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Research Article

Comparative Study of Phytochemicals Composition of *Mangifera Indica* (Mango) and *Vernonia Amygdalina* (Bitter Leaf) Leaves and Their Antimicrobial Activities

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Abstract—Mangifera indica and Vernonia amygdalina leaves are utilized for the treatment of various illnesses in folklore medicine. This research aimed to look at the antimicrobial effects and phytochemical compositions of leaf extracts of two studied plants obtained within the Katsina metropolis using different solvent extraction techniques (ethanolic and aqueous extraction) and their antimicrobial activities. The powdered leaves of the examined plants were extracted with water and ethanol solvents using the percolation method. Phytochemical studies were conducted following standard protocols to detect secondary metabolites. Phytochemical screening revealed the presence of several compounds including alkaloids, flavonoids, saponins, glycosides, steroids, terpenoids, tannins, and phenols. This work showed that the two different solvent extraction methods (ethanolic and aqueous) have varying abilities to liberate active compounds, and the presence of these secondary metabolites in the selected plants has high healing potential. Thus, these phytochemicals enhance the medicinal value of the studied plants. The current data also showed that the two extracts (Mangifera indica and Vernonia) had a range of antimicrobial effects on the different strains that were tested. The microorganisms that were tested were sensitive to different amounts of Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. The highest zone of inhibition was observed in Vernonia amygdalina ethanolic extract with 18.0mm (250mg/ml) for Staphylococcus followed by ethanolic extract against Salmonella typhi with 16.0mm (250mg/ml). The lowest concentration of 31.25 mg/ml had minimal or no effect on the test bacteria. The findings of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against bacterial isolates showed that Vernonia amygdalina exhibited the lowest minimum inhibitory concentration (MIC) values compared to the other extracts while the extracts of Vernonia amygdalina also exhibited the lowest minimum bactericidal concentration (MBC) values.

Keywords— Phytochemicals, antimicrobial activities, solvent extraction, bactericidal concentration, percolation, leaves

1. Introduction

Plants have long been utilized in a diverse range of medicines, serving therapeutic purposes in the treatment of various maladies. This trend is expected to persist, particularly in rural regions of developing nations [15]. The utilization of medicinal plants has had a significant surge in recent decades as a substitute method for enhancing the standard of living and preserving excellent health, approximately 80% of the global population depends on traditional medicines as their main source of basic healthcare, with a majority of these treatments involving the use of plant extracts [17].

Mangifera indica, often known as Mango, is a sizable perennial tree characterized by a substantial, rounded crown [18]. It is a member of the Anacardiaceae family. The plant is widely distributed throughout tropical regions worldwide, where it serves as both a horticultural and medicinal species. Fruits are composed of protein, fat, carbs, minerals, vitamins A, B, and C, as well as amino acids. The fruits also yield a resin that is reported to include mangiferin, mangiferin acid, retinol maniferol, and others [18].

The leaves contain the glucoside mangiferine.The mango tree bark has a tannin content of 16-20% [18].
Vernonia amygdalina, commonly known as bitter leaf, is obtained from the foliage of a compact perennial shrub that is widely distributed throughout Africa and belongs to the Asteraceae family. It is well known as a medicinal herb for diabetes and fever [2]. Vernonia amygdalina, sometimes known as a bitter leaf, is the predominant species of the Vernonia genus, which comprises over 1,000 shrub species

Vernonia amygdalina, also known as a bitter leaf, contains phytochemical compounds that are commonly found in non-

nutritive plants. These compounds have been traditionally used in medicine to treat various ailments. Additionally, extracts from the bitter leaf have been studied for their ability to inhibit the growth of organisms that cause spoilage in fresh catfish (Clarias gariepinus), thereby prolonging their shelf life [13].

