World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Assessment on the Phytochemicals, Mineral composition and Antimicrobial activity of avocado pear (*Persea americana*) seeds, soursop (*Annona muricata*) leaves and bitter leaf (*Vernonia amygdalina*) samples

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World Journal of Advanced Research and Reviews, 2025, 28(01), 482-492

Publication history: Received on 21 August 2025; revised on 01 October 2025; accepted on 04 October 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.1.3354

Abstract

The emergence of multi-drug resistant strains coupled with the limited availability of affordable and effective antimicrobial agents especially in developing countries poses a great challenge to healthcare providers and affects the successful management of diseases. These have resulted in the need to seek for alternative therapies in handling such infections. This study was carried out to investigate and compare the phytochemical, mineral, and antimicrobial properties of the ethanolic extracts of avocado pear (*Persea americana*) seeds, soursop (*Annona muricata*) leaves and bitter leaf (*Vernonia amygdalina*) samples. Ethanolic samples were obtained by the cold maceration method. The antimicrobial activities of the samples were screened using agar well diffusion assay methods. The presence of minerals and heavy metals were determined in the samples using previously described methods to characterize the samples. The qualitative presence of phytochemicals was also analyzed in the extract samples using previously described standard methods**.** The bitter leaf ethanolic extract (200mg/ml) was the only sample that showed antimicrobial activity on an *Escherichia coli* isolate with 20mm zone diameter. The phytochemical tests of the extract samples revealed the presence of flavonoids, steroids, alkaloids, terpenoids, cardiac glycosides in the three samples analyzed. The mineral analysis of the extract samples showed the presence of essential and trace elements. Our study showed good sources of phytochemicals and essential minerals for avocado pear seeds, soursop leaves and bitter leaf samples. This indicates their potential of having pharmacological activity if properly processed to serve as a medicinally vital material in animal health and probably humans.

Keywords: Antimicrobial Activity; Avocado Pear; Bitter Leaf; Soursop; Phytochemicals; Mineral Composition

1. Introduction

The utilization of medicinal plants for the treatment of infections is deeply rooted in traditional African medicine systems which have evolved over centuries to address the healthcare needs of local communities. In traditional African medicine, medicinal plants have been used for centuries to treat various ailments, including microbial infections. Soursop (*Annona muricata*), *Persea americana* (avocado pear) and bitter leaf (*Vernonia amygdalina*) are examples of such plants that have been extensively employed in Nigerian traditional medicine for its perceived antimicrobial properties [1, 2, 3, 4].

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The leaves of the soursop tree have been extensively used in Nigerian traditional medicine for their therapeutic benefits against various ailments, including microbial infections, fever, and inflammation [1, 2, 5, 6]. *P. americana* seeds have been used to cure a wide range of diseases, including toothaches, skin infections, and dysentery [7]. The leaves and seed oil of *Persea americana* have been reported to be useful in a variety of ways in ethnomedicine, including the treatment of intestinal parasites, toothaches, diarrhea, and dysentery [7, 8]. Bitter leaf (*Vernonia amygdalina*) has been employed for its purported medicinal properties in managing digestive issues, bleeding, malaria and other health-related problems [4].

Soursop leaves are known to possess a rich phytochemical composition, including alkaloids, flavonoids, tannins, and other bioactive compounds. These constituents have been extensively studied for their diverse pharmacological activities, including notable antimicrobial properties [9]. Tannin and carotenoids have been reported to be both abundant in the seeds of *Persea americana* [10]. Bitter leaf (*Vernonia amygdalina*) extracts have also been reported to contain bioactive compounds with significant antibacterial effects [11]. Several studies conducted in Africa, including Nigeria, have highlighted the antimicrobial efficacy of soursop leaf extracts, avocado pear (*Persea americana*) seeds and bitter leaf (*Vernonia amygdalina*) samples against a wide range of microbial pathogens [1, 2, 5, 6, 12, 13, 14]. Such findings provide a strong scientific basis for investigating the microbiological activity of soursop leaves, avocado pear (*Persea americana*) seeds and bitter leaf (*Vernonia amygdalina*) samples. These findings indicate the potential of these samples as a natural alternative to conventional antimicrobial agents.

