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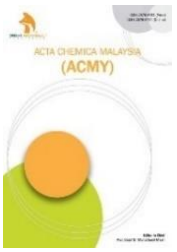

RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF PHYTOCHEMICALS, ANTIOXIDANT, AND ANTIMICROBIAL PROPERTIES IN SEED AND STEM BARK METHANOL EXTRACTS OF MORINDA CITRIFOLIA

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ABSTRACT

This study investigates the phytochemical composition, elemental make-up, and antioxidant properties of methanol extracts from the seed and stem bark of *Morinda citrifolia* (Noni). The phytochemical screening revealed the presence of glycosides, phenolics, eugenols, terpenoids, alkaloids, flavonoids, and tannins in both extracts, with steroids absent in the seed but present in the stem bark. The quantitative analysis showed higher alkaloid content in the stem bark (2.960%) compared to the seed (1.460%), while the seed exhibited a higher total phenolic content (24.430 mg/ml) than the stem bark (7.830 mg/ml). Antioxidant assays demonstrated significant activities in both extracts, with the stem bark extract displaying greater antioxidant potential, which doesn't correlates with its higher phenolic content but the lower IC_{50} value (higher antioxidant activity) of the stem bark, which could be based on the composition, effectiveness, bioavailability of the phenolic and other bioactive compounds present in the stem bark which could have enhanced its antioxidant activity. The antimicrobial activity assays indicated that both extracts possess substantial antimicrobial properties, with the stem bark extract showing a slightly higher inhibitory effect. These findings suggest that different parts of *Morinda citrifolia* can be utilized strategically based on their specific phytochemical compositions to maximize therapeutic benefits, highlighting the potential of the stem bark as a natural antimicrobial and antioxidant agent.

KEYWORDS

antioxidant, antimicrobial, phytochemicals.

1. INTRODUCTION

Medicinal herbs can also be denoted as therapeutic or medicinal plants. Since the beginning of time, they have gained strong acceptance and used customarily in traditional medicine practices. Medicinal herbs have been used for years to treat a variety of human ailments because of the powerful chemicals constituents they contain. (Ogbeide et al., 2022; Aghedo Ogbeide., 2022). Our ancestors were compelled to look about them for answers as a result of their quest for healthy living long life, and distress-relieving medicines. Some of these bioactive substances with therapeutic potential present in medicinal plants includes tannins, alkaloids, essential oils, saponins, flavonoids, terpenoids and so on (Ogbeide and Akhigbe., 2019). Herbal medicine has a long history of usage in developing countries, and medicinal plants continue to play a significant part in healthcare systems for vast segments of the global population. In many parts of the world, there are a lot of different types of plants that are flourishing in bleak conditions, but nature has also provided quite rich botanicals. As a result, traditional medicine is receiving increasing attention, and there is a strong need for more medications made from plants (Ogbeide et al., 2022). The screening of plant extracts is a continuous effort in compounds isolation, enhancing the alleviation of life-threatening diseases mostly caused by microbes (Alka and Padma., 2013). However, the escalating costs of prescription drugs and the quest for new plant-derived medicines have rekindled interest in medicinal plants as valuable health aids. Furthermore, the clinical effectiveness of many synthetic antibiotics are threatened by the rise of microorganisms that are resistant to several drugs, including bacteria, fungi, and viruses.

The fruit of Morinda citrifolia Linn (Rubiaceae) is known in Hawaiian as noni. Morinda citrifolia (Noni), a plant species of southeast Asia native and Australia, is found in Vietnam, India, Polynesia, Cambodia, Malaysia, Costa Rica, China and South America. It thrives in tropical and subtropical temperatures (Adilah and Hanani, 2016; Dussossoy et al., 2011; West, et al., 2008). It is commonly referred to as cheese fruit, mengkudu, noni, nuna, nhaut, and painkiller bush throughout the world (Bui and Bacic., 2006). When unripe, the Noni fruit is light green, but when mature, it turns yellowish-white. It is soft and meaty. The culinary and medicinal uses of noni leaves, flowers, fruits, roots, and stems have been well documented. Antioxidant, antibacterial, anti-inflammatory and anti-cancer effects have been demonstrated by these plant parts (Wang et al., 2002). Cultivation of Noni has spread widely to areas like Mexico, Central America, and South America. This is due to claims about its health advantages, its commercial value has increased dramatically in recent years globally. Morinda citrifolia (Noni) products are mostly sold in North America, Europe, Japan, Mexico, Asia, Australia and recently started gaining popularity in Nigeria.

