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Phytochemical and Antibacterial Investigation of Leaf Extracts of *Vernonia amygdalina*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AIY designed the study, wrote the first draft of the manuscript. Authors LS and AN managed the sample collection, laboratory analysis and literature review. All authors read and approved the final manuscript.

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ABSTRACT

Vernonia amygdalina was assessed for phytochemicals. The results showed that the leaf extract of the plant possessed the biologically active substances; cardiac glycosides, alkaloids, saponins and tannins. The presence of these bioactive constituents has been linked to the antimicrobial activity of the plant. The disc diffusion method was used to determine the antimimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli, Pseudomonas aeroginosae*, *Streptococcus species*, *Klebsialla pneumonia* and *Salmonella typhi*. Aqueous extract of the leaves showed antimicrobial activity against all the tested bacteria with the exception of *Proteus vulgaris* in the order of sensitivity as *Klebsialla pneumonia*> *Pseudomonas aeroginosae*> *Staphylococcus aureus*> *Salmonella typhi*> *Escherichia coli*> *Streptococcus species*. The leaves of *Vernonia amygdalina* can be used as a source of oral drugs to fight infections caused by susceptible bacteria.

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Keywords: Vernonia amygdalina; antimicrobial activity; phytochemical; alkaloids; saponins; tannins; cardiac glycosides.

1. INTRODUCTION

Medicinal plants are the backbone of traditional medicine [1]. Traditional medical practice has been known for centuries, in many parts of the world [2]. It has, however, been observed that these practices vary from one country to another [3]. The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but may be due to the combined action of different compounds originally in the plant [4].

Since the discovery of resistance to antibiotics by bacteria, attention was focused on finding new substances or alternatives that will have broad spectrum activity against bacteria [5]. Interest in natural products with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics [6]. There is an increased advocacy for the integration of traditional medicine in health care programme in Nigeria [7].

Many investigators have demonstrated the antimicrobial activity of the constituents of some plants [8 -12]. A number of chemical compounds of plant origin have been shown to possess antimicrobial activities [13].

Vernonia amygdalina belongs to the family Compositae; the family is the largest family of the flowering plants comprising 950 genera and about 23,000 species. The family is of cosmopolitan distributions covering almost all habitats [14,15]. Economically Compositae family is of considerable importance. They are used for food, as insectides, in medicinal preparation and as ornamentals [15].

In Nigeria, *Vernonia amygdalina* leaves are popularly employed daily in food and in the general traditional medicine whole or in combination with other herbs for the treatment of various diseases [5]. In the present study, *Vernonia amygdalina* leaves extract were screened for phytochemicals constituents and antibacterial activity.

2. MATERIALS AND METHODS

2.1 Sampling

Fresh samples of the leaves of *Vernonia amygdalina* were collected in August 2014 from Katsina town, Katsina state north western Nigeria. Identification was carried out in the Biological science Department, Faculty of Natural and Applied science, Umaru Musa Yar'adua University Katsina. The samples were air dried in the laboratory before powdering.

2.2 Test Microorganism

Clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeroginosae, Streptococcus species*, *Proteus vulgaris*, *Klebsialla pneumonia* and *Salmonella typhi* used in the study were obtained from the Microbiology Department, Federal Medical Centre Katsina.

2.3 Preparation of Plant extract

The aqueous extract of the leaves of *Vernonia amygdalina* was obtained using the hot water extraction technique in order to stimulate the local procedure as described by Akinloye and Olorede [16]. 400 g of the sample was soaked in 2 litres of distilled water and boiled for 5 minutes. This was shaken for 10 minutes and allowed to cool and filtered. The filtrate was evaporated to a residue in a drying cabinet.

2.4 Phytochemical Analysis of Extract

The methods described by Harbone [17] with slight modifications were used to test for the presence of the active ingredients in the test sample.

2.4.1 Test for tannins

5 g of extract was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for 5 minutes. 2 drops of 5 % $FeCl_3$ were then added. Production of greenish precipitate indicated the presence of Tannins.

2.4.2 Test for alkaloids

0.5 g of the extract was stirred with 5 ml of 10 % HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with 2 drops of Meyer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Meyer's reagent was regarded as evidence for the presence of alkaloids in the extract [17].

