



## Antidermatophytic Effect of *M Lucida* Part Extracts (Ethanollic and Aqueous) on Selected Clinical Isolates

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### Abstract

*Dermatophytoses are the most frequent fungal infections worldwide, affecting individuals in various age groups and heading to worse quality of life and economic burden due to treatment expenditure. Hence this study was carried out to determine the effect of Morida lucida parts extract on dermatohytes.*

*Twenty-five gram (25g) of the plant sample was weighed into a 250ml sample bottle, 300ml of the different solvents were added, the solvents used were ethanol and water. The mixture was allowed to stand for 72 hours (3days) then filtered. The filtrates were concentrated using a rotary evaporator. Phytochemical screening was carried out on the extracts, Anti-dermatophytic effect of the Morinda lucida extracts was evaluated by the well plate agar diffusion method. A sterile cork borer was used to make wells (6mm in diameter) on the agar plates. Aliquots of 0.1ml of extract, was filled into each of the wells in the culture plates previously inoculated with the test organisms. Each well was labeled appropriately. Control experiments were also carried out where the holes were filled with 0.1ml of Fluconazole (200mg) as positive control. Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration (MFC) of Morinda lucida parts extracts were respectively carried out by micro tube dilution method on the isolates. Phytochemical screening analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, steroids, and glycoside. The result of MIC and MFC showed higher effect of aqueous bark extract on all the isolates, followed by ehanolic bark extract.*

*Aqueous bark extract was observed with the highest MIC and MFC effects on all the isolates.*

### Introduction

Cutaneous mycoses are among the most common fungal infections and are mostly caused by filamentous keratinophilic fungi that use keratin as a nutrient during skin, hair, and nail infection. These fungi are called dermatophytes and are classified into three genera, *Trichophyton*, *Microsporum* and *Epidermophyton*, based on the

formation and morphology of their conidia (structures of asexual reproduction). In addition, species of dermatophytes are divided into zoophilic, geophilic or anthropophilic, depending on their primary habitat (animals, soil or humans, respectively). Zoophilic species are responsible for about 30% of human dermatophytoses and they often provoke acute inflammation;

anthropophilic species represent about 70% of infections on these hosts, causing a chronic infection of slow progression, suggesting that the fungus has adapted to the human host. So far, about 30 species of dermatophytes have been identified among human pathogens.<sup>1</sup> The transmission of dermatophytoses or tinea occurs by direct contact with infected animals and humans or by indirect contact with contaminated fomites. The clinical forms vary according to the etiologic agent (species) and the anatomical site involved. Symptoms may be mild or severe based on the host's immunologic condition, and invasion of subcutaneous tissues or internal organs normally does not occur. Typical lesions of skin infection are circular, erythematous and pruritic, and are the result of direct action of the fungus or hypersensitivity reactions to the microorganism and/or its metabolic products. In nail infections (onychomycosis) the nail may separate from its bed, may become thick and have white spots or even become dystrophic.<sup>2</sup> Although infections by dermatophytes are usually restricted to the superficial epidermis, these fungi may be invasive and cause a severe and disseminated infection in immunocompromised patients, with the development of dermatophytic granulomas.<sup>3</sup> According to the World Health Organization (WHO), dermatophytes affect about 25% of the world population. It has been estimated that from 30 to 70% of adults are asymptomatic hosts of these pathogens and that the incidence of the disease increases with age. Generally, dermatophytes exhibit a cosmopolitan profile, that is, they are found in different regions of the world with variations in the frequency of particular species, as geoclimatic and social conditions interfere with the distribution of dermatophyte species.<sup>4</sup> Climatic factors, social practices, migration and individual characteristics may influence the epidemiology of dermatophytoses. Moreover, some risk factors have been associated with onychomycoses, such as age, abnormalities in nail morphology, genetic factors, poor hygiene conditions, *diabetes mellitus*, and

immunodeficiency.<sup>4</sup> Reports suggest that hormonal rates affect the frequency of these infections in men and women and that steroid hormones may influence the growth of these pathogens. Some species of *Trichophyton* and *Microsporum* have cytosolic proteins that bind exclusively and with high affinity to progesterone; the latter, in pharmacological and physiological concentration, inhibits the growth of dermatophytes in a dose-dependent manner. Anthropophilic species respond better to the action of steroids than geophilic species.<sup>5</sup>