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The fruit is mostly offered for sale as juice, although it is also frequently sold in both official and informal markets in its raw, unprocessed state (Wang et al., 2002).

Noni seeds

Noni plant (stem, leave and fruits)

The majority of the wastes from the manufacturing of noni juice, puree, and powder are seeds that are thrown away as garbage (Jahurul et al., 2021). For example, only Polynesians produce more than 150 metric tons of noni seed a year in French (West et al., 2008). Oil, dietary fiber, carbohydrates, protein, and are all abundant in this noni seed (Jahurul et al.,2021). However, because noni seeds are regarded as food by-products, their economic value is limited. Furthermore, food manufacturers typically have to pay a lot of money to discard these by-products (dry, store, and ship), which could raise the fruit products' ultimate cost (Ayala-Zavala et al., 2011). The residues from the industrial manufacture of noni juice, powder, and puree. The expense of waste management would therefore be decreased by turning these food byproducts into affordable and healthful products. In an effort to cut down on waste, Numerous bioactive compounds (proteins, fiber, vitamins, minerals, oils, polysaccharides, polyphenols, and so on) that have been thoroughly studied for their possible health benefits, such as anti-inflammatory, anti-carcinogenic, antioxidant, and antimicrobial properties, have been found to be present in these by-products (Echegaray et al., 2018; Jahurul et al., 2020).

Very little research has been done on the noni plant's stem bark, which similarly appears to be a waste (Mahesh et al., 2010). However, assessed the analgesic effects of Noni stem bark. In analgesic models for tail flick, tail clip, and tail immersion, the methanolic extract of stem barks was able to lessen pain. Similar to aspirin and other NSAIDs, the study showed that the methanol extract of stem bark at dose levels of 50, 100, and 200 mg/kg body weight significantly produced an antinociceptive effect. This effect may have been caused by the inhibition or release of endogenous chemicals that activate pain receptors. Numerous chemical kinds, such as polysaccharides, fatty acids, flavonoids, iridoids, anthraquinones, coumarins, and amino acids, have been isolated from M. citrifolia L. (Chan-Blan-co et al., 2006). Scopolettin, a derivative of coumarin, is one of them and a representative constituent of M. citrifolia L. Its function in antioxidative, anti-inflammatory, and antimicrobial qualities has been fully described (Deng et al., 2007).

Scopolettin was suggested as a component identifier for M (Samoylenko et al., 2006). Citrifolia L. quality control. Among the twenty compounds are quercetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, 3-methylbut-3-enyl-6-O-β-D-glucopyranosyl-β-D-glucopyranoside, 5-hydroxymethyl-2-furancarboxaldehyde, 5-ethyl-2-hydroxyl-succinate, Americanin D, Americanin, deacetyl-asperulosidic acid, Americanin A, loganic acid, asperulosidic acid, rhodolatouside, isoprincepin, scopoletin, 1,5,15-trimethylmorindol, 5,15-dimethyl-morindol, 5,15-dimethyl-morindol, 5,15-dimethyl-dimethyl-lactone, scopoletin, and 3,3′4′-bisdemethylpinoresinol (Yang et al., 2009).

Gram-positive bacteria (S. aureus) and Gram-negative bacteria (Salmonella spp., Escherichia coli, Pseudomonas aeruginosa, Helicobacter pylori, and Shigella spp.) have been reported to be susceptible to the antibacterial properties of M. citifolia seed methanol extract. The presence of phenolic compounds such alizarin scopoletin, acubin, L-asperuloside, and other anthraquinones may be the cause of the reported antibacterial effect in Noni (De La Cruz-Sánchez et al., 2019). Research has indicated that extracts from Noni seeds and stem bark may offer several advantages, such as: Activity of antioxidants: Antioxidants, which can aid in shielding the body from harm brought on by free radicals, are not present in high concentrations in any preparations (Jahurul et al., 2021). Mineral content: Noni extracts include good levels of calcium, magnesium, and potassium, among other minerals. After proximate investigation, it was discovered that Noni extracts have good levels of protein, carbohydrates, and fiber (Chaiyasut et al., 2011). The stem bark has not received much attention in the literature. However, the purpose of this study is to compare the phytochemical, antioxidant, and antibacterial qualities of the methanol extract of Noni seed and stem bark.

