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# Phytochemical analysis, mineral composition and antimicrobial activities of ethyl acetate and ethanol extracts of *Vernonia amygdalina* and *Morinda lucida* against some clinical isolated pathogenic microorganisms: A comparative study

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## ABSTRACT

**Background & Aim:** Medicinal plants with antimicrobial activities have been used extensively in the West Africa regions. These plants of medicinal important have been proven to be very effective even where treatments with antibiotics failed. This study was aimed to evaluate the antimicrobial activities of *Morinda lucida* and *Vernonia amygdalina* leaves extracts on some pathogenic microorganisms isolated from clinical samples.

**Experimental:** The antimicrobial activities were assayed using the agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated using standard microbiological techniques. Phytochemical and mineral compositions of all the extracts were determined.

**Results:** Phytochemicals such as saponins, tannins, flavonoids, glycosides, anthraquinone and alkaloids were present in both extracts of the plants with alkaloids having a higher percentage. Minerals such as K, Ca, Fe, Na and Fe were presented in appreciable quantities in both extracts of the plants with the K and Ca levels in the ethyl acetate and ethanol extracts of *Morinda lucida* significantly higher than that of *V. amygdalina*, while Na and Fe levels of *Vernonia amygdalina* was found to be higher than that of *Morinda lucida*. Heavy metals like Pb, Cd and As were presented in significant quantities in both extracts of the plants. All the extracts exhibited varying degrees of concentration-based antimicrobial activities against the tested pathogens. Both ethanol and ethyl acetate extracts of *Morinda lucida* showed a high significant antifungal activity against *Trichophyton sp* and *Candida sp* while the ethanol extract of *Vernonia amygdalina* showed a high significant antimicrobial activity against *Klebsiella sp* and *Candida sp*.

**Recommended applications/industries:** The study provides information on the antimicrobial activities, phytochemical and mineral components of *Morinda lucida* and *Vernonia amygdalina* leaves. Also, it contributes to the development of alternative therapeutic agents against the pathogens tested in this study.

## 1. Introduction

Due to its phytochemical and phytonutrient components, plants had played a significant part in the overall health and wellbeing of many people throughout the world for millennia and they are generally recognized for their superior medicinal potential (Iheagwam *et al.*, 2019). Recently, the rise in antibiotic resistance has prompted scientists and researchers to look for additional potential antimicrobials. As a result of various searches, plants have been identified as a reliable source of antimicrobial agents to battle or combat microorganisms. The screening of several medicinal plants for their potential antimicrobial activity was prompted by the growing failure of chemotherapeutic and antibiotic resistance exhibited by pathogenic microbial agents (Akerele *et al.*, 2007). In a review of plant contribution to drug expansion, medicinal plants have continued to play an important role in the development of novel medications and effective healthcare systems in many industrialized and impoverished nations (Tchinda *et al.*, 2016).

*Vernonia amygdalina* is a perennial shrub belonging to the family Asteraceae. It is found in Africa and Asia with green coloured leaves which are petiolate in shape with a characteristic odour and bitter taste (Akpaso *et al.*, 2011), which its name "bitter leaf" spring up. In Nigeria, it is locally called Shuwaka (Hausa), Ewuro (Yoruba), Oriwo (Edo), and Olubu (Igbo) (Gashe and Zeleke, 2017). It is reported to have phytochemicals (Adeleye *et al.*, 2018; Inusa *et al.*, 2018). Its richness in vitamins and minerals had made it important diet for both human and animal where it leaves are consumed as a green leafy vegetable (Sobukola *et al.*, 2007). It has been reported to be effectively used for the treatment of diabetes, yellow fever, dysentery, constipation, malaria and stomachache (Suleiman *et al.*, 2018).

