

Evaluation of Antibacterial Potency of Ethyl acetate and Ethanolic Extracts of *Morinda lucida* Leaf, Stem, and Bark on *Mycobacterium* Species, isolated from the chest Hospital, Obafemi Awolowo University, Ile-Ife, Osun State Nigeria.

ABSTRACT

The plant *Morinda lucida* falls under the family Rubiaceae known to have wide usage in traditional medicine. *Morinda lucida* is a tropical West African tree of medium-size about 18–25 m tall, the bark is grey to brown in colour, flowers are white in colour, the fruit is a drupe, seed is ellipsoid, yellowish and soft. The purpose of this research work is to determine the antimicrobial properties of *Morinda lucida* against *Mycobacterium* species, a very virulent and infectious organism, isolated from the chest Hospital, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The method used to determine the antimicrobial potency of the plant extracts is the Agar well diffusion method. The antibacterial potency of ethyl acetate and ethanol extracts of *Murinda lucida* leaf, stem, and bark were tested against mycobacterium species including *Mycobacterium fortuitum* (ATCC 6841), *Mycobacterium smegmatis* (ATCC 19420), *Mycobacterium abscessus* (ATCC 19977), and *Mycobacterium phlei* (ATCC 19240). All extracts exhibited various degree of antibacterial potency against the test organisms with the ethyl acetate extracts of the leaf and bark being the most active and ethanol extracts were the least active. The zone of inhibition of ethyl acetate leaf, bark and stem extracts range between 3.0mm to 18.0mm and the zone of inhibition of ethanol leaf, bark and stem extracts ranges between 1.0mm to 10.0mm respectively. The results of this research work shows that *Morinda lucida* plant extracts has antimicrobial activities at various degrees of concentrations which is a good potentials antibiotics drug and may be used in the control of infection from *Mycobacterium* species.

Keywords : Agar well diffusion ,Clinical Isolate *Mycobacterium* species , Antimicrobial assay *Morinda lucida* , Ethyl acetate and Ethanol extracts.

INTRODUCTION

The plant *Morinda lucida* falls under the family Rubiaceae known to have wide usage in traditional medicine (Osuntokun 2015). *Morinda lucida* is a tropical West African tree of medium-size about 18–25 m tall, the bark is grey to brown in colour, flowers are white in colour, the fruit is a drupe, seed is ellipsoid, yellowish and soft (Zimudzi and Cardon, 2005). *Morinda lucida* which commonly known as the Brimstone tree, is known in Ghana as Konkroma in Twi (Addo-Fodjour *et al.*, 2008) while in Nigeria it is known as Nfia in Igbo (Nweze, 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 antiinfections (Abbiw, *et al.*,1990).The use of *Morinda lucida* has always been part of human culture and is wide spread in Africa. In some countries, like Ghana, government encourages the use of indigenous forms of medicine rather than expensive imported drugs. Also in Nigeria, a large percentage of the populace depends on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach (Adeshina *et al.*, 2005).

The leaves have also been reported to possess strong trypanocidal and aortic vasorelaxant activities (Asuzu, *et al.*, 1990). Further studies have shown that leaf and stem bark of *M. lucida* posses anticancer (Sowemimo, *et al.*, 2007), Hepatoprotective (Oduola, *et al.*, 2010), Cytotoxic and genotoxic (Akinyemi, *et al.*, 2005), Antispermatogetic (Raji, *et al.*, 2005), Hypoglycemia and antidiabetic activity. (Sarker and Nahar, 2007).(Abo *et al.*,1991,Liu 2004,Nweze *et al.*,2004; Doughari *et al.*, 2009). (Doughari and Obidah, 2008).

In Nigeria, *Morinda lucida* is one of the four most used plants in the preparation of traditional medicines against fever. Decoctions and infusions or plasters of root, bark and leaves are recognized remedies against different types of fever, including yellow fever, malaria, trypanosomiasis and feverish condition during childbirth. In some cases, the plant is employed in the treatment of diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, ulcers, leprosy and gonorrhea (Adesida, *et al.*, 1972),(Adeneye *et al.*, 1984).

Morinda lucida is used generally for febrifuge, analgesic and laxative (Bever, 1996) while the decoction of the stem bark is used for the treatment of severe jaundice. As reported by Adjanohoun *et al.*,(1991) *Morinda lucida* is used locally in the treatment of irregular menstruation, insomnia and jaundice. (Akinyemi, *et al.*, 2008).*Morinda lucida* is used in the treatment of wound infections, abscesses and chancre (The primary syphilitic ulcer associated with swelling of local lymph glands and is painless, indurated, solitary and highly infectious) Also amongst the Igede People in Benue State, Nigeria, it was reported by Oduola *et al.* (2005), that the decoction of the *Morinda lucida* is used twice or thrice daily as anti – diarrhea, while the leaves are used for treatment of infertility in women.