2. Materials and Methods

2.1 Plant collection and preparation Noni leaf and fruit

Noni seed and stem bark were obtained from Ekiadolor Community in Ovia North East Local Government Area of Edo State, Nigeria. It was identified by Dr. Akinnibosun from the Department of Plant Biology and Biotechnology University of Benin, with voucher number UBH-N427. The fresh seed and stem bark of the plant dried at room temperature till complete dryness. The dried seed and stem bark were pulverized into powder using the British milling machine. The weight of the powdered leaves was taken.

2.2 Extraction of Morinda citrifolia

Extraction of the seed and stem bark of Noni was carried out using maceration method as reported by (Aghedo and Ogbeide., 2022). Five hundred of the each sample was soaked differently in methanol (1:2 of sample and methanol) and allowed to stay for 3 days (72 hours) with constituent stirring. Using filter paper Whatman no. 1, the sample was filtered to separate the extract from the residue and gets a residue-free extract. The extract was then concentrated using rotary evaporator, dried and stored in a refrigerator for further use with percentage yield calculated.

3. Phytochemical Screening

The Phytochemical examination of the plant extract were carried out using standard methods as employed by with little modification (Tiwari et al., 2001; Trease and Evans., 2002).

3.1 Total Phenolic Contents Determination

Using tannic acid as a reference, the Folin–Ciocalteu reagent was used to measure the amount of total phenolic in the extract, slightly altering (Singleton and Rossi's., 1965). Method in summary, 1.0 ml of extract solution (250 ug/ml) was put into a test tube. The contents of the flask were well mixed after 1.0 ml of the Folin-Ciocalteu reagent was added. Five minutes later, 15.0 ml of 20% Na2CO3 were added, and the mixture was left to stand for two hours. A UV-Vis spectrophotometer (Janay 6100, Dunmow, Essex, U.K.) was used to measure the absorbance at 760 nm. An algorithm based on the standard calibration graph of tannic acid was used to calculate the total phenolic content, which is expressed as Ug of tannic acid equivalent (TAE).

3.2 Determination Of Total Alkaloids Content

The method was used to measure the total alkaloid content. After weighing 5g of the extract into a 250 mL beaker, 100 mL of 20% acetic acid in ethanol was added, and the mixture was let to stand for two hours (Harborne., 1973). After filtering, a water bath was used to concentrate the extract to a quarter of its initial volume. Once the precipitation was complete, concentrated ammonium hydroxide was added drop by drop to the extract. The precipitate was then filtered out, washed with 1% ammonia solution, dried, and weighed after the entire solution had been given time to settle. Every sample was analyzed three times.

Alkaloid (%) = Weight of residue x 100

Weight of sample

3.3 Flavonoid Content Determination

Three separate aliquots of the homogeneous plant extract (1.5 g) were used to measure the flavonoid concentration (Ilahy et al., 2011). For the purpose of determining the flavonoids, thirty microliter aliquots of the methanolic extract were utilized. 90 µL of methanol was used to dilute the samples, followed by the addition of 6 µL of 10% aluminum chloride ($AlCl_3$), 6 µL of 1mol/l sodium acetate ($CH3CO2Na$), and 170 µL of methanol. After 30 minutes, the absorbance was measured at 415 nm. Quercetin was used as a standard for calculating the flavonoid content (Ug Qe/g).

3.4 Estimation Of Tannins Content

After adding precisely 0.20 mL of the sample to 20 mL of 50% methanol, the mixture was agitated and kept in a water bath between 77°C and 80°C for an hour. After quantitatively filtering the extract using a double-layered Whatman No. 1 filter paper, 10 mL of 17% $Na2CO3$, 2.5 mL of Folin-Denis reagent, and 20 mL of distilled water were added and combined. For twenty minutes, the mixture was left to stand. After color development, the absorbance of the samples and a series of standard tannic acid solutions made in methanol were measured using a UV/visible spectrophotometer set to 760 nm. The calibration curve was used to determine the total tannin concentration.

