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Possible antimicrobial activity of *Morinda lucida* stem bark, leaf and root extracts

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Inhibitory activities of both aqueous and methanolic extracts of the root, stem bark, and leaf of *Morinda lucida* on *Escherichia coli, Salmonella typhi, Salmonella paratyphi*, and *Salmonella typhorium* was investigated *in vitro*. *In vitro* experiment was carried out using the agar well diffusion and disc diffusion methods with Gram-negative enterobacteria. *M. lucida* extracts were more active against all the tested bacteria than the standard antibiotics, chloramphenicol and ciprofloxacin even at the same concentrations of 5, 10 and 20 mg/ml. The results of this study show that the extracts of *M. lucida* has the potentials of inhibiting the growth of *E. coli* and *Salmonella* species, thereby suggesting its potency in the treatment of infections in which *E. coli* and *Salmonella* species are implicated.

Key words: Gram-negative, inhibitory activity, *Escherichia coli*, *Salmonella* species, enterobacteria, infections, antibiotics.

INTRODUCTION

In the world, mostly in the rural areas of the developing countries, people depend on local medicinal plant as remedy for their diseases and illness probably either because of the absence of modernized functional health facilities or because of ancestral and traditional beliefs. Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicines (Nair et al., 2005).

In different parts of Nigeria, different varieties of plants are used in the treatment of different types of diseases. Roots, barks or leaves of *Newbolbea leavis* are used in the treatment of dysentery, syphilis, ear ache, ringworm and scrotal elephantiasis (Azoro, 2002.) *Morinda lucida* known as Oruwo in the South-Western part of Nigeria is a medium sized tree with a crooked hole and rather short twisted branches. It belongs to the family *Rubiaceae*. It has a rough bark, grey in colour, flaking off in irregular patches. Its leaves are about 7 to 15 cm long by 3.5 to 7.5 cm broad, and flowers are white with a narrow glabrous corolla-tube about 2.5 cm. Stem bark, roots and leaves infusion is used as an antimalarial, antidiabetic, jaundice and dysentery treatment (Burkill, 1997), ad it is used in antimalarial activity (Tona et al., 1999; Agomo et al., 1992; Asuzu and Chineme, 1990; Koumaglo et al., 1992), anti-*Salmonella typhi* activity (Akinyemi et al., 2005), effect on contractility of isolated uterine smooth muscle of pregnant and non-pregnant mice (Elias et al., 2007), toxicity and mutagenic studies (Sowemimo et al., 2007; Akinboro and Bakare, 2007; Raji et al., 2005), and it has anti-diabetic property (Olajide et al., 1999). *M. lucida* extracts have been reported to have antioxidant and reducing activities (Ogunlana et al., 2008), and anti-microbial activity (Ogundare and Onifade, 2009; Adomi, 2006, 2008).

Several drugs including some antibiotics are no longer active against targeted organisms. It has been reported

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Table 1. Effects of aqueous stem bark extract of *Morinda lucida* on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	12.5	13.0	14.0	10.0	11.0	12.2	13.5	15.0	16.1	11.2	12.1	13.5	16.5	17.0	18.0	12.4	13.0	14.2
S. typhi	14.0	14.0	14.5	10.1	11.1	11.6	16.0	16.1	16.1	11.0	12.0	13.0	17.5	18.0	18.1	12.2	13.1	14.1
S. paratyphi	14.0	14.1	14.2	10.0	11.2	11.5	15.0	15.0	16.0	11.1	12.2	13.4	16.0	17.5	18.0	12.1	13.0	14.0
S. typhorium	14.5	14.0	14.0	10.2	12.0	11.2	15.5	15.0	16.0	11.0	12.0	13.1	17.0	17.0	18.2	12.0	13.0	14.0

that the effective lifespan of these therapeutics agents are limited (Cowan, 1999). Therefore, we experience antibiotic resistant organisms, and ineffective malarial drugs. Moreso, majority of the orthodox drugs are both expensive and display dangerous side effects in the users. Hence, discovering and identifying new safe drugs without severe side effects has become an important goal of research in biomedical science. It is in this context that the aqueous and ethanolic extracts of stem bark, leaves and roots of *M. lucida* were screened for possible anti-typhoid effect *in vitro* in comparison with known standard antimicrobial or anti-typhoid agents.

MATERIALS AND METHODS

Samples

The root, leaf and stem bark from *M. lucida* plants were collected from the ground of the Federal Polytechnic, Ede. The plant was authenticated at the Department of Botany, University of Ibadan, Ibadan, Nigeria.

The pure cultures of four Gram-negative bacteria were obtained from the Medical and Parasitology Laboratories of University College Hospital, Ibadan. These included *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella typhorium* and *Escherichia coli*. They were collected in slants in McCartney bottles containing nutrient agar. They were then stored until required.

