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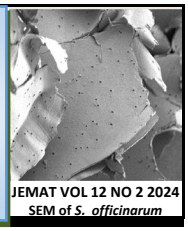

Anti-Trypanosomal Activities of Methanolic Extracts of Morinda Lucida Fruit, Stem Bark and Root Against *Trypanosoma brucei brucei*

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ABSTRACT

The study evaluated the anti-trypanosomal effects of methanolic extracts from the fruit, stem bark, and root of *Morinda lucida* against *Trypanosoma brucei brucei*. Each plant part (100 g) was macerated in 1000 mL of methanol for 72 hours. Phytochemical assays revealed the presence of saponins, flavonoids, phenols, tannins, terpenoids, alkaloids, anthraquinone, and cardiac glycosides. Extract yields were 1.81 g (fruit), 2.22 g (root), and 3.71 g (stem bark). Quantitative analysis showed polyphenols, flavonoids, and alkaloids were most abundant in the root. In vitro tests demonstrated concentration-dependent trypanocidal effects, with median lethal concentrations (LC_{50}) of 0.463, 0.406, and 0.664 mg/mL for fruit, root, and stem bark extracts respectively. The anti-infectivity test indicated that extracts inhibited the ability of *T. b. brucei* to initiate infection in mice. This study suggests that *M. lucida* extracts possess significant in vitro anti-trypanosomal activity and inhibit infectivity, highlighting their potential as alternative treatments for trypanosomiasis.

INTRODUCTION

African trypanosomiasis is a serious disease caused by *Trypanosoma* parasites and spread by tsetse flies (*Glossina* species). It causes sleeping sickness in humans and "Nagana" in animals. The disease is a major problem in sub-Saharan Africa and affects both human health and livestock production. In humans, the disease is mainly caused by *Trypanosoma brucei rhodesiense* and *T. brucei gambiense*, and it affects around 500,000 people every year [1]. If not treated, the disease can be fatal. In animals, it causes symptoms like fever, weight loss, and anemia, which lead to serious economic losses for farmers [2]. The treatment for trypanosomiasis depends on chemical drugs, but these drugs have problems such as high toxicity, drug resistance, and high cost. Because of this, many researchers are now looking for natural alternatives. Plants are a good option because they produce many different chemical compounds that may be effective and less harmful [3]. Several tropical plants have already shown activity against parasites, including trypanosomes, malaria parasites, and nematodes [4,5]. One plant of interest is *Morinda lucida*, which is commonly used in traditional medicine in West Africa. It belongs to the same family as *Morinda citrifolia* (also known as noni), which has been studied for its antibacterial and antioxidant properties [6]. Other species such as *Morinda elliptica* have also shown useful properties. For example, researchers have produced anthraquinones—compounds known for their biological activity—from *M. elliptica* using cell cultures [7,8]. These compounds have shown antioxidant and anti-cancer potential [9,10]. In addition, essential oils from *Morinda citrifolia* have been used to control plant diseases and support the plant's own defense system [11].

Based on these findings, *M. lucida* may also have antitrypanosomal properties. In this study, we test the methanol extracts from the fruit, stem bark, and root of *M. lucida* against *Trypanosoma brucei brucei* in the laboratory. This parasite is often used in experiments to study African trypanosomiasis. The

aim of this work is to explore whether *M. lucida* can be a source of new, plant-based treatments for this disease.

MATERIALS AND METHODS

Collection of Plant Materials: *Morinda lucida* samples were collected from Kaduna State, Nigeria, and identified at the Biological Science Department herbarium, Nigerian Defence Academy.

Preparation and Phytochemical Screening of Extracts

The dried plant parts (100 g each) were macerated in 1000 mL of methanol for 72 hours. Extracts were filtered, evaporated, and analyzed for phytochemical content. Qualitative tests indicated the presence of various secondary metabolites were carried out using methods by Evans and Trease [12]. Quantitative assays measured concentrations of polyphenols, flavonoids, alkaloids, saponins, and tannins.

Source of Trypanosomes

Trypanosoma brucei brucei was obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria, and inoculated (*ip*) into four Wistar rats. Three days post-inoculation, the rats were examined for development of active infection using the wet mount technique [13]. For parasite estimation, the method of Herbert and Lumsden [14] was adopted.

Screening for Anti-trypanosomal Activity

Collection of Parasitized Blood

Following chloroform anaesthesia, blood was collected from the donor rats at raising parasitemia level via cardiac puncture into an Ethylene Diamine Tetra Acetate (EDTA) sample container, and was gently mixed to prevent clotting of the blood [13].