4. Elemental Analysis

An atomic absorption spectrophotometer was used to ascertain the amount of calcium, magnesium, iron, copper, and zinc, while a flame photometer was used to measure sodium and potassium (Osarumwense et al., 2022). 1 gram of the sample was placed in a beaker, 10 milliliters of each of the nitric and perchloric acids were added to a beaker, and the sample was then placed inside. After the solution had been digested, 10 milliliters of distilled water were added, filtered into a volumetric flask after being shaken. Distilled water was used to dilute the filtrate to 100 mL. Following preparation, the sample was examined for minerals using an atomic absorption spectrophotometer and a flame photometer.

4.1 Proximate Analysis

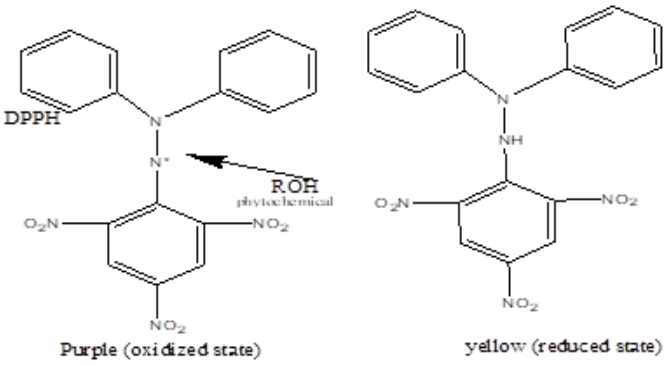
Moisture content, total Ash content crude fibre determination, crude fat determination, crude protein determination (micro-Kjeldahl method as described by (Aghedo and Ogbeide., 2022; AOAC 1980; Pearson 1976; AOAC., 1990). estimation of total carbohydrate were carried out respectively using the ascribed standard methods (Adamu et al., 2017).

4.2 Estimation of Antioxidant activity

The scavenging action of M. citrifolia seed and stem bark crude methanol extracts, respectively (Unuigbe et al., 2021). Described a method for determining the DPPH radical three milliliters (3ml) of each extract were combined with one milliliter (1 ml) of a 0.2 mM DPPH in methanol solution that contained 0.001-0.200 mg/ml of the extracts. After giving it a thorough vortex, It was left in the dark for 30 minutes at room temperature. A spectrophotometer was used to measure the absorbance at 518 nm. The standard was ascorbic acid.

The ability to scavenge DPPH was calculated using the following equation: DPPH radical Scavenging Activity% = $Ao - A_1$

Where, Ao=absorbance of DPPH radical plus methanol; A1=absorbance of DPPH radical plus sample



5. Antimicrobial Activity

4.1 Bacteria Isolates

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus, Candida albicans, and Aspergillus flavus were among the bacterium isolates employed in this investigation. These organisms were obtained from the University of Benin Teaching Hospital's microbiology lab in Benin City, Nigeria. Their identities were verified through morphological, biochemical, and cultural tests. The bacteria cultures were cultivated in nutrient agar at 4°C and kept on nutrient agar slopes.

5.2 Determination of antibacterial activity

The antibacterial activity was assayed by the agar-well diffusion method as reported by (Aghedo and Ogbeide, 2022). Where the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) was investigated.

5.3 Statistical Analysis

Some of the experiments were carried out in triplicate, some in duplicate and some once. Average values of triplicate and duplicate determinations were used and Standard deviation was calculated for the results.

6. Result and Discussion

Emergence of new active drugs used in combating drug-resistance ailment is mostly traceable to plant source. In this study, methanol extract of Noni seed and stem bark were subjected to phytochemical screening, Elemental analysis, proximate analysis, antioxidant study and antimicrobial analysis. The results of the analysis of the methanol extract of Noni seed and stem bark were compared as reported below.

6.1 Phytochemicals Activity

Table 1: Result of phytochemical screening of Morinda citrifolia (Noni) seed and Stem bark

