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Phytochemical analysis, antimicrobial and antioxidant activities of leaf extract of *Vernonia tigna* Klatt (Asteraceae)

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Abstract

The aim of this study was to determine the phytochemical composition, antimicrobial and antioxidant activities of *Vernonia tigna* leaf extracts used in folk medicine and to validate its therapeutic claim. Powdered plant material from *Vernonia tigna* was extracted by microwave-assisted extraction using n-hexane, chloroform, ethyl acetate and methanol and subjected to phytochemical screening using standard methods. The antimicrobial activity was determined by agar diffusion method as outlined by the NCCLS. The Free radical scavenging activity was determined by the DPPH method. The phytochemical screening of the crude extract revealed the presence of tannins, saponins, flavonoids, and alkaloids. The antimicrobial activity was determined by minimum inhibitory concentration (MIC) against four bacterial strains (*S. aureus, B. subtilis, E. coli and P. aeruginosa*). The result showed moderate inhibition against bacterial strains. The values of the minimum inhibitory concentration (MIC) ranged from 1.25 – 3.125 mg/mL for all the organisms studied. The radical scavenging activity of the extracts showed significant inhibition at different concentrations of 50 – 250 µg/mL. All the microbes were inhibited to varying degrees. The results of this study give credence to the ethno-medicinal use of *Vernonia tigna.*

Keywords: *Vernonia tigna*; Microwave-assisted extraction; Phytochemical screening; Antimicrobial activity; DPPH method

1. Introduction

Throughout the history of medicine, many plants have been used by traditional medicine practitioners as a primary source to treat diseases. Due to the abundance of active chemical principles present in these plants and the remarkable medicinal properties of these plants, they have been used extensively in traditional medicine, especially in traditional systems of medicine such as Ayurveda, Siddha and Unani (1,2). There have been many threats to human existence, and currently, reducing the effectiveness of antibiotics poses a new challenge for our world.

Ever since the discovery of antibiotics, there has been an ingrained belief in the eradication of infectious diseases through the use of antibiotics and the intensive development of new classes of antibiotics, but unfortunately the overuse of antibiotics has led to multidrug resistance in several classes of microbial strains (3). Negative effects of available antibiotics and the constant development of bacterial resistance motivate the search for new antimicrobial agent (4).

Several studies have been conducted of the Asteraceae family demonstrating medicinal properties, such as species of the genus *Vernonia*, which are rich in bioactive substances like sesquiterpene lactones, flavonoids, tannins and steroids (5). Phenolic compounds are the most abundant secondary metabolites in plants, showing a wide range of distinct biological activities, have received more and more attention in recent years (6).

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The genus consists morphologically of annuals, herbaceous perennials, lianas, shrubs, and trees. The genus *Vernonia* is known to have several species with food, medicinal and industrial uses. For example *Vernonia amygdalina* and *Vernonia colorata* are eaten as leafy vegetables [7], [33]. *Vernonia amygdalina* is the most studied member of the *Vernonia* genus as well as one of the most studied plants in Africa [8].

The medicinal value of some plants lies in some chemical substances that produce certain physiological effects in the human body. Examples of these key bioactive components are alkaloids, tannins, flavonoids and phenolic compound [9]. The phytochemical profile of various *Vernonia* species have revealed numerous phytochemicals [10]–[12].

The antimicrobial properties of many plants have been studied by a number of researchers around the world [13], [14]. Chondrillasterol isolated from *V. adoensis* has shown antibacterial properties against *S. aureus, K. pneumoniae and P. aeruginosa*. Chondrillasterol inhibited biofilm formation in *P. aeruginosa* as well as disrupted already formed mature biofilms [15].

Antioxidant properties of medicinal plants have been investigated. Due to increasing safety concerns associated with the consumption of synthetic antioxidants, the use of cheaper and safer sources of antioxidants of natural origin, and in particular from plants, are of interest today. The main plant compounds characterized by antioxidant activity are polyphenols [16]. Therefore, plant antioxidants are very effective in preventing the destructive processes caused by oxidative stress [17].

