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# Evaluation of Antibacterial Potency of Crude Ethyl Acetate and Ethanol Extracts of *Morinda lucida* Leaf, Stem and Bark on Mycobacterium Species, Isolated from the Chest Hospital, Obafemi Awolowo Teaching University, Ile-Ife, Osun State, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author OOT is the sole author who designed, analyzed, interpreted and prepared the manuscript for publication. Author OOT is a researcher and has researched into the antimicrobial and phytochemical properties of various medicinal plants in Nigerian and Africa. Author AOO helped in proof reading the entire manuscript in preparation for publication. Authors OVO and TOD are the authors, who helped in designing the materials and methods of the manuscript. All authors read and approved the final manuscript.*

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## 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

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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 *Morinda 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 3.0 mm to 18.0 mm and the zone of inhibition of ethanol leaf, bark and stem extracts ranges between 1.0 mm to 10.0 mm respectively.

*Keywords: Agar well diffusion; clinical isolate; mycobacterium species; antimicrobial assay Morinda lucida; ethyl acetate and ethanol extracts.*

## 1. INTRODUCTION

The plant *Morinda lucida* falls under the family Rubiaceae known to have wide usage in traditional medicine [1]. *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 [2]. *Morinda lucida* which commonly known as the Brimstone tree, is known in Ghana as Konkroma in Twi [3] while in Nigeria it is known as Nfia in Igbo [4].

The leafs of *Morinda lucida* are used as oral teas in some part of West Africa, like Nigeria in the treatment of malaria, and as a general febrifuge, analgesic, laxative and anti-infective agent [5].

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 [6].

The leaves have also been reported to possess strong trypanocidal and aortic vasorelaxant activities [7]. Further studies have shown that leaf and stem bark of *M. lucida* possess anticancer [8], hepatoprotective [9], cytotoxic and genotoxic [10], and antispermatogenic activity [11].

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, 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 [12]. The purpose of this research work is to determine the antimicrobial potency 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.

## 2. MATERIALS AND METHODS

### 2.1 Samples Collection

The leaf, stem and bark from *Morinda lucida* were collected from Akungba-Akoko, Ondo State at exactly 7:02 am, 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, the voucher number 17702.

### 2.2 Clinical Isolates Collection

The clinical isolate used for this research work were isolated from the Chest hospital, of the obafemi Awolowo Teaching Hospital, Obafemi Awolowo University, Ife, ile-Ife, Osun State, Nigeria. The 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℃.

### 2.3 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 parts were soaked in 1200mL ethyl acetate in an air-tight container [10,13]. This was allowed to stand for 14 days to permit full extraction of the active ingredients. The fluids were then filtered using Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge at- 4℃ prior to use [14,1].

### 2.4 Standardization of Inoculum

One isolated colony of each clinical isolates was transferred into 5 mL of sterile Lowenstein-Jesen broth and incubated for 24 hours. After incubation, 0.1 mL broth of each species was transferred using a sterile syringe into 9.9 ml 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 [1].

### 2.5 Standardization of Extracts

The extract was reconstituted with 0.6 mL of each extract and 0.01 mL of Di-methyl-sulphoxide (DMSO) and 7.5 mL of sterile distilled water. Serial dilutions up to a factor of three were carried out for each extracts, according to the methods described by [1].

### 2.6 Antibacterial Assay on *Morinda lucida*

The susceptibility testing was investigated by the Agar well diffusion method [15,1]. 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, (2 mL per well) extracts with concentrations between 60 to 7.5 mg/mL were introduced into the wells till it was filled. Ciprofloxacin and Metronidazole 2 mg/mL were used as the control experiment. The plates were allowed to stand on the bench for an hour, to allow pre-diffusion of the extracts before incubation at 37℃ for 24 hours. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. All experiments were performed in duplicates [14].