In Vitro Anti-Trypanosomal Assay

Extracts were tested against *T. brucei brucei* in a concentration-dependent manner: initially, 10 mg/mL stock concentration of each extract was reconstituted in serum free RPMI-1640 media (supplemented with 5% w/v D-glucose and 40 µg/mL gentamycin). Similarly, 10 mg/mL stock solution of diminazine acecurate; each stock solution was serially diluted (2x) to yield concentrations ranging from 10 to 0.3125 mg/mL. 100 µL of each of these dilutions were dispensed in triplicates into a 96 well micro-titre plate, followed by the addition of 30µl of the blood suspension. A set of controls containing only media (100 µL) and blood (30 µL) was also included. The titre plate was placed in the incubator at 37 °C for six hours, after which the numbers of motile trypanosomes were determined for each well of the titre plate [13]. For each concentration of extract/drug, as well as the control, the average number of surviving trypanosomes was calculated, and the values used for the estimation of median lethal concentration (LC_{50}).

Drug Incubation Infectivity Test (DIIT)

After the six-hour incubation period, 0.2ml of the contents of each of those wells of the titre plate was inoculated intraperitoneally into Wistar rats each of which was differentiated by a unique identifier label. The rats were then observed daily for the development of parasitemia for 30 days.

Data Analysis

Data generated from the study was subjected to statistical analysis. Results was presented in tables. The infection rate post inoculation for the drug incubation infectivity test experiment was determined. The least significance difference (LSD) was obtained by one-way analysis of variance (ANOVA) using statistical package for social science (SPSS).

RESULTS

Extract Yields and Phytochemical Content

The percentage yields of the methanolic extracts from different parts of *Morinda lucida* (fruit, root, and stem bark) varied significantly, indicating differences in the concentration and solubility of phytoconstituents among the plant parts. As shown in **Table 1**, the highest yield was obtained from the stem bark, which produced 3.71 g of extract from 50 g of dried plant material, corresponding to a yield of 7.42%. This suggests that the stem bark of *M. lucida* is particularly rich in methanol-soluble compounds, potentially including alkaloids, flavonoids, or other secondary metabolites known for their therapeutic effects. The root extract yielded 2.22 g, representing a 4.44% extraction efficiency. This intermediate value indicates a moderate concentration of extractable bioactive compounds in the root, which may still contribute significantly to the plant's medicinal properties. In contrast, the fruit produced the lowest extract yield, with only 1.81 g of extract obtained, equivalent to a 3.62% yield. This lower percentage suggests that the fruit contains fewer methanol-soluble phytochemicals compared to the root and stem bark.

Table 1. Percentage yield of the methanol extracts of the fruit, root and stem-bark of *M. lucida.*

Plant part	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Fruit	50	1.81	3.62
Root	50	2.22	4.44
Stem-bark	50	3.71	7.42

Qualitative Phytochemical Screening

Saponins, flavonoids, phenols, tannins, terpenoids, alkaloids, anthraquinone, and cardiac glycosides were all present in detectable quantities in the methanol fruit, root, and stem-bark extracts (**Table 2**).

Table 2. Qualitative phytochemical screening.

Phytochemical	Stem-bark	Root	Fruit
Saponins	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Anthraquinones	+	+	+
Cardiac glycosides	+	+	+

Key: (+): detected; (-): not detected

Quantitative Phytochemical Screening

There was statistically significant difference ($p < 0.05$) in the polyphenol concentrations in the roots (527.5 ± 3.42 µg/mL), the fruit (465.6 ± 10.1 µg/mL), and stem-bark (113.9 ± 5.25 µg/mL) extracts of the plant.

The flavonoid concentrations in the methanolic extract of the root (0.42 ± 0.04 µg/mL), fruit (0.17 ± 0.01 µg/mL), and stem-bark (0.07 ± 0.02 µg/mL) of *M. lucida* also differed significantly ($p < 0.05$). However, alkaloid concentrations in the roots (1.19 ± 0.01 mg/mL), fruit (0.88 ± 0.57 mg/mL), and stem-bark (0.60 ± 0.01 mg/mL) extracts of *M. lucida* did not differ significantly ($p > 0.05$) Saponins concentration in the methanolic extracts of the fruits (441.1 ± 7.02 µg/mL), roots (131.1 ± 5.03 µg/mL), and stem-bark (399.7 ± 10.3 µg/mL) of *M. lucida* varied significantly ($p < 0.05$). The tannin concentrations in the fruits, roots, and stem-bark extract of *M. lucida* also varied significantly ($p < 0.05$), with the highest concentration of tannins recorded in the fruit with a mean value of 36.8 ± 5.68 mg/mL, followed by the tannin concentration in the roots with a mean value of 32.9 ± 9.02 mg/mL. The least concentration of tannins was recorded in the stem-bark extract with a mean value of 28.0 ± 2.19 mg/mL (**Table 3**).