Preparation of extract

Fresh leaves, barks and roots of *M. lucida* were grounded separately and then air-dried. The hammer milled dried samples (200 g) each were soaked in 1000 ml each of water and absolute ethanol for 72 h and filtered first using muslin cloth and then No. 1 Whatman filter paper. The filtrates were concentrated and later freeze dried. The samples were stored at 4°C until required for use.

Bactericidal screening

A known weight of each extract (ethanol and water) of stem bark, leaves and roots *M. lucida* was dissolved in a little volume of sterile distilled water to give the desired concentration of extract in milligram per millilitre (mg/ml). The bacterial suspensions were cultured. 0.1 ml of 10^5 cfu/ml was used as inoculum for all the bacterial. Each inoculum was used to prepare a seeded culture in a nutrient agar plate and allowed to solidify in Petri dishes. Wells (6 mm in diameter) were punched in the agar medium using sterile stainless cork borer before being filled with 0.1 ml each of 5 to 20 mg/ml plant extracts. Also, filter paper disc method was used. The filter paper discs were impregnated with 5 to 20 mg/ml of the extracts respectively. The plates were incubated at 37°C for 24 to 72 h and the diameter of any resulting zone of inhibition was measured. The results were recorded by measuring the zones of growth inhibition surrounding the wells and discs. Clear inhibition zones around the discs and wells indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. Ciprofloxacin and chloramphenicol (5 to 20 mg/ml) were used as reference standards, as was recommended by the National Committee for Clinical Laboratory standards (NCCLS).

RESULTS AND DISCUSSION

The effects of both aqueous and ethanolic crude extacts of stem barks, roots and leaves of *Morinda lucida*, and standard antibiotics on test bacterial are presented in Tables 1 to 8. Both water and ethanolic extracts of the plant showed antibacterial activity at concentrations from5 to 20 mg/ml. All the extracts exhibited antimicrobial activity against all tested bacteria bacterial in a concentration dependent manner. Judging by the diameter of the zones of inhibition obtained, all the plants extract showed more activity against all the tested bacteria when compared with the two standard antibiotics; chloramphenicol and ciprofloxacin. 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 *E. coli* both *in vitro* and *in vivo* studies. It has been reported by Nwinyi et al. (2008) and Adejumobi et al. (2008) that the plant contains saponins, tannins, antraquinones and alkaloids.

These phytochemicals present in *M. lucida* and

Table 2. Effects of aqueous leaf extract of *Morinda lucida* on test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	16.5	17.0	18.2	12.0	12.5	15.0	18.0	19.0	20.1	14.0	15.0	22.0	21.0	22.0	23.1	19.0	20.0	21.0
S. typhi	13.5	14.0	18.0	12.1	12.2	13.2	15.0	19.2	20.0	14.1	15.1	21.0	16.5	17.2	22.0	18.2	19.0	20.0
S. paratyphi	13.0	14.0	18.0	12.0	12.1	13.0	14.0	19.0	19.5	14.0	15.0	20.0	160.	17.0	21.0	18.0	19.0	20.1
S. typhorium	13.1	14.0	18.0	12.1	12.3	13.1	14.0	19.1	19.0	14.0	15.2	20.1	16.2	17.4	21.0	18.1	19.2	20.0

Table 3. Effects of aqueous root extract of *Morinda lucida* on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	13.5	14.0	16.4	9.2	11.2	12.0	15.5	16.0	22.4	10.1	13.2	13.2	18.5	19.0	22.0	12.0	15.1	16.0
S. typhi	13.5	14.0	16.0	9.0	11.0	11.3	14.5	15.1	20.0	10.0	13.0	13.1	16.5	16.5	21.0	11.0	15.0	15.0
S. paratyphi	13.0	13.0	15.0	9.1	10.5	11.2	14.1	15.0	18.2	10.0	12.0	12.2	15.0	16.1	19.0	10.5	14.0	15.1
S. typhorium	12.5	12.5	15.1	9.0	10.2	11.1	14.0	15.0	8.0	9.0	12.2	12.2	15.0	15.5	19.1	10.0	14.0	15.0

Table 4. Effects of ethanolic stem bark extract of *Morinda lucida* on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	16.5	17.0	18.0	12.0	13.0	14.0	18.5	19.0	20.1	15.1	15.1	16.2	22.2	22.2	22.2	17.0	18.0	19.0
S. typhi	13.0	15.1	16.0	10.0	11.0	12.0	13.0	16.0	20.0	13.0	14.1	16.0	15.1	17.0	21.0	16.0	16.0	19.1
S. paratyphi	13.1	15.0	16.2	11.0	11.2	12.3	13.2	16.1	20.0	13.1	14.0	16.0	15.0	17.1	21.1	16.0	16.1	19.1
S. typhorium	13.0	15.1	16.1	12.0	12.0	12.2	13.1	16.0	20.0	13.0	14.0	16.0	15.1	17.0	21.0	16.1	16.0	19.0

their antioxidant potential could be responsible for the antimicrobial activity. Ogunlana et al. (2008) reported that *M. lucida* had antioxidant activity.

