



Antibacterial Activity and Cytotoxic Analysis of Leaf Extracts of *Morinda Lucida* (Brimestone Leaf) on Selected Gastrointestinal Bacteria

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Abstract

Medicinal plants have been used for ages as remedies for human diseases because they contain bioactive components of therapeutic value. This study was undertaken to investigate the antibacterial efficacy and cytotoxicological effects of ethanol leaf extract of *Morinda lucida* against medicinally important bacterial strains (*Klesbsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typharium*). Agar well diffusion method, with varying concentrations of each extract (20, 40 60 and 80 mg/ml) were used. *Morinda lucida* leaf extract revealed zone of inhibitions against all tested bacteria species at 60 and 80mg/ml concentrations and no inhibition was observed against the isolates at 20 and 40mg/ml. The cytotoxicity effects of the *Morinda lucida* was evaluated using root tip cells of *Allium cepa*. In this study, root length were used to determine the 72hrs effective concentration (72hrs EC₅₀) and root growth inhibition. *Allium cepa* was treated with the *Morinda lucida* leaf extracts at different concentrations (0, 2, 10, 20, 50 and 100%) and the control; dechlorinated tap water which was kept in dark cupboard for 72hrs. The root growth was concentration dependent and statistically significant at tested concentration, the result of this study indicated that at higher concentrations of the extracts, it inhibits the *Allium cepa* root cells. Based on the outcome of the present study, it is suggested that *Morinda lucida* leaf extract has inhibitory effects against all the tested organisms and can be used in management of diseases caused by them. More research should be carried out on phytochemicals and anti-oxidative potential of *Morinda lucida* stem and root.

Key words: Cytotoxicity, *Morinda lucida*, *Allium cepa*, *Salmonella typharium*.

Introduction

Traditional medicine has always been a part of the culture even though this form of medicine is not as well organized (Ogunshe *et al.*, 2006). Plants have the major advantage of still being the most effective and cheapest alternative sources of drugs (Adedokun *et al.*, 2019). The local use of natural plant as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha *et al.*, 2002). The use of medicinal plants for treatment of microbial diseases is well known and has been documented since ancient times. In different part of the world, different varieties of plants are used in the treatment of different types of diseases. Root, barks or leaves were used in treatment of dysentery, ringworm, sore eyes and ear ache (Azoro, 2002). Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day (Barboza *et al.*, 2009). The situation has further been complicated by the rapid development of multi-drug resistance by the microorganisms to the antimicrobial agents

available. In the world, mostly in the rural areas of the developing countries, people depend on local medicinal plants remedy for their diseases and illness probably either because of the absence of modernized functional health facilities or because of ancestral or traditional beliefs. Plants produce adverse range of bioactive molecules, making the rich sources of different types of medicines. (Nair *et al.*, 2005). The Secondary metabolism of higher plants has been shown to be an almost inexhaustible source of compound with possible biological activities (Santos *et al.*, 2006). Higher plants used extensively in traditional medicines are increasingly being screened for their role in modulating the activity of environmental geotaxis eats (Sreeranjini and Siril, 2011). Moreover, there is little information on the potential risk to health of such herbs. It is known that, in general, green plant are a primary source of antimutagens as well as natural toxic agent (Plewa and Wagners, 2006) and many plants contain cytotoxic and genotoxic substances resulting from the long term use of such plants. In many places in



Argentina, there is a rich traditional use of herbal medicine for the treatment of various infections, diseases and injurer (Barboza *et al.*, 2009). *Morinda lucida* is an evergreen tree or rarely a shrub 2.4 – 1.8m height. The bitter tasting roots are used as flavouring in food and alcoholic beverages and in Nigeria they are popularly use as chewing sticks (Neuwinger,2000). In West Africa *Morinda lucida* is an important plant in traditional medicine, widely applied against various kinds of fevers and infections, different types of fevers including yellow fever, malaria, trypanosomiasis and feverish condition during child birth. Plant is also employed in cases of diabetes, hypertension, cerebral congestion, dysentery, stomachache, ulcers, leprosy and gonorrhea (Neuwinger, 2000). In Nigeria, *Morinda lucida* is one of the foremost used plants in traditional medicines against fever .Likewise, in Côte d'Ivoire the bark or leaf decoction is applied against jaundice and in Democratic Republic of Congo, it is combined with a dressing of powdered root bark against itch and ringworm (Zimudzi and Cardon, 2005). Cytotoxicity is one of the most important methods for biological evaluation as it has a series of advantages, along with the preferred and mandatory items. Given this information, the ability to accurately measure cytotoxicity can prove to be a very valuable tool in identifying compounds that might pose certain health risks in humans (Ogunshe *et al.*, 2012). The cytotoxicity test is one of the most important indicators of the biological evaluation system in vitro to observe the cell growth, reproduction and morphological effects by chemicals, and with the progress of modern cell biology; experimental methods to evaluate cytotoxicity are also continuously being developed and improved (Piao *et al.*, 2011). However, health for all is a dream and a goal which humanity at large shares and strives for, but is clear that modern pharmaceutical will remain out reach a large proportion of the human population for the foreseeable future. This gap has created the need for the use of alternatives and traditional medicines, largely herbal in nature to solve human health need. Hence, this study is aimed at evaluating the antibacterial activities and cytotoxic analysis of *Morinda lucida* leaf extract on selected gastrointestinal bacteria.