Microbial infections pose a significant health burden in Africa, and Nigeria is no exception. Limited access to healthcare resources, coupled with the emergence of drug-resistant strains, further complicates the effective treatment of infections. As a result, there is an urgent need to explore alternative sources of antimicrobial agents. Traditional medicinal plants offer a promising avenue for the discovery of novel antimicrobial compounds. With the increasing global burden of antimicrobial resistance resulting in a need to explore alternative antimicrobial agents, avocado pear (*Persea americana*) seeds, soursop (*Annona muricata*) leaves and bitter leaf (*Vernonia amygdalina*) samples have the potential to provide new sources of natural antimicrobial compounds. This study was carried out to investigate and compare the phytochemical, mineral, and antimicrobial properties of the ethanolic extracts of avocado pear (*Persea americana*) seeds, soursop (*Annona muricata*) leaves and bitter leaf (*Vernonia amygdalina*) samples. The study was also carried out to validate their reported usefulness.

2. Materials and Methods

2.1. Collection and preparation of samples

Fresh and healthy leaves of soursop tree were obtained from a farm in Okada, Edo state, Nigeria. Matured avocados and bitter leaf samples were purchased at the Okada central market, Ovia North East Local Government Area of Edo State. Nigeria. The purchased items were transported to the pharmaceutical microbiology laboratory, Igbinedion University, Okada. The soursop leaves, avocado fruit and bitter leaf samples were identified and authenticated at the Department of Pharmacognosy Igbinedion University Okada as *Annona muricata* (Annonaceae), *Persea americana* (Lauraceae), *Vernonia amygdalina* (Asteraceae) respectively with the respective herbarium ID as IUO/13/051, IUO/17/171, IUO/16/022.

The leaves of *Annona muricata* and *Vernonia amygdalina* were carefully washed with water and dried at room temperature for 10 days after which they were grounded with a milling machine to obtain the powdered form of the leaves. The powdered form of the leaves was stored in sterile containers for extraction. Avocado fruits were first washed with distilled water, using a sterile knife, the fruit was then divided into two equal halves and the seed was then separated from the flesh of the fruit. Using a sterile knife, the seeds were cut into small sizes and then dried at room temperature. After, drying the dried seeds was milled into powder form. The powders were stored in separate bottles for extraction.

2.2. Extraction

500g of grounded *Annona muricata* leaves and avocado seed powder respectively was weighed and introduced into a clean jar. The ethanolic extract of the grounded leaves was prepared by soaking 500g of the powdered sample in 1500ml and 2000 ml of solvent (99.7% ethanol) respectively for the avocado seed and soursop leaves samples for 72 hours, during which the mixture was continuously mixed at intervals. It was then filtered using a Whatman filter paper. The residue gotten after filtration was macerated again in 750 ml of 99.7% ethanol for 72 hours and occasionally stirred during the period of extraction. The sample was filtered afterwards. The extract was concentrated and final solvent

elimination was done using a water bath at 60°C. After concentration of the extract, the resultant extracts were stored in clean, sterile, well labeled glass tubes and kept in a refrigerator (4°C) till further used.

For the bitter leaf sample, 250g of the powdered avocado seed was weighed into a clean jar. 1000ml of 99.7% ethanol was added to the jar containing the sample while stirring using a glass rod. The jar for extraction was tightly covered to avoid evaporation of the solvent. The cold extraction was carried out for 3 days and occasionally stirred during the period of extraction. After 3 days the sample was filtered using Whatman filter paper (No 1). The residue gotten after filtration was re macerated with 750ml of 99.7% ethanol and stirred for 3 days consistently. It was filtered using Whatman filter paper after 3 days. The filtrate gotten from the first and second maceration was added and concentrated using water bath at 60^{o}C. The bitter leaf extract gotten was kept in a sample bottle and stored in a refrigerator (4°C) until used in determining the phytochemical and antimicrobial activity of selected isolates.

