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Phytochemical composition and antimicrobial potential of ethyl acetate extract of *Morinda citrifolia* L. seeds

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Morinda citrifolia (commonly known as noni) is widely recognised for its diverse pharmacological properties. Several researchers have focused on the use of its root, leaf and fruit. This study aims to evaluate the phytochemical composition and antimicrobial activity of the ethyl acetate extract of *M. citrifolia* seeds. The ground seeds were extracted using ethyl acetate. The ethyl acetate extract underwent phytochemical screening and it was tested for antimicrobial activity against selected bacterial strains (*Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Staphylococcus epidermidis*) and fungal strains (*Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*). Phytochemical analysis revealed the presence of flavonoids and steroids, while alkaloids were absent. The antimicrobial assay showed limited activity, with mild inhibition against *Salmonella* spp. at higher concentrations (9 mm inhibition zone at 20 mg/mL). Compared to standard antibiotics, the extract exhibited weak antimicrobial effects. Although *M. citrifolia* seed extract contains bioactive constituents which could be explored for pharmacological purpose, its antimicrobial activity is relatively weak. Further studies are warranted to isolate, characterize and enhance the therapeutic potential of its active compounds.

Keywords: *Morinda citrifolia, noni seed, phytochemicals, antimicrobial activity, medicinal plants*

Introduction

Medicinal plants play a pivotal role in global healthcare, particularly in low- and middle-income countries, where they often serve as first-line treatments for a broad range of diseases. In previous reports of Ekor (2014) and Das & Aruna (2021), over 80% of the global population relies on traditional medicine for the management of infectious and chronic conditions. Most people living in the rural areas relies on the use of medicinal and aromatic plants as major sources of orthodox medicine (Paramanya et al., 2020). This heavy dependence emphasises the significance of identifying and validating medicinal plants as sources of novel therapeutic agents. Among these plants, *Morinda citrifolia* L., commonly referred to as noni, has attracted substantial scientific interest due to its wide array of pharmacological and therapeutic effects. Traditionally used for more than 2,000 years in Polynesia, Southeast Asia, and other tropical regions, the noni plant has been employed in the treatment of ailments such as arthritis, infections, diabetes, hypertension, cancer, and gastrointestinal disorders (de Oliveira et al., 2022; Nayak et al., 2015). The extensive use of the plant in ethnomedicine has been attributed to its diverse phytochemical composition, which includes over 200 bioactive compounds such as alkaloids, flavonoids, phenolics, sterols, saponins, and terpenoids (Mulat et al., 2019). Recently, some phenolic compounds identified from medicinal plants fractions has been shown to possess good pharmacokinetics making them drug-like molecules (Ozioko et al., 2025). Numerous studies have confirmed the therapeutic relevance of noni and its potential as a functional food. West et al. (2018) highlighted the increasing use of noni fruits and leaves due to their antioxidant, anti-inflammatory, and antimicrobial properties, along with their application in managing chronic diseases