	Morinda citrifolia (Noni) seed	Morinda citrifolia (Noni) stem bark
Glycoside	+	+
Saponin	-	-
Phenolic	+	+
Eugenols	+	+
Terpenoid	+	+
Steroids	-	+
Alkaloids	+	+
Flavonoids	+	+
Tannins	-	+

means present - means absent

The phytochemical screening of the methanol extract of Noni stem bark and Noni seed showed the presence of glycoside, phenolics, eugenols,

terpenoids, alkaloids, Flavonoids and tannins, both showed the absence of saponins. It was observed that there was absence of steroids in the methanol extract of Noni seed but present in the stem bark extract. Tannins which was absent in the Noni seed was found present in the stem bark extract. Phytochemicals are bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods involved in reducing the risk of major chronic diseases. Drugs in the glycoside group are typically used to boost appetite and facilitate digestion; examples include chalcone glycoside (anticancer), anthracene glycosides (purgative and used to treat skin conditions), and cardiac glycosides (act on the heart). (Sarker and Nahar., 2007). The phenolic compounds present in medicinal plants are important secondary metabolites with wide range of pharmacological activities like anticancer, anti-inflammatory. Flavonoids as well as some polyphenolic compounds have been known to possess the ability to scavenge free radicals by working against oxidative cell mutilation which makes them much nutritionally important (Ogbeide et al., 2022). The pharmacological significance of alkaloids includes antihypertensive (various indole alkaloids), antiarrhythmic (quinidine, spareien), antimalarial (quinine), and anticancer (dimeric indoles, vinblastine, vincristine) properties. Nicotine, caffeine and other stimulants found in certain alkaloids are used as analgesics, while quinine is utilized as an anti-malarial medicine (Mamta., 2013).

Table 2: Result of total Alkaloids, phenolic, tannins, and flavonoids of Morinda citrifolia (Noni) seed and stem bark

	Alkaloids (%)	Phenolic (mg/ml)	Tannins (mg/ml)	Flavonoids (mg/ml)
Morinda citrifolia (Noni) seed	1.460	24.436	8.750	59.630
Morinda citrifolia (Noni) stem bark	2.960	7.830	3.030	56.840

This study have shown that the total alkaloids content of Noni seed extract (1.460 mg/ml) is lower than that of Noni stem bark extract. Alkaloids are nitrogenous bases found in plants, and many of them have a noticeable physiological impact on people. Morphine and caffeine are two examples of alkaloids that are utilized as medications. The neurological system is stimulated by the caffeine in tea (Begg et al., 2007). The results of previous research on medicinal plants are consistent with the increased alkaloid concentration in the stem bark as opposed to the seeds. Alkaloids are a complicated family of naturally occurring compounds that primarily function as a defense mechanism against herbivores and pathogens. The plant component, the environment, and genetic factors can all affect the concentration of these chemicals. According to recent research, plants' stem and root barks usually contain larger quantities of alkaloids than their seeds. For example, a study by highlighted that secondary metabolites, including alkaloids, accumulate more in the stem and root bark due to their protective roles against pathogens and herbivores (Mazid et al.,2011). The presence of alkaloids suggests an antimicrobial activity on microorganisms thus plants that possess alkaloids are known to be effective in decreasing blood pressure and nervous system balancing in the case of mental illness. Research by demonstrated that alkaloid-rich extracts from the stem bark of various medicinal plants exhibited significant antimicrobial activity, which could be advantageous for developing new treatments from Noni stem bark. Conversely, the seeds, with their lower alkaloid content, might be more suitable for applications where lower toxicity is required (Wang et al., 2002).

The phenolic content in different parts of Noni exhibits a notable variation. The seed of Noni contain significantly higher phenolic content (24.430 mg/ml) compared to the stem bark (7.830 mg/ml). This difference can be attributed to the varying roles and metabolic activities in different plant tissues. Similar trends are observed in other plants where different parts exhibit varying phenolic concentrations based on their functional requirements. For instance, studies on various medicinal plants indicate that seeds and fruits often have higher phenolic content compared to stems and leaves due to their critical role in reproduction and protection of the next generation (Zhou et al., 2016). However, while seeds often have higher phenolic content compared to stems and leaves, this does not always translate to greater antioxidant activity. Antioxidant potency depends not only on the total phenolic content but also on the specific types of phenolic compounds, their bioavailability, and synergistic interactions with other phytochemicals. Phenolic compounds are vital secondary metabolites in plants, playing crucial roles in defense against pathogens, UV radiation, and oxidative stress. They are also important for the plant's structural integrity and pigmentation. The higher phenolic content in Noni seeds suggests greater potential for antioxidant applications, antimicrobial, anticancer and anti-inflammatory activity, making them valuable for developing health supplements and natural antioxidants (Shahidi and Ambigaipalan., 2015). However, The stem bark, despite its lower phenolic content, still holds significance for its antimicrobial and protective properties, making it useful for various therapeutic purposes.

