

PHYTOCHEMICAL STUDY AND PHARMACOLOGICAL ACTIVITIES OF DIFFERENT PARTS OF BRIMSTONE TREE (*MORINDA LUCIDA*)

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ABSTRACT

Global interest has been shown in the therapeutic potential of medicinal plants with the goal of creating an alternative, healthy, efficient and accessible complement with little to no side effects that could be in the form of medicinal drugs, food or nutritional supplements, in the treatment and management of different diseases. *Morinda lucida* is a rainforest tropical tree commonly referred to as the Brimstone tree, Indian mulberry and Hog tree apple. Different plant components are used for medicinal purposes, including stem barks, roots and leaves. Leaves are traditionally used for the treatment of malaria, analgesic, laxative, anti-infection and as a general febrifuge. Stem bark infusion is used as an antimalarial and anti-diabetic. For malaria management, the root of the plant is used in combination with other plants. The existence of various secondary metabolites such as alkaloids, anthraquinones, anthocyanins, carbohydrates, cardenolines, coumarins, flavonoids, glycosides, cyanide hydrogen, phenols, compound reducers, resins, saponins, steroids, tannins, terpenoids and triterpenoids was shown in the majority of the preliminary phytochemical screening reviewed in this paper. Numerous pharmacological activities have been responsible for the existence of these phytoconstituents, including antiviral, antibacterial, antidiabetic, antimalarial, anti-inflammatory, anti-cancer, antioxidant, anti-leishmanial, antitrypanosomally, hepatoprotective, cytotoxic and genotoxic, antispermatogenic, anticonvulsant among others.

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1. INTRODUCTION

Global interest has been shown in the therapeutic potential of medicinal plants with the goal of creating an alternative, healthy, efficient and accessible complement with little to no side effects that could be in the form of medicinal drugs, food or nutritional supplements, in the treatment and management of different diseases [1]. Medicinal plants are plants in which one or more of their organs contain substances that can be used for medicinal purposes or are precursors to the semi-synthesis of chemo-pharmaceutical products [2,3]. Medicinal plants play a vital role in people's health care needs around the world especially in developing countries [4-6]. According to [7] medicinal plant species, in many developing countries, are a valuable alternative to traditional medicine, especially in poor communities living in rural areas, which lack access to health services. According to the World Health Organisation, about 80% of the people of many developed countries use conventional medicines for their health care [3,6,8-27]. The use of medicinal plants in the field of natural products is very beneficial in terms of resources for chemical and biological research [7]. Certain secondary metabolites such as alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenes, tannins, resins, lactones, volatile oils and others [3] are naturally synthesized and accumulated by these plants.

Morinda lucida a member of Rubiaceae family [25,28-37], is a tropical rainforest tree [36] widely known as the Brimstone tree [32,33,38,39], the Indian mulberry and the Hog tree apple [33,39]. The plant is known locally as Njisi in Hausa [6], Huka, Eze-ogu [40] and Nfia in Igbo [40], Oruwo [34,40-42] or Ruwa in Yoruba [40,43], Ogele in Igalaland in Kogi state, Ewe amake (Ghana), Ewe amaka or Atakake (Togo) [40], Sangogo or Bondoukuoalongua (Cote d'Ivoire) [40].

The plant is a medium-sized tree [28,29,41,44-50], about 15m tall [29,34,37,38], roughly 20-30 cm in diameter with a thick crown of slender crooked branches [46]. The colour of the bark is grey to brown, the colour of the flowers is white, the fruit is drupe, the seed is ellipsoid, yellowish, soft [51]. Most parts of the plant are used for medicinal purposes, including the stem, bark, roots and leaves [52,53].

2. TRADITIONAL USES

In herbal medicine, plants have long been used in the treatment of various human body diseases in many parts of the African continent [54]. The leaves are used as oral teas, typically taken by mouth for the conventional treatment of malaria, and as febrifuge, analgesic, laxative and anti-infective drugs in general [36,40,42]. The use of leaf extract to treat hypertension, malaria, ulcers, and gonorrhoea was observed by [35]. The root of the plant is used in Nigeria for the treatment of malaria in combination with *Mangifera indica*, *Carica papaya* and *Cassia podocarpa* leaves [58]. Usage of cold decoction of plant leaves for the treatment of fever, as well as plant bark, root and leaf bark, as a bitter tonic and as an astringent, bitter water decoction has been recorded for dysentery, abdominal colic, and intestinal worm infestation [48,59]. In the Igede people of Benue State, Nigeria, decoction of *M. Lucida* is used as antidiarrhea two to three times every day [28,60]. Leaf decoction is often added to women's breasts while their babies are weaned to avoid infection [28]. In suspected diabetic patients among people in the south-western part of Nigeria, new leaves of *M. lucida* is macerated in fresh palm wine and filtrate taken by mouth for blood sugar control [42,61]. The infusion of stem bark is used as an antimalarial and antidiabetic medication [62]. Lawal *et al.*, [28] reported the used of *M. lucida* locally in the treatment of irregular menstruation, insomnia and jaundice.