2.3. Phytochemical screening

The phytochemical screening of the extracts for the presence of secondary metabolites was carried out using the standard phytochemical methods with slight modifications [15-17].

2.3.1. Test for saponins

5 ml of extract was placed into a test tube and 5 ml of distilled water was added to the test tube. The test tube was shaken vigorously. The presence of persistent frothing indicated the presence of saponins.

2.3.2. Test for flavonoids

Few drops of lead acetate were added to 2 ml of extract. A milky precipitate indicated the presence of flavonoids. A volume of 2 ml of dilute ammonia was also added to 2 ml of extract. The presence of flavonoids was observed by a yellowish colouration.

2.3.3. Test for steroids

A volume of 0.5 ml chloroform was added to 1ml of extract followed by 3 or 4 drops of acetic anhydride and 1 drop of concentrated sulphuric acid. Steroids were indicated by a brown ring at the interface.

2.3.4. Test for tannins

A volume of 2 ml of extract was added into a test tube followed by 10 ml distilled water and a few drops of 0.5% Iron (III) chloride. An intense blue black, green, purple coloration indicated the presence of tannins. A formation of yellow precipitate when few drops of 1% lead acetate were added to 5 ml extract confirmed presence of tannins.

2.3.5. Test for terpenoids

In a test tube, 5 ml of extract was combined with 2 ml of chloroform. A volume of 3 ml of concentrated H_2SO_4 was carefully added along the side to form a layer. The presence of terpenoids can be indicated by a reddish-brown colouration at the interface.

2.3.6. Test for alkaloids

A volume of 1ml of extract was put in five separate test tubes and 1ml 1% sulphuric acid was added to each of the test tubes containing the extract. A volume of 1ml of an alkaloidal reagent [Dragendoffs reagent, Mayer's reagent, Hager's reagent (saturated solution of picric acid), Wagner's reagent or tannic acid] was added into each tube and was observed for precipitation. The formation of an orange red precipitate (with Dragendorffs and Wagner's reagents), creamy precipitate (Mayer's reagent), yellow precipitate (Hager's reagent), buff precipitate (tannic acid) suggested the presence of an alkaloid.

2.4. Test for cardiac glycosides

2.4.1. Salkowski test for cardiac glycosides

A volume of 2 ml chloroform was added to 2 ml of extract in a test tube and put in an ice bath in a fume cupboard. The tube was tilted and 1-2 ml conc. H_2SO_4 was carefully added to form a lower layer. The formation of a reddish-brown colour at the interface indicated the presence of a steroidal ring (i.e. the aglycone portion of the cardiac glycoside).

2.5. Test for anthraquinones

2.5.1. Combined anthraquinones (free and glycosidic forms)

A volume of 5-10 ml 10% H_2SO_4 was added to 5 ml plant extract in a separatory funnel. It was heated on a boiling water bath for 5 min and allowed to cool. It was then diluted with 10-20 ml water. Chloroform (3x5 ml) was added and it was shaken twice. The organic layer was separated into another separatory funnel and 5-10 ml 10% ammonia solution was added and shaken again. The formation of a pink, red or violet colour in the ammonical (lower) layer indicated the presence of combined anthraquinones.

