

Full Length Research Paper

# Systematic study on comparing phytochemicals and the antimicrobial activities from different parts of *V. amygdalina*

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The leaf, stem and root bark of *Vernonia amygdalina* were subjected to phytochemical screening for the presence of alkaloids, saponins, cardiac glycosides, flavonoids, anthraquinones, steroids, polyphenols, and phlobatannins. The root bark possessed all the phytochemical components tested. Stem bark possessed all the phytochemical components except flavonoids, while the leaf lacks anthraquinones and polyphenols. The result showed no significant difference in the presence of the phytochemical components between leaves, stems and root bark ( $P > 0.05$ ). The *V. amygdalina* parts were screened for antibacterial activities at 100 mg/ml against *Shigella* sp., *S. aureus*, *S. typhi*, *E. coli*, *P. mirabilis*, *K. pneumoniae* and *P. aeruginosa*. All the isolates were susceptible to ethanolic extract of leaves in varying degree. The antibacterial activity of the leaves extract was significantly higher than those of stem and root bark ( $P < 0.05$ ). There was no significant difference in the antibacterial activity of both stem and root bark ( $P > 0.05$ ). The result further revealed the effectiveness of ethanolic extract over aqueous extract ( $P < 0.01$ ). The MIC of the ethanolic extract of *V. amygdalina* parts were particularly high for all the isolates (125 to 250 mg/ml), and were significantly higher than that of ciprofloxacin ( $P < 0.01$ ).

**Key words:** Phytochemical, antibacterial activity, *Vernonia amygdalina*.

## INTRODUCTION

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). 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. One of such plant with promising medicinal principle is *Vernonia amygdalina*. *V. amygdalina*, a member of the family Asteraceae is a tropical shrub, 1 to 3 m in height with petiole leaf of about 6 mm in diameter and elliptic in shape (Igile et al., 1995). All parts of the plant are pharmacologically useful. Both the roots and the leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort (Hamowia and Saffat, 1994). The plant has acquired relevance recently, having been proven in human medicine to possess potent antimalarial and antihelminthic properties (Abosi

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and Raseroka, 2003) as well as antitumorigenic properties (Izevbogie et al., 2004). 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). This paper compares the phytochemical components and antibacterial activity of leaves, stem bark and root bark extracts of *V. amygdalina* on some clinical isolates.

## MATERIALS AND METHODS

### Collection of plant materials

The leaves, stems and roots of *Vernonia amygdalina* were collected around Fadama area of Federal Polytechnic Mubi, Adamawa State, Nigeria and was identified by comparing their morphological and anatomical characteristics with the standard description.

### Extraction procedure

The *V. amygdalina* parts (leaves, stem bark and root bark) were air-dried at room temperature and ground to fine powder. 50 g of each of the powdered plant part was soaked in 200 ml distilled water and was allowed to stand for 48 h at room temperature after thorough vortexing. Each mixture was filtered using whatman no.1 filter paper. The filtrate were concentrated in vacuum using rotary evaporator. Similar procedure was followed to obtain ethanolic extracts of the plant parts using ethanol as extracting solvent. All the aqueous and ethanolic dried extracts of the plant parts were stored in sample bottles at 4°C prior to use.

### Phytochemical screening

Qualitative phytochemical tests were carried out on the aqueous extract and on the powdered specimen using standard procedure to identify the constituents (Sofowora, 1993; Evans, 2002).

#### Test for free anthraquinones

5 ml of chloroform was added to 0.5 g of the powdered dry seeds of each specimen. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10% ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

#### Test for cardiac glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

#### Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the phlobatannins.

#### Test for polyphenols

2 g of powdered sample was boiled with distilled water for 30 min, and then 1 ml of 5% ferric chloride (iron iii chloride) and 1 ml of 1% potassium ferric cyanides was added to the solution. It was filtered and observed for the formation of blue-green colour.

#### Test for saponins

1 g of each powdered dried stain was separately boiled with 10 ml of distilled water in a bottle bath for 10 min. The mixture was filtered while hot and allowed to cool. The following tests were then carried out. Demonstration of frothing: 2.5 ml of filtrate was diluted to 10 ml distilled water and shaken vigorously for 2 min (frothing indicated the presence of saponins in the filtrate).

#### Test for alkaloids

A small portion of crude extract was dissolved in 5 ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayer's reagent separately. Any precipitate or turbidity with the reagent suggests the presence of alkaloids.

