ANTIBACTERIAL EFFECTS OF BITTER LEAF PLANT (*VERNONIA AMYGDALINA*) ON SOME SELECTED PATHOGENS

BY

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Abstract

Antibacterial activity of aqueous and ethanol extracts from Vernoniaamygdalina was carried out. Using different parts (leaves and roots) its effects on Escherichia coli, Staphylococcus aurous, Staphylococcus albus, Streptococcus pneumonia, Klebsiellapneumonia, Pseudomonas aeruginosa was investigated. Agar well diffusion and filter paper disc methods were used, upon which a minimum inhibitory concentration was determined. The widest zone of inhibition (25 mm) was observed by ethanol extract of Vernonia amygdalina leaf extract on Streptococcus pneumonia and Klebsiella pneumonia. The study confirmed that the Vernonia amygdalinahad a stronger effect on Gram positive bacteria than the Gram-negative bacteria with exception of E. coli. This finding providesthe evidence that supports the use of Vernoniaamygdalina in traditional medicine.

KEYWORD: *Antibacterial Effect,Vernoniaamygdalina, Selected Pathogens.*

1. Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Tanaka, 2002). Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Makhubu, 2006). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno- medicine. In recent years, secondary plant metabolites (Phyto-chemicals) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that Phyto-chemicals with good antibacterial activity will be used for the treatment of bacterial infections. This is because, according to Arora and Keur(1999), the success story of chemotherapy lies

in the continuous search of new drugs to counter the challenges posed by resistant strains of microorganisms. Studies indicate that in some plants there are many substances such as peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others which could serve as sources for antimicrobial production. These substances or compounds have potentially significant therapeutic application against human pathogens including bacteria, fungi and viruses (Okigbo and Omodamiro 2006). Adebayo (2017) corroborated that V, amygdalina as it called Ewuro in Yoruba parlance is the mother of leaves and thus all leaves should have a respect for it.

Herbal products have been used since ancient times in folk medicine, involving both eastern and western medical traditions (Groppo*et al*. 2008). Some have been evaluated for possible use in modern medicine, while thousands of other potentially useful plants have not been tested (Tichy*et al.* 1998). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the foregoing disadvantages (Marchese*et al*. 2001).However, plant used in traditional medicines are still understudied, particularly in chemical laboratory(Kirby, 1996). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethno medicinal plants may still be needed (Damintoti*et al*.,2005). This forms the basis of not only alternative medicine but also paved way to evolution of a gamut of new and novel modern medicines. Literature have shown that several plants were known to have antimicrobial properties (Dash & Murthy, 2011). *Vernonia amygdalina* popularly called Ewuro by the Yoruba is one of the important herbs widely used in traditional medicine in many tropical and subtropical regions. It has been used in the treatment of diarrhea and abdominal pain.

The aim of this study is to use the leaves and root extract of *Vernonia amygdalina* to test for its antimicrobial activities against some clinical isolates of six bacteria namely Escherichia coli, Staphylococcus aureus, Staphylococcus Albus, Streptococcus Pneumonia, Klebsiella Pneumonia, and Pseudomonas Aeruginosa. The study further

investigates the antibacterial susceptibility levels of the test organism using the agar well diffusion and paper disc method.

2. Materials and Methods

2.1 Plant Collection

Different parts of *Vernonia amygdalina used for this study* were chiefly obtained from Kaura Namoda town in Zamfara state Nigeria.

2.2 Preparation and Extraction of Plant material

Extraction of leaf and root of the plant was done with water and 60% ethanol. The leaf powder and root (10g each) were soaked in 100 ml of each solvent. The suspended solutions were left to stand for 5 days, and labeled accordingly. The extracts were filtered and stored at 4°C until when needed.

2.3 Test Organisms

Nine microorganisms used in this study as test organisms comprising of clinical isolates of six bacteria (*Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Streptococcus pneumoniae, Klebsiellapneumoniae, Pseudomonas aeruginosa)* were obtained from the Microbiology Department of General Hospital, Kaura Namoda. The typed cultures of bacteria Nutrient agar (Oxoid) slants and stored at 4°C until required for study.

2.4 Screening for antimicrobial activity

The antibacterial and antifungal susceptibilities were tested using the agar well diffusion method and paper disc method.

2.4.1 Paper Disc Technique

Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40^0C for 30 minutes. The prepared Nutrient agar plates were seeded with each of the test bacteria and the filter paper discs were placed on each plate. The plates were incubated at 37^0C for 24 hours. The fungal isolates were similarly cultured on PDA plates and incubated at 30^0C for 48 hours.

2.4.2 Agar Well Diffusion

The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells. The open wells were filled with 0.05 ml of the extract. The plates were incubated at 37^0C for 24 hours. For the fungi, the test was carried out on PDA plates and incubated at 30^0C for 48 hours. The zones of inhibition were measured and recorded.

2.4.3 Minimum Inhibitory Concentration

Activated culture of respective organisms were inoculated (about 0.2ml) in nutrient agar (for bacteria) and potato dextrose agar (for fungi and yeast) at about 45°–50°C. After well mixing, the agar was poured in a sterile Petri plate. After the solidification of agar, the paper disc previously dipped in extract and the solvent from extract being evaporated in an oven and its control is placed over the solidified agar plate. Then the plates were incubated in the incubator at 37°C for 24 hours for bacteria and at 30^0C for 48 hours for fungi and yeast. The result was interpreted after 24 hrs. The determination of minimum inhibitory concentration (MIC) was carried out by placing the paper discs in increasing or decreasing concentration of the extract over the Petri plate (Gaurav*et al*. 2010).