An epidemiological evaluation involving 16 countries in Europe showed that between 35 and 40% of the analyzed individuals had infection of the foot (*tinea pedis*) caused by dermatophytes.<sup>6</sup> In addition, a study conducted with children in daycares in the United States revealed that between 22 to 50% of them exhibited symptoms of dermatophyte infection of the hair scalp.<sup>7</sup> In Brazil, the Southern and Southeast regions have a high incidence of infections caused by the dermatophyte *Trichophyton rubrum*, followed by *Microsporum canis* and *Trichophyton mentagrophytes*, whereas in the Northeast, *Trichophyton tonsurans*, *T. rubrum* and *M. canis* predominate.<sup>8, 9</sup> In the State of Sao Paulo, 4,500 children (0-15 years old) were evaluated to determine the incidence of *tinea capitis* from 1996 to 2000. Of the 132 children with suspicion of infection, 112 (85%) received confirmation through direct microscopy and culture. The most common isolates were *M. canis* (70.5%) and *T. tonsurans* (23.2%), followed by *T. mentagrophytes* (3.6%), *Microsporum gypseum* (1.8%) and *T. rubrum* (0.9%).<sup>10</sup>

Among the fungi isolated from skin infections, the anthropophilic dermatophyte *T. rubrum* is the most frequent in clinical cases of *tinea pedis* (feet), *tinea unguium* (nails), *tinea corporis* (body) and *tinea cruris* (groin region).<sup>4</sup> Epidemiological studies conducted with university students from Sao Paulo demonstrated the occurrence of dermatophytes in 18.2% of the studied population; *T. rubrum* was isolated in 80% of the

cases and *T. mentagrophytes*, in 20%.<sup>11</sup> Among onychomycoses, *T. rubrum* is also the most prevalent dermatophyte, affecting children and adults in about 33.2% of the cases identified in Sao Paulo, followed by *T. mentagrophytes* (6.3%).<sup>12</sup> In Fortaleza, a three-year study described the isolation of dermatophytes in 12.99% of the cases of onychomycosis; *T. rubrum* was isolated in 9.04% of the patients and *T. tonsurans* and *T. mentagrophytes*, in 2.54% and 1.41%, respectively.<sup>13</sup>

*Morinda lucida* Benth., belonging to the family Rubiaceae is a tropical rainforest tree with the English name "Brimstone tree". It is also known as Sangogo or Bondoukou alongua (in Cote d'Ivoire), Twi, Kon kroma or Ewe amake (in Ghana), Ewe amake or Atak ake (in Togo) and Oruwo or Ruwo amongst the Yoruba tribe (South-west Nigeria) and Huka or Eze-ogu amongst the Igbo speaking tribe of South-east Nigeria (Adeneye, 2013). It is a medium size tree about 15m tall with scaly grey bark, short crooked branches and shining foliage. In West Africa, *Morinda lucida* is an important plant in traditional medicine. In Nigeria, *Morinda lucida* is one of the four most used plants in the preparation of traditional medicines against fever (Lawal, Etatuvie, and Fawehinmi, 2011). The leaves are used as "oral teas", which are usually taken orally for the traditional treatment of malaria, and as a general febrifuge, analgesic, laxative and anti-infections. The major constituents of *M. lucida* extract are the various types of alkaloids, anthraquinones and anthraquinols. Two compounds (oruwalol and oruwal) and anthraquinones have been isolated and characterized from the stem of the plant. In South-West Nigeria, fresh leaves of the plant are macerated in fresh palm wine and the filtrate taken orally for blood sugar control in suspected diabetic patients. The leaves have also been reported to possess strong hypoglycemia, trypanocidal and aortic vasorelaxant activities (Adeneye, 2013). Further studies have shown that leaf and stem bark of *M. lucida* possess anticancer