Morinda lucida is used as an astringent and antiseptic for ulcerating abscess, exudate is rubbed on affected area (Adomi and Umukoro, 2010). The crude ethyl acetate extract of the leaves is said to contain alkaloids, cardenolides and saponins (Adomi and Umukoro, 2010). Ogundare and Onifade

(2009) worked exclusively and extensively on the effect of the leaves of *Morinda lucida* on *Escherichia coli*, and reported that it has potent inhibitory activity both *in vitro* and *in vivo*. This work has been done to study the antimicrobial effects of the leaves, stem and bark of *Morinda lucida* on selected *Mycobacterium* species.

MATERIALS AND METHODS

SAMPLES COLLECTION

The leaf, stem and bark from *Morinda lucida* were collected from Akungba-Akoko, Ondo State at exactly 7:02am, on the 23rd June 2015. The plant was authenticated by a botanist at the Department of Plant Science and Biotechnology, Adekunle Ajasin University Akungba-Akoko, Ondo State, Nigeria. with the voucher number 17702.

CLINICAL ISOLATES COLLECTION

Clinical isolates used included *Mycobacterium fortuitum* (ATCC 6841), *Mycobacterium smegmatis*(ATCC 19420), *Mycobacterium abscessus* (ATCC 19977), *Mycobacterium phlei* (ATCC 19240). They were collected in slants in McCartney bottles containing nutrient agar and then stored in a refrigerator at -4°C.

PREPARATION OF EXTRACTS

The extracts were prepared from three different plant parts: the leaf, stem and bark. The plant parts were washed with distilled water, sliced and air-dried. After drying, they were powdered using a grinder. Four hundred grams of each plant parts was soaked in 1200mL ethyl acetate in an air-tight container (Akinyemi *et al.*, 2005; El Astal *et al.*, 2005). It was mixed vigorously and left for nine days in the laboratory. After the ninth day, the solution contained in the bottle was filtered using Whatman (No. 1) filter paper. Then the extracts was separated from the solvent using rotary evaporator at different temperatures depending on the solvent used to obtain a pure extract. The filtrates were concentrated and later freeze dried. The samples were stored at -4°C until required for use (Osuntokun *et al.*, 2015).

STANDARDIZATION OF INOCULUM

One isolated colony of each clinical isolates was transferred into 5mL of sterile Lowenstein-Jesen broth and incubated for 24 hours. After incubation, 0.1mL broth of each species was transferred using a sterile syringe into 9.9ml of sterile distilled water contained in each test tube and then mixed properly. The resultant mixture served as a source of inoculum containing approximately 10^6 cfu/mL of bacterial suspension (Osuntokun *et al.*, 2015).

STANDARDIZATION OF EXTRACTS

Using aseptic condition, the extract was reconstituted by added in 0.6mL of each extract with 0.01mL of Dimethyl sulphoxide (DMSO) and 7.5mL of sterile distilled water. Serial dilutions up to a factor of three were carried out for each extracts, according to the methods described by Osuntokun *et al.* (2015).

ANTIMICROBIAL ASSAY ON *MORINDA LUCIDA*.

The susceptibility testing was investigated by the Agar well diffusion method (Adeniyi *et al.*, 2006; Osuntokun 2015). A 0.1 ml of 1:10,000 dilutions (equivalent to 10^6 cfu/mL) of fresh overnight culture of the non-tuberculous mycobacteria species grown in Lowenstein-Jesen broth, was seeded into 40 mL of Lowenstein-Jesen broth, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork borer of 4 mm diameter, equidistant wells were made in the agar. Drops of the re-suspended,(2mL volume per well) extracts with concentrations between 60 to 7.5 mg/mL were introduced into the wells till it was filled. Ciprofloxacin and Metronidazole 2mg/mL were used as the control. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37°C for 24hours. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates (Osuntokun and Olajubu 2014).

RESULTS

Table 1,Shows the antibacterial activity of ethyl acetate leaf extract of *Morinda lucida* on clinical organism. It was observed that the extract of *Morinda lucida* ethyl acetate has antimicrobial potency against the clinical isolate *Mycobacterium smegmatis* and *Mycobacterium fortuitum* between the range of 5.0 and 18.0mm.