## 3. RESULTS

Table 1 shows the antibacterial activity of ethyl acetate leaf extract of *Morinda lucida* on test organism. It was observed that, the ethyl acetate extracts of *Morinda lucida* has antimicrobial activity against the clinical isolate *Mycobacterium smegmatis* and *Mycobacterium fortuitum.* Also, antibacterial activity of ethyl acetate stem extract of *Morinda lucida* was found on test organism. It was observed that *Morinda lucida* stem extract has antibacterial activity against the clinical isolate. It was observed that *Mycobacterium fortuitum* exhibits the lowest and highest zone of inhibition of 4.0 mm and 18.0 mm starting from 15 mg/ml up to 60 mg/ml, respectively. Table 1 also shows the antibacterial activity of ethyl acetate bark extract of *Morinda lucida* on test organism. It was observed that the bark extract exhibits antibacterial properties, with zones of inhibition between 3.0 mm and 18.0 mm for *Mycobacterium fortuitum* and *Mycobacterium smegmatis* respectively.

Table 2 shows the antibacterial activity of ethanol stem extract of *Morinda lucida* on test organism. It was observed that exhibits antibacterial activity against *Mycobacterium smegmatis,* with 1.0 mm area of inhibition, and 9,0 mm for *Mycobacterium fortuitum*. Table 2 also shows the antibacterial activity of ethanol leaf extract of *Morinda lucida* on the test organism. It was observed that *Mycobacterium smegmatis* has the lowest value of inhibition of 1.0 mm and *Mycobacterium abscessus* and *Mycobacterium smegmatis* has the highest value of the zones of inhibition of 8.0 mm respectively. It also shows the antibacterial activity of ethanol bark extract of *Morinda lucida* on the test organism .It was observed that *Mycobacterium fortuitum* has the lowest value of zone of inhibition of 2.0 mm and *Mycobacterium phlei* and *Mycobacterium abscessum* had the highest value of zones of inhibition of 10.0 mm respectively.

**Table 1. Antibacterial activity of crude ethyl acetate leaf, stem and bark extracts of *Morinda lucida* on clinical isolates**

| Clinical isolates | Leaf | | | | | | Stem | | | | | | Bark | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 60 mg\mL | 30 mg/mL | 15 mg/mL | 7.5 mg/mL | Ciprofloxacin 20 mg/mL | Metronidazole 20 mg/mL | 60 mg\mL | 30 mg/mL | 15 mg/mL | 7.5 mg/mL | Ciprofloxacin 20 mg/mL | Metronidazole 20 mg/mL | 60 mg\mL | 30 mg/mL | 15 mg/mL | 7.5 mg/mL | Ciprofloxacin 20 mg/mL | Metronidazole 20 mg/mL |
| *Mycobacterium abscessus* | 11.0 | 6.0 | 4.0 | 4.0 | 11.0 | 12.0 | 18.0 | 12.0 | 8.0 | 4.0 | 11.0 | 17.0 | 17.0 | 14.0 | 8.0 | 4.0 | 15.0 | 15.0 |
| *Mycobacterium phlei* | 10.0 | 7.0 | 5.0 | 2.0 | 12.0 | 14.0 | 16.0 | 14.0 | 9.0 | 5.0 | 22.0 | 12.0 | 12.0 | 9.0 | 5.0 | 3.0 | 12.0 | 12.0 |
| *Mycobacterium smegmatis* | 14.0 | 8.0 | 5.0 | 4.0 | 12.0 | 13.0 | 13.0 | 11.0 | 5.0 | 2.0 | 14.0 | 11.0 | 17.0 | 12.0 | 8.0 | 4.0 | 18.0 | 12.0 |
| *Mycobacterium fortuitum* | 18.0 | 12.0 | 8.0 | 4.0 | 11.0 | 17.0 | 9.0 | 6.0 | 4.0 | 1.0 | 22.0 | 16.0 | 18.0 | 14.0 | 14.0 | 0.0 | 17.0 | 16.0 |

*Zone of Inhibition -mm*

**Table 2. Antibacterial activity of crude ethanol leaf, stem and bark extracts of *Morinda lucida* on the clinical isolates**