2.6. Mineral analysis

The method used by Fernandez [18] was employed with slight modifications. Using the conventional wet acid digestion method, 0.200g of *Annona muricata*, avocado pear seeds and bitter leaf samples was accurately weighed separately into a beaker (250 ml). About 20 ml of a freshly prepared mixture of concentrated HCl-HNO3 (3:1 v/v) was added to a flask and kept for 10 minutes at room temperature, and then the content of the flask was heated on an electric plate at 80°C until a clear solution was obtained. The content of the flask was evaporated and the semidried mass was dissolved in 5 ml of distilled water. It was filtered using Whatman filter paper and made up to a final volume of 50 ml in a volumetric flask with distilled water and kept as a stock sample solution. The blank was also prepared by digesting the HCl-HNO3 (3:1) reagent mixture. The digest was analyzed for minerals and heavy metals, using Perkin Elmer Analyst 200 atomic absorption spectrophotometer.

2.7. Microorganism used

Gram positive and gram negative bacteria which include *Staphylococcus aureus, Escherichia coli,* and *Klebsiella* spp. of clinical origin (ear swab and urine) were obtained from Igbinedion University Teaching Hospital (IUTH), Okada, Edo state and University of Benin Teaching Hospital (UBTH), Benin City, Edo state for the susceptibility studies. Isolates were sub-cultured on nutrient agar plates to obtain pure colonies. Previously described standard microbiological techniques [19] were used for species identification. All the isolates were maintained on nutrient agar slants at 4°C.

2.8. Preparation of extracts

For the antimicrobial sensitivity testing two solvents, dimethyl sulphoxide (DMSO) and tween 80 were used to dissolve the plant extracts. In addition to DMSO and tween 80, ethanol was also used to dissolve the *Vernonia amygdalina* leaves to a concentration of 200mg/ml. To get 200 mg/ml of the plant extract with DMSO/ ethanol, 1g of the plant extract was weighed and placed in a universal bottle and labeled appropriately. A volume of 1 ml of DMSO/ ethanol was used to dissolve the extract and made up to volume with 4 ml of sterile distilled water. The total volume for the plant extract with DMSO/ ethanol solvent was 5 ml. To get 200 mg/ml of the plant extract with Tween 80, 1g of the plant extract was weighed and placed in a universal bottle and labeled appropriately. A volume of 0.5 ml of tween 80 was added into the bottle to dissolve the plant extract and made up to volume with 4.5 ml of sterile distilled water. The total volume for the plant extract with tween 80 solvent was 5 ml.

2.9. Preparation of positive control

A volume of 1 ml of gentamicin injection (80 mg/2 ml) was added to 99 ml of sterile distilled water to make 400 µg/ml concentration. A volume of 1 ml was taken from the 400 µg/ml concentration and added to 39 ml of sterile distilled water to make the final concentration of 10 µg/ml.

2.10. Preparation of negative control

There were two negative controls used. For the plant extract with DMSO solvent, the negative control was prepared by adding 1 ml of DMSO to 4 ml of sterile distilled water to make 20% DMSO. For the plant extract with tween 80 solvent, the negative control was prepared by adding 0.5 ml of tween 80 to 4.5 ml of sterile distilled water to make 10% tween 80.

2.11. Antimicrobial sensitivity testing

Antimicrobial sensitivity testing was conducted using the agar diffusion technique with slight modifications [20]. The inoculum was adjusted to McFarland 0.5 and used for inoculation on Mueller Hinton agar plates. A sterile cork borer was used to make equidistant holes on the plates. With the aid of a pasteur pipette, 0.1 ml of the 200 mg/ml DMSO, ethanol and tween 80 extracts and 0.1 ml of their respective positive and negative controls were added into the bored

holes in the agar plates. The plates were incubated for 24 hours at room temperature and the diameter of the zone of inhibition was measured with a calibrated meter rule and recorded.

3. Results and Discussion

3.1. Phytochemical screening

Table 1.0 summarizes the results obtained from the qualitative phytochemical analysis of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves. The results of the screening reveal that the *Annona muricata* and *Vernonia amygdalina* leaves contain some phytochemical constituents which include tannins, steroids, flavonoids, terpenoids, cardiac glycosides and alkaloids. Saponin was also detected in the *Vernonia amygdalina* leaves. The result of the phytochemical screening of the *Persea americana* seeds showed that the extract contained saponin, flavonoids, steroids, alkaloids, cardiac glycoside, terpenoids.