*Morinda lucida* is widely known as brimstone tree, it belongs to the family Rubiaceae (Chokki *et al.*, 2020). In Nigeria, it is locally known as Njisi (Hausa), Huka, Eze-ogu and Nfia (Igbo), Oruwo or Ruwa (Yoruba) (Odoh *et al.*, 2020). It is used in the treatment of diverse disease due to its distinct therapeutic benefits that is derived from different parts of the tree. The plant has been reported to have phytochemicals such as saponins, phenols, steroid, alkaloids, tannins, and flavonoids (Babalola *et al.*, 2020; Chokki *et al.*, 2020; Odoh *et al.*, 2020), and antioxidants such as vitamin A and E (Adeleye *et al.*, 2018). Previous studies have demonstrated that different extracts of *M. lucida* possess numerous pharmacological properties such as antibacterial (Fakoya *et al.*, 2014; Adeleye *et al.*, 2018), antiviral (Chokki *et al.*, 2020), antidiabetic (Adeleye *et al.*, 2018; Chokki *et al.*, 2020), antimalarial (Umar *et al.*, 2013; Chokki *et al.*, 2020), anti-inflammatory (Aborisade *et al.*, 2017; Chokki *et al.*, 2020), anticancer, hepatoprotective, cytotoxic and genotoxic, antispermatogenic, hypoglycemia (Adeleye *et al.*, 2018). However, these distinguished therapeutic and nutritional value of these medicinal plants has make it necessary for this study to be carried out with the aim of comparatively investigating the phytochemical analysis, mineral composition and the antimicrobial effects of ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves on some pathogenic microorganisms.

## 2. Materials and Methods

### *2.1. Collection, identification and extraction of plant materials*

Fresh leaves of *Morinda lucida* and *Vernonia amygdalina* were collected in October, 2021 from a local farm in Iyana Camp, Itori, Ewekoro Local Government Area, Ogun State, Nigeria. The identification and authentication of the plant materials was done at Forest Research of Nigeria, Jericho Hill, Ibadan, Oyo State, with the voucher number of FHI 113358 and 113359 respectively. The collected samples were air dried at room temperature for twenty-one (21) days. The samples were ground into powder using a pulverizer and stored in an air tight bottle. After weighing, 200 g of the ground sample of the leaf was dissolved in 500 mL each of ethanol and ethyl acetate and was kept in the refrigerator for 72 h with intermittent shaking. The extract was filtered using a chess cloth and Whatman filter paper No. 1 (125 mm), to obtain filtrates of the respective solvents of ethanol and ethyl acetate (which was use for the antimicrobial assays). However, some amount of the filtrates was evaporated to dryness using a water bath (at 40 $^{o}$C) to obtain the slurry, which was persevered in a phial, labelled appropriately and stored in the refrigerator at 4 $^{o}$C and was used for phytochemical analysis.

### *2.2. Collections and maintenance of test organisms*

The test organisms used for this study were all clinical isolates from the Department of Medical Microbiology and Parasitology, Federal Medical Center, Idi-Aba, Abeokuta, Ogun State. The isolates include; *Klebisella sp.*, *Pseudomonas sp.*, *Staphylococcus aureus*, *Trichophyton sp.*, and *Candida sp*. The organisms were collected on a sterile agar slant and incubated at 37 $^{o}$C for 24 h.

### *2.3. Antimicrobial assay (agar well diffusion method)*

Antimicrobial activities of ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves were evaluated by the agar well diffusion method (Bauer *et al.,* 1966) using ciprofloxacin as positive control. The microbial culture was adjusted to 0.5 McFarland turbidity standards. The plate was flooded with 1ml each of the standardized test organism, swirled and excess inoculums was carefully decanted. A sterile cork borer was used to make wells (6 mm in diameter) on the agar plates. Aliqouts of 0.2 mL of dilution were applied on each well in the culture plates previously inoculated with the test organisms. The holes were filled with the plant extract. Each well was labeled approximately; control experiment was also carried out where the hole was filled with ciprofloxacin as positive control for bacteria. However, each extract was tested in triplicates. These were then left on the bench for 1 h for proper diffusion of the nanoparticles. Thereafter the plates were incubated at 37 $^{o}$C for 24 h for bacteria. Antimicrobial activities were determined by measuring the zone of inhibition around each well (excluding the diameter of the well) for the extract.

### *2.4. Determination of minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) of the ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves for both the bacterial and fungal isolates were evaluated according to the method of Ochei and Kolhatkar (2008), using microtubes dilution method described by National Committee for Clinical Laboratory standards (NCCLS, 2000).

### *2.5. Determination of minimum bactericidal concentration (MBC)*

The minimum bactericidal concentration (MBC) was determined by first selecting the tubes that showed no growth during the MIC determination. A loopful from each of the tube was sub-cultured on the sterile nutrient agar and incubated for 24 h at 37 $^{o}$C. The bactericidal effect was demonstrated when no growth occurred on the medium.