like hypertension, diabetes, and cancer. The fruit's recognition as a novel food by the European Union has stimulated global production and incorporation into products like dairy foods, baked goods, and nutritional supplements (Deng et al., 2010). For instance, Farias et al. (2024) identified milk-clotting enzymes in noni puree, suggesting its utility in cheese production. Beyond its nutritional uses, *M. citrifolia* has been reported to stimulate immune responses, accelerate wound healing, and exhibit anticancer potential, placing it at the intersection of nutraceutical and pharmaceutical development (Chaudhury et al., 2024). As such, countries like French Polynesia have expanded noni cultivation, particularly for puree export (Hou et al., 2025). However, while much research has focused on the fruit and leaves, less attention has been paid to the seeds and other by-products, which may harbour equally potent bioactive compounds. Phytochemical analysis of different parts of the noni plant roots, bark, leaves, fruit, and seeds has revealed a complex mix of secondary metabolites with antimicrobial potential (Anand et al., 2019; Mancuso et al., 2021). In particular, compounds such as pentacetyl-β-D-glucopyranose and iridoid acubin have demonstrated antimicrobial activity (Gajdacs, 2019). O'Neill (2016) and Selvam et al. (2025) reported the efficacy of noni-derived compounds against pathogenic microbes, emphasising its potential as a source of plant-based antimicrobials. Moreover, Vyas et al. (2012) stressed the growing importance of exploring such natural alternatives due to the global rise in antimicrobial resistance. Recent studies have confirmed the antimicrobial spectrum of *M. citrifolia*. Selvam et al. (2025) found that the aqueous noni fruit extract significantly inhibited the growth of bacterial strains like *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Streptococcus pyogenes* with zone of inhibition ranging from 9.1 - 25.3 mm while the growth of fungal species such as *Aspergillus flavus*, *A. niger*, *Neurospora crassa*, and *Trichophyton rubrum* were inhibited within the zone of inhibition ranging from 7.15 - 19.2 mm. Obeng-Boateng et al. (2024) reported a moderate antibacterial activity against all the bacteria pathogens while Shigella spp. and Klebsiella spp. showed resistance to most extract. Additionally, the root extract of noni showed a significant antibacterial activity followed by its fruit while the least was noted in the leaf extract. The 100% ethanol extracts were found to yield significant higher zones of inhibition compared to other solvents and most inhibitory activity was against *Campylocabter* spp. (Obeng-Boateng et al., 2024). Although many studies have examined noni's fruits and leaves, phytochemical screening of its seed extracts, particularly those obtained using solvents like ethyl acetate, has remained limited. Imieje et al. (2024) reported the presence of flavonoids and steroids in noni seeds, with an absence of alkaloids, alongside observed anti-inflammatory and antipyretic effects in animal models. However, despite extensive research on *M. citrifolia* fruits and leaves, the phytochemical and antimicrobial properties of its seed extracts, particularly those obtained using moderately polar solvents like ethyl acetate, remain largely uncharacterised. This study therefore, seeks to characterise the phytochemical constituents and evaluate the antimicrobial potential of the ethyl acetate extract of *M. citrifolia* seeds, with the goal of identifying novel bioactive compounds that could contribute to the development of plant-derived antimicrobial agents.

Materials and Methods

Plant Collection and Extraction

Noni seeds were obtained from Kawo Market in Kaduna Metropolis. The seeds were authenticated by a Botanist in the Biology Unit, Air Force Institute of Technology, Kaduna, with the authentication reference numbers AFITBIO/NO-001-0001. The seed was pounded in a wooden mortar to a consistent rough powder (Figure 1).

Figure 1. **Pulverised seeds**

About 200 g of *M. citrifolia* seeds were macerated in 6 liters of ethyl acetate with intermittent shaking for three days at room temperature. The residue (marc) was re-macerated for an additional 72 hours to obtain a more concentrated extract for further analysis (Figure 2 and Figure 3).

Figure 2. Extraction Process

Figure 3. Extraction Process using Ethyl Acetate

Phytochemical Screening

Phytochemical screening of the extract was conducted using standard qualitative procedures as described by Trease & Evans (1989), Wallis (1989), Pandey & Tripathi (2014), Beena et al. (2016), and Dhawan & Gupta (2017). The analysis was aimed at detecting the presence of major classes of secondary metabolites, including alkaloids, tannins, flavonoids, saponins, and steroids. The procedures are outlined below:

- **Test for Alkaloids:** A portion of the extract was treated with a few drops of Dragendorff's reagent. The appearance of a reddish-brown precipitate was taken as a positive indication of alkaloid presence.
- **Test for Tannins:** To another portion of the extract, a few drops of 1% gelatin solution containing sodium chloride were added. The formation of a yellowish precipitate indicated the presence of tannins.
- **Test for Flavonoids:** A few drops of 10% sodium hydroxide (NaOH) solution were added to the extract. A yellow colouration that disappeared upon the subsequent addition of 10% hydrochloric acid (HCl) indicated the presence of flavonoids.
- **Test for Saponins:** The extract was diluted with distilled water and vigorously shaken. The formation of stable froth upon standing was considered indicative of saponin presence.
- **Test for Steroids:** The extract was dissolved in chloroform, and an equal volume of concentrated sulfuric acid (H_2SO_4) was carefully added along the side of the test tube. The formation of a reddish ring at the interface indicated the presence of steroidal compounds.