Table 1 Phytochemical analysis of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves**.**

Phytochemical constituents	***Persea americana* seeds**	***Vernonia amygdalina* leaves**	***Annona muricata* Leaves**
Saponins	+	+	-
Flavonoids	+	+	+
Steroids	+	+	+
Tannins	-	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Cardiac glycosides	+	+	+
Anthraquinones	-	-	-

KEY: + = Present **,** - = Absent

3.2. Mineral Analysis

Table 2.0 gives the mineral composition of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves. The mineral analysis showed the presence of essential minerals (Calcium, potassium, iron, magnesium, zinc, manganese) at varying quantities in all the samples. Copper was only detected in *Annona muricata* and *Vernonia amygdalina* leaves. Toxic heavy metals (Cadmium, Chromium and Lead) were also detected in the samples at a negligible range (Table 2.0). Cadmium was not detected in the *Vernonia amygdalina* leaves.

Table 2 Mineral analysis of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves

MINERALS	***Persea americana* seeds**	***Vernonia amygdalina leaves***	***Annona muricata* leaves (mg/g) (MEAN±SD)**
Calcium	8.053±0.051	0.385±0.001	13.773±0.022
Cadmium	0.003±0.002	0.000±0.000	0.002±0.001
Chromium	0.015±0.001	0.015±0.001	0.009±0.001
Copper	0.000±0.000	0.011±0.001	0.002±0.000
Iron	0.093± 0.003	7.758±0.019	0.284±0.003
Potassium	32.554±0.085	41.986±0.022	27.264±0.015
Magnesium	1.631±0.004	3.494±0.014	2.977±0.004
Manganese	0.005±0.001	0.016±0.001	0.021±0.001
Lead	0.056±0.002	0.015±0.002	0.076±0.002

Zinc	0.045±0.002	0.070±0.002	0.069±0.001

3.3. Antimicrobial Sensitivity Test

Table 3.0 and 4.0 show the results of the antimicrobial sensitivity test of the ethanolic extract of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves using DMSO and tween 80 solvents on the clinical isolates. All the extracts had no significant antimicrobial activity on the growth of the clinical isolates tested. The negative controls also did not show any activity. Only *Vernonia amygdalina* leaves dissolved with ethanol solvent had activity on an *Escherichia coli* isolate with a diameter zone of inhibition of 20mm (Table 5.0). The positive control 10 µg/ml gentamicin also showed activity on some of the isolates.

Table 3 Antimicrobial activity of ethanolic extract of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves using dimethyl sulphoxide (DMSO) as solvent

ORGANISM	***Persea americana* seeds (200 mg/ml)**	***Annona muricata* leaves (200 mg/ml)**	***Vernonia amygdalina* leaves (200 mg/ml)**	**POSITIVE CONTROL (10 µg/ml)**	**NEGATIVE CONTROL (20% DMSO)**
Staphylococcus aureus	R	R	R	R	R
Escherichia coli	R	R	R	15mm	R
007 - *Escherichia coli*	R	R	R	15 mm	R
09 - *Escherichia coli*	R	R	R	18 mm	R
12 - *Klebsiella spp.*	R	R	R	15 mm	R
16 – *Klebsiella spp.*	R	R	R	20 mm	R

KEY: R – Resistant

Table 4 Antimicrobial activity of ethanolic extract of *Persea americana* seeds, *Annona muricata* and *Vernonia amygdalina* leaves using tween 80 as solvent

ORGANISM	***Persea americana* seeds (200 mg/ml)**	***Annona muricata* leaves (200 mg/ml)**	***Vernonia amygdalina* leaves(200 mg/ml)**	**POSITIVE CONTROL (10 µg/ml)**	**NEGATIVE CONTROL (10% tween 80)**
Staphylococcus aureus	R	R	R	R	R
Escherichia coli	R	R	R	15mm	R
007 - *Escherichia coli*	R	R	R	15 mm	R
09 - *Escherichia coli*	R	R	R	18 mm	R
12 - *Klebsiella spp.*	R	R	R	15 mm	R
16 – *Klebsiella spp.*	R	R	R	20 mm	R