The total tannin content of Noni seed extract shown in Table 2 above (8.750mg/ml) is higher than that of Noni stem bark extract (3.030 mg/ml) . This means that Noni seed extract may have a stronger antioxidant, antimicrobial, and anti-inflammatory activity than Noni stem bark extract. Tannins possess antioxidants, with anti-inflammatory, antidiarrheal, cytotoxic, antiphrastic, antibacterial, antifungal and antiviral activities (Michael., 2015). The study has also shown that the total flavonoids content of Noni seed extract (59.63mg/ml) is higher than that of Noni stem bark extract (56.84 mg/ml), though slightly. This means Noni seed extract may have a stronger antioxidant, antimicrobial, and cholesterol-lowering activity than Noni stem bark extract**.** Flavonoids, a polyphenolic substance, are responsible for the presence of many hues in nature, especially the orange and yellow of petals. They have multiple biological activities, including antioxidative, anticarcinogenic, antibacterial, vasodilatory, anti-inflammatory, immune– stimulating, anti-allergic, estrogenic and antiviral effects, as well as being inhibitors of phospholipase, cycloxygenase, lipoxygenase, xanthine oxidase and glutathione reductase (Igwe and Okwu., 2013).

6.2 Elemental Analysis

Table 3: Result of elemental analysis of methanol extract of Morinda citrifolia (Noni) seed and stem

ELEMENTS	MORINDA CITRIFOLIA (NONI) SEED (mg/ml)	MORINDA CITRIFOLIA (NONI) STEM BARK (mg/ml)
Sodium (Na)	4.04 ±2.305	7.51 ±1.595
Potassium (K)	20.46 ±4.910	91.12 ±16.122
Calcium (Ca)	2.35 ±0.131	7.59 ±0.737
Magnesium (Mg)	21.33 ±6.600	129.00 ±47.842
Iron (Fe)	1.30 ±0.497	2.80 ±0.648
Copper (Cu)	0.01 ±0.005	0.02 ±0.005
Zinc (Zn)	0.19 ±0.056	0.26 ±0.086

The elemental analysis revealed the presence of Sodium, iron, calcium, magnesium, Potassium, copper and zinc in methanol extract of Noni stem bark and Noni seed as shown in the table below.

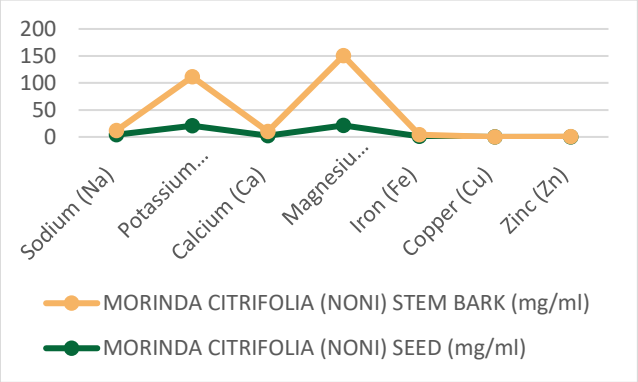


Figure 6: Comparative graph of elemental analysis of Morinda citrifolia (Noni) stem bark and seed

The methanol extract of Noni stem bark was observed to show more presence of minerals/elements than methanol extract of Noni seed, when compared graphically. Minerals are required in the body cells for the proper functioning of the enzymes. Their absence is known to affect the metabolism of many macromolecules. Iron supplementation helps in reducing the oxidative stress (Mamta et al., 2014). Deficiency of magnesium reduces GR (Glutathione reductase) activity and GSSG (Glutathione disulfide) does not reduce to GSH (Reduced Glutathione), hence causing oxidative damage to the cells (Fang et al., 2002). The results were corresponding to the mineral analysis carried out by (Ani et al., 2013). Minerals are required in the body cells for the proper functioning of the enzymes.

6.3 Proximate Analysis

Using a methanol extract of Noni seed and stem bark, the proximate analysis's findings shows the percentage of the moisture content, crude fiber, ash content, crude fat, crude protein and total carbohydrates of the samples as shown in the table below.