Sample Adsorption

The concentrated extract (61.4 g) was pre-adsorbed by dissolving it in ethyl acetate and adsorbed onto silica gel (15 g) *in vacuo* until a free-flowing powder was obtained.

Column chromatography of the ethyl acetate extract

The ethyl acetate extract was subjected to column chromatography for the separation of constituent compounds. A glass column (3.5 cm × 70 cm) was packed with silica gel (stationary phase), and the pre-adsorbed extract slurry was carefully applied onto the column. Elution was performed using a stepwise gradient solvent system of increasing polarity, beginning with 100% hexane, followed by mixtures of hexane and ethyl acetate, then ethyl acetate alone, and finally ethyl acetate–methanol mixtures up to 100% methanol. This gradient system facilitated the effective partitioning of compounds according to their polarity. A total of 21 fractions (200 mL each) were collected, labelled M1 to M21, concentrated under reduced pressure (*in vacuo*) and stored in clean, labelled vials for further analysis (Figure 4).

Figure 4. Pre-Column Fractions (200 mL) of Noni Seed

Fraction selection for antimicrobial analysis

Column chromatography was employed to separate and purify the compounds present in the *Morinda citrifolia* (noni) seed ethyl acetate extract, based on their differential interactions with the silica gel (stationary phase) and the stepwise hexane–ethyl acetate–methanol gradient solvent system (mobile phase). Fractions M2 to M12 yielded dense, milky encrustations with a sweet, fruity aroma, suggesting the presence of complex phytochemical constituents. Notably, fraction M10 appeared whiter and exhibited an acrid odour, indicative of a distinct chemical composition and higher purity. On this basis, fraction M10 was selected for antimicrobial analysis.

Preparation of media (Nutrient and Potato Dextrose Agar) for bacterial and fungal sensitivity tests

Nutrient agar (NA) and potato dextrose agar (PDA) were used for the antimicrobial testing of the extracts of noni seed extract. The media were prepared according to the manufacturer's instructions, where 28 g and 39 g of NA and PDA, respectively, were each weighed and dissolved in 1000 mL of sterile distilled water in a conical flask. This was loosely sealed with cotton wool wrapped with aluminium foil and autoclaved at 121°C for 15 minutes. This was allowed to cool down to 40°C after which they were aseptically poured onto sterile plastic petri dishes and allowed to cool and solidify (Kirby et al., 2019).

Test organisms

The test organisms (*S. aureus, S. epidermidis, Salmonella sp, Shigella sp, E. coli, A. niger, A. flavus* and *C. albicans*) were obtained and confirmed at the Microbiology and Zoology laboratories of the Departments of Microbiology and Biological Sciences respectively at The Faculty of Life Sciences, Kaduna State University, Nigeria. *E. coli* was confirmed on EMBA by its characteristic green metallic sheen, *S. aureus* and *S. epidermis* on MSA, *Salmonella* and *Shigella* on SSA, while *A. niger*, *A. flavus* and *C. albicans* were confirmed on PDA following stain with lactophenol cotton blue.

Preparation of working stock and concentrations of plant extract

A working stock solution of 100 mg/mL of the extract was prepared by dissolving 100 mg of the extract to 1 mL of ethyl acetate. This was extrapolated to obtain two separate concentrations of 20 mg/mL and 25 mg/mL by calculating their respective dilution factors (100 mg/mL/20 and 100 mg/mL/25) and adding 1 mL of the 100 mg/mL to 4 mL of ethyl acetate and 1 mL of the 100 mg/mL to 3 mL of the ethyl acetate to obtain concentrations of 20 mg/mL and 25 mg/mL concentrations respectively (Adefuye & Ndip, 2013).