### *2.6. Quantitative screening of phytochemical constituents*

#### *2.6.1. Determination of alkaloid*

Alkaloid was determined according to the method of Obadoni and Ochuko (2001)**.** About 5 g of the samples was weighed into 250 mL beaker and 200 mL of 10 % acetic acid was added and covered, then allow to stand for 4 hours. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath (Grant-Model: 600303003). Concentrated ammonium hydroxide was added drop-wise until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is the alkaloid, which will be dried and weighed. The percentage of total alkaloid content was calculated as:

$$\text{Total alkaloids (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken}}$$

#### *2.6.2. Determination of flavonoid*

The total flavonoid content was determined using the procedure described by Ejikeme *et al*. (2014). About 10 g of the samples was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath (Grant-Model: 600303003) and weighed to a constant weight. The total flavonoid content was calculated as:

$$\text{Total flavonoid (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken}}$$

### *2.6.3. Determination of saponin*

Saponin quantitative determination was carried out using the method described by Obadoni and Ochuko (2001). 20 g of the sample was weighed and put into a conical flask, 100 mL of 20 % aqueous ethanol was added. The mixture was heated using a water bath (Grant-Model: 600303003) for 4 hours with continuous stirring at about 55 $^{o}$C. The residue of the mixture was re-extracted with another 100 mL of 20 % aqueous ethanol after filtration and heated for 4 hours at a constant temperature of about 55 $^{o}$C. The combined extract was evaporated to 40 mL over water bath (Grant-Model: 600303003) at about 90 $^{o}$C. The concentrated is transferred into a 250 mL separator funnel and 20 mL of diethyl ether is added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. This purification process was repeated twice. 60 mL of n-butanol was added and extracted twice with 10 mL of 5 % sodium chloride. After discharging the sodium chloride layer, the remaining solution was heated in a water bath (Grant-Model: 600303003) for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content is calculated as percentage:

$$\text{Total saponin (\%)} = \frac{\text{Weight of residue} \times 100}{\text{Weight of sample taken}}$$

### *2.6.4. Determination of tannin*

Tannin content was determined using the method of Ejikeme *et al*. (2014), using tannic acid as standard. 0.20 g of sample was measured into a 50 mL beaker. 20 mL of 50 % methanol was added and covered with parafilm film and placed in a water bath (Grant-Model: 600303003) at 77-80 $^{o}$C for 1 hour. It was shaken thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No I. filter paper into a 100 mL volumetric flask, 20 mL water added, 2.5 mL Folin-Denis reagent and 10 mL of 17 % $Na_2CO_2$ were added and mixed properly. The mixture was made up to mark with water mixed well and allow to stand for 20 min. The bluish – green colour will develop at the end of range 0-10 ppm were treated similarly as 1 mL sample above. The absorbance of the tannic acid standard-solutions as well as samples were read after colour development on a digital spectrophotometer (DRE 3000 HACH) at a wavelength of 760 nm. The percentage tannin content was calculated using the formula:

Total tannin (%) =

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

### *2.6.5. Determination of glycoside*

Glycoside content was determined using the method of Sofowora (1995). 10 mL of extract was pipette into a 250 mL conical flask, 50 mL chloroform was added and shaken on a Vortex mixer for 1 h. The mixture was filtered into l00 mL conical flask and 10 mL pyridine, 2 mL of 2 % sodium nitroprusside were added, shaken thoroughly for 10 minutes. 3 mL of 20 % NaOH was later added to develop a brownish-yellow colour. Glycoside standard of concentrations which range from 0–50 mg/mL were prepared from 100 mg/mL stock glycoside standard. The series of standards 0-50 mg/mL treated similarly like sample above. The absorbance of sample as well as standards were read on digital spectrophotometer (DRE 3000 HACH) at a wavelength of 510 nm. The glycoside content is calculated as percentage using the formula:

Total glycoside (%) =

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

### *2.6.6. Determination of anthraquinones*

Anthraquinones was determined using the method described by Bussmann *et al*. (2013). 0.50 g of sample was weighed into a 250 mL beaker and 60 mL benzene added and stirred with a glass rod to prevent lumping. This was filtered into 100 mL volumetric flask using Whatman No.1 filter paper. 10 mL of filtrate was pipetted into another 100 mL volumetric flask and 0.2 % zinc dust was added followed by the addition of 50 mL hot 5 % NaOH solution. The mixture was heated just below boiling point for five minutes and then rapidly filtered and wash once in water. The filtrate was again heated with another 50 mL of 5 % NaOH to develop a red colour. Standard anthraquinone solution of range 0– 5 mg/L were prepared from 100 mg/L stock anthraquinone and treated in a similar way with 0.2 % zinc dust and NaOH like sample. The absorbances of sample as well as that of standard concentrations were read on a digital spectrophotometer (DRE 3000 HACH) at a wavelength

of 640 nm. The percentage anthraquinone is calculated using the formula:
Total anthraquinones (%) =

