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Antibacterial and antifungal activities of crude extract and fractions of *Morinda citrifolia* leaf

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ABSTRACT
Background: *Morinda citrifolia* (Noni) is an evergreen plant used by traditional healers in Africa and beyond to treat infectious and non-infectious diseases. **Methods**: The fresh leaves were harvested, air-dried, pulverized and extracted with methanol and distilled water in a Soxhlet's apparatus. The extracts were filtered using Whatman's no 1 filter paper. The crude methanolic extracts were subjected to phytochemical analysis and fractionation using ethyl acetate, butanol, distilled water and n-hexane. The isolates were collected from diarrhoiec stool using standard procedure. The antimicrobial susceptibility test was carried out using agar well diffusion assay. Phytochemical analysis revealed the presence of flavonoids, alkaloids, and other phenolic compounds. **Results**: The results showed that the MIC of the methanolic extract and fractions ranged from 3.125 mg/mL to 12.5 mg/mL. **Conclusion**: Bioactivity of *Morinda citrifolia* leaf tested revealed its antimicrobial properties which could be exploited to formulate novel drugs from *M. citrifolia* origin against pathogenic bacteria and fungi.

Key words: *Morinda citrifolia*, antibacterial, antifungal, extract, fraction

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INTRODUCTION

Morinda citrifolia is one of the numerous medicinal plants explored for ages for the treatment of human ailments [1–3]. For centuries, traditional healers in Africa and beyond have utilized various parts of *M. citrifolia* to treat diseases of known and unknown aetiology. This has led to the surge in the number of pharmaceutical and medical researchers turning attention to African herbs as possible alternative to orthodox medicine [4–7].

Despite the array of antibiotics that are available for clinical use, the idea of new molecules with antimicrobial properties continues to prevail in clinical research [8 – 10]. This could be as a result of several concerns with chemical compounds already in use. Some of the very potent antibiotics used to treat infectious diseases are known to have relatively high toxicity profile especially at high doses [5, 11-13]. This has impacted their clinical use and only deployed as last resort to save patients.

Antibiotics of low toxicity profile are preferred as first and second lines of treatment. Some of these antibiotics are relatively available over the counter. This has led to their misuse and decline in their potency. Abuse of antibiotics is fingered as one of the major enabler of antibiotic resistance amongst clinically important microorganisms [14 – 18]. For instance, *Staphylococcus aureus* isolated from diarrhoeic stool sample was resistant to cefixime and amoxicillin/clavulanic acid, *Salmonella spp* was resistant to ceftazidime and cefuroxime while *Escherichia coli* was resistant to gentamicin and cefixime in previous studies by several researchers [1, 19-21]. If this trend continues, the future of antibiotic use in clinical practice is bleak.

Morinda citrofolia (Noni) being a green plant and widely distributed especially in the tropical regions are ecofriendly [22, 23]. Several studies have shown their phytochemical composition and proven medicinal values [24-29]. Yhang *et al* has shown their antibacterial and antioxidant effects [27]. García-Vilas *et al*. demonstrated the antitumor effect of *M. citrifolia* [30]. As more resources are committed into Noni research, its clinical benefits will continue to mount. Therefore, this study focuses on antimicrobial profiling of crude extract and solvent fractions of Noni on our indigenous clinical isolates.

MATERIALS AND METHODS

Preparation of plant material

Fresh leaves of *M. citrifolia* were harvested from the farmlands at Okigwe in Imo State, Nigeria and were identified in the Department of Botany, Nnamdi Azikiwe University Awka by a curator. The plant sample was prepared following a method described by Obaji *et al* with little modification [4]. The leaves of *M. citrifolia* were dried under shade after washing thrice with clean water at room temperature and pulverized using a grinding machine. A measure of 500 g of pulverized sample was macerated in 1.6 Liter of methanol and was intermittently shaken for 3 days. The mixture was sieved using muslin cloth and further filtered with Whatman No. 1 filter paper. The resultant filtrate was concentrated using rotary evaporator and further concentrated with water bath at 50^0C. The crude extract was stored in refrigerator at 4 – 8^oC.