Table 2, Shows the antibacterial activity of ethyl acetate stem extract of *Morinda lucida* on clinical organism. it was observed that *Morinda lucida* stem extract has antibacterial activity against the clinical isolate. It was observed that *Mycobacterium fortuitum* has the lowest and highest zone of inhibition of 4.0mm and 18.0mm respectively.

Table 3, Shows the antibacterial activity of ethyl acetate bark extract of *Morinda lucida* on clinical organism. it was observed that *Morinda lucida* bark extract exhibit antibacterial properties, the range in the zones of inhibition between *Mycobacterium fortuitum* and *Mycobacterium smegmatis* are 3.0mm and 18.0mm respectively.

Table 4, Shows the antibacterial activity of ethanol stem extract of *Morinda lucida* on clinical isolates. it was observed that *Mycobacterium smegmatis* has 1.0mm and *Mycobacterium fortuitum* has 9.0mm zone of inhibition on the *Morinda lucida* ethanol extract.

Table 5. Shows the antibacterial activity of ethanol leaf extract of *Morinda lucida* on the clinical isolate. It was observed that *Mycobacterium smegmatis* has the lowest value of inhibition of 1.0mm and *Mycobacterium abscessus* and *Mycobacterium smegmatis* has the highest value of the zones of inhibition of 8.0mm respectively.

Table 6, Shows the antibacterial activity of ethanol bark extract of *Morinda lucida* on the clinical isolate. it was observed that *Mycobacterium fortuitum* has the lowest zones of inhibition of 2.0mm and *Mycobacterium phlei* and *Mycobacterium fortuitum* has the highest zones of inhibition of 10.0mm against the plant extract of *Morinda lucida* respectively.

TABLE 1: ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE LEAF EXTRACT OF MORINDA LUCIDA ON CLINICAL ISOLATES.

TEST ORGANISM	ZONES OF INHIBITION (mm)					Metronidazole 20mg/mL
	60 mg/mL	30mg/mL	15mg/mL	7.5mg/mL	Ciprofloxacin 20mg/mL	
<i>Mycobacterium abscessus</i>	11.0	6.0	4.0	4.0	11.0	12.0
<i>Mycobacterium phlei</i>	10.0	7.0	0.0	0.0	12.0	14.0

<i>Mycobacterium smegmatis</i>	14.0	8.0	5.0	4.0	12.0	13.0
<i>Mycobacterium fortuitum</i>	18.0	12.0	8.0	4.0	11.0	17.0

TABLE 2 : ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE STEM EXTRACT OF MORINDA LUCIDA ON THE CLINICAL ISOLATES.

TEST ORGANISM	ZONES OF INHIBITION (mm)					
	60 mg/mL	30mg/mL	15mg/mL	7.5mg/mL	Ciprofloxacin 20mg/mL	Metronidazole 20mg/mL
<i>Mycobacterium fortuitum</i>	18.0	12.0	8.0	4.0	11.0	17.0
<i>Mycobacterium abscessus</i>	16.0	14.0	9.0	5.0	22.0	12.0
<i>Mycobacterium plei</i>	13.0	11.0	5.0	5.0	14.0	11.0
<i>Mycobacterium smegmatis</i>	9.0	6.0	0.0	0.0	22.0	16.0

TABLE 3: ANTIBACTERIAL ACTIVITY OF ETHYL ACETATE BARK EXTRACT OF MORINDA LUCIDA ON CLINICAL ISOLATES.

TEST ORGANISM	ZONES OF INHIBITION (mm)					
	60 mg/mL	30mg/mL	15mg/mL	7.5mg/mL	Ciprofloxacin 20mg/mL	Metronidazole 20mg/mL
<i>Mycobacterium fortuitum</i>	17.0	14.0	8.0	4.0	15.0	15.0
<i>Mycobacterium abscessus</i>	12.0	9.0	5.0	3.0	12.0	12.0
<i>Mycobacterium plei</i>	17.0	12.0	8.0	4.0	18.0	12.0
<i>Mycobacterium smegmatis</i>	18.0	14.0	14.0	0.0	17.0	16.0

Table 4: ANTIBACTERIAL ACTIVITY OF ETHANOL STEM EXTRACT MORINDA LUCIDA ON THE CLINICAL ISOLATES.