Materials and methods

Collection of material

Fresh leaves of *Morinda lucida* found growing naturally on ground of Igboora in Oyo State. The plant were carefully harvested. Identification of plant was confirmed by a botanist in the department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora. The leaves were washed thoroughly with clean water to remove dirt resident on the surface. The leaves were air dried for two weeks, after which they were milled into powder with a dry sterilized blender. The powdered were sieved, and stored in an air tight sterile container until it was used.

Ethanol leaves extract method

The cold extraction method was used. One hundred grammes (100g) of the dried sample (*Morinda lucida*) was weighed using weighing balance. Then added with 300ml of the extracting solvent (Ethanol) in the extraction tank. It was stirred immediately for 30mins. Then covered with foil paper with continuous agitation for 4 days. After 4 days, the whole process was terminated and was filtered to have our filtrate (extract). The filtrate was concentrated using rotary evaporator and finally opened to the atmosphere to get our dried extract. The extract was kept in a sample for further use.

Collection of Test Organisms

The organisms used for the investigation were clinical isolates obtained from Oluyole Laboratory, Igboora, Oyo State. The cultured *Salmonella typhi*, *Staphylococcus aureus*, *Klebselia pneumoniae* and *Escherichia coli* were obtained in McCartney bottles and introduce to prepared Muller Hinton agar in a sterile petri – dish plate. They were then stored at ambient temperature until required.

Method for Antimicrobial Bacterial Susceptibility Assay

The method of RajaKaruna *et al.* (2008) was used for the standardization of inoculum. The antimicrobial activity of *Morinda lucida* extract was determined by using a modification of the agar well diffusion techniques described by Perez *et al.* (2010). The prepare organisms on petri – dishes allow to set at room temperature router of 40°C. The Petri-dishes were bored with a sterile 8mm cork borer to create 5 wells on the agar plate which is about 10mm deep.



The wells were filled with 200mg/ml, 400mg/ml, 600 mg/ml and 800mg/ml of *Morinda lucida* extract. The plates were incubated for 24hr at 37°C. The results of inhibition zone in mm were then measured using transparent ruler.

Minimum Inhibitory Concentration of Extract

The Minimum Inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms (Kumar *et al.*, 2007). The MIC value were determined by varying concentrations of ethanol leaf extracts (20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml) and added to prepared Muller Hinton Agar that contain the bacterial isolate. The plates were then incubated at 37°C for 72hrs. The lowest concentration preventing all visible growth was taken as the minimal inhibitory concentration.

Preparation of Aqueous Leaf Extract

Extraction was carried out as practiced locally by Adedokun *et al.* (2019). 640g *Morinda lucida* leaf was weighed, washed and boiled in 2 liters of tap water until it ferments. The fermented liquid was taken into a clean bottle and was considered as stock solution.

Allium cepa Assay

Onion bulb (*Allium cepa* L. Zn=16) were obtained commercially at Igboora market. They

were sun dried for two (2) weeks and the dried outer scales were carefully removed leaving the ring of root primordial intact. These were used for bioassay according to Standard procedures of Adedokun *et al.* (2019) for the growth of root inhibition, five (5) concentrations of each extract were considered: i.e 2, 10, 20, 50 and 100% were used. Six onion bulb were utilized for each of concentration of each extract and the control (Tap water). The bases of the bulb were suspended on the extract inside 100ml beakers in the dark cupboard for 72hrs and avoid sunlight. The test extracts were being changed daily. At the end of the exposure period, the length of the roots of five onion bulb with the best growth at each concentration was measured in centimeter (cm) with a ruler from the weighted average. The percentage of root growth inhibition in relation to control and EC50 (effective concentration) was determined using the procedure of Adedokun *et al.* (2019). The colour was observed and root growth was examined.

Statistical Analysis

The length of the grown cell in the onion bulb was calculated using standard deviation method. The EC50 was determined from a plot of root length as a percentage of control against the sample concentrations, by using a Microsoft Excel computer program.

