Evaluation of Antibacterial and Antifungal Activities of Crude Extract and Fractions of *Morinda citrifolia* Fruit on Multiple Drug Resistant Clinical Isolates

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Morinda citrifolia* fruits are consumed by humans in tropical areas of Africa due to their nutritional and medicinal values.

Methods: The fresh fruit of *M. citrifolia* were harvested, washed with clean water, dried under a shade, pulverized and extracted with methanol in a Soxhlet’s extractor. The crude methanolic filtrate was subjected to phytochemical analysis and fractionation using ethyl acetate, butanol, distilled water and n-hexane. The isolates, which comprises *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans*, were collected from diarrhoeic stool of patients aged 0-5 years using standard procedure. Antibiotic susceptibility assay was carried out using disc diffusion method. The antimicrobial susceptibility test of the fruit extract and fractions was carried out using agar well diffusion method.

Results: All the isolates were susceptible to the extract and the fractions with MICs range from 3.125 mg/mL to 12.5 mg/mL. *S. aureus* and *S. typhi* were resistant to more than two conventional antibiotics.

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Conclusion: Crude and fractions of *M. citrifolia* have excellent antibacterial and antifungal activities against multiple antibiotic resistant strains of *S. aureus*, *S. typhi* and *C. albicans*.

Keywords: *Morinda citrifolia*; antibacterial; antifungal; extract; fruit.

1. INTRODUCTION

*M. citrifolia* (also known as noni) is an economic shrub, which grows in some parts of Africa, Australia and Asia. All parts of its body including fruits, seeds, barks, leaves, and flowers have nutritional and therapeutic values [1]. The fruit is rich in most valuable phytochemical compounds [2]. Traditional healers squeeze the fruit juice for the treatment of arthritis and high blood pressure. Sundrarajan *et al* opined that *M. citrifolia* fruits are currently being utilised in the field of nanotechnology, pharmaceutical and food industries [3]. Ripe *M. citrifolia* fruit contains about 90% of water, which is essential to human body. The remaining 10% comprises dietary fiber, protein and simple sugar such as glucose, sucrose and fructose [4]. This makes noni a veritable fruit for consumption.

The search for alternative to conventional antibacterial and antifungal agents for the treatment of multidrug-resistant microorganisms has intensified in the pharmaceutical research sector in Nigeria and around the globe in the recent years [5–7]. Efforts are being channeled towards ameliorating the menace of antimicrobial resistance and improve chances and duration of patients’ recovery [8]. The dangers posed by antimicrobial resistance to public health are quite enormous. Almost all the microorganisms implicated in nosocomial infections have strains that are resistant to one or more antibiotics [9–12]. For instance, *Staphylococcus aureus* is resistant to Ciprofloxacin, erythromycin, and Amoxicillin/clavulanic acid, *Samonella typhi* is resistant to erythromycin, Cefuroxime, amoxicillin/clavulanic acid and oxacillin [13–16]. This is an indication that these antibiotics cannot guarantee efficacy against future infections.

*M. citrifolia* fruits have proven to be a potent source of phyto compounds, which could be used in the formulation of antimicrobial agents [17]. Many studies have reported the presence of flavonoids, terpenes, anthraquinones, phenols and carotenoids in noni fruits [18–22]. These plant metabolites are known to have both bacteriostatic and bactericidal effects against clinical pathogens [23]. The focus of this study was to evaluate the antibacterial and antifungal profiles of crude noni fruit extract and fractions against multiple drugs resistant organisms isolated from diarrhoeic stools.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Material

Fresh fruits of *M. citrifolia* were harvested from the bush and taken to the Department of Botany, Nnamdi Azikiwe University Awka for the purpose of identification. The sample was prepared according to the method described by Oli *et al* with little modification [24]. The fruits were washed with clean water, air-dried and ground using a grinding machine. A measure of 500 g of powdered sample was macerated in 2.5 Litres of methanol and was shaken intermittently for 72 hours. The resulting mixture was sieved using muslin cloth and then filtered with Whatman No. 1 filter paper. The filtrate was then sieved using muslin cloth and then filtered with Whatman No. 1 filter paper. The filtrate was then concentrated using rotary evaporator and the crude extract stored in the refrigerator at temperature < 8 °C.