TEST ORGANISMS	ZONES OF INHIBITION;mm					
	60mg/mL	30mg/mL	15mg/mL	7.5mg/mL	Ciprofloxacin 20mg/mL	Metronidazole 20mg/mL
<i>Mycobacterium fortuitum</i>	8.0	7.0	3.0	0.0	13.0	12.0
<i>Mycobacterium smegmatis</i>	5.0	3.0	3.0	1.0	8.0	10.0
<i>Mycobacterium abscessus</i>	9.0	8.0	6.0	0.0	14.0	12.0
<i>Mycobacterium phlei</i>	7.0	6.0	4.0	1.0	10.0	10.0

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149 **TABLE 5: ANTIBACTERIAL ACTIVITY OF ETHANOL LEAF EXTRACT**
150 **OF MORINDA LUCIDA ON THE CLINICAL ISOLATES.**

TEST ORGANISMS	ZONES OF INHIBITION;mm					
	60mg/mL	30mg/mL	15mg/mL	7.5mg/mL	Ciprofloxacin 20mg/mL	Metronidazole 20mg/mL
<i>Mycobacterium fortuitum</i>	7.0	4.0	3.0	0.0	12.0	10.0
<i>Mycobacterium smegmatis</i>	8.0	5.0	2.0	1.0	12.0	8.0
<i>Mycobacterium abscessus</i>	7.0	6.0	5.0	2.0	10.0	10.0
<i>Mycobacterium phlei</i>	8.0	6.0	5.0	3.0	12.0	11.0

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152 **TABLE 6: ANTIBACTERIAL ACTIVITY OF ETHANOL BARK EXTRACT OF**
153 **MORINDA LUCIDA ON THE CLINICAL ISOLATES .**

TEST ORGANISMS	ZONES OF INHIBITION-mm					
	60mg/mL	30mg/mL	15mg/mL	7.5mg/mL	Ciprofloxacin 20mg/mL	Metronidazole 20mg/mL

<i>Mycobacterium fortuitum</i>	10.0	8.0	5.0	2.0	13.0	10.0
<i>Mycobacterium abscessus</i>	8.0	7.0	5.0	4.0	9.0	12.0
<i>Mycobacterium phlei</i>	10.0	8.0	5.0	3.0	12.0	11.0
<i>Mycobacterium smegmatis</i>	5.0	4.0	3.0	0.0	9.0	6.0

DISCUSSION

Antibacterial potency of ethyl acetate leaf extract of *Morinda lucida* on the clinical isolates. It were observed that ethyl acetate extract of *Morinda lucida* has antibacterial activity on *Mycobacterium fortuitum* in which *Mycobacterium fortuitum* were the most sensitive ,with zones of inhibition between 18mm and 12mm at 60mg/mL and 30mg/mL concentration respectively. Ethyl acetate leaf extract of *Morinda lucida* on *Mycobacterium plei* had the lowest zones of inhibition of 10mm and 7mm. The control shows the zones of inhibition between 11mm and 17mm of Ciprofloxacin and Metronidazole. The evidence of the potency of *Morinda lucida* was demonstrated in Table 1. It can be used to control the effect of deleterious organism like mycobacteria which is the cause of tuberculosis infection. This is to say that medicinal plants is a very important tool in human well being and it can be used to bambat infectious diseases (Osuntokun2015).

It was observed that antibacterial activity of ethyl acetate stem extract of *Mycobacterium fortuitum* at 60mg/mL and 30mg/mL concentration has the highest zone of inhibition between 12mm and 18mm.9mm and 6mm were observed as the lowest zones of inhibition for *Mycobacterium smegmatis*. The control experiment recordings were between the range of 17mm and 22mm at 20mg/mL concentration. The effect of ethyl acetate stem extracts of *Morinda lucida* on the test organisms were demonstrated in Table 2. This observation is in accordance with Balandrin *et al.* (1985), who demonstrated the effect of natural medicinal plants on different test organisms. It also shows that medicinal plants like *Morinda lucida* can be considered for potential use in the control of mycobacterial infections.Many researchers have advocated for the use of medicinal plant like *Morinda lucida* in our daily human activities (Daziell, 1973; Cowan, 1999; Chukwujekwu, 2005). Fabricant and Farnsworth (2001) described in details the use of some of these medicinal plants in the

production and discovery of efficacious drugs. This is not to say that conventional synthetic drugs are not efficacious, but the use of medicinal plants must be encouraged. The effect of nature of extracting solvent is another factor that must be discussed. Ethanol, distilled water, ethyl acetate and methanol are various solvent used for extraction, but it must be clearly stated that all of the solvents used have no effect on the activities of the medicinal plant (Facey et al., 1999).