(Sowemimo, Fakoya, Awopetu, Omobuwajo, and Adesanya, 2007), hepatoprotective (Oduola, T., Bello. I., Adeosun, Ademosun, Raheem, Avwioro, 2010), antispermatogenic (Raji, Akinsomisoye, and Salman, 2005), and antidiabetic activity. The stem bark, roots and leaves infusion of *M. lucida* is used as an antimalarial, antidiabetic, jaundice and dysentery treatment and it is used in antimalarial activity, anti-*Salmonella typhi* activity, effect on contractility of isolated uterine smooth muscle of pregnant and nonpregnant mice, toxicity and mutagenic studies and it has anti-diabetic property. *M. lucida* extracts have been reported to have antioxidant and reducing activities, and antimicrobial activity. The major constituents of *M. lucida* extracts were found to be: essential oils, anthraquinones and anthraquinols. Oruwalol, oruwal, ursolic acid, and oleanolic acid were also isolated from this plant. The red colorants of *M. lucida* were confirmed to be 1-methyl-ether-alizarin, rubiadin and derivatives, lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin and purpuroxanthin, anthraquinones, oruwacin, tannins, flavonoids, digitolutein, and saponosides were isolated from different parts of *M. lucida*. The root of *M. lucida* is used as chewing sticks are for oral hygiene in Nigeria. The aqueous extracts of different parts of this plant were found to be effective against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

## Material and Method

### Collection and Authentication of Sample

Twenty five gram (25 g) of fresh stem bark of *Morinda lucida* were collected from Gateway (ICT) Polytechnic Saapade botanical garden. The harvested plant materials were processed for voucher referencing as previously described by Adeneye and Agbaje (2008). The fresh stem bark peels were gently rinsed under tap water and dried under room temperature protected from direct heat and sunlight for 4 weeks. Afterwards, the dried samples were pulverized using laboratory

hammer-mill in the King Hospital, Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University Teac, Ogun State, Nigeria.

### Extraction

Twenty-five gram (25g) of the plant sample was weighed into a 250ml sample bottle, 300ml of the different solvents were added, and the solvents used were ethanol and water. The mixture was allowed to stand for 72 hours (3days) then filtered. The filtrates were concentrated using a rotary evaporator.

### Phytochemical Screening

This was done on the different extracts to ascertain the presence of bioactive components present in leave. The presence of alkaloids, resins, Saponin, glycoside, tannins, Flavonoids, cardiac glycoside steroidal ring, steroidal terpenes, Anthraquinone and carbohydrates were determined as described by (Okwu 2005, Sofowora 1993; Trease and Evans 2002)

### Test for Tannins

About 5ml portion of the of the sample was stirred and shaken with 10ml of distilled water and then filtered, few drops of 1% ferric chloride solution were added to 2ml of the filtrate, occurrence of a blue – black, or blue – green precipitate indicated the presence tannins (Trease and Evans, 2002).

### Test for Anthraquinone

About 2ml of some portion to be tested was shaken with 10ml of benzene and then filtered. 5ml of the 10% ammonia solution was then added to the filtrate and thereafter was shaken appearance of a pink, red or violet colour in the ammonical (lower) phase showed as the presence of free anthraquinones (Sofowora, 1993)

### Test for Saponin

Two (2) ml of the sample was boiled with 20ml distilled water in the water bath. The mixture filtered, way about 5ml of distilled water was added to 10ml of the filtrate, this was shaken

vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously and then observed for the formation of emulsion (Okwu, 2005).

### Test for Flavonoids

5ml of 10% dilute ammonia solution was added to a portion of the plant extracts followed by addition of concentrated  $H_2SO_4$ . A yellow coloration observed in the extracts indicated the presence of Flavonoids (Okwu, 2005).