Phytochemicals can be chemical compounds found naturally in plants that are accountable for many of the plant's characteristics, including smell, taste, colour, and other organoleptic aspects. despite the fact that some phytochemicals may have biological importance due to their recognised basic nutritional values, several have been proposed as medications due to their therapeutic potential. Plants containing such phytochemicals are classified as medical plants since their effects are comparable to those of traditional pharmaceutical drugs.

2. Related work

According to [17] It is classified under the Asteraceae family. The plant is propagated through vegetative cultivation using stem cuttings angled at 45 degrees. This method is widely practiced in several West African nations, such as Nigeria, Cameroon, Gabon, and the Democratic Republic of the Congo. While mostly utilised for culinary purposes, it has also historically been employed for its therapeutic attributes [17].

Bitter leaf lives up to its name, as it has a bitter flavor but is unexpectedly delightful when used in meals [1]. They are typically utilised as vegetables and spices due to their aromatic properties, enhancing the flavour of various types of soup (such as pepper soup) and other delectable dishes. The entire plant possesses numerous applications in traditional medicine, particularly in Africa and India [15]. The applications encompass the management of ringworms, gout, fungal infections, malaria, catarrh, aches, and colon pain. The juice extracted from compressing the leaf of this plant possesses medicinal properties that can effectively treat many gastrointestinal ailments, such as cholera, diarrhea, dysentery, vomiting, and convulsions [15].

According to [[2] The widespread utilization of indigenous plants as primary therapeutic agents according to their pharmacological attributes is prevalent [4]. In many parts of the world, especially in West Africa, plant extracts are still widely used in the treatment of malaria and other ailments. Every plant is medicinal based on the contents of its phytochemicals; hence, pharmaceutical industries rely heavily on their therapeutic purpose to be used as precursors for drug synthesis [5].

phytochemical screening is a method used to identify the therapeutic properties of plant components by testing them on a chemical substance that has a specific physiological effect on the human body. The primary bioactive compounds found in plants include alkaloids, flavonoids, tannins, saponins, glycosides, cardenolides, bufadienolides, and polyphenolic compounds. Understanding the chemical components of plants is important for two reasons. Firstly, it aids in the identification of medicinal compounds. Secondly, it helps in the exploration of new sources of economically valuable materials such as tannins, oils, gum, and precursors for the synthesis of complex chemical substances.

SCIENTIFIC CLASSIFICATION

- Kingdom: - Plantae
- Subkingdom: - Tracheobionta
- Superdivision - Spermatophyta
- Divisio: - Magnoliophyta
- Class: -Magnoliopsida
- Subclass: -Rosidae
- Order: -Sapindales
- Family: -Anacardiaceae
- Genus: -Mangifera
- Species: -*M.Indica*

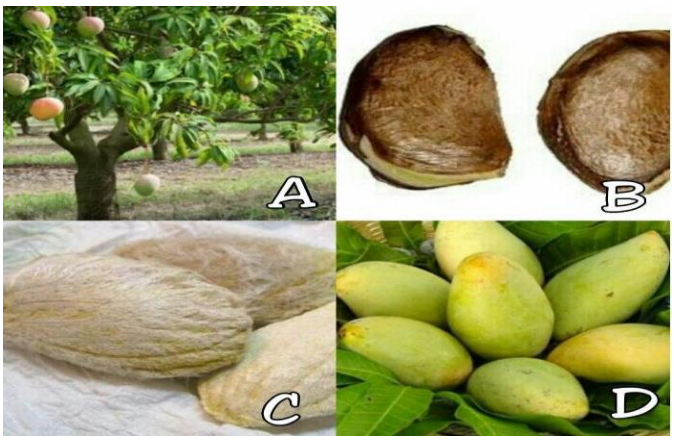

Plate 1: (A) Whole plant of mango, (B) Seed kernel, (C) Seed, (D) Fruits of mango

Botanical Description of *Vernonia amygdalina* (Bitter Leaf)

The plant is taxonomically classed as a member of the Kingdom Plantae. The plant belongs to the order Asterales, family Asteraceae, genus Vernonia, and species V. amygdalina. It is classified as an angiosperm. The complete binomial nomenclature for this organism is Vernonia amygdalina.