#### Test for steroids

A small portion of the extract was dissolved in 1 ml of chloroform and filtered. To the filtrate on ice, 1 ml of acetic acid was added and then a few drops of concentrated sulphuric acid were run down the side of the test tube. The appearance of blue, bluish-green or a rapid change from pink to blue colours indicates the presence of steroids.

#### Test for flavonoids

1 g of the powdered dried leaves of each specimen was boiled with 10 ml of distilled water for 5 min and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

### Test organisms

The test organisms used for this study were clinical isolates collected from National Veterinary Research Institute (NVRI) Vom Jos, Nigeria. The organisms were maintained on nutrient agar slants and preserved in refrigerator at 4°C prior to use.

#### Preparation of Inoculum

The overnight cultures of the test organisms were inoculated onto peptone water and vortex thoroughly. The turbidity of the bacterial suspensions were then adjusted and compared with 0.5 McFarland standard. The 0.5 McFarland standards was prepared by adding 0.5 ml of 1.2% (wt/vol) barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ) solution to 99.5 ml of 1% sulphuric acid. The turbidity standard was then aliquot into test tubes identical to those used to prepare the inoculums suspension.

#### Determination of antibacterial activity

The dried aqueous and ethanolic extracts of *V. amygdalina* parts were reconstituted in glycerol to obtain a final concentration of 100

**Table 1.** Phytochemical screening of leaves, stem and root bark of *Vernonia amygdalina*.

Phytochemical components	Leaves	Stem bark	Root bark
Anthraquinones	-	+	+
Cardiac glycosides	+	+	+
Phlobatannins	+	+	+
Polyphenols	-	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Steroids	+	+	+
Flavonoids	+	-	+

Key: + presence, - = absence.

**Table 2.** Diameter of zones of inhibition produced by ethanolic and aqueous extracts of *V. amygdalina* plant part.

Organisms	Diameter of zones of inhibition (cm)					
	Leaves		Stem bark		Root bark	
	E.E	Aq. E	E.E	Aq. E	E.E	Aq. E
<i>Shigella</i> sp	3.5	1.0	3.0	1.7	3.0	1.5
<i>S. aureus</i>	3.0	-	-	1.4	1.5	1.5
<i>S. typhi</i>	3.4	2.0	2.6	2.0	-	1.7
<i>E. coli</i>	3.0	1.1	1.5	-	1.4	1.8
<i>P. mirabilis</i>	3.5	-	1.8	1.5	1.6	-
<i>K. pneumoniae</i>	4.0	2.0	1.4	1.2	1.4	1.6
<i>P. aeruginosa</i>	3.0	1.0	1.6	1.3	1.7	1.5

Key: E.E = Ethanolic extract, Aq.E = Aqueous extract, - = no zone of inhibition.

mg/ml for this test. The susceptibility test was done using agar well diffusion method. 0.1 ml aliquot of each test organism suspension was transferred onto dried agar plates in duplicate and was spread evenly with a sterile bent glass rod. After drying, three (3) wells were bored (using 6 mm diameter cork borer) into the dried nutrient agar plates and 0.5 ml each of the extracts (leaves stem bark and root bark) was aseptically introduced into two wells. Glycerol was introduced into the third well as control. The plates were then incubated at 37°C for 24 h after which zones of inhibition were measured in centimetres and recorded appropriately.

#### Determination of Minimum Inhibitory Concentration (MIC) of Ethanolic extracts (leaves, stem bark and root bark)

The MIC of the ethanolic extracts was estimated for all the test organisms using the broth dilution method. To each test tube containing 5.0 ml of nutrient broth, 0.5 ml of varying concentrations (50 to 300 mg/ml) of the reconstituted extract was added and inoculated with 0.1 ml of each test isolate. The same procedure was repeated using ciprofloxacin of varying concentration (5.0 to 25 mg/ml) for each of the test organisms. After incubation of the tubes at 37°C for 24 h, the lowest dilution of the extracts and antibiotic that prevented visible growth of the test organisms was taken as MIC (De et al., 2002).

#### Statistical analysis

Anova and Student T-test was used to test for significance difference in all the data obtained. All statistical analyses were

carried out using the SPSS 17.0 window based program. Significance difference and non-significance difference was defined when  $p \leq 0.05$  and  $p \geq 0.05$  respectively (Ogbeibu, 2005).