3. PHYTOCHEMICAL COMPOSITION

Plants have different phytochemical properties, such as glycosides, saponins, alkaloids, flavonoids, tannins, which provide pharmacological importance because plants use them to combat disease-causing pathogens [63]. In general, the search for these compounds from medicinal plants ends with the isolation of novel compounds and, finally, the production of drugs [64]. The study [65] documented the presence of ethanol, acetone and aqueous extracts of *M. lucida* with flavonoids, tannins and sugar reduction. Whereas saponins were only found in the water extract. Qualitative and quantitative preliminary screening of the phytochemical constituents on some of the leaves tested showed that alkaloids, tannins, anthraquinones and steroids were present in the leaves of the plant [42]. Ethyl acetate and ethanolic extracts prepared from the bark, leaves and stem of *M. lucida* was screened for phytochemical constituents and the result reported the presence of saponin, tannins and alkaloids in the leaves extract [66]. Similar study revealed the presence of alkaloids, saponins, tannins [48,67] and anthraquinones [67]. It was reported that the leaves, stem barks and roots extract of *M. lucida* contained alkaloids, tannins, glycosides, terpenoids, saponins,

Phenols, hydrogen cyanide, flavonoids and steroids [33]. The result also showed higher values of alkaloids, terpenoids, phenols and steroids in all the plant parts screened [33]. [68] studied the phytochemical composition of the aqueous leaf extract of *M. lucida* and result revealed the presence of alkaloids and flavonoid as the predominant secondary metabolite [68]. While the phytochemical screening of methanolic leaf extracts showed the present of secondary metabolite including tannins, anthocyanine, steroid, anthraquinones, terpenoids and saponin [69]. An investigation of ethanolic extracts prepared [70] from the bark, leaves and root of *M. lucida* were evaluated for the presence of different secondary metabolites [70]. The finding, revealed the presence of saponin, tannins, anthraquinone, alkaloids, and cardiac glycosides in the leaves, bark and root extracts, while flavonoids was presence in the ethanol stem extract. The finding of [25] on the ethanol extract of the plant revealed the presence of tannins, glycosides, alkaloids and flavonoids. Addy *et al.*, [51] tested and confirmed the presence of important phytochemical compositions (saponins, anthraquinones, cardenolides, alkaloids, sterols and tannins) on the leaves of *M. lucida* using ethanol and dichloromethane as a solvent.

The result of preliminary phytochemical screened by [27], showed that the aqueous stem bark extract of *M. lucida* possess catechic tannins, triterpenes, flavonoids, alkaloids, saponins and phenols, whereas sterols and galic tannins were absent. Reference [40] investigated the phytochemical composition of the crude methanol extract of *M. lucida* the result showed that the plant contained alkaloids, glycosides, reducing sugar, flavonoids, resins, carbohydrates, steroids, terpenoids, tannins and saponins. The preliminary phytochemical analysis performed on the leaf powders of *M. lucida* showed different secondary metabolites such as alkaloids, flavonoids, triterpenoids, steroids, coumarins, anthocyanins, tannins cardenolins and reducing compounds [36]. The phytochemical screening of both aqueous and methanolic extracts of *M. lucida* carried out [43] revealed the presence of glycosides, saponins, flavonoids and steroids [43]. *M. lucida* leaf extract analysed revealed that the extract contains saponins, terpenoids, flavonoids, glycosides, and tannins [71]. Another study screened the *HEML* and showed the presence of saponins, reducing sugars, polyphenols and flavonoids, with absence of polyuronides, cyanogenic glycosides, alkaloids, anthraquinone sides, triterpenes and phytosterols [72].