Table 1: Botanical Description of ***Vernonia amygdalina***

Scientific classification	
Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Asterales
Family:	Asteraceae
Genus:	Vernonia
Species:	V. amygdalina
Binomial name	
Vernonia amygdalina	

Plate 2: Bitter leaves *(Vernonia amygdalina)*

3. Experimental Method

Mangifera indica (Mango) and *Vernonia amygdalina* (bitter leaf) leaves were collected from the same stands of bitter leaf tree in Unguwar Madawaki Quarters Katsina, Katsina State-Nigeria. The reagents used includes: deionized water, ethyl acetate, ethanol, chloroform,1% sodium hydroxide (NaOH), dilute 1% hydrochloric acid, Mayer's reagent (potassium iodide), concentrated H_2SO_4, 1% lead acetate, ferric chloride ($FeCl_3$) solution and All chemicals and reagents are analytical reagent (AR) grade. equipment used includes conical flask, beaker, measuring cylinder, stirrer, foil paper, filter paper, evaporating dish, Petri dish, Siring

3.1 Collection and Identification of Plant Materials

The plants were obtained in the Katsina metropolis and their authenticity was verified by a botanist from the Department of Biology at Umaru Musa Yar'adua University in Katsina, Katsina State, Nigeria. The collected leaves were dried naturally in the shade. The dried leaves were subsequently pulverized into a fine powder to facilitate the extraction of active chemicals [3].

3.2 Sample Preparation of Leaf Extracts

Leaves were collected from the same stands of bitter leaf and mango trees in one. The leaves of Vernonia amygdalina were cleansed with water to eliminate any dust and grime and thereafter dried naturally in the shade by laying them out on a table for a number of days. Then, it was crushed and ground to a mesh size of 2 mm and soaked in 95% ethanol for 72 hours. The bottles underwent shaking at regular intervals and were then filtered using Whatman filter paper. The resulting filtrate was then evaporated using a rotary evaporator to obtain the crude extract.

3.3 Extraction

25 grams of dried plant leaf powder for each Vernonia amygdalina and *Mangifera indica* were individually placed into separate glass containers. The powdered leaves were progressively extracted with 250 ml of Ethanol each. A total of 500ml of water was utilized. The extraction process utilized percolation for a duration of 3 days (72 hours), during which the bottles were periodically shaken. The extracts underwent filtration using the Whatman No. 1 filter paper. Subsequently, each filtrate was concentrated through the entire evaporation of the solvent using a rotary evaporator. The resulting concentrated filtrate was then meticulously labelled for subsequent analysis [8].

3.4 Phytochemical Methods

Qualitative Analysis of Phytochemical Compounds

Phytochemical tests for alkaloids, terpenes, steroids, saponins, phenolics, and Flavonoids were carried out and fractions by standard methods. The methods were based on those reported [9]

3.4.1 Flavonoid Testing

Alkaline Reagent Test: A portion of the unrefined extract was subjected to the addition of a little amount of 1% sodium hydroxide (NaOH). The occurrence of a yellow hue signifies the existence of flavonoids.

3.4.2 Alkaloid Testing

The undiluted extract was dissolved in a solution of 1% hydrochloric acid and subsequently subjected to filtration.

Mayer's Test: 3 ml of the crude extract filtrate was mixed with a small amount of potassium iodide (Mayer's reagent). The occurrence of a white or pale-yellow solid indicates the existence of alkaloids.

3.4.3 Terpenoid Testing

The 2 ml crude extract was combined with 2 ml of chloroform, followed by the cautious addition of 1 ml of concentrated H_2SO_4 to create a distinct layer. The occurrence of a reddish-brown hue near the boundary signifies the existence of terpenoids.