Sensitivity tests using the disk diffusion (Kirby-Bauer method)

The procedure of Kirby-Bauer disk surface diffusion technique as described in Hudzicki (2009) was adopted with modifications to test for the antimicrobial potential of the different extracts of noni seed against eight different microorganisms (*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella* sp., *Shigella* sp., *Aspergillus niger, Aspergillus flavus* and *Candida albicans*). Commercially prepared multi-antibiotic discs (Maxi Care Medical Laboratory, Nigeria) were used as positive controls. The disc size of 8 mm was aseptically cut using a sterile sharp cork borer. Twenty-four-hour cultures of the test organisms were prepared on sterile nutrient broth with turbidity corresponding to the 0.5 McFarland standard. Each test organism was picked with a sterile wire loop (flamed on the burner) and aseptically streaked evenly to give a uniform and thin layer of the organism on the NA and PDA. The discs

were dipped into each concentration of the extracts and gently placed on the surface of the plates. This was covered and incubated at 37°C for 24 hours. Diameters of zones of inhibition (ZI) for susceptibility were measured and recorded against each organism and extract concentration. Interpretation of results was done based on the guidelines by the Clinical and Laboratory Standards Institute (CLSI) as described by CLSI (CLSI, 2019; 2020; 2022).

Data analysis

Data were analyzed using descriptive statistics, and results were presented as mean values of triplicate determinations.

Results

Phytochemical composition of *Morinda citrifolia* seed extract

The phytochemical screening (Table 1) revealed low levels of tannins and moderate levels of saponins. The levels of flavonoids and steroids were high but alkaloids were absent.

Table 1. Test Result for Phytochemical Screening

Test	Extract
Alkaloid	–
Tannin	+
Flavonoid	++++
Saponins	++
Steroids	++++

Key: ++++ High; ++ Moderate; + Low; – Absent

Anti-microbial susceptibility of *Morinda citrifolia* seeds

Using the Surface Disc Diffusion method with a disc size of 8 mm, the antimicrobial activity of noni was investigated (Table 2) against the following organisms: *Escherichia coli, Salmonella* sp., *Shigella* sp., *Staphylococcus aureus, Staphylococcus epidermidis, Aspergillus niger, Aspergillus flavus* and *Candida albicans*. *Escherichia coli* had a resistance of 8 mm against *M. citrifolia* seed extract for all the studied concentrations. Using ciprofloxacin and pefloxacin as control drugs for gram-positive and gram-negative bacteria, respectively, the zone of inhibition for *Escherichia coli* was 16 mm. *Salmonella sp* had a resistance of 8 mm at concentrations of 25 mg/mL and 100 mg/mL while at a concentration of 20 mg/mL, there was a susceptibility of 9 mm for the same bacteria. Using ofloxacin (tarivid) as the control drug for gram-positive bacteria, there was a susceptibility of 14 mm for the zone of inhibition, and for Rocephin against gram-negative bacteria, there was a susceptibility of 13 mm for the zone of inhibition.

Table 2. **Anti-microbial susceptibility profile of *Morinda citrifolia* seeds by surface disc diffusion**

Test Organism	Concentration (mg/mL)			Control			
	20	25	100	G +ve		G -ve	
				Drug	ZI (mm)	Drug	ZI (mm)
Escherichia coli	R(8)	R(8)	R(8)	CPX	S(16)	PEF	S(16)
Salmonella sp.	S(9)	R(8)	R(8)	OFX	S(14)	RO	S(13)
Shigella sp.	R(8)	R(8)	R(8)	STM	S(14)	SXT	S(11)
Staphylococcus aureus	R(8)	R(8)	S(9)	GN	S(12)	E	S(12)
Staphylococcus epidermidis	R(8)	R(8)	R(8)	AM	S(11)	CPX	S(16)
Aspergillus niger	R(8)	R(8)	R(8)				
Aspergillus flavus	S(9)	R(8)	R(8)	Control drug **(CLT)**			
Candida albicans	R(8)	R(8)	R(8)	**S**(14)			

