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EVALUATION OF THE PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF *Vernonia amygdalina* AGAINST SELECTED CLINICAL ISOLATES.

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ABSTRACT

Phytochemical properties and antimicrobial activities of *Vernonia amygdalina* leaves extracted in cold water and ethanol against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were investigated using standard microbiological methods. Aqueous extracts showed higher antimicrobial activity against *Escherichia coli,* with a zone diameter of 1.2±0.05cm at extracts concentration of 12.5mg/ml, but the organism was resistant to the ethanolic extract, at all exact concentrations, *Staphylococcus aureus* and *Pseudomonas aeruginosa* recorded the same zone diameter of 6.0±0.05cm with extract concentration of 12.5mg/ml. The weight percent yield of extracts was higher in the aqueous form (50%) than the ethanolic extract (40%). The qualitative phytochemical composition of *V. amygdalina* revealed the presence of saponins, flavonoids, glycosides, alkaloids, tannins, terpenes, anthracenosides, reducing sugars, and phytosteroids. This study has shown that *Vernonia amygdalina* leaves had antimicrobial activity against some common medically important isolates. Their use as potential source of alternative antimicrobial medicine holds pharmacological potentials for antibiotic therapy.

Keywords: *Vernonia amygdalina,* Phytochemicals, antimicrobials.

1. INTRODUCTION

There has been a growing worldwide concern on the alarming increase in the rate of infections caused by antibiotic resistant microorganisms (Udobi and Onaolopa 2009). Antibiotics, though effective, have been known to induce resistance in bacteria, which has brought about increase in morbidity, mortality and healthcare cost due to bacterial infections (Takon *et al.*, 2013). As a result, there has been growing interest among researchers for natural plant products for new antimicrobial and antioxidants agents (Takon *et al.*, 2013). The efficacies and little or no known side effects and low cost of these plant products as well as their availability and ability make them a drug of choice to succeed where most synthetic or conventional agents have made little progress (Udobi and Onaolopa, 2009).

Vernonia amygdalina also known as bitter leaves (English), Olugbu (Igbo), Oriwo (Edo), etidot (Efik), and ewuro (Yoruba) is widely used in Nigeria for both therapeutic and nutritional purposes (Arhoghro *et al*; 2009, Oboh and Masodje, 2009). *Vernonia amygdalina* is indigenous to tropical Africa and could be wild or cultivated all over sub-Saharan Africa (Bonsi, et al; 1995, and Obo and Masodje 2009). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Aka and Okafor 1992). The macerated leaves are consumed as vegetables and condiments after washing to remove the bitterness, while the water extract serves as tonic for the prevention of certain illnesses (Bonsi *et al.*, 1995, and Arhoghro *et al.*, 2009). All parts of the plant are pharmacologically useful (Oboh and Masodje, 2009). The roots and leaves are used in phytomedicine to treat fever, hiccups, kidneys disease and stomach discomfort (Takon *et al.*, 2013). Also antihelmitic, antibacterial and antimalarial properties (Abosi and Raseroka, 2003), as well as anti-tumourigenic properties (Izevbigie *et al.*, 2008) have been associated with the plant extracts. Hypoglyceaemic and hypolipidaemic effects of the leaf extract in experimental animals have been reported, as such could be used in managing diabetes (Ulhayarasa *et al.,* 2010). This study is concerned with the phytochemical properties and antimicrobial activity of *Vernonia amygdalina* (bitter leaf) against selected clinical isolates.

2. MATERIALS AND METHODS

2.1 Collection of samples

Mature, healthy and fresh leaves of *Vernonia amygdalina* were obtained from Botanical garden, University of Calabar and identified by Mr. Frank Adepoje a plant taxonomist in the Botany Department, University of Calabar.

2.2 Reagents

All reagents used in the study were of analytical grade.

2.3 Plant extracts

The aqueous and ethanolic extracts of *V. amygdalina* were prepared based on the method described by Adeshina *et al*., (2010) and Awofisayo *et al*., (2010)

Fresh leaves were weighed to determine the initial weight, 1400g, later sun dried, pulverized and sieved. The loss in weight was recorded as moisture content (AOAC, 1984).

2.3.1 Preparation of aqueous extract

A 25g of ground leaves was dissolved in 250ml distilled water. The mixture was shaken and kept for 24hr before filtering through Whatman No 1 filter paper. The filtrates were evaporated to dryness in a water bath at 60^{o}C to obtain a solid mass and stored away, for antimicrobial activity tests.

2.3.2 Preparation of Ethanolic Extract

A 20g of ground leaves of *V. amygdalina* was measured into 200ml of 95% ethanol in a conical flask and mixed properly and later filtered using Whatman No 1 filter paper. It was evaporated to dryness to remove the ethanol and dried extract stored away for antimicrobial activity tests.

2.2.3 Preparation of sensitivity discs

The discs were prepared according to the method described by Adeshina *et al*., (2010). Sterile discs of 6mm diameter were punched from Whatman No 1 filter paper. These discs were placed in McCartney bottles and sterilized by autoclaving at 120^{o}C from 30min. The sterile blank discs where soaked in 2.0ml of the extracts at varying concentrations per milliliter (10.0mg/ml, 5.0mg/ml, 2.5mg/ml, 1.3mg/ml and 0.6mg/ml) per disc in sterile petridishes for 6hrs for proper absorption and later allowed to dry.

2.3.4 Standardization of known clinical isolates

Test organisms were obtained from the University of Calabar Teaching Hospital (UCTH), Nigeria. These were Bacillus subtilis, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeniginosa*. Inoculun of pure culture of each test organism on solid agar plate was transferred to sterile nutrient broth and incubated at 37^{o}C for 18h for growth and later used for antimicrobial test.