3.4.4 Steroids Testing

Salkowski Tests: Here 5 ml of chloroform was added to 5 ml of the crude extract in a test tube, and this was followed by 5 ml of concentrated sulphuric acid added by the side of the test tube. The formation of a red color on standing is indicative of the presence of steroids.

3.4.5 Saponins Testing

Froth Test: A portion of the crude extract was diluted to 5 ml with distilled water, and then shaken in a graduated cylinder for about 5 minutes. The formation of honeycomb froth indicates the presence of Saponins.

3.4.6 Tannin Testing

Lead Acetate Test: In this procedure, 2 ml of the filtrate was combined with 3 drops of a 1% lead acetate solution in a test tube. The occurrence of a blue-black hue is a clear indication of the existence of tannins.

3.4.7 Phenol Testing

A fraction of the crude extract was subjected to treatment with 3 drops of ferric chloride ($FeCl_3$) solution. The occurrence of a cyan hue signifies the existence of phenolic compounds.

3.5 Antibacterial Method

Table 2: The following table presents list of microbes used in the evaluation of the antibacterial activity of the leaf extracts.

S/NO.	DESCRIPTION	TYPE
1.	Staphylococcus aureus	+
2.	Salmonella typhi	-
3.	Pseudomonas aeruginosa	-

3.5.1 METHOD:

Serial Dilution

0.25g of the ethanol extract was measured with a weighing balance and transferred into a sterile and clean tube containing 1ml of distilled water to obtain a concentration of 250mg/ml, followed by transferring into another containing 0.5ml to obtain 125 mg/ml, 62.5 mg/ml, and 31.25mg/ml, and stored for further analysis.

The procedure is repeated with aqueous extract.

Discs preparation

The filter paper was punched using a puncher dispensed into each concentration and allowed to be absorbed for 1 hour.

Sensitivity test using disc diffusion method

Twenty grammes of MHA were produced, and 0.2 ml of each bacterium was transferred to the solidified Mueller Hinton agar. The dish remained on the bench set. Discs with varying concentrations of 250mg/ml, 125mg/ml, 62.5 mg/ml, and 31.25 mg/ml were seeded and Ciprofloxacin was used as a control. The plate was then placed in an incubator set at 37 degrees Celsius for 24 hours. The diameter of the zone of inhibition was then measured and documented [15].

Determination of Minimum Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined using four containers per test organism. In brief, 1g of the ethanol extract was diluted in 2 ml of distilled water to reach a concentration of 250 mg/ml. Serial dilution was carried out to obtain a concentration of 125mg/ml, 62.5mg/ml, and 31.25mg/ml, respectively. 0.25ml were transferred into containers containing 0.25ml of nutrient broth each. The containers were then inoculated with the required amount of standardized test organisms. Another container containing 0.25ml of nutrient broth and 0.25ml of ciprofloxacin solution was inoculated with the required amount of the test organism, which served as a positive control. Finally, all the tubes were placed in an incubator set at a temperature of 37 degrees Celsius for a duration of 24 hours. the lowest concentration showing visual inhibition of growth is the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was obtained by sub-culturing the minimum inhibitory tubes that did not grow on the nutrient broth on the surface of freshly produced nutrient agar. This was followed by a 24-hour incubation at 370 degrees Celsius. The minimum bactericidal concentration (MBC) is the lowest concentration at which no growth occurs.

4. Results and Discussions

4.1 Physical Properties of the Extracts

Table 3: The following table presents the physical properties of *Mangifera indica (*Mango) extracts

Solvent	Colour	Odor	Texture	Quantity (gram)
Ethanol	Dark green	Chemicals	Slightly sticky	25g
Aqueous	Reddish brown	Odorless	Slightly sticky	25g

Table 4: The following table presents the physical properties of *Vernonia amygdalina (*bitter leaf) extracts

Solvent	Colour	Odor	Texture	Quant.(gram)
Ethanol	Dirty green	Chemicals	Slightly sticky	25g
Aqueous	Light brown	----	Gummy/sticky	25g

4.2 Qualitative Analysis

A Qualitative examination was conducted to determine the presence of several phytochemical substances in the leaves. The following part presents the utilized methodology and the outcomes of the experimental findings.

