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ANTIBACTERIAL ACTIVITYOF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF VERNONIA AMYGDALINA ON SELECTED SPECIES OF GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Abstract
The phytochemical screening and antibacterial activity of ethanolic and Methanolic leaves extract of *Vernonia amygdalina* against five clinical isolates (*Staphylococcus aureus, E. coli, Pseudomonas species, Salmonella species and Proteus species*) was determined using standard method of analysis. The results of the antibacterial activity of ethanol, methanol and aqueous extract of leaves of *V. amygdalina* have diameters ranging between 0.4 to 10mm. The plant extracts from the plants had profound activities against gram-positive than gram negative bacteria. From the above studies, it has clearly indicated that *V. amygdalina* extract may represent new sources of antibacterial drug, if the phytoactive components are purified and proper dosage are determined for administration.
Key Word: Phytochemical, Antibacterial, V. Amyglidana, Leaves and Isolates

Introduction

Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play important roles in drug development in the pharmaceutical industry (Preethi *et al.,* 2010). The use of plant continues to play essential roles in traditional medicine for the treatment or management of various human diseases, especially in rural Africa where infectious diseases are endemic due to poverty and poor sanitations. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of the children with high fevers, is the use of herbal medicines at home. Despite the great technological boom, man has continually been battled by emerging and reemerging infectious diseases (Nwoke, 2004: Nwachukwu *et al.,* 2012). Responding effectively to these health challenges requires the immobilization of knowledge and energy; and a synergy between man and its environment (biotic and abiotic). More than 50% of all current clinical drugs are of plant origin. Therefore, plant products play an important role in drug development activities of pharmaceutical industry (Baker *et al* 1995; Nwachukwu *et al.,* 2012). The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents (Oteo *et al.,* 2002; Tula *et al.,* 2012). The slow pace in the

development of newer antibiotics has provided the need to explore nature in search of phytotherapeutic agents with novel targets and mode of action (Ibrahim *et al.,* 2011). Plants have the major advantage of still being the most effective and cheaper alternative source of drugs. Yedjou *et al.* (2008) estimated that 80% of the population of Africa depends on medicinal plants to satisfy their health care requirements.

Vernonia amygdalina commonly called bitter leaf is a perennial shrub of 2-5 m in height that grows throughout tropical Africa. It belongs to the family Asteraceae and it's a highly appreciated vegetable in west and central Africa where it's commonly used in traditional medicine. Leaf decoctions are used to treat fever, malaria, diarrhoea, dysentery, hepatitis and cough as a laxative and as fertility inducer (Ijeh *et al.,* 1996). The plant has acquired relevance recently, having been proven in human medicine to possess potent antimalarial and antihelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties (Izevbigie *et al.,* 2004: Tula *et al.,* 2012). Various workers had reported the phytochemical and antibacterial activity of the plant parts against food borne pathogen (Ibrahim *et al.,* 2009), urinary tract pathogens (Uzoigwe and Agwa, 2011) and other clinical isolates (Oboh and Masodje, 2009; Ibrahim *et al.,* 2011: Tula *et al.,* 2012). This paper compares the phytochemical components and antibacterial activity of leaves, stem bark and root bark extracts of *V.* amygdalina (Tula *et al.,* 2012).

Material and Method

Collection of plant materials

The leaves of *Vernonia amygdalina* were collected from the Ramat Polytechnic Maiduguri Agriculture garden, Maiduguri, Borno state, Nigeria. The fresh leaves materials were shade dried and homogenized to fine powder. The taxonomy of the plant was carried out at the Department of Science Laboratory Technology, Ramat Polytechnic Maiduguri.

Extraction of *Vernonia amygdalina* plant leaf

Seventy grams (70g) of the leaf powder was added to 350ml of the ethanol and methanol until super saturation for a period of 72 hours at room temperature (Oladunmoye, 2007). The leaf extract obtained was protected from sunlight and stirred several times with a sterile glass rod. The resultant suspension was then filtered using muslin cloth. The filtrates were then evaporated under reduced pressure and concentrated in-vacuo using a rotary evaporator at 85°C. The concentrated extracts are then stored in labeled sterile screw cap bottles at 4°C in the refrigerator, until when required for use.

Aqueous extraction of *Vernonia amygdalina* leaf

Seventy grams (70g) of the leaf powder was weighed out and soaked in 350 ml of distilled water in 500ml conical flask which was thereafter stopper with a rubber cork and left for 24 hours. After this period, it is filtered using a sterile Whatman no. 1 filter paper into a clean conical flask. It is then subjected to water-bath evaporation where the liquid was evaporated at its boiling temperature of 100°C. The remaining extract obtained was stored in a refrigerator at 4˚C until when required for use (Akueshi *et al.,* 2002).