| Clinical isolates | Leaf | | | | | | Stem | | | | | | Bark | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 60 mg/mL | 30 mg/mL | 15 mg/mL | 7.5 mg/mL | Ciprofloxacin 20 mg/mL | Metronidazole 20 mg/mL | 60 mg/mL | 30 mg/mL | 15 mg/mL | 7.5 mg/mL | Ciprofloxacin 20 mg/mL | Metronidazole 20 mg/mL | 60 mg/mL | 30 mg/mL | 15 mg/mL | 7.5 mg/mL | Ciprofloxacin 20 mg/mL | Metronidazole 20 mg/mL |
| *Mycobacterium fortuitum* | 8.0 | 5.0 | 3.0 | 1.0 | 23.0 | 22.0 | 7.0 | 4.0 | 3.0 | 1.0 | 22.0 | 20.0 | 10.0 | 8.0 | 5.0 | 2.0 | 23.0 | 20.0 |
| *Mycobacterium smegmatis* | 5.0 | 3.0 | 3.0 | 1.0 | 18.0 | 20.0 | 8.0 | 5.0 | 2.0 | 1.0 | 22.0 | 20.0 | 8.0 | 7.0 | 5.0 | 4.0 | 20.0 | 22.0 |
| *Mycobacterium abscessus* | 9.0 | 8.0 | 6.0 | 0.0 | 24.0 | 22.0 | 7.0 | 6.0 | 5.0 | 2.0 | 20.0 | 20.0 | 10.0 | 8.0 | 5.0 | 3.0 | 22.0 | 21.0 |
| *Mycobacterium phlei* | 7.0 | 6.0 | 4.0 | 1.0 | 20.0 | 20.0 | 8.0 | 6.0 | 5.0 | 3.0 | 22.0 | 21.0 | 5.0 | 4.0 | 3.0 | 0.0 | 20.0 | 22.0 |

*Zones of Inhibition -mm*

## 4. DISCUSSION

Antibacterial activity of crude ethyl acetate leaf extracts of *Morinda lucida* on the clinical isolates. It was observed that crude ethyl acetate extracts of has antibacterial activity on *Mycobacterium fortuitum. Mycobacterium fortuitum* was the most sensitive with the zone of inhibition between 18mm and 12 mm at 60 mg/mL and 30 mg/mL concentration respectively. Crude ethyl acetate leaf extracts of *Morinda lucida* also exhibits antimicrobial activity on *Mycobacterium phlei* which has the lowest zones of inhibition of 10 mm and 7 mm. In the control experiments, in which two antibiotic were used for this research work, which are Ciprofloxacin and Metronidazole. The two antibiotic has their zones of inhibition between 11 mm and 17 mm. The evidence of the antibacterial activity of *Morinda lucida* was demonstrated in Tables 1 and 2, in which two solvent namely ethyl acetate and ethanol were used for its extraction. It was observed that different parts of the plants exhibits various antibacterial activities due to the fact that, the section expose to photosynthesis process differs from one another and the content of bioactive components were concentrated at different parts of the plants. The bioactive compoments dictates the antibacterial activities of theplants against various clinical isolates. They are what is reffers to as the chemical contents of the plants and examples of the bioctive component are tannin, alkalods,terpenoids,flavonoids, steroids and etc [1,16].

Some bioactive components of the plants are used to control the effect of deleterous organism like mycobacterium species which is the cause of tuberculosis infection, the subject matter of this research work. This can be used to prove the high antibacterial activities exhibited by the leaf extracts of *Morinda lucida* in all the sovent used for extraction.This is to say that medicinal plants is a very important tool to human well being and it can be used to combat infectious diseases [14,1,17].

It was also observed that antibacterial activity of crude ethyl acetate stem extracts of *Morinda lucida on Mycobacterium fortuitum* at 60 mg/mL and 30 mg/mL concentration has the highest zone of inhibition between 12 mm and 18 mm, 9 mm and 6mm were observed as the lowest zones of inhibition for *Mycobacterium smegmatis*. The control experiment recordings were between the range of 17 mm and 22 mm at 20 mg/mL concentration. The effect of crude ethyl acetate stem, leaf and bark extracts of *Morinda lucida* on the test organisms were demonstrated in Table 1. This observation is in accordance with [18,19] who demonstrates the effects of natural medicinal plants on different test organisms. It also shows that medicinal plants like *Morinda lucida* can be considered as potential use in the control of mycobacterial infections. Many researchers has advocated for the use of medicinal plant like *Morinda lucida* in our daily human activities [20,21,22]. [23] 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 effects 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 [24].