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

### *2.7. Determination of the mineral composition*

The mineral contents of the samples were determined by the procedure of AOAC (2000). Calcium, potassium, magnesium, phosphorus, iron and other elements were measured with Atomic Absorption Spectrophotometer (Thermo scientific S Series Model GE 712354) after digesting with perchloric-nitric acid mixture. Prior to digestion, 5 mL of the samples were measured into a 125 mL Erlenmeyer flask with the addition of perchloric acid (4 mL), concentrated $HNO_3$ (25 mL) and concentrated sulphuric acid (2 mL) under a fume hood. The contents were mixed and heated gently in a digester at low to medium heat on a hot plate under perchloric acid fume hood and heating was continued until dense white fume appeared. Heating was continued strongly for half a minute and then allowed to cool followed by the addition of 50 mL distilled water. The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was then read on Atomic Absorption Spectrophotometer.

### *2.9. Statistical Analysis*

Statistical analysis was carried out using one-way analysis of variance (ANOVA) to access the difference between means followed by Tukey's multiple comparisons. Resulting data were represented as mean± standard deviation of triplicate determinations. GraphPad Prism 9® (Version 9.0.1, GraphPad Software Inc., San Diego, United States of America) software was used for the statistical analysis and statistical significance was obtained at $P<0.05$.

## 3. Results and discussion

Phytochemicals are majorly responsible for the biological activities of medicinal plants such as antioxidant, hormonal action, stimulation of enzymes and antibacterial effect among others (Usin and Daramola, 2022). The preliminary phytochemical screening of the ethanol and ethyl acetate extracts of *M. lucida* and *V. amygdalina* leaves revealed the presence of compounds like flavonoids, saponins, tannins, alkaloids, glycoside and anthraquinone. This observation is similar to several studies with regards to the phytochemical constituents of these plants (Adeleye *et al.*, 2018; Inusa *et al.*, 2018). Tannins, alkaloids, flavonoids and anthraquinone were significantly higher in ethanol and ethyl acetate extracts of *V. amygdalina* than that of *M. lucida*. However, saponin and glycoside were found to be higher in ethyl acetate extract of *M. lucida* than that of *V. amygdalina*, and lower using ethanol as the solvent for extraction (Table 1). The health benefit of these phytochemicals has been documented; flavonoids are known to inhibit formation of plagues in arteries and so prevent atherosclerosis, hypertension and other cardiovascular diseases. They are also very important antioxidants that mop up reactive oxygen radicals known to be involved in many conditions that cause cancers, diabetes, inflammatory diseases and neurodegenerative diseases. Saponins lower cholesterol and glucose level. They are also involved in ulcer protection and certain antimicrobial activity (Ukwe *et al.*, 2010). Alkaloids are involved in antimicrobial and hypoglycemic activities (Punithal *et al.*, 2005). Cardiac glycosides are useful for treatment of heart conditions (Enemor *et al.*, 2014). It has been validated that the bioactive constituents of these plants are responsible for its antimicrobial potentials (Al-Harbi *et al.*, 2017).

**Table 1.** Phytochemical analysis of ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves (%).

| Phytochemicals | *Morinda lucida* | | *Vernonia amygdalina* | |
|---|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Ethyl acetate extract | Ethanol extract |
| Tannin | 3.11±0.995$^{a}$ | 2.56±1.010$^{ab}$ | 4.37±0.995$^{abc}$ | 6.15±0.990$^{c}$ |
| Saponin | 8.92±0.990$^{a}$ | 6.76±1.031$^{a}$ | 7.21±0.990$^{a}$ | 8.84±0.981$^{a}$ |
| Alkaloid | 6.22±1.010$^{a}$ | 10.12±0.560$^{b}$ | 12.11±0.990$^{bc}$ | 14.02±0.990$^{c}$ |
| Flavonoid | 5.91±0.995$^{a}$ | 8.11±1.005$^{a}$ | 11.21±0.990$^{b}$ | 10.92±0.990$^{b}$ |
| Glycoside | 1.03±0.040$^{b}$ | 0.72±0.200$^{a}$ | 0.83±0.010$^{a}$ | 0.96±0.093$^{b}$ |
| Anthraquinone | 0.56±0.010$^{b}$ | 0.31±0.100$^{a}$ | 0.72±0.010$^{d}$ | 0.64±0.010$^{c}$ |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript on the same row means there is no significant difference between the comparing groups (P>0.05). Non– identical superscripts on the same row means there is significant difference between the comparing groups at P<0.05.

