



Original Article

ANTI-TYPHOID POTENTIALS OF CRUDE AND FRACTIONS OF DEFATTED CHLOROFORM EXTRACT OF *Morinda lucida* LEAVES

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ABSTRACT

Morinda lucida leaves are used in Igala ethnomedicine for the treatment of typhoid fever. *In vitro* antibacterial effects of defatted crude chloroform extract of *Morinda lucida* leaves, collected from Anyigba, North-Central Nigeria, and its fractions were evaluated on *Salmonella* Typhi. Three standard antibiotics, gentamicin, amoxicillin and chloramphenicol, were used as positive Controls. Determination of antibacterial activity using the agar diffusion method showed that the crude chloroform extract had identical antibacterial activity as the most active standard antibiotic used, gentamicin (17mm at 100 mg/ml concentration). There were six distinct fractions of the crude extract, four of which exhibited varying degrees of antibacterial activity against *S. Typhi* (2.00 - 11.00 mm at 100 mg/ml concentration). The minimum inhibitory concentration (MIC) values for the crude and the most active fraction were comparable to that of gentamicin (≤ 12.50 mg/ml). Result of the minimum bactericidal concentration (MBC) revealed that the crude extract was only bacteriostatic at the concentration range tested (12.50 to 100 mg/ml), whereas the most active fraction was bactericidal at 100 mg/ml concentration. These findings

validates the folkloric application of the plant for the treatment of typhoid fever and provides evidence for the potentials of defatted crude chloroform extracts *M. lucida* leaves and its partially purified fraction for the development of (novel) anti-typhoid therapy.

Keyword: Antibacterial, Ethnomedicine, Igala, MBC, MIC, *Morinda lucida*, Typhoid fever,

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INTRODUCTION

Gastroenteritis-causing pathogens such as *Escherichia coli*, *Shigella* spp. and *Salmonella* spp. are the second leading cause of morbidity and mortality worldwide (Asrat, 2008). *Salmonella enterica* serotype Typhi (*S. typhi*) previously known as *Salmonella typhi*, a human- adapted pathogen, is the cause of typhoid fever, a systemic infection (Rhen *et al.*, 2007; Crump and Mintz, 2010). Typhoid fever is a global infection with a fatality rate of 10% (Doughari *et al.*, 2007). The multidrug resistance (MDR) phenotype of *S. typhi* has been shown to be widespread for many years (Rowe *et al.*, 1997). There has been gradual shift from the use of synthetic drugs in treatment of some diseases to plant products due to emergence of MDR phenomena (Dawang and Datup, 2012). The Igala people of North-Central Nigeria use medicinal plants for the treatment of typhoid fever with empirical pharmacological basis (Musa *et al.*, 2010). One of such plants used in Igala ethnomedicine for the treatment of typhoid fever is *Morinda lucida* (Musa *et al.*, 2011a).

Morinda lucida is a medium-sized tree about 15 m tall (Yinusa *et al.*, 2005) and is widely used in Igala traditional folk medicine for several febrile ailments and other infective disorders. Studies have shown that water extract of leaves of *Morinda lucida* produced antibacterial effects comparable to those of standard antibiotics against *S. Typhi*, *S. aureus* and *E. coli* (Musa *et al.*, 2013), and the chloroform extract has been shown to have antibacterial activity against *S. typhi* (Musa *et al.*, 2011a). Ajayi (2012) also reported that crude extracts of the leaf have been recommended in the treatment of hypertension and cerebral complications showing distinct diuretic and tranquilizing effects.

This study is aimed at investigating the antibacterial activity of defatted crude chloroform extract of the leaves of *Morinda lucida* and bioactivity guided fractionation of the chloroform extract.

MATERIALS AND METHODS

The plant materials were collected from Anyigba, North-Central Nigeria. They were identified by Prof. C. O. C.