4. PHARMACOLOGICAL ACTIVITIES

Previous studies have demonstrated that leaf and stem bark extracts of *M. lucida* possess numerous pharmacological matters to do such as antibacterial [31,52,67], antiviral [57], antidiabetic [25,28,49,57], antimalarial [19,57], anti-inflammatory [53,57], anticancer, hepatoprotective, cytotoxic and genotoxic, antispermatogenic, hypoglycemia [28,49]. This review focused on antidiabetic/hyperglycemic, antimalarial. Diabetes mellitus is a chronic disease of carbohydrate, lipid and protein metabolism characterised by using chronic elevations of fasting blood glucose above 200 mg dL⁻¹ due to insulin insufficiency or whole cessation of insulin synthesis or secretion and/or insulin resistance [43]. Olajide et al. [73] studied the hypoglycemic and antihyperglycaemic activities of a methanol extract of *M. lucida* leaves in normal and streptozotocin-diabetic rats. The outcomes recommended that the leaves extract of the plant have a sturdy glucose decreasing property when administered to streptozotocin-treated rats. Possible defensive feature and mechanism of action at 125-500 mg/kg/day of *M. lucida* aqueous stem bark extract (MLASE) on renal and hepatic features in alloxan-induced hyperglycemic rats for 8 days was once investigated [74].

The finding confirmed that intraperitoneal injection with one hundred twenty mg/kg of alloxan in bloodless 0.9% ordinary saline reliably and notably precipitated a normally sustained hyperglycemia which have been ameliorated by using the short-term oral therapy with 125-500 mg/kg/day of MLASE. Antidiabetic, antihyperlipidaemic, and antioxidant activities of the blended aqueous extracts of *M. lucida* and *Saccharum officinarum* leaves was also investigated [38]. A significant reduction at $P < 0.001$ in the fasting blood glucose of diabetic rats dealt with the plant extracts, both one via one and the extract mixture when compared to the untreated diabetic crew was observed. Diabetic treated groups showed a significant decrease ($P < 0.001$) in the levels of Total cholesterol and low-density lipoprotein cholesterol when compared to the diabetic untreated group.

Methanol leaf extract of *M. lucida* (MLEML) was assessed for hypolipidemic activity on Alloxan induced diabetic wistar rats [74]. Result obtained from the study, showed that the two groups orally administered with different doses of the extract at 100 mg/kg and 200 mg/kg, have their blood glucose levels significantly lowered ($p < 0.05$) when compared with the diabetic control but higher than that of the normal control rats. Results of the study indicate that MLEML can be potentially used for diabetics to control glucose and lipid levels [75]. Extracts of *M. lucida* and *Nauclea latifolia* caused a significant reduction in the rats' glucose level after treatment as evaluated [37], while the status of reduced glutathione and antioxidant enzyme activities was significantly increased. The total cholesterol, low-density lipoprotein, triglyceride status showed reduction compare to the control. Also, the concentration of malondialdehyde in the groups administered with *M. lucida* (2.88 ± 0.00) and with *N. latifolia* leaves extracts (3.85 ± 0.12) was reduced compare to the diabetic control group (7.37 ± 0.10) [37]. Investigation of aqueous and 50% methanolic leaf extracts of *M. lucida* performed [76] for hypoglycemic and toxicological effects in alloxan-induced hyperglycemia in rats. The aqueous and 50% methanolic extracts significantly ($P < 0.05$) reduced the blood glucose level of the hyperglycemic treated rats compares with the untreated group. The aqueous extract showed more hypoglycemic effects compared with the 50% methanolic extract. The hypoglycemic activities of the extracts are concentration dependent.

Malaria is the most common prima health hassle in tropical and developing global locations of sub-Saharan Africa and South East Asia, accountable for the dying of one-two million human beings every year and about 300-500 million human beings being infected [77]. It is a parasitic health problem transmitted as a quit result of the bites of Anopheles mosquitoes infected with Plasmodium species. Four species of plasmodium which infect human beings are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* [78]. An in vivo activities of ethanol extracts of two vegetation in particular *A. boonei* and *M. lucida* at unique doses (400, 600, and 800 mg/kg and chloroquine at 10 mg/kg) was carried out against established *P. berghei* NK65 [79]. The results revealed that the percentage parasitemia was higher in the infected untreated mice (18.40%) than the treated mice in which highest percentage parasitemia (6.0%) was obtained in mice treated with 800 mg/kg of *A. boonei* while the lowest percentage parasitemia (0.0%) was obtained in mice treated with 10 mg/kg of chloroquine. Also, antiplasmodial activity was highest with the combined recipe of *M. lucida* and *A. boonei* at 800 mg/kg (92%) and lowest at 800 mg/kg (45%) of *M. lucida*. However, the optimal antiplasmodial activity (83%) of *M. lucida* was obtained at 400 mg/kg and that of *A. boonei* was obtained at 600 mg/kg (85%). The finding of [69] concluded that methanolic leaf extract of *M. lucida* at 400, 600 and 800 mg/kg body weight of suppressed *P. berghei* NK65, therefore the extracts of the plant could be used in the management of malaria.