Minerals play vital metabolic and physiologic roles in the living system (Balogun and Olatidoye, 2012). Minerals are activator and component of many plant enzymes (Paul *et al.*, 2013). The deficiency of the minerals or excess might disturb normal biochemical functions of the body. Investigation of the minerals showed that sodium (Na), potassium (K) and calcium (Ca) and iron (Fe) were presented in appreciable quantities in both extracts of the plants. It was observed that the ethyl acetate extract yielded a higher percentage of sodium, potassium and calcium in both plants with exception of iron (Fe). However, potassium was found to be in the highest proportion in both extracts of the plants and sodium as the least (table 2). Previous studies by Alara *et al.* (2017), and Zaharaddeen and Oviosa (2019) validate this finding.

Iron is important in immune function, cognitive development, temperature regulation and energy metabolism. It is also required for the synthesis of haemoglobin and myoglobin while its deficiency causes anaemia (Geissler and Singh, 2011). Calcium is required for formation and maintenance of bones and teeth. It is also required in blood clothing and muscle contraction (Wardlaw *et al.*, 2004). Calcium plays a vital role in the conversion of prothrombin to thrombin during blood coagulation (Murray *et al.*, 2000). It activates large number of enzymes and it is also required for membrane permeability, normal transmission of nerve impulses and in neuromuscular excitability (Murray *et al.*, 2000). Reduced extracellular blood calcium will increase the irritability of nervous tissue and really low levels might cause spontaneous discharge of nerve impulses resulting to tetany and convulsions (Murray *et al.*, 2000). Sodium is the major element of the extracellular fluid and is a key factor in retaining body fluid. In conjunction with potassium, through creation of electrical potential, nerve impulses are conducted and the contraction of muscles is enabled. It helps to make it easier for the small intestine to absorb nutrients like glucose and amino acids. However, high levels of sodium are associated with hypertension and high blood pressure. Potassium has been implicated as the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and $Na^+/K^+$-ATPase (Paul *et al.*, 2013). Potassium is required during glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid and probably has a role in many basic cellular enzymatic reactions. Calcium and potassium are collectively known to reduce hypertension and blood pressure as well as used in the prevention and treatment of high blood pressure (Wardlaw *et al.*, 2004). So, their presence in these leaves gives a positive report to their nutritional reputation.

**Table 2.** Mineral compositions of ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves (mg/L).

| Minerals | *Morinda lucida* | | *Vernonia amygdalina* | |
|---|---|---|---|---|
| | Ethyl Acetate Extract | Ethanol Extract | Ethyl Acetate Extract | Ethanol Extract |
| Sodium (Na) | 0.758±0.001$^b$ | 0.453±0.003$^a$ | 2.986±0.006$^d$ | 2.459±0.116$^c$ |
| Potassium (K) | 2965.0±0.999$^a$ | 2214.4±2.001$^b$ | 2416.8±1.000$^c$ | 2016.3±0.012$^a$ |
| Calcium (Ca) | 97.67±1.000$^d$ | 92.57±0.997$^c$ | 85.12±0.007$^b$ | 79.86±0.999$^a$ |
| Iron (Fe) | 221.7±1.000$^a$ | 234.6±0.996$^a$ | 248.9±10.000$^b$ | 266.1±0.997$^c$ |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript on the same row means there is no significant difference between the comparing groups (P>0.05). Non– identical superscripts on the same row means there is significant difference between the comparing groups at P<0.05.

More so, lead (Pb), cadmium (Cd) and arsenic (As) were presented in insignificant quantities in both extracts of the plants. Arsenic (As) was found to be the highest in ethanol and ethyl acetate extracts of both plants, while lead (Pb) was the lowest (Table 3). The insignificant amounts of heavy metals make it not to exhibit any toxic effect. Lead is considered as a toxic pollutant of concern, partly because of it has been

prominent in the debate concerning the growth of anthropogenic pressure on the environment. Cadmium is a toxic metal that occurs naturally in the environment (RezaeiKahkha *et al.*, 2015). Humans are exposed to cadmium mostly through the plant derived food. There is no safe margin of cadmium exposure and the need to lower human exposure is desperate. The cadmium metal produces number one health problems and is a known carcinogen. Cadmium is of no use to the human body and is toxic even at low levels of exposure. The negative effects of cadmium on the body are numerous and can impact nearly all systems in the body including cardiovascular, reproductive, the kidneys, eyes and even the brain. It affects blood pressure, prostate function and testosterone levels (Pizent *et al.*, 2012). Exposure to cadmium can affect renal and dopaminergic systems in children (Pizent *et al.*, 2012).