## RESULTS AND DISCUSSION

Table 1 showed the results of the phytochemical analysis of leaves, stems and root barks of *Vernonia amygdalina*. The phytochemical component screened includes anthraquinones, cardiac glycosides, phlobatannins, polyphenols, saponins, alkaloids, steroids and flavonoids. The root bark possessed all the bioactive components tested. Stem bark possessed all the bioactive components tested with the exception of flavonoid, while the leaves lack anthraquinones and polyphenols. However, there was no significant difference in the presence of the phytochemical components between the leaves, stems and roots ( $P > 0.05$ ).

The result of the antibacterial activity of ethanol and aqueous extract of leaves, stem bark and root bark of *V. amygdalina* was presented in Table 2. The ethanolic extract of leaves produced impressive antibacterial activity against all the tested organisms with zones of inhibition ranging from 3.0 to 4.0 cm. *Staphylococcus aureus* was not susceptible to aqueous extract of leaves and ethanolic extract of stem bark. *Staphylococcus typhi*,

**Table 3.** Minimum inhibitory concentration (MIC) of ethanolic extract of *V. amygdalina* plant parts and ciprofloxacin (mg/ml).

Organisms	Leaves	Stem bark	Root bark	Ciprofloxacin
<i>Shigella</i> sp	150	150	175	5.0
<i>S. aureus</i>	125	175	150	5.0
<i>S. typhi</i>	175	200	250	5.0
<i>E. coli</i>	150	150	125	5.0
<i>P. mirabilis</i>	150	150	175	5.0
<i>K. pneumoniae</i>	150	175	150	5.0
<i>P. aeruginosa</i>	150	175	175	5.0

*P. mirabilis* and *E. coli* were not susceptible to ethanolic extract of root bark, aqueous extract of leaves and aqueous extract of stem bark respectively. Statistically, the antibacterial activity of the leaves extract of *V. amygdalina* was significantly higher than those of stem and root bark extract ( $P < 0.05$ ). However, there was no significant difference in the antibacterial activity of both stem and root bark extract ( $P > 0.05$ ). The result further showed significant higher antibacterial activity of ethanolic extracts than aqueous extracts ( $P < 0.01$ ).

The minimum inhibitory concentration (MIC) of ethanolic extract of leaves, stem bark and root bark of *V. amygdalina* was high for all the isolates and ranges from 125 to 250 mg/ml (Table 3). There was no significant difference in the MIC of ethanolic extract of all the plant parts on the isolates ( $P > 0.05$ ). However, the MIC of *S. typhi* was significantly higher than those of other isolates ( $P < 0.05$ ). Also, the MIC of crude ethanolic extract of the plant parts were significantly higher than that of ciprofloxacin ( $P < 0.01$ ).

The findings that *V. amygdalina* plant (leaves, stem and root) possessed alkaloids, saponins, glycosides, flavonoids, steroids and polyphenols as reported by Ibrahim et al. (2009); Item et al. (2010) and Eyong et al. (2011) was confirmed in this study. Although, the findings of this study showed that the leaves and stem bark extract polyphenols and flavonoid respectively. Both polyphenols and flavonoid contain antioxidants which help to protect cells and body chemicals against damage caused by free radicals and reactive atoms that contribute to tissue damage in the body. Also, the presence of steroids in the leaves extract contradicts the report of Ibrahim et al. (2009) who reported the absence of steroids in the same plant part. Item et al. (2010) showed that the leaves, stems and roots of *V. amygdalina* do not possess phlobatannins and anthraquinones. This study however, showed that only the leaves of *V. amygdalina* lack phlobatannins and anthraquinones. The leaves, stems and roots of *V. amygdalina* showed no significant difference in the presence of the phytochemical components ( $P > 0.05$ ). Several studies have reported that these bioactive components as shown in this study possess antimicrobial activities (Subrahmanyam et al., 2001; Osman et al.,

2003; Farombi, 2003).

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 other researchers (Okoh et al., 1995; 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). 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 antibacterial activity of the leaves extract was significantly higher than those of stems and root bark ( $P < 0.05$ ). This observation agrees with the report 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* sp. than the stem extracts of the same solvent.

The effects of ethanolic and aqueous extracts have been demonstrated in this study. The results showed significant higher antibacterial activity of ethanolic extract than aqueous extract ( $P < 0.01$ ). This finding agrees with earlier reports on the 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 reported 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). The minimum inhibitory concentrations (MIC) of ethanolic extract of *V. amygdalina* parts were particularly high for all the isolates. Several other researchers have reported high MIC in their investigation on medicinal plants (De et al., 2002; EL-Mahmood and Ameh, 2007). The ethanolic extracts of the *V. amygdalina* showed a better antibacterial activity than aqueous extracts but not comparable to the standard antibiotic (ciprofloxacin) used. Ciprofloxacin showed a better antibacterial activity than the crude preparation of *V. amygdalina* plant as shown in the MIC.