Umar et al. [19] assessed the therapeutic effects of the methanolic root extract and a combination of extracts of the leaf and root parts against *P. berghei* infection in mice. Percentage suppression of parasitaemia for the methanolic root extract was found to be 56.30, 59.84, 67.72 and 81.80% for doses of 100, 200, 400 mg/kg body weight of the extract, and 5 mg/kg chloroquine respectively. The study suggested the use of the plant parts for development of antimalarial drug. Antimalarial activity of stem bark extract of *M. lucida* was performed against *P. falciparum* strain 3D7 using the parasite lactate dehydrogenase (pLDH) assay [80]. The result indicated that some fractions reduced the malaria parasite viability by approximately 50% at 100 µg/mL.

The study of [81] investigated the antiplasmodial activities of the combined aqueous extract of *M. lucida*, *Phyllanthusamarus*, *Vernonia amygdalina* and *Newbouldialaavis* at variable doses in Swiss albino mice infected with *P. berghei* (NK65). The results of the 4 days suppressive test revealed that the test extract achieved percentage suppression of 9.8%, 58.3% and 60.2% for 200mg/kg, 400mg/kg and 800mg/kg concentration respectively. While, the curative test achieved the highest parasitaemia reduction with 800mg/kg ($29.5 \pm 29.1 \times 10^3$) which was comparable to the standard antimalarial drug. In another study, [82] combined the aqueous extracts of *Morinda morindiodes* (Mm) root, *Morinda lucida* (ML) leaf and *V. amygdalina* (Va) (leaf) were combined and assessed for anti-malarial activities in *Plasmodium berghei* infected mice. The finding showed that the combination of ML with Va (ML+Va) and Mm(ML+Mm) produced 40.4% and 70% suppression respectively. While combination of *M. morindiodes* with *V. amygdalina* (Mm+Va) produced 50.63% suppression on day six of treatment. Lastly,

Combination of the three plants extract produced 53.21% suppression compared to the untreated group which recorded no suppression at the end of the sixth day ($p < 0.05$). These results were significantly different ($p < 0.05$) when compared with the infected non-treated group. Also, results obtained from the study revealed that, the extracts of Mm and ML possess antiplasmodial effect when administered singly while the combination of these extracts reduced their chemosuppression. Prophylactic efficacy of a crude aqueous extract of the leaves of *M. lucida* on *P. falciparum* was studied using sixteen albino rats grouped into curative, suppressive, prophylactic and control [29]. The result showed that CAEML has a prophylactic efficacy in addition to its curative activity against *P. parasites* and could be used to mitigate the impact of plasmodium parasites. *In vitro* antitrypanosomal activity was tested against *Trypanosoma brucei brucei* S427 [83]. An investigation of [84] identified three novels tetracyclic iridoids, molucidin, ML-2-3, and ML-F52, from the CHCl₃ fraction of *M. lucida* leaves which possess activity against the GUTat 3.1 strain of *T. brucei brucei* the 50% inhibitory concentrations (IC₅₀) of molucidin, ML-2-3, and ML-F52 were 1.27 μ M, 3.75 μ M, and 0.43 μ M, respectively. ML-2-3 and ML-F52 suppressed the expression of paraflagellum rod protein subunit 2, PFR-2, and caused cell cycle alteration, which preceded apoptosis induction in the bloodstream form of *Trypanosoma* parasites. The aqueous leaf extract of *M. lucida* showed the possession of trypanocidal properties and could be useful as a source of new trypanocidal agent from medicinal plants [58]. The study of [85] was designed to evaluate the trypanocidal properties of hot and cold aqueous leaf extracts of *M. lucida* in mice infected with *T. brucei brucei*. The hot aqueous extracts of the leaf at 100, 200, 300, 400 and 500 mg kg⁻¹ body weight per day were administered to the tested groups intraperitoneally for 3 days.