Table 3 shows the antibacterial activity of ethyl acetate bark extracts of *Morinda lucida* on the clinical isolates. Ethyl acetate bark extracts of *Morinda lucida* observed that *Mycobacterium smegmatis* and *Mycobacterium fortuitum* has the highest zones of inhibition at 60mg/mL and 30mg/mL at 14mm and 18mm respectively and 12mm and 9mm zones of inhibition value were recorded for *Mycobacterium abscessus* at the same value of concentration. Zones of inhibition of 4mm and 8mm were observed at 15 and 7.5mg/mL concentration of *Mycobacterium fortuitum* and *Mycobacterium plei* but a sharp value were recorded at 15mg/mL concentration.

Antibacterial activity of ethanol stem extract of *Morinda lucida* were demonstrated in table 4 .it was observed that the *Mycobacterium abscessus* has the highest zones of inhibition of 9mm and 8mm at 60mg/mL and 30mg/mL and *Mycobacterium smegmatis* had the lowest zones of inhibition of 3mm and 5mm at the same concentration of 60 and 30mg/mL concentration on the stem extract of ethanol. *Mycobacterium abscessus* had the highest zones of inhibition of 6mm at 15mg/mL and lowest zones of inhibition of 3mm at the same concentration. The control antibiotics zones of inhibition are between 12mm and 14mm at 20mg/mL of Ciprofloxacin and Metronidazole. This present study has shown that all the plant parts used have some considerable levels of antimicrobial activities. This is corroborated by the findings of Indu(2006) who reported that all plant parts are useful for one purpose or the other. The increasing resistance to antibiotics has also resulted in search for leads for new organic molecules from plants with antimicrobial properties (Sofowora, 1993). Some plants which are of vital importance have been fully exploited and utilized in the pharmaceutical industry.

In table 5, Antibacterial activity of ethanol leaf extract of *Morinda lucida* were demonstrated in table 5.it was observed that *Mycobacterium smegmatis* and *Mycobacterium phlei* had the highest zones of inhibition of 5mm and 8mm at the same concentration of 60mg/mL and 30mg/mL concentration. *Mycobacterium phlei* had the highest concentration of between 3mm and 5mm at 15mg/mL and 7.5mg/mL concentration and *Mycobacterium smegmatis* had the lowest concentration of 1mm and 2mm at the same concentration The control of Ciprofloxacin and Metronidazole at 20mg/mL were between 10 and 20mg/mL. El Astal et al. (2005), reported the use of some medicinal plants extracts

in Palestine. It was observed that the plant has no relationship with the solvent used in extraction but plant has antimicrobial potency against some clinical organisms. A clue can be taken from this in the production of drugs and drugs potent against multi-resistant microorganism. Another factor that must be put into consideration is the concentration used for the research work, in this research work, gradation of different concentrations were used like 60,30,15 and 7.5mg/mL this will give us the ability to detect the level of antimicrobial potency of the medicinal plant *Morinda lucida*. This is most likely why it is used as folk medicine in Nigeria. These results support that of Ogundare and Onifade (2009) where the extract inhibited the growth of *Escherichia coli* both *in vitro* and *in vivo* studies. This has also been reported by Nwinyi *et al.* (2008) and Adejumobi *et al.* (2008).

Antibacterial activity of ethanol bark extract of *Morinda lucida* were demonstrated in table 6. It was observed that at 60mg/mL and 30mg/mL concentration, *Mycobacterium fortuitum* and *Mycobacterium phlei* has the highest zones of inhibition of 8mm to 10mm compared with the concentration at 15mg/mL and 15mg/mL which recorded the lowest zones of inhibition of 2mm and 5mm respectively. Totte (1999) and Sowenimo *et al.* (2007) reported that ethanolic extract of *Morinda lucida* has a better antimicrobial potency compared to other extracting solvents. Judging by the activity of *Morinda lucida* in this research work, it is clear that all the solvent and their extract has considerable antimicrobial properties, and can be used to formulate new antibiotics.

CONCLUSION

In conclusion, it is reasonable to state that the *Morinda lucida* plant bears antimicrobial activity based on the results obtained from this study. The extracts are additional answers to man's quest to his health problems showing that *Morinda lucida* possesses potentials as an additional source of antibacterial agent to fight against or inhibit pathogenic microorganisms.

RECOMENDATION

It is thereby recommended that *Morinda lucida* and other types of medicinal plants should be studied and exploited for use. However, it is necessary to further investigate these plant extracts *in vivo* to ascertain their efficacy in living cells.

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