KEY

EE =Ethanolic Extract AE = Aqueous Extract
VA= *Vernonia amygdalina* M.I = *Mangifera indica*
+ = Present - =Absent

Table 5: The following table presents comparative analysis of *Vernonia amygdalina* and *Mangifera indica* phytochemical results for ethanolic extracts

Phytochemicals	Flav	Sap	Tan	Alk	Ter	Gl.	St	Ph
V.A	+	+	+	-	+	+	-	+
M.I	+	-	+	+	-	+	+	+

Table 5 displays the phytochemical components found in the extracts of Vernonia amygdalina leaves obtained by both ethanolic and aqueous extraction methods. Flavonoids, Saponins, tannins, terpenoids, and phenols were all present in both solvent extractors employed, aqueous and ethanol extracts, while alkaloids and steroids are only present in the aqueous extract and absent in ethanolic extract. Furthermore, a glycoside is present in ethanolic extract and found absent in aqueous extract.

Table 6: The following table presents comparative analysis of *Vernonia amygdalina* and *Mangifera indica* phytochemical results for aqueous extracts

Phyt.	Flav.	Sap.	Tan.	Alk.	Terp.	Gly.	Ste.	Phe.
V.A	+	-	+	+	+	-	+	+
M.I	+	+	+	+	-	+	+	+

Table 6 displays the phytochemical substances found in the extracts of *Mangifera indica* leaf for both solvent extractors, i.e. ethanolic extraction and aqueous extraction. Flavonoids, tannins, alkaloids, steroids, and phenols were all present in the extracts (both aqueous and ethanol extracts), while saponins and glycoside were present in aqueous extract and found absent in ethanol extract. Also, terpenoids are absent in aqueous extract and found to be present in ethanolic extract.

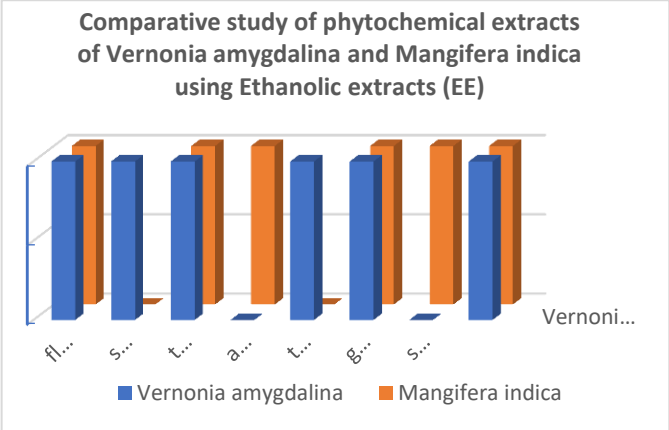

Figure 1: Comparative study of phytochemical extracts of Vernonia amygdalina and Mangifera indica using Ethanolic extracts (EE)

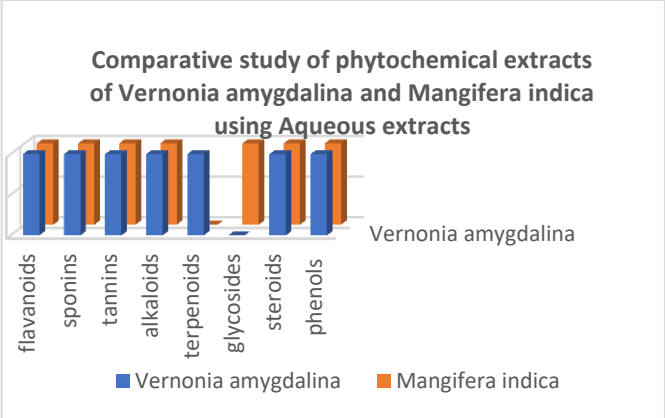

Figure 2: Comparative study of phytochemical extracts of Vernonia amygdalina and Mangifera indica using Aqueous extracts

