The study indicates that *M. lucida* leaf is a rich source of bioactive phytochemicals and carbohydrates. Thus, if adequately harnessed, it will offer medicinal, as well as nutritional benefits to its users.

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