Table 4: Result of proximate analysis of methanol extract of Morinda citrifolia (Noni) seed and stem bark

	MORINDA CITRIFOLIA (NONI) SEED (%)	MORINDA CITRIFOLIA (NONI) STEM BARK (%)
Moisture content	18.330 ±0.057	18.993 ±0.007
Crude fiber	49.940 ±0.055	37.900 ±0.002
Ash content	13.400 ±0.050	6.400 ±0.039
Crude fat	13.780 ±0.055	9.120 ±0.002
Crude Protein	5.690 ±0.005	4.717 ±0.043
Total carbohydrates	17.190 ±0.020	22.870 ±0.018

Comparing the results on the proximate analysis of the two samples shows that the methanol extract of Noni seed has higher percentage of crude fiber, ash content and crude fat while it has a lower moisture content and total carbohydrate. Both samples have the same percentage of crude protein. The moisture in animal feeds is critical in its overall nutritional value and thus is an important factor in agricultural commerce and management (Thiex and Richardson., 2003). Chemical deterioration and microbiological infection are often impeded or avoided by low moisture content. However, excessive moisture level in crude drugs can also cause essential chemicals to break down quickly and promote the growth of bacteria, particularly when the medicine is being stored (Aghedo and Ogbeide., 2022). Ash values are important qualitative indicators that may be used to define the authenticity and purity of a sample (Aghedo and Ogbeide., 2022). Protein is very essential to livestock ensuring energy supply required by the animal for maintenance, growth, pregnancy and lactation. The protein content of the noni seed (5.690 ±0.005) is not significantly greater than that of the noni stem bark (4.717 ±0.043) which indicates that both parts of the M. citrifolia plant will be an essential protein source relevant for medicinal use. The lipid materials present in crude fat may include triglycerides, diglycerides, monoglycerides, phospholipids and Steroids. Crude fiber is a measure of the quantity of indigestible cellulose, lignin, and other components of this type in present foods. Adequate dietary fiber intake has a number of health benefits, including maintenance of healthy laxation and the reduced risk of cardiovascular disease and cancer (Chandaka et al., 2022).

6.4 Anti-Oxidant

The table below shows the % inhibition (scavenging capacity) of the two samples, both of the samples showed variation at different concentration as shown in the table below.

%inhibition (scavenging capacity)

Table 5: result of antioxidant analysis of Morinda citrifolia (Noni) stem bark and seed

Concentration (µg/ml)	Seed (%)	Stem (%)	Ascorbic acid (%)
500	67.299	40.236	87.777
400	34.034	34.034	83.547
300	15.992	16.966	43.977
200	19.510	16.930	14.370
100	18.760	18.110	16.430

The comparison showed that the percentage of inhibition (scavenging activities) of methanol extract of Noni seed and Noni stem bark are close in percentage at concentration of 500mg/ml, 200mg/ml and 100 mg/ml to the percentage of ascorbic acid, which means they are good antioxidants. Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Vitamins C and E are two examples, along with enzymes like catalase (CAT), superoxide dismutase (SOD), and other peroxidases (Hamid et al.,2010). Numerous human diseases, including cellular necrosis, cardiovascular disease, cancer, neurological disorders, Parkinson's dementia, Alzheimer's disease, inflammatory diseases, muscular dystrophy, liver disorders, and even aging, are mostly caused by oxidative stress (Amit, 2011).

Table 6: IC_{50} result for the methanol extract of Morinda citrifolia (Noni) seed and stem

	IC_{50} (mg/mL)
Morinda citrifolia seed	129.300
Morinda citrifolia stem	49.478
Ascorbic acid	46.548

IC_{50} is a measure of the concentration of a substance that is required to inhibit 50% of a biological process. It is commonly used to measure the potency of drugs and other bioactive compounds. In the context of antioxidant activity, IC_{50} is the concentration of an antioxidant that is required to inhibit 50% of the oxidation of a free radical. The lower the IC_{50}, the more potent the antioxidant (Ekhator et al., 2022; Unuigbe et al., 2021). From the results, The IC_{50} of Morinda citrifolia stem bark extract is **49.478 mg/mL**, which is significantly lower than the IC_{50} of the seed extract at **129.300 mg/mL**. Ascorbic acid, a standard antioxidant, has the lowest IC_{50} value of **46.548 mg/mL**, indicating the highest potency among the tested samples. These findings demonstrate that the methanol extract of Morinda citrifolia stem bark is a more potent antioxidant than the seed extract. Specifically, the stem bark extract exhibits approximately **1.5 times** greater antioxidant activity than the seed extract. Conversely, this result contradict the suggestion based on the higher concentration of phenolic, tannin, and flavonoid content of seed extract of M. citrifolia compared to the stem bark extract, being that the stem bark extract has a lower IC_{50} value (higher antioxidant activity) compared to the seed extract. This suggests that despite seeds typically having higher phenolic content for protective and reproductive purposes, the composition and effectiveness of the phenolic and other bioactive compounds in the stem bark can sometimes make it a more potent antioxidant source. This emphasizes the need to assess antioxidant activity experimentally rather than relying solely on general trends. The superior antioxidant activity of the stem bark extract is likely attributable to its higher concentration of phytochemicals, including tannins, saponins, and phenolics, which are