Test of Isolates

Pure cultures of bacterial isolates for the in-vitro antimicrobial assay were obtained from the Medical Microbiology Laboratory, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria after re-identification. The cultures were maintained on MacConkey agar slants for a period of 48 hours in a refrigerator before they were subcultured into freshly prepared MacConkey agar slants for nutrient replenishment and finally

transferred on to the Muller Hinton agar for well diffusion method. The isolates were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus.*

Table 1: Antimicrobial assay of the leaves of *Vernonia amygdalina* using Disc diffusion Techniques

Test Organism	Methanol	Ethanol	Aqueous	Ciprofloxacin (Control)
Pseudomonas species	0.4	6.0	-	4.0
Staphylococcus aureus	5.0	8.0	10.0	14.0
Salmonella species	4.0	8.0	-	-
Proteus species	4.0	6.0	6.0	13.0
E. coli	4.0	6.0	4.0	5.0

Result and discussion

The result of the antibacterial activity of ethanol, methanol and aqueous extract of leaves of *V. amygdalina* was presented in Table 1. The ethanolic extract of the leaves showed potential activity against all the tested organisms with the zone of inhibition ranging between 6 to 8mm. *Pseudomonas species* and *Salmonella species* were resistance to aqueous extract of the leaves. All the three tested extract of *V. amyglidalina* as presented in table 1 showed antimicrobial activity against *E. coli, Proteus species* and *S. aureus* with the average diameter ranging between 4 to 10mm. This study revealed that bitter leaf (*Vernonia amygdalina*) has very high bactericidal action on the common clinical isolates tested during this study. The isolates tested were *Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Klebsiella spp., Proteus mirabilis, Salmonella typhi and Shigella dysenteriae.* The susceptibility of these organisms to these extracts explains their use in native medicine for the treatment of infections such as dysentery, sore throat, cough and wound. The extracts were shown to exhibit a broad spectrum of antimicrobial property against the tested organisms (Akinjogunla *et al*., 2011). The bacterial isolate that showed different levels of susceptibility to leaf extracts of *V. amygdalina* was *Klebsiella* species while *Pseudomonas species* was not susceptible to either of the extracts except ethanolic extract. However, in previous studies by Iwalokun (2003), showed *V. amygdalina* leaf extract exhibit a higher degree of antimicrobial activity on the organisms tested. Even at considerably lower concentration, bitter leaf extract still exhibited a moderately antimicrobial effect on the clinical isolates (Preethi *et al.,* 2010). The result of this study is in line with the report of Tula *et al.* (2012) who reported higher activity of ethanolic extract of the leaves of *Vernonia amygdalina* against the organism tested in this study while *Pseudomonas species,* was resistance to aqueous extract of the leaves as reported in this study.

The results of this study revealed that both gram positive and gram negative bacteria are susceptible to *V. amygdalina* extracts. This finding agrees with reports from Okoh *et al.* (1995) and Taiwo *et al.* (1999). It has also been reported that bitter leaf could be effectively used against drug resistant micro-organisms (Iwalokun *et al.,* 2003: Tula *et al.,* 2012). This observation agrees with the study of Iwalokun *et al.* (2003), who reported on the effectiveness of *V. amygdalina* leaf extract. Uzoigwe and Agwa (2011) observed in their study that leaf extracts of *V. amygdalina* was more effective against *Klebsiella* species. The varying degree of sensitivity of the bacterial strains may be due to the intrinsic tolerance of the bacterial and the nature and combinations of phyto-compounds present in the extracts as observed by Suree and Pana (2005). It could also be attributed to physical factors, extracting solvents and method of extraction. The result in (Table 1) also showed that *Staphylococcus aureus* was found to be the most susceptible to the ethanolic extract with an inhibition zone diameter ranging between 5mm and 10mm at 100% concentrations followed by *Proteus mirabilis and Escherichia coli* with an inhibition zone diameter ranging between 4mm and

6mm at concentrations between 100mg/ml, then *Pseudomonas aerugenosa* with an inhibition zone diameter of 6mm at 100mg/ml. none of the strains were resistant to the ethanol extract (Akinjogunla *et al*., 2011). The plant extracts from the plants had profound activities against gram-positive than gram negative bacteria. This difference in susceptibility of gram positive and gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls, the amount of peptidoglycan, presence of receptors and lipids, nature of cross linking, activity of autolytic enzymes that determined the penetration, binding and activity of the antimicrobial agents.

The effects of ethanolic, methanolic and aqueous extracts have been demonstrated in this study. The results showed significant higher antibacterial activity of ethanolic extract than Methanolic and aqueous extract. This finding agrees with the report of Tula *et al.* (2012) that shows effectiveness of ethanolic extract of *V. amygdalina* than aqueous extract of the same plant (Akinpelu, 1999; Minetesnot and Mogessie, 2004; Ibrahim *et al.*, 2009), due to its better extraction power as an organic solvent. Eloff (1998) also showed that most active components of plants are not water soluble. The high activity of ethanolic extracts verifies the use of the ethanolic extraction method by local herbalists (Allero and Afolayan, 2006).

In conclusion *V. amygdalina* extract has excellent antibacterial activity against both gram positive and gram negative bacteria. From the above studies, it has clearly indicates *V. amygdalina* extract may represents new sources of antibacterial drug, if the phytoactive component are purified and proper dosage are determine for administration.

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