Fractionation

The methanolic crude extract was fractionated using n-hexane, ethyl acetate and butanol, aqueous solvents by liquid-liquid fractionation method with the aid of a separating funnel. The concentrated crude extract was reconstituted with 100ml of methanol. Then 300ml of n-hexane was added and shaken vigorously releasing the pressure at intervals. The mixture was then allowed to stand for two hours for proper separation, then n-hexane fraction was collected into a clean beaker. Another 300ml of n-hexane was added to the extract in the funnel and the fraction was collected again. Then 300ml of ethyl acetate was added to the residue in the separating funnel and shaken vigorously, then allowed to stand for 2 hours. The ethylacetate fraction was then collected into a clean beaker. Another 300ml of ethylacetate was added and the fraction was collected. The above procedure was repeated for butanol to obtain the butanol and aqueous fractions. The fractions were further concentrated using rotary evaporator and their weights were obtained and stored in the refrigerator [31].

Preparation of test isolates

Bacteria and yeast isolates which include one Gram negative bacillus (*Salmonella spp),* one Gram positive coccus (*Staphylococcus aureus*) and a yeast (*Candida albicans*) were isolated from diarrhoeic stool samples obtained from Chukwuemeka Odimegwu Ojukwu Teaching Hospital, Awka. The isolates were confirmed, prior to use for this study, in the Medical Microbiology Unit in WeCare Diagnostics Laboratories Awka, Anambra State Nigeria. The method described by Oli *et al.* [2] was used to isolate the test organisms. The test isolates were maintained on nutrient agar slopes in a refrigerator at temperature 4 – 8 ^{0}C.

Primary screening of extracts for antibacterial activity

Stock solutions of the methanolic extract and fractions were prepared by dissolving 100 mg of the extracts in 2 mL of DMSO respectively (to make 50 mg/mL) in screw capped tubes for primary screening of plant solvent extracts. Antimicrobial activity was assayed according to agar well diffusion method described by Ghamba *et al.*, [33]. A two - fold dilution of 50, 25, 12.5, 6.25 and 3.125 mg/mL were prepared from 100 mg/mL stock solutions of the crude extracts and four fractions. A 20 mL of molten Mueller Hinton agar 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. Holes of 4mm diameter were made in the agar plates using a sterile metal cork-borer. A 60 µl of the various dilutions of each extract and controls were aseptically dispensed into each hole and kept at room temperature for 1 hour to allow the agents diffuse into the agar medium before incubation at 37^{o}C for 24 hours. Fluconazole (15 µg/mL) and Tetracycline (25µg/mL) were used as positive controls while sterile water and methanol were used as negative controls for aqueous and methanolic extracts of plant parts under study. The positive controls were prepared by dissolving 150mg of Fluconazole and 250mg of Tetracycline in 10 mL distilled water respectively. The zones of inhibition were measured and extracts and fractions that yielded significant activities against test organisms were further tested against the organisms to determine the minimum inhibitory concentrations (MICs).

RESULTS

Percentage yield of the plant Leaf extracts and the fractions

The percentage yields of the methanolic crude leaf extracts (MCLE) revealed 15.08% yield while the N-hexane leaf fraction exhibited 41.67% of the entire leaf fractions, percentage for aqueous crude extract is 4.3.(Table 1)

Qualitative and quantitative analyses of the phytochemical constituents of the plant parts.

Quantitatively, saponin constituted 12.5% of the estimated phytochemical components of the leaf followed by flavonoid (Table 2)

Antibiotic susceptibility profile of test isolates

The bacterial isolates were found to be resistant to some of the standard antibiotics used. *Salmonella spp* was not susceptible to augmentin, ofloxacin and erythromycin but susceptible to ciprofloxacin and oxacillin. *S. aureus* was not susceptible to oxacillin and erythromycin but susceptible to augmentin, ofloxacin and ciprofloxacin.(Table 3)

Minimum inhibitory concentration (MIC) of the methanolic crude extracts on test isolates

The methanolic crude extracts and the fractions that showed activity in the preliminary antimicrobial screening were subjected to further study to determine their MIC against test isolates as shown on Table 4. The MICs of the methanolic crude extract and fractions range from 3.125 µg/mL to 12.5 µg/mL.

Bactericidal and fungicidal activity of the crude extracts on test isolates

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 5.