4.3 Antimicrobial Analysis

Table 7: The following table presents the antibacterial activity of *Vernonia amygdalina* leaves extract against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*

Antibacterial activity of *Vernonia amygdalina* leaves extract against *Staphylococcus*					
Extract	**250mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.25 mg/ml**	**Control**
Ethanol	18	15	13	ND	29
Aqueous	12	9	ND	ND	28
Antibacterial activity of *Vernonia amygdalina* leaves extract against *Salmonella typhi*					
Ethanol	16	10	9	ND	25
Aqueous	11	ND	ND	ND	27
Antibacterial activity of *Mangifera indica leaves extract* against *Pseudomonas aeruginosa*					
Ethanol	14	12	7	ND	29
Aqueous	11	9	ND	ND	32

Key
ND = Not detected

Table 8: The following table presents the Minimum Inhibitory Concentration (MIC) of *Vernonia amygdalina and Mangifera indica* against Bacterial Isolates.

Bacterial Isolates	**100 mg/ml**	**50 mg/ml**	**25mg/ml**	**12.5 mg/ml**	**CONTROL (Ciprofloxacin)**
***Vernonia amygdalina* extracts**					
Staphylococcus aureus.	+	+	+	-	+
Salmonella typhi.	+	-	-	-	+
Pseudomonas aeruginosa	+	+	-	-	+
***Mangifera indica* extracts**					
Staphylococcus aureus.	+	+	-	-	+
Salmonella typhi.	+	-	-	-	+
Pseudomonas aeruginosa	+	+	-	-	+

Key
- = Absence of turbidity (Clear)
+ = Presence of turbidity (growth)

Table 9: The following table presents Minimum Bactericidal Concentration (MBC) of against Bacterial Isolates.

Bacterial Isolates	**100 mg/ml**	**50 mg/ml**	**25mg/ml**	**12.5 mg/ml**	**CONTROL**
Vernonia amygdalina extract					
Ciprofloxacin					
Staphylococcus aureus.	+	+	+	-	+
Salmonella typhi	+	-	-	-	+
Pseudomonas aeruginosa	+	+	-	-	+
***Mangifera indica* Extract**					
Staphylococcus aureus.	+	+	-	-	+
Salmonella typhi	+	-	-	-	+
Pseudomonas aeruginosa	+	-	-	-	+

Key
- = Absence of turbidity (clear)
+ = Presence of turbidity (growth)

Discussion

The result of the antibacterial activity of *Mangifera indica* and *Vernonia amygdalina* leaf extract against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa (Table 7) Shows the highest zone of inhibition was observed in *Vernonia amygdalina* ethanolic extract with 18.0mm (250mg/ml) for Staphylococcus. This is followed by ethanolic extract against Salmonella typhi with 16.0mm (250mg/ml). It is important to note that the lowest concentration of 31.25 mg/ml had minimal or no effect on the test bacteria. Therefore, the bioactivity of the extracts might be ranked in the following order: Ethanolic extract is obtained by combining the combined extract, followed by obtaining the aqueous extract. The susceptibility pattern of the examined

bacteria to the extract follows the hierarchy: Staphylococcus aureus is the most susceptible, followed by Pseudomonas aeruginosa, and then Salmonella typhi. [12] A study demonstrated that extracts from two plants, Mangifera indica and Citrus aurantifolia, significantly inhibited the growth of Staphylococcus albus, Pseudomonas aeruginosa, Aspergillus terreus, Aspergillus niger, and Penicillium oxalicum. In their study. [15] found that various doses of methanolic extracts from *Mangifera indica* leaves showed antibacterial effects against all tested bacterium isolates, including Bacillus cereus, Bacillus subtilis, Escherichia coli, and Salmonella typhi.