Antimicrobial sensitivity screening of extracts was carried out using the agar disc diffusion method (Ulhayarasa et al, 2010). Mueller Hinton Agar plates were used. A 0.1ml aliquot each standardized culture was inoculated on the plates and spread evenly. Their disc impregnated with extracts of different concentrations were placed on the agar surface and incubated at 37^{o}C for 24hrs. After incubation, zones of inhibition were observed and zone diameters measured. Ethanol and distilled water served as control.

2.3.5 Phytochemical analysis of extracts

The different extracts were analysed qualitatively for the presence of certain phytochemicals, such as alkaloids, cyanogenic glycosides, saponins, flavonoids, tannin, anthracenosides, polyphenoids, terpenoids, reducing sugars, anthraquinones, phytosteroids, hydroxyl methyl anthraquinones using standard methods, Trease and Evans (1989).

3. RESULTS

The weight and percentage yield of the different solvent extracts of *V. amygdalina* is presented in Table 1.The percentage yield of the different extracts of *V. amygdalina* varied between the different solvents. Aqueous extract had a higher yield of 50% as compared to 40% with ethanolic extract.

Table 2 shows the antibacterial activity of the two extracts of *V. amygdalina* against the different clinical isolates used in the study

Escherichia coli and *Bacillus subtilis* exhibited high sensitivity to the aqueous extract, with 1.2cm and 0.7cm zone diameters respectively.

Staphylococcus aureus and *Pseudomonas aeruginosa* had similar zone diameters of 0.6cm respectively.

4. DISCUSSION

The phytochemical composition and antimicrobial activity of *Vernonia amygdalina* against some clinical isolates have been investigated, using the aqueous and ethanolic extracts of the leaves. The weight and percentage yield of the crude extracts were 25g and 50% for aqueous and 20g and 40% for ethanol respectively. Although this variation exists it has been reported by Udobi and Onaolopa (2009) that the amount of yield does not influence the ability to inhibit growth of pathogens, but rather the active ingredients in extract. Similarly, the result of the antimicrobial activity of *V. amygdalina* extracts obtained in this work has revealed that both extracts exhibited antimicrobial activity.

The plant had maximum activity against *Escherichia coli* and *Bacillus subtilis* in the aqueous extract, at 1.2±0.05cm and 0.7±0.05cm zone diameter respectively. This result is similar to that observed by Takon *et al*., (2013). But in the ethanolic extract, *Escherichia coli* and *Pseudomonas aeruginosa* were resistant. The results obtained had revealed that *V. amygdalina* antimicrobial property was dependent on the type of solvent used for the extraction, extract concentration and test organisms. This result agrees with that reported by Ulhayarasa *et al*., (2010), that polar solvent extracts showed higher activity than low polar solvent extract. This is due to solubility of active component in the former than the latter; hence the polarity of the solvent is important in antimicrobial activity of the plant. Statistical analysis of the two extracts revealed high significant difference ($P<0.05$) in the degree of activity between extracts and concentration of extracts.

The results of the qualitative phytochemical analysis revealed the presence of nine (9) compounds. These include Saponins, flavonoids, glycosides, alkaloids, tannins, reducing sugar anthracenosides, terpenes and phytosteroids. This result showed that *V. amygdalina* had high level of medical value. This result agreed with that obtained by Aka and Okafor (1992) and Arhoghro *et al*., (2009), where the presence of these phytochemicals in *V. amygdalina* has been reported.

In conclusion, the study has shown that *Vernonia amygdalina* extracts have broad spectrum antibacterial activity traceable to the broad spectrum mechanisms of active phytochemicals. These results justify some of the pharmacological claims about the plant in the treatment of infections caused by these isolates. *Vernonia amygdalina* holds pharmacological potential for antibiotic therapy.

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Table 1: Weight and percentage yield of crude extracts of *Vernonia amygdalina* leaves

Extract	Weight yield (g)	Percentage (%)
Aqueous	25.02	50
Ethanol	20.02	40

Table 2a: Antibacteiral activity of aqueous extract of *V. amygdalina leaves*

Microorganism	Extract Concentration (mm)/ Zones of inhibition (mm)				
	100	50	25	12.5	Mean value
Bacillus subtilis	-	0.7±0.05	0.7±0.05	0.8±0.05	0.7±0.05
Staphylococcus aureus	0.9±0.01	0.8±0.05	0.6±0.05	-	0.6±0.05
Escherichia coli	1.4±0.05	1.0 ± 0.12	1.2±0.05	-	1.2±0.05
Pseudomonas aeruginosa	-	0.6±0.022	0.6±0.022	-	0.6±0.022

Legend: Zone of inhibition means ± SD
- = Resistance or no clearing

Table 2b: Antibacterial activity of ethanolic extract of *V. amygdalina* leaves

Microorganism	Extract Concentration (mg/ml) Inhibition Zones (mm)				
	100	50	25	12.5	Mean value
Bacillus subtilis	-		0.7±0.05	0.7±0.05	0.7±0.63
Staphylococcus aureus	-	-	0.8±0.04	0.9±0.05	9±0.05
Escherichia coli	-	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-	-

Legend: Zones of inhibition mean ± SD
- = Resistance or no zones of inhibition
Extract concentration – mg/ml.

Table 3: Qualitative phytochemical composition of ethanol and aqueous extracts of *V. amygdalina* leaves

Phytochemicals	Ethanol extract	Aqueous extract
Saponins	+	++
Flavonoids	+	-
Glycosides	+	-
Alkaloids	+	-
Tannins	+	-
Reducing sugar	+	+
Anthracenosides	+	+++
Terpenes	+	-
Phytosteroids	+	-
Anthraquinones	-	-

Legend:
+ = small amount
++ = moderate amount
+++ = excessive amount
- = absence

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