2.2 Fractionation

The solvents utilized for fractionation of methanolic are n-hexane, ethyl acetate, butanol and aqueous solvents using liquid-liquid fractionation method with the aid of a separating funnel. 100 ml of methanol was poured on concentrated crude extract before adding 300 ml of n-hexane. The resulting mixture was shaken vigorously while releasing the pressure at intervals. The mixture was allowed to stand for 2 hours before n-hexane fraction was collected into a clean beaker.300 ml of ethyl acetate was added to the resulting residue in a separating funnel and shaken vigorously and allowed to stand for 2 hours. The ethyl acetate fraction was then collected into a clean beaker. Another 300 ml of ethyl acetate was added and the fraction was collected. This procedure was repeated with all the solvents. Each fraction was stored in a refrigerator [25].

2.3 Preparation of Test Isolates

*Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans* were isolated from diarrhoeic
stool samples of children aged 0-5 years who were admitted into Children ward at Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka. The organisms were isolated and confirmed following standard procedures as described by Oli et al at the medical microbiology pharmaceutical science Agulu, Anambra State Nigeria [24]. A loopful from the isolates were subcultured to obtain pure cultures of the test isolates which were verified through biochemical test [25]. The identified *S. typhi* and *S. aureus* were maintained on nutrient agar slopes while *Candida albicans* were maintained in Sabouraud dextrose agar (SDA) in a refrigerator at temperature <8 °C [26].

### 2.4 Antibiotic Susceptibility Test

The following antibiotics were selected for this study: ciprofloxacin (10µg), erythromycin (30µg), cefuroxime (30µg), amoxicillin/clavulanic acid (25µg) and oxacillin (5µg). The antibiotic discs were purchased from Oxoid. The method described by ICCL was employed in evaluating the antibiotic susceptibility pattern of the test isolates [27]. 20ml of sterilized molten MHA was dispensed in a petridish under aseptic condition. 0.1 ml of test isolates were applied on the surface using a sterile swab stick. The antibiotic disc was carefully placed on the agar and incubated for 18 hours at 35 °C. The procedure was carried out in triplicate. The inhibition zone diameters (IZD) were measured and mean plus standard deviations were calculated.

### 2.5 Primary Screening of Extracts for Antibacterial Activity

Antimicrobial activities of the extract and fractions were evaluated using agar well diffusion method described by Ghamba et al. [28]. 100 mg of stock solutions of the methanolic fruit extract and fractions were dissolved in 2 mL of DMSO respectively to make 50 mg/mL. Dilutions of 25, 12.5, 6.25 and 3.125 mg/mL were prepared from 50mg/mL of the resulting solutions. Mueller Hinton agar (MHA) was media of choice for bacteria isolates and SDA for yeast. A 20 mL of molten MHA was dispensed into sterile petri dishes (90 mm) and inoculated with 0.1 mL fresh cultures of test isolates at McFarland 0.5 concentration standard aseptically and allowed to set. Using a sterile metal cork-borer, holes of 6mm diameter were strategically made in the agar plates containing the media with equal consistency. Various dilutions of the extract, fractions and controls were introduced into each hole kept at room temperature for to allow the agents to diffuse into the agar medium before incubation inverted at 37°C for 24 hours. The procedure was repeated three times and zones of inhibition were measured using the transparent plastic rule. To determine the minimum bactericidal/fungicidal concentrations of the extracts and fraction, the isolates were incubated with the antimicrobial agents for 48 hours. The procedure was repeated in triplicate and the zones of inhibition were recorded.

### 3. RESULTS

The percentage yields of methanol crude fruit extracts and the fractions are shown in Table 1. Of the entire fraction, aqueous yielded 3.78g, which was the highest while ethyl acetate yielded 0.5g, which was the lowest. *S. aureus* used for this study showed remarkable susceptibility to cefuroxime and oxacillin but was significantly resistant to amoxicillin, erythromycin and ciprofloxacin as shown in table 3. *Styphi* showed resistant to amoxicillin, erythromycin and cefuroxime but susceptible to ciprofloxacin (Table 2).