3. Results and Discussion

Table 1 showed that the ethanol extract of *V. amygdalina* leaves and root has higher zone of inhibition than the aqueous extract. In paper disc method the aqueous extract of *V. amygdalina*root and leaves have the same effect on *S. aureus* by having the same zone of inhibition, but the ethanol extract of the root is not active.it is so significant that the ethanol root extract of *V. amygdalina* is more active than leaf extract , and this also is distinct in aqueous extract of the plant as the root extract is more active than the leaves extract. *P. aeruginosa* showed the same zone of inhibition in both root and leaves aqueous extracts of *V. amygdalina* this result shows that the aqueous extract of the plant is very active against the *P. aeruginosa*but in ethanol extract the root is more active than the leaf extracts. *S. albus* shows higher zone of inhibition in aqueous and ethanol extract of the plant root than the leaf extract. *S. pneumonia* is more active to ethanol extract of root than leaves extract but the zone of inhibition is the same in aqueous extract of the plant. The root ethanol extract is not active against *K. pneumonia.*

The present study revealed that the bacteria isolates used were susceptible to both the ethanoic and water extracts of *V. amygdalina*at varying degree using agar well diffusion method and paper dics method although the effect of water extract was not as much as that of ethanol. It was found that ethanol extract is the best extractive solvent for the extraction of *V. amygdalina which* stated that ethanol is the best extractive solvent for extracting antimicrobial resistance in plants (Rahman et. al. 2004)

Significantly, *E. coli* and *P. aeruginosa* are affected equally by *V. amygdalina* ethanol extract of leaf and root as they produce 5mm and 7mm respectively. Leaf extract of *V. amygdalina* affected *S. albus* and *S. pneumonia* by*V. amygdalina* leaf extract by producing 9mm zone of inhibition. *S. aureus* and *E. coli* have the same zone of inhibition in ethanol extract of *V. amygdalina* root with 11mm and *P. aeruginosa* and *S. albus* were also affected equally by ethanol root extract of the plant with 10mm zone of inhibition.

In table 2 which showed agar well diffusion result, *S. aureus* does not react to both aqueous extracts of root and leaf but produce zone of inhibition in ethanol extract of the plant with the root extract having a better zone of inhibition of 20mm. *E. coli* does not produce much significant difference between aqueous and ethanol leaf extract of the plant as they both have the same effect of 11mm, but little difference in root and the root is more active than the leaf. It has been reported that plant roots have more bioactive compounds than the leaf (Nasar-Abbas and Halkman, 2004).Interestingly enough both leaf and root of both ethanol and aqueous extracts of the plant have the same effect on *P. aeruginosa*by producing 15mm zone of inhibition in all. *S. pneumonia* and *K. pneumonia* are both affected equally by ethanol extract of *V. amygdalina* by producing 25mm zone of inhibition each.

Plants have been model source of medicines as they are sources of chemical agent with therapeutic properties they provide a good source of anti-infective agents for example emetine, quinine and berberine which still remain to be highly effective instrument in the fight against microbial infections, various publications have documented the antimicrobial activity of plant extracts(Hoffman, 1987).

Table 1 Antimicrobial properties of Vernonia amygdalinausing paper disc method

Micro organisms	Zone of inhibition (mm)			
	Aqueous extract		Ethanol extract	
	Leaf	Root	Leaf	Root
S.aureus	11	11	9	NI
E. coli	8	11	5	7
P. aeruginosa	10	10	5	7
S. albus	9	10	9	14
S. pneumonia	14	14	9	15
K. pneumonia	13	19	11	NI

NOTE; NI –no inhibition

Table 2 Antimicrobial properties of V, amygdalina using agar well diffusion method

Micro organisms	Zone of inhibition (mm)			
	Aqueous extract		Ethanol extract	
	Leaf	Root	Leaf	Root
S.aureus	NI	NI	12	20
E. coli	11	12	11	13
P. aeroginosa	15	15	15	15
S. albus	11	10	20	11

S. pneumonia	20	NI	25	23
K. pneumonia	20	19	25	NI

NOTE; NI-no inhibition

Table 3: Minimum Inhibitory Concentration (mg/ml) of aqueous and ethanol extract of V. amygdalina.

Microorganism	Aqueous extract		Ethanol extract	
	Leaf	Root	Leaf	Root
S. aureus	11.0	8.5	8.5	NA
E. coli	11.0	10.0	10.0	13.0
P. aeroginosa	11.0	11.0	11.0	10.0
S. albus	11.0	11.0	11.0	8.0
S. pneumonia	12.5V	12.5	12.5	11.0
. pneumonia	11.0	12.0	12.0	NA

NOTE; NA- not active

4. Conclusion and Recommendation

The experimental study concluded that Vernonia amygdalina (Ewuro) has been fully justified to have antibacterial properties that can inhibit the growth of the selected bacteria pathogens. The paper therefore, recommended that Vernonia amygdalina (Ewuro) be subjected for both NAFDAC and other regulatory agency's approved on drug efficiency implementation policy.

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