Table 1 shows the antibacterial activity of crude ethyl acetate bark extracts of Morindalucida on the clinical isolates. *Mycobacterium smegmatis* and *Mycobacterium fortuitum* has the highest zones of inhibition at 60 mg/mL and 30 mg/mL at 14 mm and 18 mm respectively and 12 mm and 9 mm zones of inhibition value were recorded for *Mycobacterium abscessus* at the same value of concentration. Zones of inhibition of 4 mm and 8 mm were observed at 15 and 7.5 mg/mL concentration of *Mycobacterium fortuitum* and *Mycobacterium phlei* but a sharp value were recorded at 15 mg/mL concentration.

Antibacterial activity of crude ethanol stem extracts of *Morinda lucida* were demonstrated in Table 2. It was observed that the *Mycobacterium abscessus* has the highest zones of inhibition of 9 mm and 8 mm at 60 mg/mL and 30 mg/mL and *Mycobacterium smegmatis* has the lowest zones of inhibition of 3 mm and 5 mm at the same concentration of 60 and 30 mg/mL concentration of crude ehanol stem extracts and *Mycobacterium abscessus* had the highest zones of inhibition of 6 mm at 15 mg.mL and lowest zones of inhibition of 3 mm at the same concentration. This present study has shown that all the plant parts used has some considerable levels of antibacterial activities and corroborated by the findings of [25,26] 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 new organic

molecules from plants with antibacterial properties [27,7].

Some plants which are of vital importance have been fully exploited and utilized in the pharmaceutical industry and in herbal medicine practice, this were supported by [28] where *Morinda lucida* and other medicinal plants were used to inhibit the growth of *Escherichia coli* both *in vitro* and *in vivo* studies.

Antibacterial activity of crude ethanol leaf, stem and bark extracts of *Morinda lucida* were demonstrated in Table 2. It was observed that *Mycobacterium smegmatis* and *Mycobacterium phlei* has the highest zones of inhibition of 5 mm and 8 mm at the concentration of 60 mg/mL and 30 mg/mL. *Mycobacterium phlei* has the highest zones of inhibition which ranges between 3mm and 5 mm at 15 mg/mL and 7.5 mg/mL and *Mycobacterium smegmatis* has the lowest zones of inhibition of 1 mm and 2 mm.

This results corroborates the report of [13], who reported, that the use of some *Morinda lucida* extracts in Palestine. He reported that, the leaves of *Morinda lucida* have been used for the treatment of splenomegaly, an enlargement of spleen usually associated with viral, bacterial infections and sexually transmitted diseases. He also report that the bark and stem, has antibacterial activity against upper respiratory tract infections, cough and infection associated with *Mycobacterium* species like *Mycobacterium fortuitum*, *Mycobacterium smegmatis*, *Mycobacterium abscessus*, and *Mycobacterium phlei* which causative agent of tuberculosis. This may be attributed to the presence of tannins as these compounds inhibit the growth of many fungi, yeast, bacteria, and viruses [29]. The results also shows the leaves and bark of this plant to be rich in alkaloids, compounds that have diverse useful bioactivity component like anti-inflammatory activity and anticancer [30,31,4].

It was observed that, the plant has no relationship with the solvent used for extraction but plant has antimicrobial activities against some clinical organisms. A clue can be taking from this in the production and discovery of drugs active against multi-resistant microorganism. Another factor that must be put into consideration is the purity of the extracting solvents which has a multiple effects on the activity of the plants extracts; this was also demonstrated by [32,33].

Antibacterial activity of Crude ethanol bark extracts of *Morinda lucida* were demonstrated in Table 2. It was observed that, at 60 mg/mL and 30 mg/mL concentration, *Mycobacterium fortuitum* and *Mycobacterium phlei* has the highest zones of inhibition of 8 mm to 10 mm compare with the concentration at 30 mg/mL and 15 mg/mL which recorded the lowest zones of inhibition of 2 mm and 5 mm respectively. [2] and [8] reported that ethanolic extracts of *Morinda lucida* has a better antimicrobial activities. Judging by the activity of *Morinda lucida* in this research work, the bark are rich in flavonoids and tannins, which are reported to be a well-known potent phytochemical and antioxidants. This may be the reason why this plant has been used for superficial bacterial infections, cough, gonorrhea and tuberculosis infection. This was also reported by [34,35].

## 5. CONCLUSION

In conclusion, it is reasonable to state that the *Morinda lucida* plant bear 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 pathogenic microorganisms like mycobacterium species.

## 6. RECOMMENDATION

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.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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