Vernonia tigna belongs to the family Asteraceae, the plant is an upright subshrub that can reach 1.8m in height. It has a woody rootstock. The stems are sparsely branched and leafy. The flowers are moderately dense, terminal on branches; capitate usually 13 mm long [18], [31].

Figure 1 Image of *Vernonia tigna*

In traditional medicine, *Vernonia tigna* is used to treat fever, menstrual irregularities and arthritis and the leave decoction is taken to treat malari**a.** It is also traditionally recommended as a powerful aphrodisiac and as an abortifacient. The leaves are used against hookworm infection of the small intestine [19], [20]. There was no report on pharmacological or phytochemical study on this *Vernonia* specie; therefore, this study was designed in order to evaluate the phytochemical as well as antibacterial properties of the plant to validate its therapeutic properties in ethno-medicinal practices.

2. Material and methods

2.1. Reagents and Chemicals

The reagents and chemicals used in this study; chloroform, ethyl acetate, n-hexane methanol, 1,1-diphenyl-2-pycrylhydrazyl, ascorbic acid and other reagents were of analytical grade.

2.2. Collection and preparation of Plant material

Aerial part of *Vernonia tigna* used in this study was collected from Benue State Nigeria. The plant was authenticated at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State. The fresh leaves were washed and allowed to dry at room temperature for a period of about two weeks. The dried leaves were reduced to fine coarse powder particles using an electronic blender. The pulverized plant material was kept sealed until when required.

2.3. Extraction

Microwave Assisted Extraction (MAE) was performed using household multi-mode microwave oven (Panasonic NN-SD681) at 900 W. Exactly 250 g of aerial parts in Mason jar was soaked in separate in n-hexane, ethyl acetate, chloroform and methanol. Each mixture of solvent and sample was extracted for 5 minutes using an irradiation cycle of 20 seconds intervals. The solvent in the Mason jar was evaporated and cooled by a condenser. After filtration, each sample was concentrated in a rotary evaporator at 38–40 °C [32].

2.4. Phytochemical screening

Preliminary phytochemical screening of the powdered leaves of *Vernonia tigna* for the detection of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, glycosides, anthraquinones, cardiac glycosides, steroids and triterpenes was carried out according to standard laboratory procedures reported by [21] –[23], [29], [30].

2.4.1. Test for Alkaloids

To about 0.5 g of each extract, 1% diluted HCl (20 mL) was added in a conical flask, heated on a steam bath and then filtered. The filtrate was made alkaline with 28% NH_3 solution and then extracted with chloroform (3 - 5 cm^3). The combined $CHCl_3$ extracts were concentrated and treated with equal volume of 1% HCl. Dragendorff's reagents (2 mL) were added and the appearance of an orange-red precipitate indicated the presence of alkaloids.

2.4.2. Test for Anthraquinones

A small portion of the extract was shaken with 10 mL of benzene and filtered. 5 mL of 10% NH_3 solution was added to the filtrate and stirred. The production of a pink-red or violet colour indicates the presence of free anthraquinones.

2.4.3. Test for Flavonoids

About 0.5 g of the extract was dissolved in 1.5 mL of 50% methanol and warmed on steam bath. Metallic magnesium and 5 drops of concentrated hydrochloric acid were added. A red or orange color indicates the presence of flavonoids.

2.4.4. Test for Glycoside

To about 0.5 g of the extract, 5 mL of concentrated H_2SO_4 was added and boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Three drops of ferric chloride solution was added to one of the portions, and a green to black precipitate indicated phenolic aglycone as a result of hydrolysis of glycoside.

2.4.5. Test for Saponins

A small amount of the extract was dissolved in 10 mL of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A honeycomb froth formed for more than 30 min indicates saponins.

2.4.6. Test for Steroids and triterpenes

An equal volume of acetic anhydride was added to the extract. 1 mL of concentrated H_2SO_4 was added downside the tube and the colour change was observed immediately and later. Red, pink or purple colour indicates the presence of triterpenes while blue or blue-green indicates steroids.

2.4.7. Test for Tannins

About 0.5 mL of extract was dissolved in 10 mL of distilled water and then filtered. Few drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicates hydrolysable tannins and green precipitate indicates the presence of condensed tannins.