2.4.3 Test for saponins

0.5 g of the extract was introduced into a tube containing 5 ml of distilled water; the mixture was

vigorously shaken for 2 minutes. Formation of froth which persists on warming indicated the presence of Saponins.

2.4.4 Test for phlobatinnins

Extract of the sample was boiled with 1% aqueous HCl. Deposition of a red precipitate indicated the presence of Phlobatinnins [18].

2.4.5 Test for anthraquinones

5 g of the extract was shaken with 10 ml benzene and filtered, and then 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink colour in the ammoniacal (lower) phase indicated the presence of free anthraquinones.

2.4.6 Test for cardiac glycosides

5 g of the extract was dissolved in pyridine and 2 drops of 2% sodium nitroprusside together with 2 drops of 20% NaOH were added. A deep red colour which faded to a brownish yellow indicated the presence of cardiac glycosides.

2.5 Antimicrobial Assay

Antibacterial activity of the extract was determined by the disc diffusion method as described by Lennete [19]. The microorganisms were cultured overnight at 35°C in nutrient agar. Suspension of the bacteria with an optical density of McFarlend 0.5 was made in isotonic sodium chloride solution.

Petri dishes with 60 ml of sterile Miselle Hinton agar were seeded with appropriate bacterial suspension. Sterile, 6 mm diameter filter paper discs were impregnated with the extraction of different concentrations, gently tapped to remove excess liquid and positioned on seeded plates at 45^0 opposite each other into each petri dish, respectively. After incubation for 24 h at 35°C, the plates were observed for zones of inhibition and the diameter of these zones measured in millimeters.

3. RESULTS

The results of the phytochemical screening of *Vernonia amygdalina* leaf extract are shown in Tables 1 and 2, respectively. The phytochemical constituents of the extract included cardiac glycosides, alkaloids, tannins and saponins. Anthraquinones and phlobatinnins were not detectable at the tested assay condition.

Table 1. Phytochemical screening of *Vernonia amygdalina* leaves extract

Phytochemical group	Result
Alkaloids	Present
Anthraquinones	Absent
Cardiac glycosides	Present
Phlobatinnins	Absent
Saponins	Present
Tannins	Present

Determination of the inhibition zones by means of the disc diffusion method shows that the leaf extract exhibited an antibacterial effect against all the seven tested bacteria except *Proteus vulgaris*. *Klebsialla pneumonia* was sensitive at 80, 120, 140, 160 and 180 $mgml^{-1}$ of extract with 0.2, 0.3, 0.6, 0.8 and 1.0 mm zones of inhibition. While *Pseudomonas aeroginosae* was sensitive at 120, 140, 160 and 180 $mgml^{-1}$ of extract with 0.2, 0.2, 0.2 and 0.4 mm zones of inhibition. Also, *Staphylococcus aureus* was sensitive at 140, 160 and 180 $mgml^{-1}$ of extract with 0.2, 0.2 and 0.4 mm zones of inhibition. Meanwhile *Salmonella typhi* was sensitive at 140, 160 and 180 $mgml^{-1}$ of extract with 0.2, 0.2 and 0.4 mm zones of inhibition. While *Streptococcus species* was only sensitive at 160 and 180 $mgml^{-1}$ of extract with 0.2 and 0.2 mm zones of inhibition.

Table 2. Antibacterial activity of *Vernonia amygdalina* leaves extract

	Zone	of	inhibition	(mm)	
Test bacteria conc. of extract ($mgml^{-1}$)	80	120	140	160	180
Test bacteria					
Staphylococcus aureus	-	-	0.2	0.2	0.4
Proteus vulgaris	-	-	-	-	-
Escherichia coli	-	-	-	0.4	0.6
Pseudomonas aeroginosae	-	0.2	0.2	0.2	0.4
Streptococcus species	-	-	-	0.2	0.2
Klebsialla pneumonia	0.2	0.3	0.6	0.8	1.0
Salmonella typhi	-	-	0.2	0.2	0.4