known for their free radical scavenging abilities.

6.5 Anti-Microbial

6.5.1 Zone of inhibition at concentration of (500mg/ml) of Morinda citrifolia (Noni) seed and stem bark

The antimicrobial test of methanol extract showed antimicrobial activity only with Staphylococcus aureus as shown in the table below.

Table 7: result of antimicrobial activity of Morinda citrifolia (Noni) stem bark and Morinda citrifolia (Noni) seed

Organism	Morinda citrifolia stem bark (500 mg/ml)	Morinda citrifolia (Noni) seed (500 mg/ml)
Staphylococcus Aureus	16mm	14mm
Escherichia Coli	Nz	Nz
Pseudomonas Aeruginosa	Nz	Nz
Streptococcus	Nz	Nz
Candida Albicans	Nz	Nz
Aspergillus Flavus	Nz	Nz

The comparison of the antimicrobial activity of both samples showed a slight difference in their antimicrobial activities. According to the study's findings, Morinda citrifolia stem bark and seed methanol extracts exhibit selective antibacterial activity. The growth of the Gram-positive bacterium (Staphylococcus aureus) was inhibited by both extracts; however, the stem bark extract had a greater effect (16 mm inhibition zone) than the seed extract (14 mm inhibition zone). This implies that the stem bark might have stronger antibacterial substances or higher concentrations that are effective against Staphylococcus aureus.

6.5.2 MIC and MBC (mg/ml) of Noni stem bark and Noni seed at different concentration (Staphylococcus Aureus).

Furthermore, MIC and MBC tests were also carried out at different concentration, the table below displays the findings.

Morinda citrifolia (Noni) seed

Table 8: Result of MIC methanol extract of Morinda citrifolia (Noni) seed and stem bark

Organism	200mg/ml	100mg/ml	50mg/ml
Staphylococcus Aureus	NG	G	G

Morinda citrifolia (Noni) stem bark

Organism	200mg/ml	100mg/ml	50mg/ml
Staphylococcus Aureus	NG	NG	G

The comparative result of the MIC of methanol extract of Noni stem showed no growth at concentration of 200mg/ml and 100mg/ml, but showed growth at 50mg/ml for the stem bark extract while the MIC of methanol extract of Noni seed showed no growth at concentration of 200mg/ml, but showed growth at 100mg/ml and 50mg/ml for the seed extract.

Table 9: Result of MBC and Morinda citrifolia (Noni) seed and stem bark

Organism	200mg/ml	100mg/ml	50mg/ml
Staphylococcus Aureus	G	G	G

The comparative result of the MBC of methanol extract of Noni stem and Noni seed showed growth at concentration of 200mg/ml, 100mg/ml and 50mg/ml.The study reported active antibacterial activities against E(Noviana et al.,2021). coli and Staphylococcus aureus of the Noni seed using diffusion method. This effect was traceable to the secondary metabolites of flavonoid and phenol present in the seed. The stem bark extract of noni plant has also been reported to be active against Gram + and Gram – bacteria (Sunder et al., 2012).

7. Conclusion

This study reveals the therapeutic potential of Morinda citrifolia (Noni) stem bark and seed extracts, rich in phytochemicals with antioxidant and antimicrobial properties. The stem bark demonstrated superior antioxidant and antimicrobial activity, particularly against Staphylococcus aureus, despite the seed's higher phenolic content. These findings highlight the stem bark's potential for health supplements and natural antimicrobials. Further research on active compounds, broader microbial testing, and dose optimization is needed to fully harness its medicinal benefits

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