The MICs of the methanolic crude fruit extract and fractions range from 3.125 µg/mL to 12.5 µg/mL as shown on Table 3. The bactericidal and fungicidal activity of the methanolic crude extract and fractions against the test isolates ranged from 3.125 µg/mL to 12.5 µg/mL as shown on Table 4.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Yield in gram(g)</th>
<th>Percentage yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanolic crude Fruit</td>
<td>102.0</td>
<td>25.55</td>
</tr>
<tr>
<td>N-hexane fraction fruit</td>
<td>0.70</td>
<td>0.69</td>
</tr>
<tr>
<td>Ethyl acetate fraction fruit</td>
<td>0.5</td>
<td>0.49</td>
</tr>
<tr>
<td>Butanol fraction fruit</td>
<td>1.4</td>
<td>1.37</td>
</tr>
<tr>
<td>Crude aqueous fruit</td>
<td>3.78</td>
<td>3.7</td>
</tr>
</tbody>
</table>
Table 2. Antibiotic susceptibility pattern (mm) of the test isolates to selected antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Salmonella typhi</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SEM</td>
<td>X ± SEM</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>19 ± 0.33</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>0 ± 0.00</td>
<td>22 ± 0.33</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0 ± 0.00</td>
<td>18 ± 0.33</td>
</tr>
</tbody>
</table>

X=Mean, SEM= Standard Error of mean

Table 3. MICs of the Methanolic crude extract and the various fractions of the Morinda citrifolia fruit against the test isolates in mg/ml

<table>
<thead>
<tr>
<th>Crude/Fractions</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Methanolic crude</td>
<td>6.25</td>
</tr>
<tr>
<td>N-Hexane fraction</td>
<td>6.25</td>
</tr>
<tr>
<td>Ethyl-acetate fraction</td>
<td>3.125</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>3.125</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Table 4. MBCs /MFCs of the Methanolic crude extract and the various fractions of the Morinda citrifolia Leaf against the test isolates in mg/ml

<table>
<thead>
<tr>
<th>Crude/Fractions</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td>Methanolic crude leaf</td>
<td>6.25</td>
</tr>
<tr>
<td>N-Hexane fraction</td>
<td>6.25</td>
</tr>
<tr>
<td>Ethyl-acetate fraction</td>
<td>3.125</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>3.125</td>
</tr>
<tr>
<td>methanol</td>
<td>0.0</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Pathogenic microorganisms are dangerous to human, especially for children below 5 years and immunocompromised individuals [28,29]. Antibiotics are used as antidotes to tame the spread of these unfriendly organisms. However, many antibiotics have been rendered useless due to the emergency and spread of resistant strains [30-34]. Bacterial isolates with a high resistant profile were selected for this study. For instance, S. typhi was resistant to Cefuroxime, Amoxicillin/clavulanic acid, oxacillin and erythromycin, S. aureus isolates showed resistance to ciprofloxacin, Amoxicillin/clavulanic acid and erythromycin. This resistance pattern is in agreement with the study carried out by Akinpelun and Kolawole [35].

Plants are known to contain phyto-compounds such as secondary metabolites which can be exploited for formulations of novel candidate drugs. In line with this assumption, the phytochemical analysis of M. citrifolia reveals the presence of phenol, alkaloid, flavonoids, glycosides, tannins, saponins and steroids at various levels [36-40]. These said plant metabolites are known to have antimicrobial characteristics [41].

The noni fruit extract and fractions showed diverse activities against various strains of bacteria and yeast isolate. The fractions showed higher activities and wider zone of inhibitions than the crude extract. This could be as a result of more yields of alkaloids, which has shown lethal effect against bacteria and yeasts in another study [42-45]. M. citrifolia extracts and fractions showed significant activities against test isolates that were resistant to more than one antibiotics. This adds to numerous data suggesting that herbal extracts hold the key for the formulations of antimicrobial agents in no distant future.
5. CONCLUSION

*M. citrifolia* fruit extracts and fractions showed excellent activities against multiple drug resistant clinical organisms isolated from diarrhoeic stool samples. This is an indication that the fruits of *M. citrifolia* can be harnessed for the treatment of some resistant strains of bacteria and fungi.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

We thank the Pharmaceutical Chemistry Department for their assistance in the preparation, extraction and fractionation of the fruits. We are immensely grateful to Dr. Angus N. Oli, the former head of department, Pharmaceutical Microbiology and Biotechnology for granting us a space in their lab for microbiological study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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