**Table 3.** Heavy metals compositions of ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves (mg/L).

| Heavy metals | *Morinda lucida* | | *Vernonia amygdalina* | |
|---|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Ethyl acetate extract | Ethanol extract |
| Lead (Pb) | 0.182±0.0010$^{c}$ | 0.206±0.0030$^{d}$ | 0.086±0.0027$^{a}$ | 0.153±0.0056$^{b}$ |
| Cadmium (Ca) | 0.413±0.0015$^{b}$ | 0.492±0.0076$^{c}$ | 0.321±0.0010$^{a}$ | 0.412±0.0010$^{b}$ |
| Arsenic (As) | 0.841±0.0015$^{b}$ | 0.958±0.0021$^{d}$ | 0.614±0.0020$^{a}$ | 0.910±0.0095$^{c}$ |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript on the same row means there is no significant difference between the comparing groups (P>0.05). Non– identical superscripts on the same row means there is significance between the comparing groups at P<0.05.

Antibiotic resistance has become widely acknowledged as a major global health menace in recent years. As a result, efforts have been made to combat the threat of antibiotic resistance while also investigating different sources of antimicrobial agents, such as medicinal plants (Anand *et al.*, 2019). Medicinal plants eradicate pathogens and inhibit its recontamination through different mechanisms. This is achieved through inhibiting cell wall synthesis, interfering with cell membrane permeability, triggering membrane disruption, changing cellular components, and inducing cell mutation (Rajakumar *et al.*, 2022). The ability of plant extracts to inhibit the growth of several bacteria is an indication of the antimicrobial efficacy of such plant, this was the case as the ethanol and ethyl acetate extracts of *M. lucida* and *V. amygdalina* leaves were comparatively studied for their antimicrobial properties, alongside their mineral composition and phytochemical constituents.

The antimicrobial evaluation from this study revealed that the ethanol extracts of *M. lucida* and *V. amygdalina* have significant antimicrobial effect on the clinical isolates studied (Table 4 and 5). *Morinda lucida* and *V. amygdalina* extracts were tested at different concentrations (25 mg/mL to 200 mg/mL). The results of this study showed that increase in the concentration of the extracts resulted in decrease in the growth of the microorganisms. All organisms recorded high susceptibility to the positive control (ciprofloxacin) at 200 mg/mL. They were also resistant to the negative control (ethanol and ethyl acetate). This result is in accordance with the previous study which also reveals that ethanol extract exerts a very strong antimicrobial effect when compared to other solvent (Eloff, 1998), and this ability could be attributed to a very high polarity of the solvent (Wang, 2010), with a potential of extracting intracellular ingredient most especially the phytochemicals which are mostly uncharged thereby making it easy to use polar solvent which can penetrate the cell membrane and yield more phytochemical which generate a greater pharmacological activity against microbes. *Vernonia amygdalina* and *M. lucida* were effective against all the microbial species, as previous studies by Alara *et al.* (2017) and Omachi, (2021), show that they possess antimicrobial activity against several microbial species.

**Table 4.** Antimicrobial activity of *Morinda lucida* leaf extracts on some pathogens.

| Organisms | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Control (Ciprofloxacin) |
| *Pseudomonas sp.* | 26.00±3.606$^{c}$ | 22.33±2.517$^{b}$ | 32.33±4.509$^{b}$ |
| *Klebsiella sp.* | 17.67±2.517$^{a}$ | 20.00±2.000$^{a}$ | 37.67±2.517$^{c}$ |
| *Trichophyton sp.* | 21.00±1.000$^{b}$ | 30.00±4.583$^{c}$ | 30.67±2.082$^{a}$ |
| *Candida sp.* | 28.00±3.606$^{c}$ | 28.67±2.309$^{c}$ | 28.00±4.000$^{a}$ |
| *Staphylococcus aureus* | 23.33±4.163$^{b}$ | 19.00±1.732$^{a}$ | 36.67±3.512$^{c}$ |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript means there is no significant difference between the comparing groups (P>0.05). Non – identical superscripts means there is significant difference between the comparing groups at P<0.05.