Also, antioxidant activity has been directly linked with or to the presence of phenolic moieties present in the molecular structure of natural antioxidants. Many phytochemicals with phenolic moieties exhibit antioxidant activity (Kahkonen et al., 1999; Frankel et al., 1995).

Table 5. Effects of ethanolic leaf extract of *Morinda lucida* on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	16.5	17.0	18.4	14.2	15.1	16.4	18.5	20.1	23.0	17.2	18.2	19.3	22.5	23.0	25.1	18.7	19.2	20.2
S. typhi	13.2	15.0	16.0	14.1	15.0	16.1	14.0	19.0	20.0	17.0	18.1	19.1	16.0	20.0	25.0	18.0	19.0	20.0
S. paratyphi	13.0	15.1	16.1	14.0	15.1	16.0	14.0	19.1	20.1	17.0	18.0	19.0	16.0	20.0	25.0	18.1	19.0	20.0
S. typhorium	13.1	15.0	16.0	14.0	15.0	16.2	14.0	19.0	20.0	17.1	18.0	19.0	16.0	20.1	25.0	18.1	19.0	20.0

Table 6. Effects of ethanolic root extract of *Morinda lucida* on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	13.5	14.2	15.1	12.1	13.2	14.1	16.5	18.2	18.3	14.0	15.1	16.3	20.0	21.0	22.0	17.0	18.3	19.1
S. typhi	13.5	14.0	15.0	12.0	13.1	14.0	15.0	17.0	18.1	13.0	15.0	16.0	16.5	18.1	19.0	15.0	16.2	19.2
S. paratyphi	13.5	14.1	15.0	12.2	13.0	14.2	15.0	17.2	18.3	13.0	15.0	16.1	16.2	18.0	19.0	15.0	16.0	19.0
S. typhorium	13.2	14.0	15.1	12.0	13.1	14.0	15.0	17.1	18.0	13.0	15.0	16.0	16.0	18.3	19.1	15.1	16.0	19.1

Table 7. Effects of ciprofloxacin on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	4.5	8.5	12.0	4.0	8.0	11.0	5.0	9.1	13.0	4.5	8.7	12.1	6.0	11.0	15.0	5.0	10.0	13.5
S. typhi	4.5	8.1	11.0	4.1	7.9	10.5	5.0	9.0	12.5	4.5	8.5	12.0	7.5	12.0	14.0	6.9	9.5	13.0
S. paratyphi	4.5	8.1	11.0	4.1	7.9	10.5	5.0	9.0	12.5	4.5	8.5	12.0	7.5	12.0	14.0	6.9	9.5	13.0
S. typhorium	4.5	8.1	11.0	4.1	7.9	10.5	5.0	9.0	12.5	4.5	8.6	12.0	7.5	12.0	14.0	6.9	9.5	13.1

Table 8. Effects of chloramphenicol on the test bacteria.

Bacteria	Zones of Inhibition (mm)																	
	5.0 mg/ml						10.0 mg/ml						20.0 mg/ml					
	Cork method			Disc method			Cork method			Disc method			Cork method			Disc method		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
E. coli	4.0	7.0	11.0	3.5	6.5	10.0	4.5	9.0	12.5	4.0	8.0	12.0	5.5	11.0	14.0	5.0	10.0	13.0
S. typhi	3.9	6.9	10.5	3.6	6.5	10.0	4.2	8.0	11.5	4.0	8.0	11.0	5.0	10.5	13.0	4.9	10.0	12.0
S. paratyphi	3.9	6.9	10.5	3.6	6.5	10.0	4.2	8.0	11.5	4.0	8.0	11.0	5.0	10.5	13.0	4.9	10.0	12.0
S. typhorium	3.9	6.9	10.6	3.6	6.5	10.0	4.2	8.0	11.7	4.0	8.0	11.0	5.0	10.5	13.0	4.9	10.0	12.0

Conclusion

Crude extracts of *M. lucida* had active antimicrobial activity, and a promising natural antibiotic when compared to the standard antibiotic drugs chloramphenicol and cipro-floxacin. The results strongly suggest that it can be used to treat infections with especially *Salmonella* species.

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