The results obtained in the study show that aqueous extract of the plant possesses antitrypanosomal activity [85]. The result of the antitrypanosomal screening *M. lucida* crude extract, fractions 13-18, 19-28 and 33-45 showed antitrypanosomal activities with MIC values of 25, 6.25, 25 and 12.5 μ g/ml, respectively [86]. Abubakar et al. [48] explored the potential of *M. lucida* and *Tridaxprocumbens* individually as single therapy and as combination therapy in the treatment of trypanosomiasis. The finding showed that methanol leaves and stem bark extracts of *M. lucida* gave significant mean survival of 7.0 ± 3.3 and 9.7 ± 3.7 days respectively when compared to the untreated control ($P < 0.05$). The combination of *T. procumbens* and methanol leaves extract of *M. lucida* at 1:2 gave significant mean survival of 10 ± 2.2 days ($P < 0.05$) at 200 mg/kg body weight. An investigation of the activity of crude leaf extract of *Morinda lucida* on *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* *in vitro* using the agar well diffusion method. *P. aeruginosa* and *S. aureus* showed inhibitory activity at a concentration of 10mg/ml, with no activity against *S. typhi* at a concentration of 10mg/ml [51]. Antimicrobial study on the ethanol extract of *M. lucida* using the micro broth dilution method against strains of *S. aureus*, *S. pyogenes*, *Escherichia coli*, *P. aeruginosa*, *Salmonella typhi* and *Candida Albicans*, demonstrated a minimum inhibitory concentration (MIC) with values ranging from 10 to 30 mg/mL [25]. *In vitro* and *in vivo* antimicrobial activity leaf extract of *M. lucida* on *Escherichia coli* was investigated and the results showed that 25mg/ml of the extract inhibited *E. coli* with a zone of inhibition measuring 5 mm [67]. Therefore, this plant extract can be used for the treatment of the symptoms of infections caused by enteropathogenic *E. coli*. While, aqueous and methanolic extracts of the root, stem bark, and leaf of *M. lucida* was tested *in vitro* for inhibitory activities on *E. coli*, *S. typhi*, *S. paratyphi*, and *S. typhorum*. Both extracts showed significant inhibition growth of *E. coli* and *S. species* [31].

Antioxidant effects of the aqueous stem bark extract of *Morinda lucida* in mice was evaluated [27]. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and Folin-Ciocalteu tests showed that the aqueous stem bark extract of *M. lucida* has a significant antioxidant activity. While, aqueous extract of the leaves of *M. lucida* demonstrate strong analgesic, anti-inflammatory and antipyretic potency comparable in a time and dose dependent manner to a nonsteroidal anti-inflammatory drug [83]. Anticonvulsant activity of the methanol extract of *M. lucida* using the suppression test against pentylenetetrazole (PTZ) and isoniazid (INH) induced convulsion was evaluated [40]. The result showed a significant dose-dependent delay of the time of onset, peak of seizure, recovery and mortality from seizure at different doses (200, 400 and 800 mg/kg). The anticonvulsant activity observed may be attributed to the presence of alkaloids, flavonoids, saponins, steroids, terpenoids and tannins.

Table 1: Phytochemical composition of different parts of *M. lucida*

		Aqueous	Methanol	Ethanol	Parts	References
1	Alkaloids	+	+	+	Leaves, stem bark	[25,27,33,40,43,66,70]
2	Anthraquinones	+	+	+	bark, leaves and root	[33,42,70]
3	Flavonoids	+	+	+	bark, leaves and root	[25,27,66,68,70]
4	Coumarins	+	+	+		
5	Saponins	+	+	+	bark, leaves and root	[66]
6	Terpenoids	+	+	+	Leaves	[69]
7	Triterpenoids	+	+	+		[27]
8	Tannins	+	+	+	bark, leaves and root	[66]
9	Cardiac glycoside	+	+	+	bark, leaves and root	[33,40,66]
10	Resins	+	+	+		[40]
11	Reducing sugar	+	+	+		[40]
12	Carbohydrates	+	+	+		[40]
13	Phenols	+	+	+	bark, leaves and root	[33]

Key: + =presence

5. CONCLUSION

Morinda lucida is a rainforest tropical tree commonly referred to as the Brimstone tree, Indian mulberry and Hog tree apple. Different components of this plant are used for medicinal purposes, including stem barks, roots and leaves. Leaves are traditionally used for the treatment of malaria, analgesic, laxative, anti-infection and as a general febrifugation while stem bark infusion is used as an antimalarial and antidiabetic. The root of the plant is used for malaria management in combination with other plants. The presence of various secondary metabolites such as alkaloids, anthraquinones, anthocyanins, carbohydrates, cardenolines, coumarins, flavonoids, glycosides, cyanide hydrogen, phenols, compound reducers, resins, saponins, steroids, tannins, terpenoids and triterpenoids has been demonstrated. The pharmacological activities of *M. Lucid* may be attributed to the presence of alkaloids, flavonoids, saponins, steroids, terpenoids and tannins identified in different extracts of the plant.

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