### Test for Alkaloids

Few quantities of the samples was stirred with 5ml of 1% aqueous HCL on water bath and then filtered, 1ml was placed into 2 test tubes, to the first portion, a few drops of Dragendorff's reagent were added; occurrence of orange – red precipitate was taken as positive. To the second 1ml, Mayer's reagent was added and appearance of buff – color, showed the presence of alkaloids (Sofowora, 1993).

### Test for Cardiac Glycosides

Five milliliters (5ml) of the extracts were treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This was underplayed with 1ml of concentrated  $H_2SO_4$ , a brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring may appear below the ring. While in the acetic layer a greenish ring may form just gradually throughout their layer (Okwu, 2005).

### Test for Steroids

Two milliliters (2ml) of acetic anhydride was added to 0.5ml of each extract this was followed by the additional off with 2ml  $H_2SO_4$ . The colour changed from violet to blue or green in some sample indicating the presence of steroids (Okwu, 2005).

### Determination of antifungal activity (Agar well diffusion method)

Anti-dermatophytic effect of the *Morinda lucida* extracts was evaluated by the well plate agar

diffusion method. The dermatophyte cultures were adjusted to equal 0.5 McFarland turbidity standards and inoculated on PDA ( Oxoid) plate (diameter 9cm).The plates were flooded with 1ml of each of the standardized test organism, swirled and excess inoculum was carefully decanted. A sterile cork borer was used to make wells (6mm in diameter) on the agar plates. Aliquots of 0.1ml of extract, was filled into each of the wells in the culture plates previously inoculated with the test organisms. Each well was labeled appropriately. Control experiments were also carried out where the holes were filled with 0.1ml of Fluconazole (200mg) as positive control. However, the experiment was done in duplicates. The plates were then left on the bench for 30 minutes for

proper diffusion of the extracts. Thereafter, the plates were incubated at 37 °C for 24 hours.

**Minimum Inhibitory Concentration (MIC)**

Minimum Inhibitory Concentration (MIC) of *Morinda lucida* was performed on the isolates by microtube dilution method. The 96 well microtiter plate was used for this bio-assay and each well was labelled.

**Minimum Fungicidal Concentration (MFC) assay**

Sub-culturing was made from each well showing no growth including the MIC well onto SDA plate. Following incubation at 37c for seven days, the plate was examined for growth of dermatophytes.

**Results**

**Table 1:** Phytochemical Characteristics of Root Bark and Leaf extracts of *Morinda lucida*

Parameters	Mean(±SD)					
	Root		Bark		Leaf	
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Alkaloids	0.37±0.3	0.29±0.2	0.39±0.2	0.35±0.1	0.45±0.5	0.41±0.2
Flavonoids	0.26±0.2	0.21±0.1	0.28±0.1	0.21±0.2	0.35±0.2	0.35±0.3
Saponins	0.25±0.1	0.21±0.0	0.27±0.3	0.25±0.3	0.30±0.2	0.28±0.2
Tannins	0.31±0.1	0.28±0.2	0.32±0.3	0.30±0.2	0.37±0.1	0.30±0.1
Steroids	0.24±0.2	0.18±0.1	0.26±0.0	0.24±0.1	0.29±0.2	0.25±0.2
Glycoside	0.19±0.1	0.15±0.2	0.21±0.2	0.19±0.1	0.25±0.3	0.21±0.1
Anthraquinone	0.12±0.1	0.10±0.0	0.14±0.0	0.10±0.0	0.18±0.2	0.14±0.1