2.5. Test organisms

The antibacterial activity of the various crude extracts of *Vernonia tigna* was determined using a strain of clinical bacterial isolates. The test microorganisms used for the study were;

- *Staphylococcus aureus*
- *Bacillus subtilis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

All these samples were obtained from Medical Microbiology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria. They were sub-cultured on sterile broth and incubated at 37°C for 24 hours.

2.6. Determination of Antibacterial Activity

Antibacterial and fungal screening was performed using the agar-well diffusion method as described by (20) with slight modifications. The cultures of the bacterial isolates were streaked onto Mueller Hinton Agar and Sabouraud Dextrose Agar plates, respectively, using sterile swab sticks to prepare inocula of 106 CFU/ml. The medium was inoculated with standard inoculum (0.1 ml) of the microorganisms (Mc-Farland 0.5). The inocula were spread evenly over the surface of the media using a sterile swab. The inoculated media were allowed to dry in the incubator at 37°C for 30 minutes. A standard cork borer (6 mm) was used to cut a well in the center of each inoculated medium and the solution of the extracts (100 mg/ml, 50 mg/mL, 25 mg/ml and 12.5 mg/mL) was then inserted into each hole on the surface of the medium. Sterile water (0.1 ml) was introduced into one well to serve as a negative control. The medium was incubated at 37°C for 24 hours, after which the plates were examined for zones of inhibition. Zones of inhibition were measured with a transparent ruler and the results recorded.

2.7. Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined using micro-broth dilution methods as described by NCCLS [34]. Dilutions (19 mg/ml) of extract concentrations and fractions showing sensitivity to the test organisms were prepared using test tubes containing 9 ml of double concentrated broth. The test tubes were inoculated with the suspension of the standardized inocula. These were incubated at 37°C for 24 h and observed for growth. The MIC of the extracts for each test organism was considered to be the lowest concentration that inhibited visible growth of the test organism.

2.8. Determination of antioxidant activity

The free radical scavenging activity of the extracts against the 2,2-diphenyl-1,1-picrylhydrazyl radical was determined. The scavenger activity was measured by a slightly modified method previously described by Mensor (21). The following concentrations of extracts were prepared, 50, 100, 150, 200 and 250 µg/mL in methanol. Ascorbic acid was used as standard. Using the same concentration, each extract was placed in a test tube, and 1 mL of a 0.3 mM methanol solution of DPPH was added and allowed to react at room temperature. After 30 minutes the absorbance was measured at 517 nm and the scavenger activity was calculated using the following formula;

$$\text{Radical Scavenging Activity (\%)} = \frac{A0 - A1}{A0} \times 100$$

where

A_0 = the absorbance of the control
A_1 = the absorbance of the sample

3. Results

Phytochemical analyses carried out on the plant extracts revealed the presence of components known to have both medicinal and physiological activities [20], [29]. The presences of phytochemicals in all the four extracts were observed (Table 1). The result of antimicrobial screening is presented in Table 2 and 3.

Table 1 The preliminary phytochemical screening of the extracts of *V. tigna*

Phytochemicals	Leaf extracts of *Vernonia tigna*			
	Hexane extract	**Chloroform extract**	**Ethyl acetate Extract**	**Methanol extract**
Alkaloids	-	+	+	+
Anthraquinones	-	-	-	-
Flavonoids	-	+	+	+
Glycosides	-	-	-	-
Saponins	-	+	-	+
Steroids	+	+	+	+
Triterpenes	+	+	+	+
Tannins	-	-	-	-

Key: (+) = presence of phytochemical (-) = absence of phytochemical

Table 2 Zone of inhibition of extracts of *V. tigna*

Test Organism	Diameter of zones of inhibition (mm)			
	Hexane extract	**Chloroform extract**	**Ethyl acetate Extract**	**Methanol extract**
Staphylococcus aureus	ND	14	18	20
Bacillus subtilis	ND	14	15	18
Escherichia coli	ND	16	20	16
Pseudomonas aeruginosa	ND	15	18	22