KEY: S = Susceptible/Sensitive**; R =** Resistant; **CPX** = Ciprofloxacin (30µg)**; OFX** = Ofloxacin-Tarivid (10µg)**; SP** = Sparfloxacin (10µg)**; GN** = Gentamycin (30µg)**; STM =** Streptomycin (30µg)**; E =** Erythromycin (10µg); **AM =** Amoxicillin (30µg)**; RO =** Rocephin (10µg)**; PEF =** Pefloxacin (10µg)**; SXT =** Trimethoprim/sulfamethoxazole (10µg)**; CLT** = Clotrimazole (10mg); **ZI =** Zone of inhibition (mm); **G-ve** = Gram negative; **G+ve** = Gram positive.

Shigella sp., the sample had a resistance of 8 mm for all the concentrations. Using streptomycin as the control drug, the zone of inhibition had a sensitivity of 14 mm for gram-positive bacteria, and for the gram-negative bacteria, the sensitivity was 11 mm using SXT. *Staphylococcus aureus*, the sample had a resistance of 8 mm at 20 mg/mL and 25 mg/mL, but the

resistance for 100 mg/mL was 9 mm. Using gentamycin and erythromycin as control drugs, the zone of inhibition for gram-positive and gram-negative bacteria was 12 mm, respectively. *Staphylococcus epidermidis*, the sample, had a resistance of 8 mm for all the concentrations. The zone of inhibition for the gram-positive bacteria was 11 mm using amoxicillin as the control drug, and for the gram-negative bacteria, the zone of inhibition had a sensitivity of 16 mm using CPX as the control drug. *Aspergillus niger* and
albicans had a resistance of 8 mm at all concentrations. *Aspergillus flavus* had a resistance of 9 mm at 20 mg/ml, then a sensitivity of 8 mm at 25 mg/mL and 100 mg/mL. The control drug for all three fungi was clotrimazole, and they all had a susceptibility of 14 mm as zones of inhibition (Table 2).

Minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of *Morinda citrifolia* seed extract

The results of MIC, MBC and MFC are presented in Table 3. MIC determines the lowest level at which an antimicrobial agent inhibits growth, and the MBC determines the lowest level at which an antimicrobial agent leads to microbial death (David). *Escherichia coli* and *Shigella* sp. had no susceptibility to MIC and MBC. *Salmonella* sp. had 20 mg/mL MIC and MBC. *Staphylococcus aureus* and *Staphylococcus epidermidis* had MIC and MBC of 100 mg/mL. *Aspergillus niger* and *Candida albicans* have no susceptibility to MIC and MFC. *Aspergillus flavus* had MIC and MFC of 100 mg/mL.

Table 3. Result of MIC, MBC and MFC of the extracts of *Morinda citrifolia* seeds

Test Organism	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)
Escherichia coli	NS	NS	NS
Salmonella sp.	20	20	NS
Shigella sp.	NS	NS	NS
Staphylococcus aureus	100	100	NS
Staphylococcus epidermidis	100	100	NS
Candida albicans	NS	NS	NS
Aspergillus flavus	100	NS	100
Aspergillus niger	NS	NS	NS

KEY: **MIC** = Minimum Inhibitory Concentration (mg/mL); **MBC** = Minimum Bactericidal Concentration (mg/mL); **MFC** = Minimum Fungicidal Concentration (mg/mL) and **NS** = No Susceptibility.