KEY: R - Resistant

Table 5 Antimicrobial activity of the ethanolic extract of *Vernonia amygdalina* leaves using ethanol as solvent

ORGANISM	***Vernonia amygdalina* leaves (200 mg/ml)**	**POSITIVE CONTROL (10 µg/ml)**	**NEGATIVE CONTROL (20% ethanol)**
Staphylococcus aureus	R	R	R
Escherichia coli	20mm	15mm	R
007 - *Escherichia coli*	R	12mm	R
09 - *Escherichia coli*	R	17mm	R
12 - *Klebsiella spp.*	R	R	R
16 – *Klebsiella spp.*	R	15 mm	R

KEY: R - Resistant

Plants have been reported to have an advantage of being useful and the most effective and cheaper alternative source of drugs [13]. Trease and Evans [15] reported that medicinal plants are believed to contain bioactive compounds with diverse biological effects. Certain compounds within these plants are responsible for the plant's unique fragrance, spiciness, and color, while others grant it distinct culinary, medicinal, or potentially poisonous characteristics. The results of the phytochemical screening of the ethanolic extract of *Annona muricata* leaves as seen in table 1.0 showed the absence of saponins and anthraquinone while the following phytochemical constituents: flavonoids, steroids, tannins, terpenoids, alkaloids and cardiac glycosides were present. The results from this study correlates with a similar study performed by Usunobun *et al*., [21] on the ethanolic leaf extracts of *Annona muricata,* which recorded the absence of anthraquinone and steroids and detected the presence of saponins, flavonoids, triterpenoids, alkaloids, cardiac glycosides and reducing sugars. Another study by Vinothini and Lali, [22] on aqueous and methanolic extracts of *Annona muricata* detected the presence of saponins, flavonoids, tannins, terpenoids and alkaloids on both aqueous and methanolic leaf extracts. The phytochemical screening of *Persea americana* seed showed the presence of saponin, flavonoid, steroid, alkaloids and cardiac glycosides and terpenoids. Tannins and anthraquinones were absent in the extract sample of of *Persea americana* seed. In a previous study of Egbuonu *et al*., [23], alkaloids, saponins, flavonoids and tannins were detected in the seeds of avocado. Also in another recent study of the phytochemical constituents of avocado seeds by Akpomie *et al*., [3] alkaloids, flavonoids, saponin, tannins were detected but terpenoids and steroids were not detected. The phytochemical screening result of the bitter leaf sample revealed that the extract contained saponins, tannins, flavonoids, alkaloids, phenols, steroids, cardiac glycosides and terpenoids. This compares with a previous study of Ojimelukwe and Amaechi [24] that reported phytochemical contents in bitter leaf sample to include flavonoids, tannins, alkaloids, anthraquinones, steroids and phenols. In another study by Evbuomwan et al., [25] the phytochemicals detected in bitter leaf sample included flavonoids, cardiac glycosides, reducing sugars, terpenoids, saponins, anthraquinones, alkaloids and steroids which correlates with the results in this study. The results from the phytochemical tests revealed that the plant extract contains phytochemical constituents of pharmacological importance.

Mineral elements serve as structural components of tissues and as constituents of the body fluid and vital enzymes in major metabolic pathways and are essential for the function of all cells [26].