Results and discussion

Table 1: Inhibitory Effects of Ethanol Leaf Extract of *Morinda lucida* on Selected Gastro-intestinal Bacteria

Test organisms	Inhibition Zones (mm)				
	200mg/ml	400mg/ml	600mg/ml	800mg/ml	Gentamycin
<i>Escherichia coli</i>	2.3	2.0	2.2	2.5	1.4
<i>Klebsiella pneumonia</i>	1.4	1.5	1.5	2.0	1.6
<i>Staphylococcus aureus</i>	-	1.9	2.0	2.6	0.6

Table 2: Minimum Inhibitory Concentration of *Morinda lucida* Ethanol Leaf Extract on Test Organisms

Test organisms	Inhibition zones(mm)			
	20%	40%	60%	80%
<i>Escherichia coli</i>	-	-	05.0	12.0
<i>Klebsiella pneumonia</i>	-	-	08.0	15.0
<i>Staphylococcus aureus</i>	-	-	08.0	10.0
<i>Salmonella typharium</i>	-	-	-	06.0

Table 3: Cytological Effects of Aqueous Leaf Extract of *Morinda lucida* on the Root of *Allium cepa*

Con. %	No of root	Mean of root±SE	Treated root growth(% of the root in relation to control)	Inhibition(%)	Duration (Hr.)
0 (control)	24	4.99±0.23	100	00.00	72
2	4	2.90±0.15	16.67	83.33	72
10	3	1.38±0.14	12.50	87.50	72
20	3	1.3±0.14	12.50	87.50	72
50	NG	-	-	-	72
100	NG	-	-	-	72

Key: NG means No growth

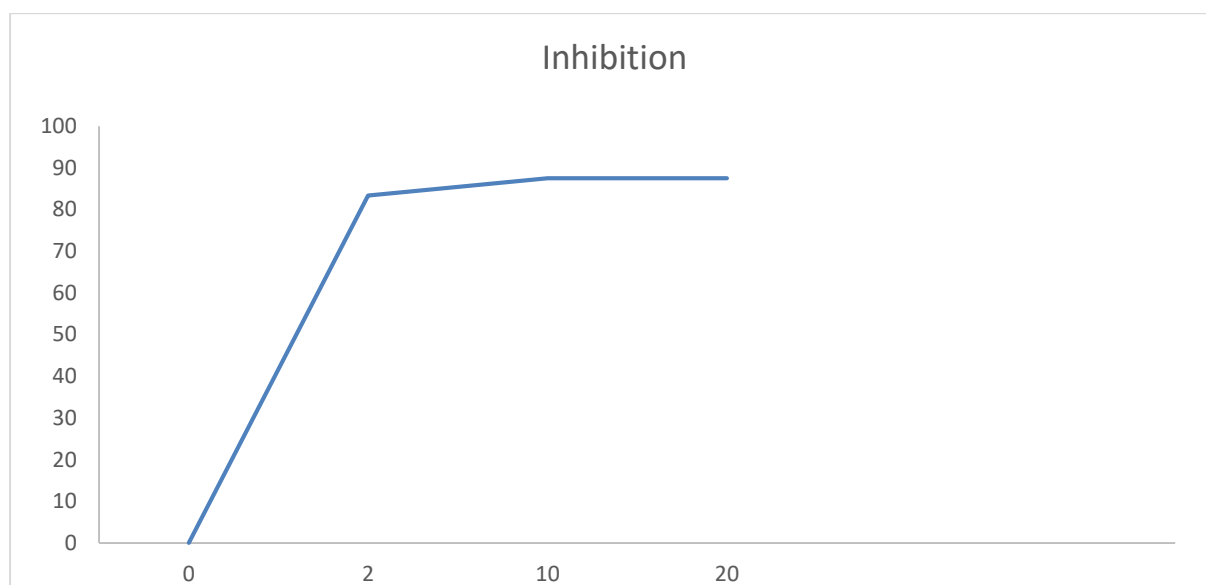


Fig 1: Inhibition Curve of Aqueous Leaf Extract of *Morinda lucida* on *Allium cepa* Root

The graph revealed the EC₅₀ of the aqueous leaf extract of *Morinda lucida* on *Allium cepa* root which was calculated to be 0.9%