**Table 5.** Antimicrobial activity of *Vernonia amygdalina* leaf extracts on some pathogens.

| Organisms | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Control (Ciprofloxacin) |
| *Pseudomonas sp.* | 34.33±4.041[a] | 38.00±2.646[a] | 45.00±5.000[b] |
| *Klebsiella sp.* | 37.33±3.055[b] | 40.33±1.528[b] | 43.00±3.000[a] |
| *Trichophyton sp.* | 38.67±1.528[c] | 37.00±1.000[a] | 45.67±4.041[b] |
| *Candida sp.* | 36.00±3.606[b] | 40.67±1.155[b] | 48.00±2.000[c] |
| *Staphylococcus aureus* | 39.00±1.000[c] | 36.67±2.887[a] | 46.67±3.055[b] |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript means there is no significant difference between the comparing groups (P>0.05). Non – identical superscripts means there is significant difference between the comparing groups at P<0.05.

Minimum inhibitory concentration (MIC) is the minimum concentration of the antimicrobial needed to inhibit at least 99% of visible growth of a microorganism, whereas minimum bactericidal concentration (MBC) is the minimum concentration of the antimicrobial needed to kill at least 99% of microbial growth. In this study, the ethyl acetate extract of both plants shows a significant minimum inhibitory concentration (MIC) on several of the microbial species than the ethanol extract (Table 6 and 7). This effect was demonstrated with a better result (low MIC value) that was obtained with the ethyl acetate extracts against the test pathogens. The low MIC is an indication of higher antimicrobial activity and this was consequently displayed by higher zones of inhibition recorded in this study, which was also the case in the study carried by Falana *et al*. (2021). More so, this finding could also be attributed to the abundant of flavonoid, a phytochemical which have been reported to exhibit antimicrobial activities via different mechanism such as inhibition of quorum sensing among bacteria, inhibition of protein synthesis, alteration in the bacteria cell wall and cell membrane (Movahhedin *et al.*, 2016), inhibiting bacteria virulence factor, reducing the extracellular polysaccharides activity, suppressing biofilm formation, and acting as extracellular polysaccharides inhibitors (Bazzaz *et al.*, 2018).

**Table 6.** Minimum inhibitory concentration activity of *Morinda lucida* leaf extracts on some pathogens.

| Organisms | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Control (Ciprofloxacin) |
| *Pseudomonas sp.* | 58.33±38.188[c] | 14.58±9.547[b] | 19.79±26.206[a] |
| *Klebsiella sp.* | 29.17±19.094[b] | 7.29±4.771[a] | 39.58±52.416[c] |
| *Trichophyton sp.* | 7.29±4.771[a] | 14.58±9.547[b] | 19.79±26.206[a] |
| *Candida sp.* | 29.17±19.094[b] | 7.29±4.771[a] | 22.92±23.662[b] |
| *Staphylococcus aureus* | 58.33±38.188[c] | 14.58±9.547[b] | 34.67±3.0550[c] |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript means there is no significant difference between the comparing groups (P>0.05). Non – identical superscripts means there is significant difference between the comparing groups at P<0.05.

**Table 7.** Minimum inhibitory concentration activity of *Vernonia amygdalina* leaf extracts on some pathogens.

| Organisms | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Control (Ciprofloxacin) |
| *Pseudomonas sp.* | 29.17±19.094[a] | 45.83±47.324[b] | 19.79±26.206[c] |
| *Klebsiella sp.* | 29.17±19.094[a] | 45.83±47.324[b] | 51.04±48.443[d] |
| *Trichophyton sp.* | 58.33±38.188[b] | 45.83±47.324[b] | 7.29±4.771[a] |
| *Candida sp.* | 29.17±19.094[a] | 14.58±9.547[a] | 11.46±11.829[b] |
| *Staphylococcus aureus* | 29.17±19.094[a] | 13.54±10.972[a] | 11.46±11.829[b] |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript means there is no significant difference between the comparing groups (P>0.05). Non – identical superscripts means there is significant difference between the comparing groups at P<0.05.