The findings of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against bacterial isolates, as presented in Tables 8 and 9, demonstrate that both *Mangifera indica* and *Vernonia amygdalina* extracts have MBC range of 100mg/ml to 12.5 mg/m and an MBC range of 100mg/ml to 12.5 mg/m for *Mangifera indica* and Vernonia amygdalina extracts, respectively. The MIC and MBC ranges of the common antibiotic, Ciprofloxacin, were identical. Based on the results of this investigation, it can be concluded that Vernonia amygdalina exhibited the lowest minimum inhibitory concentration (MIC) values compared to the other extracts while the extracts of Vernonia amygdalina also exhibited the lowest minimum bactericidal concentration (MBC) values.

5. Conclusion and Future Scope

1.Based on the experimental results, it can be concluded that ethanol and water are good solvent extractors of phytochemical compounds using standard procedures and also have varying abilities to liberate active compounds present in the studied plants. This implies that ethanol, being an organic solvent, is a highly effective molecule for releasing phytochemical compounds.

2. The phytochemical result of this research work showed that the plants under study *Vernonia amygdalina* (Bitter leaf) and *Mangifera indica* (Mango), possess several phytochemical substances such as alkaloids, steroids, flavonoids, tannins, terpenoids, glycoside, and phenols which are accountable for their diverse functions and may also contribute to pharmaceutical advantages, these selected plants contain substantial phytochemicals which are helpful in the prevention of some deadly diseases, use to preserve foods, to provide color and flavors and to make dietary supplements.

3.The study discovered that both Mangifera indica and Vernonia extracts had antimicrobial activity against various strains, with varying sensitivity to Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. Vernonia amygdalina ethanolic extract had the largest zone of inhibition against Staphylococcus at 18.0mm (250mg/ml), followed by ethanolic extract against Salmonella typhi at 16.0mm (250mg/ml). The lowest dose, 31.25 mg/ml, showed little to no effect on the test bacterium.

4.The findings of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against bacterial isolates showed that Vernonia amygdalina exhibited the lowest minimum inhibitory concentration (MIC) values compared to the other extracts while the extracts of Vernonia amygdalina also exhibited the lowest minimum bactericidal concentration (MBC) values. Therefore, the bioactivity of the extracts might be ranked in the following order: Ethanolic extract followed by aqueous extract. The susceptibility pattern of the examined bacteria to the extract follows the hierarchy: Staphylococcus aureus is the most susceptible, followed by Pseudomonas aeruginosa, and then Salmonella typhi.

The outcomes of this study were particularly fascinating because, in the conventional way of treating a bacterial infection, a decoction of plant components or boiling the plant in water is used. In contrast, the current study found that making an extract using both an organic solvent (ethanol) and an aqueous solvent resulted in higher phytochemical and antibacterial activity.

Future Scope

The next goal of this research is to investigate the molecular mechanisms behind the antibacterial effects of the phytochemicals discovered in the extracts. Furthermore, it is recommended (i)for incorporating these plant extracts into herbal formulations or combination therapy with conventional antibiotics to combat bacterial resistance. (ii) Expanding the study to include a broader range of bacterial strains and fungi could help to better understand their antibacterial properties. (iii) Detailed studies on toxicity and appropriate dosage are critical for guaranteeing safety in human and animal applications. (iv) Furthermore, integrating these extracts into nanomaterials may improve their transport, stability, and bioavailability for therapeutic applications.

Data Availability

Due to technological and time constraints, raw data required for an ongoing investigation cannot be shared.

Conflict of Interest

The authors do not have any potential conflicts of interest to disclose.

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The authors get no financial assistance from any agency or institution for their research.

Authors' Contributions

The author Auwal Bello carried out the research drafted the manuscript. Faisal Sunusi Aliyu help in drafting and reviewing the procedure/methodology to carried out the research, Mohammed Sani Galadima supervised the research and guided the researchers.

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