The results of the mineral composition of the leaf of *Annona muricata* as shown in table 2.0 shows potassium (K) and calcium (Ca) having the highest concentrations of 27.264 mg/g and 13.773 mg/g respectively and copper (Cu) and cadmium (Cd) both having the same lowest concentrations of 0.002 mg/g. Other minerals detected from the mineral analysis included chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), lead (Pb) and zinc (Zn). The value of potassium (K) was higher when compared to the value of K in an investigation by Princewill-Ogbonna *et al*., [27] and the values for Ca and Mg in the same investigation were relatively higher than the values obtained in this study. The levels of Ca, Cd, Cr, Cu, Fe, K, Mg, Mn and Zn obtained in this study were relatively low compared to an earlier research by Usunobun and Okolie [28] where the corresponding minerals were recorded in higher amounts.

Calcium is necessary for the coagulation of blood, proper functioning of the heart and nervous system and the normal contraction of muscles as well as aid in the formation of bones and teeth [21]. Iron is an essential dietary mineral used to support vital human functions, such as erythropoiesis, cellular energy metabolism, and immune system development and function [29]. According to the Regents of the University of Minnesota [30], potassium serves as the primary cation in intracellular fluid and fulfills various essential functions in the body. These functions include maintaining acid-base balance, regulating osmotic pressure, conducting nerve impulses, facilitating muscle contractions, especially in the

cardiac muscle, supporting cell membrane functions, and enabling the activity of the Na^+/K^+-ATPase pump. Its involvement in these critical processes highlights the vital role potassium plays in maintaining overall physiological health. Magnesium is a key component of chlorophyll, vital for photosynthesis in plants. It also plays essential roles in ischemic heart disease and calcium metabolism in bones [31].Toxic heavy metals detected like cadmium, chromium, and lead are not a health risk as their presence falls within permissible levels [32].

A previous study carried out on the evaluation of the chemical composition of *Persea americana* seed, by Nwaokobia et al., [33] reported in higher quantities of calcium (36.78±0.03%), magnesium (20.81±18.09%), Iron (19.56±19.88%), copper (1.09±0.13%), zinc (6.47±1.32%) compared to the quantity detected for each of the elements in this study. In another previous study, Arukwe et al., [34] investigated the mineral composition of *Persea americana* seed and the following minerals were also found in higher quantities: calcium (26.16±5.90mg/100g), potassium (100.83±5.64mg/100g), Iron (0.31±0.03mg/100g), copper (0.98±0.13mg/100g), zinc (0.09±0.01mg/100g) compared with results obtained in this study (Table2.0).

In a previous report, Ojimelukwe and Amaechi [24] detected Calcium (2.78mg/g), iron (0.14mg/g) and magnesium (2.61mg/g) in a bitter leaf sample which are lower than results obtained in this study Calcium (7.76mg/g), iron (0.39mg/g) and magnesium (3.49mg/g). Potassium detected in the bitter leaf sample (41.99mg/g) in this study was notably high compared to results from other studies. Muhammad et al., [35] reported a value of 0.61mg/g while Ojimelukwe and Amaechi [24] reported a value of 0.60mg/g for the concentration of potassium present in its bitter leaf sample. Martinez-Ballesta et al., [36] reported that the varying levels of minerals possibly present in the samples are dependent on the agricultural practices used, soil content and extraction method.