Agwu of the Biological Sciences Department, Kogi State University, Anyigba, Nigeria. The plant samples (leaves) were collected in bags and then washed to remove debris. They were air dried at room temperature for about two weeks, then pulverised using high speed Creston grinder. The pulverised samples were stored in plastic containers in the open laboratory until they were required. The test organism: *Salmonella* Typhi was obtained from the Microbiology Department of Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria. Reagents and media were obtained from reputable names like BDH Poole, England and Lab M ltd Lancashire, England.

Extraction

To obtain the chloroform extract, the leaves were first defatted with N-Hexane. One thousand gramme quantity of the pulverised plant sample was cold macerated in five litres of n-hexane in a capped vessel for 24 hours. Thereafter, the macerate was filtered through Whatman filter paper using a Speedvac vacuum pump. The residue obtained from the filtration was collected, dried and cold macerated in 5 litres of chloroform for another 24 hours, the filtrate was then concentrated using a rotary evaporator and dried on a water bath to obtain the chloroform crude extract and the yield was determined relative to the starting material.

The yields of the crude extract and active fractions were calculated as follows

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of starting material}} \times 100$$

Bioactivity Guided Fractionation

The powdered defatted chloroform extract of the plant leaves was partially purified using the Column and Thin Layer Chromatography techniques. Silica gel was activated in the oven at 105°C for 2 hours. The activated silica gel was then allowed to cool. Three and a half grammes of the extract was dissolved in 20 ml of chloroform. Ten grammes of silica gel was added to the extract to absorb the extract in solution and was allowed to dry completely. The column was then packed with 25g of silica gel (70-230

mesh) using the wet packing method; and eluted with solvents of gradually increasing polarity. Three solvents were used; hexane, ethyl acetate and methanol, starting with 100% hexane and gradually increasing the polarity, by varying the percentages of the three solvents (hexane:ethyl acetate (4:1), hexane:ethyl acetate (3:2), hexane:ethyl acetate (2:3), hexane:ethyl acetate (1:4), ethyl acetate (100%), and ethyl acetate:methanol (9:1)). Eluates were obtained as they exit the column in bands and based on their colour

separation. They were then subjected to Thin Layer Chromatography and subsequently bulked into sub-fractions based on their migrations on the plate.

Thirteen fractions, 100 ml each, were obtained from fractionation of the crude chloroform extract by column chromatography. The first five fractions had identical movement on the TLC plate so they were combined to form one sub-fraction. The last four fractions also had identical migrations on the TLC plate and so were combined to form one fraction.

Antibacterial Activity Test

Zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration tests of the crude extract and the partially purified fractions were carried out by the method of Musa *et al.* (2011b).

RESULTS

Yields

Defatted chloroform extract had a yield of 0.49%. The number of fractions with different migration pattern from the TLC experiment was six as shown in Table 1.

Table 1: Yield of fractions from defatted crude chloroform extract of *M. lucida* leaves

S/N	FRACTION	WEIGHT OF CRUDE (mg)	ELUTING VOLUME (ml)	WEIGHT (mg)	YIELD (%)
1	ML1	3.5	500	0.97	27.63
2	ML2	3.5	100	1.08	30.86
3	ML3	3.5	100	0.83	23.7
4	ML4	3.5	100	0.13	3.77
5	ML5	3.5	100	0.24	6.91
6	ML6	3.5	400	0.25	7.14

Zone of Inhibition

The crude chloroform extract as shown in Fig. 1 had identical antibacterial activity as the most active standard antibiotic used, gentamicin. Fraction ML1 was the most active fraction with antibacterial effect against *S. Typhi* with its activity slightly higher than that of amoxicillin. Fractions ML 3 and ML 4 did not produce any activity against the test organism. The standard antibiotics, crude extract and active fractions produced diameter zone of inhibition against the test organism in a concentration dependent manner.