Discussion

The findings from this study are consistent with previous reports by Das & Aruna (2021), Imieje et al. (2024), and Aondoackaa et al. (2024), who similarly identified flavonoids, saponins, and steroids as major phytochemical constituents of *M. citrifolia* extracts, although variations were noted depending on the plant part, extraction solvent, and geographical origin. Flavonoids and steroids were highly abundant in the extract, as indicated by their very intense presence (++++). *M. citrifolia* fruits have been reported to be a good source of aromatic compounds such as phenols and flavonoids which have been linked to their antioxidant properties (Das & Aruna, 2021). Flavonoids are known for their antioxidant, antimicrobial, and anti-inflammatory properties, which could contribute to the bioactivity of *M. citrifolia* (Lawal et al., 2023). Similarly, steroids play a crucial role in membrane stabilization and have potential pharmacological applications, including anti-inflammatory effects (Imieje et al., 2024). The presence of these compounds suggests that noni seeds may hold therapeutic potential, although further investigation is necessary to isolate and characterize the specific bioactive constituents. Tannins and saponins were detected at lower concentrations, with tannins showing a poor intensity (+) and saponins displaying fairly intense (++) presence. Tannins are known for their antimicrobial and astringent properties, which could contribute to the mild antibacterial activity observed in this study (Das & Aruna, 2021). The detection of saponins aligns with findings from Mubarokah et al. (2023), who reported their presence in noni leaves. Saponins have been implicated in antimicrobial and immunomodulatory activities, which may explain some of the traditional medicinal uses of *M. citrifolia* (Aondoackaa et al., 2024). Interestingly, alkaloids were absent in this study, contrasting with findings from Aondoackaa et al. (2024) and Imieje et al. (2024), who identified alkaloids in fermented noni fruit juice and seed extracts, respectively. This discrepancy may be attributed to differences in solvent polarity, extraction techniques, or variations in phytochemical distribution across different plant parts. Alkaloids are known for their potent pharmacological effects, including antimicrobial and analgesic properties, and their absence in this study suggests that noni seeds may not be a primary source of these compounds. Alternatively, the role of ethyl acetate solvent in selectively extracting nonpolar or moderately polar compounds, might be responsible for the absence of alkaloids in noni seed. The antimicrobial potential of *M. citrifolia* has been investigated in multiple studies with varying results. The ethyl acetate extract of noni seeds exhibited limited antibacterial activity, showing mild inhibition against *Salmonella* sp. (9 mm at 20 mg/mL) but weaker effects compared to standard antibiotics (Imieje et al., 2024). Contrastingly, fermented noni fruit juice extracts

demonstrated antibacterial activity against *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*, with minimum inhibitory concentrations (MIC) ranging between 25 mg/mL and 100 mg/mL, depending on the solvent used (Aondoackaa et al., 2024). Sina et al. (2021) found that noni fruit juice significantly inhibited the growth of multiple bacterial strains, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These findings suggest that while noni seeds may have weaker antibacterial effects, fruit and leaf extracts exhibit stronger antimicrobial properties which may be as a result of differences in solvent polarity or extraction techniques. The collective findings from previous studies indicate that *M. citrifolia* contains a rich array of bioactive compounds with promising pharmacological properties, most of which are aromatic compounds. However, the antimicrobial activity of noni seed extracts appears to be weaker compared to its fruit and leaf extracts. Further research is needed to isolate and characterize the specific bioactive compounds responsible for these effects. Additionally, clinical studies should be conducted to validate the therapeutic potential of *M. citrifolia* extracts in disease management.

Conclusion

Although phytochemicals were present, the ethyl acetate extract of *M. citrifolia* seeds exhibited limited antimicrobial activity. This may be so due to the possible influence of the ethyl acetate solvent. Hence, the need to use solvents of varying polarity in future studies to better isolate active compounds is recommended.

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Author contributions

MS conceived the idea for this research work and proposed the research design. MS and OJO supervised the research and were major contributors to writing the original manuscript. MS, OJO and KMM conducted the experiment, analyzed and interpreted the research data. OJO and KMM carried out a critical review of the revised manuscript and were major contributors in editing the manuscript. All authors read and approved the final manuscript.

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AI tool declaration

The authors have not used AI and it's related to tools to write this manuscript.

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