Plants constitute an important source of active ingredients known as secondary metabolite which confers to them some physical characteristic and also defense purposes (Adejumobi *et al.*, 2008). However, these metabolites have been reported for various beneficial properties to humanity (Matos *et al.*, 2008). Judging by the diameter of the zones of inhibition obtained in this study, all the plants extract showed more activity against all the tested bacteria when compared with the standard antibiotic; Gentamycin except for *Staphylococcus aureus* at 200mg/ml and *Salmonella typharium* at 200, 400 and 600 mg/ml. This is most likely why it is used as folk medicine in Nigeria. These results support the observation of Ogundare and Onifade (2000)

where the extract of *Morinda lucida* inhibited the growth of *E. coli* both in vitro and in vivo studies. It has been reported by Nwinyi *et al.* (2008) that the plant contains saponins, tannins, antraquinones and alkaloids. These phytochemicals present in *Morinda lucida* and antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to the plant tissues extracts that are placed in contact with them (Adejumobi *et al.*, 2008). The choice of organisms to be used would depend on the purpose of the investigation. *Morinda lucida* leaf extract was active against all the tested bacteria as revealed by the zones of inhibition against *Klebsiella pneumonia*, *Salmonella typharium*, *Escherichia coli* and *Staphylococcus aureus*, respectively (Table 1). This is in line with the work of Montelli and Levy (2001), which reported that the extract of *Piper guineense* had significantly inhibited the growth



of bacteria *Salmonella typhi* and *Staphylococcus aureus*.

However, *E. coli*, *Staphylococcus*, *Klebsiella* and *Salmonella* showed susceptibility to the extract of *Morinda lucida* at concentrations 20 and 40% (Table 2). Maximum inhibition (15 mm) against *Klebsiella* at 80% while low inhibition 5mm against *E. coli* at 60% were recorded. In another study carried out by Mihigo *et al.* (2015) where they studied the preliminary GC-MS profiling and anti-bacterial activity investigation of *Emilia coccinea*, and found out that the ethanol extract of the plant was most active against *S. aureus* and *E. coli*, with an MIC of 500 – 1000 µg/mL. These results support the results obtained for this study which showed similar MIC of ethanol extract of the plant against *S. aureus* and *E. coli*. *S. aureus* which has been reported to be one of the major organisms that cause diarrhea and stomach disorders in humans (Jouret-Mourin and Geboes, 2002). In contrast to the results obtained from this study, Okiei *et al.* (2009) reported activity of the ethanol extract of *Morinda lucida* on a range of bacteria including *S. aureus*, *E. coli* and *Staphylococcus*. The results obtained from this study could thus be used as a scientific support for the use of the plant for the management of stomach related disorders like diarrhea. Therefore, the activity of *Morinda lucida* on these bacteria as shown in this study may constitute a scientific support for the traditional use of this plant in the treatment and management of diseases.

Table 3 reveals that higher growth rate was recorded at 2% concentration of aqueous leaf extract and the least growth were recorded at 10 and 20% concentrations. The result of this finding showed that cytotoxic effects was dose dependent. Cytotoxicity activity on *Morinda lucida* has been reported by some researchers, among them is Berame *et al.* (2017) who carried out a study on preliminary phytochemical screening and toxicity test of leaf part of plant (*Morinda lucida*) and they found out that the leaf part was not toxic. These results corroborate that obtained from this study which indicates cytotoxicity of the aqueous leaf extracts of *Morinda lucida*. The results obtained in this study, disagreed with the results obtained by Shylesh and Padikkala (2001), in a study they carried out on *Emilia sonchifolia* which is a member of the same family and genus as *Emilia coccinea*. They found out that the methanol extract of *Emilia*

sonchifolia leaf was cytotoxic to *Daltons lymphoma* (DL), Ehrlich ascites carcinoma (EAC) and mouse lung fibroblast (L-929) cells. This study has shown that onion root growth test can be fully integrated into the whole effluents Toxicity (WET) program by giving a particular EC50 that a medicinal plant must met before being used for therapeutics.

Conclusion

In this study, the *Allium* roots consistently showed symptoms of cytotoxicity as a result of influence of *Morinda lucida* aqueous leaf extracts. Thus, this finding clearly elaborates the negative consequences of this plant. This plant also showed various medicinal and pharmacological properties against selected bacteria making it to be utilized as an attractive, alternate and cheap source of functional ingredients for the formulation of functional foods, pharmaceuticals and nutraceuticals. Therefore, it could be concluded that *Morinda lucida* can be used in treatment of bacterial infection.

Recommendation

It is recommended that *Morinda lucida* leaf extracts inhibits microbes, so it can be of great significance for therapeutic treatment. This plants has traditionally provided a source of hope for novel drug compounds, as plants herbal mixtures have made large contributions to human health and well-being. Screening the extracts on gram negative bacteria and a range of gram positive bacteria would give more insight into the antibacterial activity of these plants. More research should be carried out on phytochemicals and anti-oxidative potential of *Morinda lucida* leaves, stem and root.

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