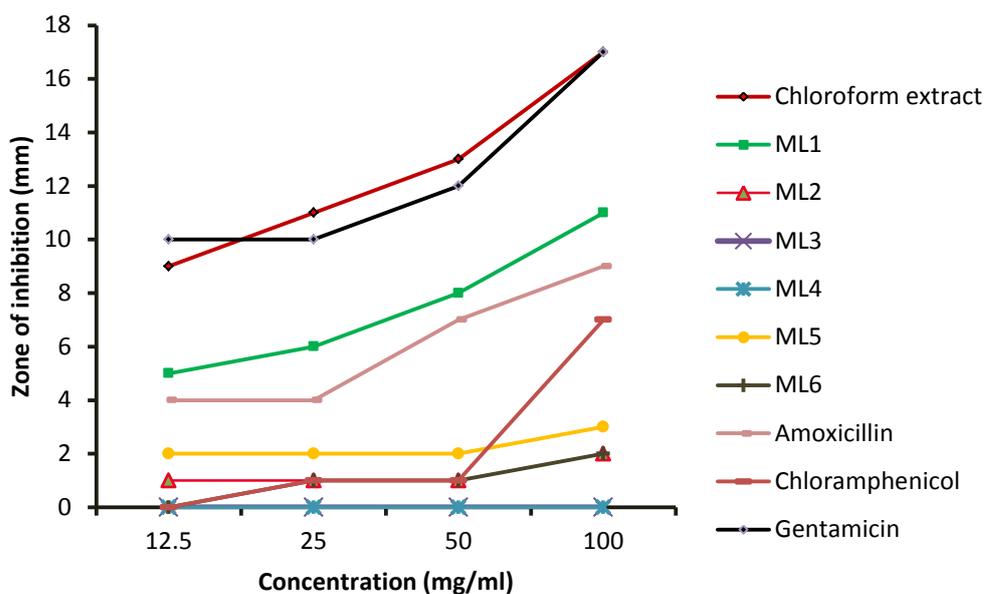


Fig 1: Zone of inhibitions of crude chloroform extract of *M. lucida*, partially purified fractions and standard antibiotics against *S. Typhi*

Minimum Inhibitory Concentration

The crude chloroform extract, the most active fraction, ML1, and gentamicin each had an MIC of 12.5 mg/ml as

shown in Fig. 2 Amoxicillin and Chloramphenicol on the other hand had an MIC of 25 mg/ml and 100 mg/ml, respectively.

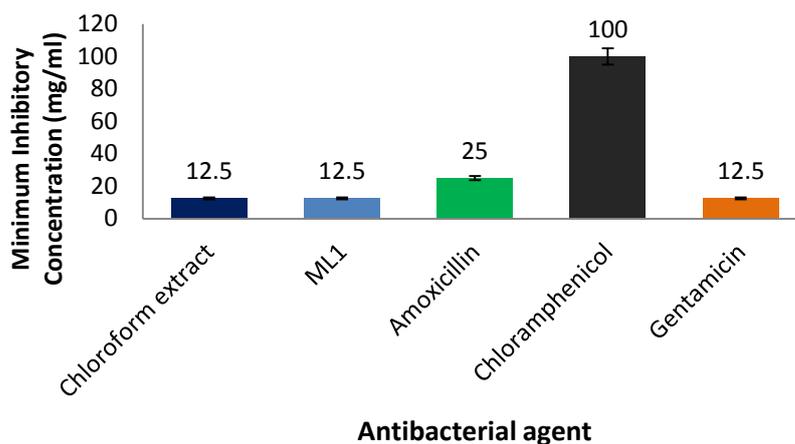


Fig 2: MIC values of crude and fraction of chloroform extract of *M. lucida* and standard antibiotics against *S. Typhi*

Minimum Bactericidal Concentration

The crude chloroform extract was bacteriostatic at the highest concentration tested, 100 mg/ml,

whereas the most active fraction had an MBC of 100 mg/ml.