Key: ND = Not determined

Table 3 Minimum inhibitory concentration (MIC) of the extracts of *V. tigna* (mg/mL)

Test organism	MIC (mg/mL)			
	Hexane extract	**Chloroform extract**	**Ethyl acetate extract**	**Methanol extract**
Staphylococcus aureus	NA	1.25	2.5	3.125
Bacillus subtilis	NA	1.25	1.25	6.25
Escherichia coli	NA	2.50	2.0	2.5
Pseudomonas aeruginosa	NA	1.25	1.25	2.5

Key: NA = No Activity

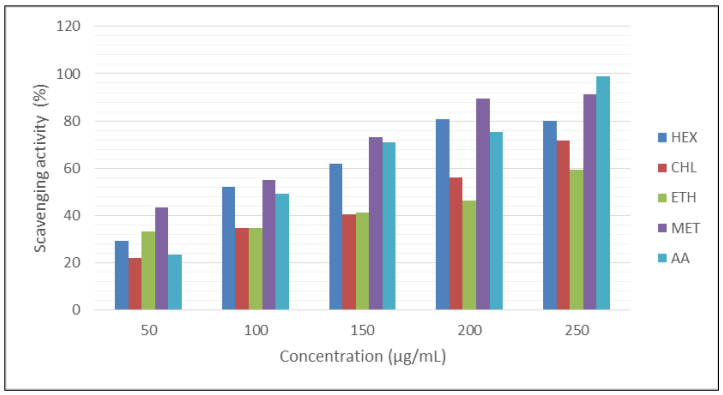

Figure 2 Inhibition DPPH Scavenging activity of different extracts of *Vernonia tigna*

4. Discussion

This study was conducted to evaluate the phytochemicals, antimicrobial activity and antioxidant of the leaf extract of *Vernonia tigna*.

The phytochemicals revealed from the various extracts of *Vernonia tigna* includes; alkaloids, flavonoids, saponins, triterpenes and steroids as shown in Table 1. It was observed that anthraquinones, glycosides and tannins were absent in all the four extracts of *V. tigna.* Currently, the search for phytochemicals has increased due to their potential use in the therapy of various chronic and infectious diseases. It has been reported that flavonoids, alkaloids and saponins are responsible for the action against rheumatoid arthritis (22,23).

The bioactive components present in the plants such as alkaloids, saponins, tannins, anthraquinones, steroids, flavonoids etc. have been reported (24,25). The result for the zone of inhibition (Table 2) against the test organisms ranges between 14 – 22 mm. The methanol extract was found to possess the highest inhibition activities, which ranges between 16 – 22 mm against all the test organisms. Hexane extract shows no inhibitory activity. The minimum inhibitory concentration (MIC) tests of the leaf extract on the organisms, as shown in Table 3 revealed that all extracts shows activity in the range between 1.25- 3.125 mg/mL except in n-hexane extract. This present study results are consistent with the work of (26,27) who also reported antimicrobial activities of different plants extract.

The antioxidant activity through the radical scavenging activity of the DPPH method of the different leaf extracts of *Vernonia tigna* was determined and compared with ascorbic acid as a standard. All extracts showed different levels of DPPH scavenger activity over the concentration range of 50, 100, 150, 200 and 250 mg/mL, respectively, as shown in (Figure 2). Methanol extract showed the strongest DPPH radical scavenging activity compared to other extracts. The radical scavenging activity of the extracts were effective in the order of MET > ETH > CHL > HEX. The polar solvents; methanol and ethyl acetate have higher DPPH activity for scavenging free radicals compared to non-polar solvents; chloroform and hexane. The different scavenging activities of extracts may be due to the diverse chemical nature of different phytochemicals, which can react in unique ways with free radicals (28).

5. Conclusion

The phytochemical screening revealed the presence of important secondary metabolites. The crude leaf extracts were subjected to antimicrobial screening against some selected strains of gram-positive and gram-negative bacteria and showed significant activity at different concentrations. The antioxidant activity of the plant extract showed different scavenging activity at different concentrations. Therefore, this study showed that the leaves of *Vernonia tigna* have medicinal value in treating bacterial infections and could be a potential source of drug lead.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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