The minimum bactericidal concentration (MBC) susceptibility of the test organisms in ethyl acetate and ethanol extracts of *Morinda lucida* and *Vernonia amygdalina* leaves are showed in Table 8 and 9. The ethyl acetate extract of *Morinda lucida* leaf showed the highest antibacterial activity against *Staphylococcus aureus* and *Trichophyton sp.*, followed by ethanol extract compared with the control. The ethanol extract showed the highest antibacterial activity against *Pseudomonas*, *Klebsiella sp.*, and *Candida sp.*, followed by ethyl acetate extract compared with the control at the same concentration (Table 8). The ethyl acetate extract of *Vernonia amygdalina* leaf show the highest antibacterial activity against *Pseudomonas sp.*, and *Staphylococcus aureus* followed by those of the ethanol extract compared with the control. The ethanol extract showed the highest antibacterial activity against *Klebsiella sp.*, *Candida sp.*, and *Trichophyton sp.*, followed by ethyl acetate extract compared with the control at the same concentration (Table 9). The minimum bactericidal concentration (MBC) activity revealed that ethanol extract of both plants had the most effect on the microbes. This trend was revealed by all the extracts against the test microbes as the concentration needed to kill them is higher than that needed to inhibit them. This means they can easily be inhibited but difficult to be killed. Zainol *et al.* (2013) discussed that there are some species which are easier to get inhibited but hard to be killed due to their adaptive ability. Henceforth, this study reveals that ethyl acetate and ethanol extracts of *M. lucida* and *V. amygdalina* leaves have antimicrobial potentials and can serve as an antimicrobial source. Also, the components of extracts of these plants justify its antimicrobial activities.

**Table 8.** Minimum bactericidal concentration activity of *Morinda lucida* leaf extracts on some pathogens.

| Organisms | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Control (Ciprofloxacin) |
| *Pseudomonas sp.* | 29.17±19.094$^{c}$ | 58.33±38.188$^{c}$ | 27.08±21.949$^{a}$ |
| *Klebsiella sp.* | 29.17±19.094$^{c}$ | 58.33±38.188$^{c}$ | 52.08±46.910$^{c}$ |
| *Trichophyton sp.* | 54.17±43.899$^{d}$ | 13.54±10.972$^{a}$ | 51.04±48.443$^{c}$ |
| *Candida sp.* | 7.29±4.771$^{a}$ | 26.04±23.452$^{b}$ | 27.08±21.949$^{a}$ |
| *Staphylococcus aureus* | 27.08±21.949$^{b}$ | 14.58±9.547$^{a}$ | 29.17±19.094$^{b}$ |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript means there is no significant difference between the comparing groups (P>0.05). Non – identical superscripts means there is significant difference between the comparing groups at P<0.05.

**Table 9.** Minimum bactericidal activity of *Vernonia amygdalina* leaf extracts on some pathogens.

| Organisms | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Ethyl acetate extract | Ethanol extract | Control (Ciprofloxacin) |
| *Pseudomonas sp.* | 58.33±38.188$^{d}$ | 29.17±19.094$^{b}$ | 51.04±48.443$^{c}$ |
| *Klebsiella sp.* | 27.08±21.949$^{b}$ | 45.83±47.324$^{c}$ | 58.33±38.443$^{d}$ |
| *Trichophyton sp.* | 29.17±19.094$^{c}$ | 58.33±38.188$^{d}$ | 7.29±4.771$^{a}$ |
| *Candida sp.* | 13.54±10.972$^{a}$ | 22.92±23.662$^{a}$ | 19.79±26.206$^{b}$ |
| *Staphylococcus aureus* | 58.33±38.188$^{d}$ | 58.33±38.188$^{d}$ | 58.33±38.188$^{d}$ |

Values were performed in triplicates and represented as means ± standard derivations. Identical superscript means there is no significant difference between the comparing groups (P>0.05). Non – identical superscripts means there is significant difference between the comparing groups at P<0.05.

## 4. Conclusion

In this study, several compounds and minerals were found in the leaves extracts of *Morinda lucida* and *Vernonia amygdalina* which support their medicinal usage in traditional medicine. Alkaloid, flavonoid and saponin were the effective compounds in the two medicinal plants studied, however, the ethanol solvent yields more of the compounds than the ethyl acetate solvent in both plants. However, iron, lead, cadmium and arsenic were copious in the ethanol extracts; while, sodium, potassium and calcium were copious in the ethyl acetate extracts. More so, these plant extracts exhibited an antimicrobial activity against the test organisms to further support their use in traditional systems of treatment of infectious diseases caused by pathogens.

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