The antimicrobial screening test of the ethanolic extract of *Persea americana* seeds *Annona muricata* and *Vernonia amygdalina* leaves showed no zone of inhibition with the DMSO and tween 80 solvents indicating that they did not possess any antimicrobial activity on the selected isolates (*Staphylococcus aureus, Escherichia coli* and *Klebsiella* spp.). Only *Vernonia amygdalina* leaves dissolved with ethanol solvent had activity on an *Escherichia coli* isolate with a diameter zone of inhibition of 20mm (Table 5.0). The antimicrobial sensitivity test carried out in this study contrasts with that of Iyanda-Joel et al., [37]. Iyanda-Joel et al., [37] analyzed the ethanolic extract of *Annona muricata* leaves and the antibacterial sensitivity tests revealed that the extract had antibacterial activity against *Klebsiella* spp. and *Staphylococcus aureus*. Akpomie et al., [3] carried out a study on the antibiotic activity of ethanolic extract of *Persea americana* seed using isolates like (*E.coli, Klebsiella* spp.) from urine and reported that *E. coli* and *Klebsiella* spp. were susceptible to the ethanolic extracts of *Persea americana* seed. Another research carried out by Egbuonu et al., [2023] showed that ethanolic extract of *Persea americana* seeds have a broad spectrum antibacterial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus*. This contrasts with result from this study. The lack of antimicrobial activity observed in the various extracts on the isolates might be attributed to the method of extraction. The Soxhlet extraction method was utilized in the extraction of *Annona muricata* ethanolic leaves extract as seen in Iyanda-Joel et al., [37]. The choice of solvent for the dissolution and extraction of plants have could also been reported to play a role in the type and amount of phytochemicals present in a plant extract and hence it's antimicrobial activity [38]. Aqueous and methanolic solvents used in the extraction of *Annona muricata* leaves have been reported to have positive antibacterial activity against *Staphylococcus aureus, Klebsiella* spp. and *Escherichia coli* [22, 37, 39]. Idris et al., [40] conducted a research on the comparison of antimicrobial activity of petroleum ether, chloroform, ethyl acetate, methanolic extract of avocado seed against *S. aureus, S. pyrogens, C. ulcerans, B. subtilis, S. typhi, E.coli, K. pneumoniae, P. aeruginosa, N. gonorrhea*. The ethyl acetate extract was found to be more potent with activity against all the organisms especially *S. aureus, S. pyogenes, C. ulcerans, E.coli,* and *S. typhi*, while petroleum ether extract however demonstrated the least activity against all test organisms. A significant limitation in this study is the limited number of isolates used in the antimicrobial study. Expanding the number of test organisms could potentially uncover some isolates that may be sensitive to the plant extracts. The geographical location and environmental factors where the plant was obtained from could also be a possible factor that attributed to the lack of antimicrobial activity of the plant.

The solubility and penetration properties of ethanol might contribute to the enhanced bioactivity of the *Vernonia amygdalina* leaves [41]. Ethanol has been reported to have better solubility for a wide range of compounds. Evbuomwan et al., [25] reported that the antibacterial activity of *V. amygdalina* in his study was found to be dependent on the nature of the solvent used for extraction and the concentration of the extract. In their study, ethanolic extract was observed to possess more antibacterial activities compared to the aqueous extract. This is attributable to the fact that ethanol extracted more of the bioactive component of the plant compared to aqueous extract. Zones of inhibition produced by ethanolic extract ranged from 7.0±0.0mm at 25mg/ml to 14.5±2.5mm at 200mg/ml against *E. coli*; 6.5±0.5mm at 100mg/ml to 9.0±2.0mm at 200mg/ml against *S. aureus*; 11.0±1.0mm at 50mg/ml to 16.0±5.0mm at 200mg/ml against *P. aeruginosa*; 7.5±1.5mm at 25mg/ml to 11.5±0.5mm at 200mg/ml against *K. pneumoniae*. Ethanolic extract was found to possess inhibitory activities against the test bacterial species compared to the aqueous extract. This finding agrees

with previous reports by Udochukwu et al., [42] and Muhammad et al., [43] who reported the phytochemical and antibacterial activity of *Vernonia amygdalina*.

4. Conclusion

Results gotten from this study validates that *Persea americana* seeds*, Vernonia amygdalina*, *Annona muricata* leaves are good sources of phytochemicals and minerals. This indicates that the plant has the potential of having pharmacological activity and can also be useful as nutraceuticals. Further studies are necessary on how the plant can be properly processed to serve as a medicinally vital material in animal health and probably humans.

Compliance with ethical standards

Acknowledgements

We thank Mr Edafe Arusi for technical assistance rendered during the course of the work.

Disclosure of conflict of interest

No conflict of Interest is declared.

Funding sources

This study did not receive any grant from funding agencies.

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