DISCUSSION

Results of the zone of inhibition indicate that the chloroform extract *M. lucida* leaves was very active against *S. Typhi*. This suggests that chloroform extraction after the more hydrophobic lipids has been removed by a less polar organic solvent may be a good form of extraction for the plant when used as an anti-typhoid agent. This agrees with the findings of Ijeh *et al.* (2005) and Junaid *et al.* (2006) who suggested non polar solvent to be the best for extraction of active substance from medicinal plants.

There were six distinct fractions of the crude extract, four of which exhibited varying degrees of antibacterial activity against *S. Typhi*. The first fraction (ML1) was however the most

active of the four fractions with a diameter of zone of inhibition of 5 mm at 12.5 mg/ml concentration). The zones of inhibition of ML1 at 12.5 to 100 mg/ml concentrations were slightly higher than those of amoxicillin but lower than those of gentamicin at the same concentration range. The crude extract had diameter zone of inhibition (9 mm at 12.5 mg/ml concentration) comparable to that of gentamicin (10 mm at 12.5 mg/ml concentration), thus suggesting that the crude extract was more active than the fractions. Compared with the different fraction, this finding also suggests a combined bioactivity.

The results of the minimum inhibitory tests confirmed the result of the agar-well diffusion method sensitivity test. Gentamicin, ML1 and the crude extract

had an MIC of ≤ 12.5 mg/ml as shown in Fig. 2. This suggests that the ML1 may be as efficacious as the crude extract and gentamicin as an anti-typhoid agent but is only slightly less potent. Efficacy refers to the inherent ability of a drug to exert an effect, while potency refers to the lowest dose that would produce the maximum effect (Ebadi, 1996). Amoxicillin had an MIC of $\leq 25 > 12.5$ mg/ml whereas chloramphenicol which did not show any appreciable activity against *S. Typhi* until 100mg/ml concentration had an MIC of 100mg/ml. It thus appears as if the strain of *S. Typhi* used was chloramphenicol resistant

Result of the minimum bactericidal concentration revealed that the chloroform extract of *M. lucida* was not bactericidal at 12.5 to 100 mg/ml concentrations. This implies that the extract is either only bacteriostatic or that the extracts require higher concentration than 100 mg/ml to achieve bactericidal activity against *S. Typhi*. ML1 is bactericidal at 100 mg/ml concentration whereas the crude extract was only bacteriostatic at the same concentration. Deducible from this study therefore, is the fact that fractionation which reduces the number of component in each fraction compared to the crude has produced two different and opposing outcomes in the active component compared to the action in the crude. The crude extract was more potent in inhibiting the growth of the bacteria but was not bactericidal against the *S. Typhi* isolates, whereas the most active

fraction which although was less potent in inhibiting growth of the bacteria, had bactericidal properties. This suggests that there are components of the crude extract apart from the active principle which might act synergistically to improve the potency of the active principle as an antibacterial which was not present in the fraction and hence the decreased potency of the fraction. Weight for weight, the amount of the active principle in the fraction would be more than in crude extract; thus, 100 mg of the fraction would contain more of the active principle than 100 mg of the crude extract. This explains why the active fraction (ML1) was bactericidal at 100 mg/ml but the crude extract could not exert bactericidal property at the same concentration. This indicates that the critical concentration of the active agent required for bactericidal activity was not attained in 100mg/ml of the crude extract but was attained in the same concentration of the active fraction, and thus, suggests that when purified, the active agent is bactericidal and may exhibit bactericidal activity even at a much lower concentration.

These findings validates the application of this plant for the treatment of typhoid fever in Igala ethnomedicine and form the basis to further studies as there was evidence for potentials of defatted crude chloroform extracts of *M. lucida* leaves and its partially purified fraction for the development of (novel) anti-typhoid therapy. These results also correlate with the findings of Akinyemi

(2000), who reported that that extracts of *M. lucida* were found to have antibacterial activity on the typhoid and paratyphoid bacilli.

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