Table 3. Quantitative phytochemical screening of the methanol fruit, root and stem-bark extracts of *Morinda lucida.*

Extract	Polyphenols (µg/ml)	Flavonoids (µg/ml)	Alkaloids (mg/mL)	Saponins (µg/ml)	Tannins (mg/mL)
Fruit	465.6±10.1^{b}	0.17±0.01^{b}	0.88±0.57^{a}	441.1±7.02^{c}	36.8±5.68^{b}
Roots	527.5±3.42^{c}	0.42±0.04^{c}	1.19±0.01^{a}	131.1±5.03^{a}	32.9±9.02^{ab}
Stem-bark	113.9±5.25^{a}	0.07±0.02^{a}	0.60±0.01^{a}	399.7±10.3^{b}	28.0±2.19^{a}
p value	< 0.001	< 0.001	0.172	< 0.001	0.024

Note: Values are given as mean ± standard deviation. In each row, superscripts with the same letters are not significantly different ($p > 0.05$)

In Vitro Anti-Trypanosomal Activity

There was a statistically significant ($p < 0.05$) effect of the fruit extract on the mean number of surviving trypanosomes, with the extract exhibiting a concentration dependent effect on the number of surviving trypanosomes: at 10 mg/mL, 5 mg/mL, and 2.5 mg/mL concentrations of the fruit extract, the mean counts of surviving trypanosomes were 0.00±0.00 trypanosomes per field each; at 1.25 mg/mL of the fruit extract, the mean count of surviving trypanosomes was 4.33±1.63 trypanosomes per field; at 0.625 mg/mL concentration of the fruit extract, the number of surviving trypanosomes per field was 7.17±1.33 trypanosomes per field; at 0.3125 and 0.156 mg/mL concentrations of the fruit extract, the mean counts of surviving trypanosomes were 18.0±2.53 and 19.5±2.6 trypanosomes per field, respectively. In the control well, the mean trypanosome count was 27.3±2.88 trypanosomes per field (**Table 4**).

The root extract of *M. lucida* also demonstrated statistically significant ($p < 0.05$) concentration-dependent effect on the survival of trypanosomes over the range of concentration tested. The negative control has a mean count of 27.3 ± 2.88 trypanosomes per field, while at 10 mg/mL, 5.0 mg/mL, and 2.5 mg/mL of the root extract, the trypanosome counts were 0.00 ± 0.00 each; at 1.25 and 0.625 mg/mL, the mean count of surviving trypanosomes were 1.17 ± 1.16 and 2.67 ± 1.63 trypanosomes per field, respectively (**Table 4**). The methanol stem bark extract also exhibited concentration-dependent effect on the number of surviving trypanosomes after 6 hours of incubation: at 10.0 and 5.0 mg/mL of the stem bark extract, the mean number of trypanosome counts were 0.00 ± 0.00 trypanosomes per field each; at 2.5 and 1.25 mg/mL of the extract, the mean counts of surviving trypanosomes per field were 0.50 ± 0.84 and 7.50 ± 1.05 trypanosomes per field, respectively; at 0.625 mg per mL of the extract, the mean number of surviving trypanosome was 11.0 ± 1.55 trypanosomes per field while at 0.3125 and 0.156 mg/mL concentrations of the stem bark extract, the mean counts of surviving trypanosomes were 17.5 ± 4.23 and 19.8 ± 1.94 per field. In the control well, the mean count of surviving trypanosome was 27.3 ± 2.88 per field (**Table 4**). The standard drug diminazine aceturate also demonstrated significant ($p < 0.05$) concentration-dependent effect on the survival of trypanosomes. Over the concentrations of 10 mg per mL to 0.156 mg/mL Diminazine aceturate, there was no surviving trypanosome seen, thus the mean counts were 0.00 ± 0.00 per field. In the negative control, the mean count of trypanosome recorded per field was 27.3 ± 2.88 (**Table 4**). The results further give the median lethal concentrations (LC_{50}) of the fruit, root and stem bark extracts of *Morinda lucida* against *T. brucei brucei in vitro*. The LC_{50} of the fruit extract was 0.463 mg/mL (95% CI: 0.272 -0.429), the root 0.406 mg/mL (95% CI: 0.189 – 0.631), and stem bark 0.661 mg/mL (95% CI: 0.401 – 0.905) (**Table 4**).

Table 4. *In vitro* effect of the fruit, root and stem-bark extracts on the number of surviving Trypanosomes.