**Table 2** Anti-dermatophytic effect of *Morinda lucida* part extracts

Dermatophytes	<i>M. lucida</i> / Mean(±SD)Zone diameter (mm)						Control
	Root		Bark		Leaf		
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	
<i>M. audouinii</i>	18.0±0.2	15.0±1.0	19.0±1.0	14.0±2.0	22.0±2.0	19.0±1.0	25.0±1.0
<i>M. ferrugineum</i>	15.0±0.0	11.0±0.0	17.0±1.0	10.0±1.0	18.0±1.0	15.0±2.0	27.0±0.0
<i>E.floccosum</i>	19.0±0.5	17.0±1.0	19.0±0.5	14.0±2.0	23.0±1.0	21.0±0.5	25.0±0.0
<i>T.schenieni</i>	12.0±1.0	10.0±0.0	15.0±1.0	11.0±1.0	17.0±1.0	14.0±1.0	26.0±0.0
<i>T.rubrum</i>	10.0±0.0	7.0±1.0	13.0±1.0	10.0±0.5	18.0±0.5	15.0±0.5	25.0±0.5
<i>T.metagrophyte</i>	12.0±0.5	9.0±1.0	15.0±0.5	13.0±1.0	21.0±1.0	19.0±1.0	25.0±0.0

Controls – Fluconazole

**Table 3** Minimum Inhibitory Concentration (MIC) of *Morinda lucida* part extracts.

Dermatophytes	<i>M. lucida</i> / MIC (% v/v)						Control
	Root		Bark		Leaf		
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	
<i>M. audini</i>	12.5	12.5	12.5	25.0	6.25	12.5	3.13
<i>M. fereginum</i>	12.5	25.0	12.5	50.0	12.5	25.0	1.57
<i>E.flocossum</i>	6.25	12.5	12.5	50.0	6.25	6.25	3.13
<i>T.schenieni</i>	25.0	50.0	25.0	25.0	12.5	25.0	3.13
<i>T.rubrum</i>	50.0	100.0	25.0	50.0	12.5	25.0	3.13
<i>T.metagrophyte</i>	25.0	100.0	25.0	50.0	6.25	12.5	3.13

Controls – Fluconazole

**Table 4** Minimum fungicidal Concentration (MFC) of *Morinda lucida* part extracts.

Dermatophytes	<i>M. lucida</i> MBC (% v/v)						Control
	Root		Bark		Leaf		
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	
<i>M. audini</i>	25.0	25.0	25.0	50.0	12.5	50.0	6.25
<i>M. fereginum</i>	25.0	50.0	25.0	100.0	25.0	50.0	3.13
<i>E.flocossum</i>	12.5	50.0	25.0	100.0	25.0	12.5	6.25
<i>T.schenieni</i>	50.0	100.0	50.0	50.0	50.0	50.0	3.13
<i>T.rubrum</i>	100.0	100.0	50.0	100.0	50.0	50.0	6.25
<i>T.metagrophyte</i>	50.0	100.0	50.0	100.0	25.0	25.0	6.25

Controls – Fluconazole

## Discussion

Table 1 shows the result of phytochemical screening of *Morinda lucida* part extracts, the following bioactive constituent were observed Saponin, alkaloid, flavonoid, tannins, steroid, and glucoside. This result corroborates the findings of (Adeneye, 2013).The root, bark, and leaf ehanolic and aqueous extracts were observed with essential bioactive constituents.

Table 2 Shows. anti-dermatophytic effect of *Morinda lucida* part extracts, all dermatophytes used in this study were sensitive to ehanolic and aqueous extract, but *Epidemophyton flocussom* was highly sensitive to aqueous and ethanolic extracts.

Table 3. Minimum Inhibitory Concentration (MIC) of *Morinda lucida* part extracts reveals susceptibility of the isolates to different concentrations of the *M lucida* part extracts with the following results 12.5on *M audounii*, (ethanolic and aqueous extract), *M audounii*, *Tmentagrophyte*, and *T rubrum* respectively. Highest susceptibility was observed in *M fereginum*( 50.aqueous extract).

Table 4. Minimum fungicidal Concentration (MFC) of *Morinda lucida* part extracts.shows high susceptibility of all the isolatess to bark,root and leaf extracts(ethanolic and aqueos).

## Conclusion

This work has demonstrated that *Morinda lucida* part extracts possesses broad spectrum antifungal activity against dermatophytes, because all the isolates used in this study were susceptible to aqueous and ethanolic extracts of *Morinda lucida* parts extract

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