Table 1: Percentage yields of methanolic crude extracts and various fractions of *M. citrifolia* Leaf (MCL)

Solvents	Yield in gram(g)	Percentage yield (%)
Methanolic crude leaf	75.4	15.08
N-hexane fraction	1.0	1.33
Ethyl acetate fraction	0.4	0.53
Butanol fraction	0.3	0.93
Crude aqueous	3.24	4.3

Table 2: Qualitative and Quantitative phytochemical constituents of *M. citrifolia* leaf

Phytochemical components	Qualitative	Quantitative %
Alkaloids	+	3.0
Saponin	+++	12.5
Tanin	+	3.5
Flavonoid	+	5.8
Steroid	-	-
Terpenoids	+	-
Cardiac glycoside	-	-
Protein	+	-
Carbohydrate	+	-
Reducing sugar	+	-

Table 3: Antibiotic susceptibility pattern of the test isolates to selected antibiotics

Antibiotics	*Salmonella spp Zones of inhibition (mm)* X ± SEM	*S. aureus Zones of inhibition (mm)* X ± SEM
Ciprofloxacin	19 ± 0.33	21 ± 0.58
Erythromycin	0 ± 0.00	0 ± 0.00
Ofloxacin	0 ± 0.00	22 ± 0.33
Augmentin	0 ± 0.00	18 ± 0.33
Oxacillin	22 ± 0.67	0 ± 0.00

X=Mean, SEM= Standard Error of mean.

Table 4: MICs of the Methanolic crude extract and the various fractions of the *M. citrifolia* leaf against the test isolates in mg/ml

Crude/Fractions	Isolates		
	Candia albicans	*Salmonella* spp	*Staphylococcus aureus*
Methanolic crude	6.25	3.125	6.25
N-Hexane fraction	6.25	6.25	12.50
Ethyl-acetate fraction	3.125	6.25	3.125
Butanol fraction	6.25	6.25	6.25
Crude aqueous	25.0	100.0	0.00

Table 5: MBCs /MFCs of the Methanolic crude extract and the various fractions of the *M. citrifolia* leaf against the test isolates in mg/ml

Crude/Fractions	Isolates		
	Candia albicans	*Salmonella* spp	*Staphylococcus aureus*
Methanolic crude leaf	6.25	6.25	6.25
N-Hexane fraction	6.25	6.25	12.50
Ethyl-acetate fraction	3.125	6.25	3.125
Butanol fraction	6.25	6.25	6.25
Crude aqueous	25.0	100.0	0.00

DISCUSSION

Misuse of antibiotics is considered the leading cause of antibiotic resistances amongst microorganisms of clinical importance. Several studies have also characterized some important clinical isolates as multidrug resistant species [34-36]. The bacterial isolates used in this study were not susceptible to some selected antimicrobial drugs. *Salmonella spp* showed resistance to erythromycin, augmentin and ofloxacin while *Staphylococcus aureus* exhibited resistance to erythromycin and oxacillin. These are in line with antibiotic susceptibility pattern described by Akinpelun and Kolawole [37].

The hope of discovering candidate drugs with low toxicity profiles hinges on information on phytochemical constituents of plants. The findings of this study is in line with several studies on qualitative and quantitative analysis of *M. citrifolia*, which showed varying compositions of phenol, alkaloid, flavonoids, glycosides, tannins, saponins and steroids at various levels [38-40]. These compounds are known to have bactericidal and fungicidal properties [41].

The potency of plant extract and fractions were different with the two bacterial strains. Among the extract and fractions tested, ethyl acetate leaf fraction exhibited significant antibacterial and antifungal activity with larger zones of inhibition and all the three microbes tested were susceptible to all the extracts and fractions. Leaf extracts and fractions have significant alkaloids which contributes to the control of bacterial growth [42, 43]. The *test* isolates are known multiresistant drug human pathogens and their inhibition by the *M. citrifolia* extracts and fractions might suggest their possible use in the treatment of infections caused by organisms isolated from diarrhoiec stool. The overall result suggests that *S. aureus* is the most susceptible strain. According to Sathish*et al.*, ethyl acetate extract of *M. citrifolia* exhibited broad spectrum of inhibition against Gram negative bacteria [44].

CONCLUSION

Bioactive substances from *M. citrifolia* leaf could be employed in the formulation of antimicrobial agents for the treatment of various bacterial and mycotic infections. The presence of antimicrobial activity in *M. citrifolia* plant extracts give credence to the traditional use in treating conditions associated with microorganisms in humans.

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