Conc (mg/mL)	Fruit extract	Root extract	Stem-bark extract	DA
10	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5.0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2.5	0.00±0.00^{a}	0.00±0.00^{a}	0.50±0.84^{a}	0.00±0.00^{a}
1.25	4.33±1.63^{b}	1.17±1.16^{a}	7.50±1.05^{b}	0.00±0.00^{a}
0.625	7.17±1.33^{c}	2.67±1.63^{b}	11.0±1.55^{c}	0.00±0.00^{a}
0.3125	18.0±2.53^{d}	20.3±2.07^{c}	17.5±4.23^{d}	0.00±0.00^{a}
0.156	19.5±2.26^{d}	20.3±1.86^{c}	19.8±1.94^{d}	0.00±0.00^{a}
Control	27.3±9.54^{e}	27.3±2.88^{d}	27.3±2.88^{e}	27.3±2.88^{b}
LC_{50} (mg/mL)	0.463	0.406	0.661	-

Note: Values (except LC_{50} values) are given as mean ± standard deviation. In each row, superscripts with the letters are not significantly different ($p > 0.05$).

Anti-infectivity Test

Rats that received the inoculum from the control became infected with trypanosomes, and survived up to the 12 days post-inoculation. On the other hand, in rat inoculated with trypanosomes and incubated with the fruit, root, stem back, as well as the diminazine, infection was not established subsequently, and the rats survived beyond 30 days of observation (**Table 5**).

Table 5. Infectivity status of mice inoculated with trypanosomes following incubation in the fruit, root, and stem-bark extract of *Morinda lucida.*

Extract	Concentration of extract (mg/mL)	Infection status	Survival time (days)
Fruit	1.25	Uninfected	> 30
Root	2.5	Uninfected	> 30
Stem-bark	2.5	Uninfected	> 30
Dim	0.156	Uninfected	> 30
Control		Infected	12

DISCUSSION

The presence of saponins, flavonoids, phenols, tannins, terpenoids, alkaloids, anthraquinone, and cardiac glycosides in detectable quantities in the stem, root, and fruit extracts of *M. lucida* were in consonance with several reports [15,16]. These findings indicate that secondary metabolites are major constituents of medicinal plants and are associated with diverse pharmacological activities [3,15,17]. The quantitative determinations of the phytochemicals in the fruit, roots and stem-bark extracts of *M. lucida* indicated that polyphenols, flavonoids, and alkaloids were most abundant in the root extract and least in the stem bark extract. These differentials correlate well with those of Oladeji *et al* [17], Ojewumi and Oluyori [18] and Dahunsi *et al*. [16]. These differences may be attributable to plant genetics, interaction of the plant with both soil and environmental

factors [19]. The results of the *in vitro* assay showed that the fruit, root and stem bark extracts of *M. lucida* demonstrated significant concentration dependent reduction in the number of surviving trypanosomes. These observations agree with those of Nweze [20], Kwofie *et al*. [21] and Amoa-Bosompem *et al*. [22]. The anti-trypanosomal activity recorded for the fruit, root, and stem bark extracts of *M. lucida* as observed in this study can be attributed to the presence of bioactive moieties resident in the plant. The present results further indicated that there was a differential in the effects of the fruit, root and stem-bark extracts on *T. brucei* as indicated by the values of the median lethal concentrations (LC_{50}) of the extracts with least value (0.406 mg/mL) given by the root extract and the highest (0.66 mg/mL) for the stem ark extract. These values indicated that the root extract was the most potent of the three extracts followed by the fruit and then the stem bark extract. These observed differentials in median lethal concentrations between the fruit, leaves and stembark extracts of *M. lucida* may be accounted for by the differences in the quantity of some of the phytochemicals that were identified in the extract: the roots extract had the highest amounts of polyphenols, flavonoids, and alkaloids, with an intermediate quantity of tannins and the least content of saponins; the relative abundance of these chemical classes in either the fruits or the stem-bark extract was also noted to correlate with their respective LC_{50} values. Thus, the in vitro activity of *M. lucida* may be attributable to the actions of these compound classes working either in concert or singly [15–18,20–22].

The anti-infectivity results indicated that the roots, roots, and stem-bark extracts of *M. lucida* inhibited the hitherto inherent ability of extract-native trypanosomes to infect mice. This may be connected with interference with the turnover of the glycoprotein surface coat that forms the outermost interface within host's internal milieu [23], which in turn renders the kinetoplastid more susceptible to the immune factors of the host organisms arrayed against the protozoa [24]. Interference with the turnover rate of the surface coat may result from a knockdown of the gene that expresses the protein coat or an impairment or blocking of the gene translation machinery [25]. *Morinda lucida* extracts especially from the root, showed promising anti-trypanosomal activity and potential for developing alternative treatments. Possible mechanism of action of *Morinda lucida* extracts on other on other species of trypanosomes should be explored.

CONFLICT OF INTEREST

The author declared no conflict of interest.

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