Table 3. F- Test Two-sample for variances of test microorganisms

Test bacteria	Mean		Variance		Obs.		df		$F_{calculated}$	P_{value}	$F_{critical}$
	M_1	M_2	V_1	V_2	O_1	O_2	df_1	df_2			
Staphylococcus aureus	136	0.16	1480	0.028	5	5	4	4	452857.14	1.07E-09	6.388233
Escheria coli	136	0.16	1480	0.068	5	5	4	4	21764. 71	6.33E-09	6.388233
Pseudomonas aeroginosae	136	0.20	1480	0.02	5	5	4	4	74000	5.48E-10	6.388233
Streptococcus species	136	0.081	1480	0.012	5	5	4	4	123333.3	1.97E-10	6.388233
Klebsialla pneumonia	136	0.58	1480	0.112	5	5	4	4	13214.29	1.72E-08	6.388233
Salmonella typhi	136	0.16	1480	0.028	5	5	4	4	52857.14	1.07E-09	6.388233

M_1= Mean1; M_2= Mean2; V_1= Variance1; V_2=Variance2; O_1= observation1; O_2= Observation2; df= degrees of freedom

4. DISCUSSION

Phytochemical screening portrays that most of the natural products tested for were present in the leaf extract. In the present study, the aqueous leaf extract of *Vernonia amygdalina* tested positive for the presence of glycosides, alkaloids, saponins and tannins.

Glycosides serve as defence mechanisms against predation by many microorganisms, insects and herbivores [20]. Saponin has detergent properties and exhibits anti-inflammatory properties [8]. Alkaloid is a plant-derived compound that is toxic or physiologically active, contains nitrogen in a heterocyclic ring with complex structure and is of limited distribution in the plant kingdom. Alkaloids are formed as metabolic by-products and have been reported to be responsible for antibacterial activity [21]. The above illustrations may therefore explain the demonstration of antimicrobial activity of the aqueous leaf extract of *Vernonia amygdalina.*

The leaf extract exhibited increasing degree of antibacterial activities against the tested microorganisms in the following order of sensitivity; *Klebsialla pneumonia* > *Pseudomonas aeroginosae* > *Staphylococcus aureus*, *Salmonella typh*i > *Escherichia coli* > *Streptococcus species*. The inhibitory effect of the extract on the growth of these microorganisms could be attributed to the presence of some of the phytochemicals that were found present in the plant extract. The mechanism of action of these phytochemicals may be via lysing the cell, increasing permeability of the cell wall and membrane, inhibition of protein and DNA synthesis and or by inhibiting the transport of nutrient across cell wall or membrane [22].

To date, penicillins (ampicillin, amoxicillin, penicillin and cloxacillin) continue to be the most widely used antibiotics in developing countries. In Nigeria alone, resistance pattern studied against the penicillins showed that there was a resistance pattern to amoxicillin of about 64 % and an overall resistance to penicillin and ampicillin of 90-98 % [23]. Similarly, such resistance exists in Ethiopia of up to 87 % of penicillins [24]. The demonstration of antimicrobial activity against bacteria by this plant may provide the basis for future use of this plant in antibacterial formulations.

However, the statistical analysis (F-Test, $P<0.05$) showed that there is a significant difference between the mean concentration of leaf extract and the zone of inhibition (Table 3 above).

This observation confirmed that as the concentration of the leaf extract increases, the antimicrobial activity of the phytochemicals contained in the extract against bacteria increases as well.

Furthermore, the correlation analysis between the leaf extract concentration and bacterial zone of inhibition has revealed that there is a strong positive correlation between the two variables in all the test microorganisms (Table 4).

Table 4. Correlation between concentrations of extract (mg/ml) with zone of inhibition (mm) for Test microorganisms

Test bacteria	Correlation
Staphylococcus aureus	0.900986
Escheria coli	0.777516
Pseudomonas aeroginosae	0.919018
Streptococcus species	0.806784
Klebsialla pneumonia	0.963123
Salmonella typhi	0.900986

This is a clear indication that the leaves of *Vernonia amygdalina* can be an effective oral drug for curing infections caused by susceptible bacteria.

5. CONCLUSION

Aqueous leaf extract of *Vernonia amygdalina* produced antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosae, Streptococcus species, Klebsialla pneumonia and Salmonella typhi*. The extract contains the phytochemicals such as cardiac glycoside, alkaloids, saponins and tannins. This study observes that *Vernonia amygdalina* has useful antimicrobial properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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