This observation agrees with earlier reports on better antibacterial activity (lower MIC) of ciprofloxacin than crude preparation of *V. amygdalina* (Jude et al., 2010; Tula et al., 2011). The significant lower MIC of ciprofloxacin comparable to crude extract of the plant parts might be attributed to strict adherence to manufacturing techniques and procedure such as proper purification, quality chemotherapeutic index which was lacking in the crude extracts. However, the fact that the crude extracts of this plant parts inhibited these medically important isolates proved that *V. amygdalina* might have some potential as an alternative source of anti-bacterial substances that might be used against infections caused by these isolates.

## REFERENCES

- Aboji AO, Raseroka BH (2003). *In vivo* antimalarial activity of *Vernonia amygdalina*. Br. J. Biomed. Sci. 60:89-91.
- Akinpelu DA (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. Fitoterapia. J. Stud. Med. Plants 70(4):432.
- Allero AA, Afolayan AJ (2006). Antimicrobial activity of *Solanum tomentosum*. Afr. J. Biotechnol. 5:269-272.
- De N, Tula MY, Sale M (2002). Antibacterial Spectrum of Extracts of *Azadirachta indica* A. Juss (Neem seed). Nig. J. Biotechnol. 1:108-118
- EI-Mahmood AM, Ameh JM (2007). *In vitro* antibacterial activity of *Parkia biglobosa* (Jacq) root bark extract against some microorganisms associated with Urinary Tract Infections. Afr. J. Biotechnol. 6(11):1272-1275.
- Eloff JN (1998). A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for Bacteria. Planta Med. 60:1-8.
- Evans WC (2002). Pharmacognosy: London, W.R. Saunders.
- Eyong UE, Agiang MA, Atemwho, IJ, Iwara IA, Odey MO, Ebong PE (2011). Phytochemicals and micronutrients composition of root and stem bark extracts of *V. amygdalina* Del. J. Med. Med. Sci. 2(6):900-903.
- Farombi EO (2003). African Indigenous Plant with Chemotherapeutic Potentials and biotechnological Approach to the production of bioactive prophylactic agents. Afr. J. Biotechnol. 2(12):662-671.
- Hamowia AM, Saffar AM (1994). Pharmacological studies on *Vernonia amygdalina* (Del) and *Tithenia diversifolia* (Gray). Vet. Med. J. Giza, 22:91-97.
- Ibrahim TA, Ajala L, Adetuyi FO, Jude-Ojei B (2009). Assessment of the antibacterial activity OF *Vernonia amygdalina* and *Occimum gratissimum* leaves on selected food borne pathogens. Elect. J. Environ. Agric. Food Chem. 8(11):1212-1218.
- Ibrahim TA, Opawale BO, Oyinloye JMA (2011). Antibacterial activity of herbal extracts against multi drug resistant strains of Bacteria from clinical origin. Life Sci. Leaflets 15:490-498.
- Igile GO, Oleszyk W, Burda S, Jurzysta N (1995). Nutritional Assessment of *Vernonia amygdalina* leaves in growing mice. J. Agric. Food Chem. 43:2126-2166.
- Item JA, Patrick EE, Godwin EE, Augustine U (2010). Extracts of *V. amygdalina* del. (African bitter leaf) can reverse pancreatic cellular lesion after alloxan damage in the rat. Aust. J. Basic Appl. Sci. 4(5):711-716.
- Iwalokun BA, Bamiro SB, Durojaiy OO (2003). An antimicrobial evaluation of *Vernonia amygdalina* (Compositae) against Gram - positive and Gram - negative bacteria from Lagos, Nigeria. West Afr. J. Pharmacol. Drug Res. 19(1&2):9-15.
- Izevbigie EB, Bryant JL, Walker A (2004). A novel natural inhibitor of extracellular signal related kinases and human breast cancer cell growth. Exp. Biol. Med. 229:163-169.
- Jude OBS, Ibrahim TA, Ola IO (2010). Comparing the antibacterial activity of ethanolic extract of *V. amygdalina* and *Occimum gratissimum* with some antibiotic of choice. Internet J. Trop. Med. 6(2):1540-1546.
- Minetesnot A, Mogessie A (2004). Assessment of the antibacterial activity of some traditional plants on some food borne pathogens. Ethiop. J. Pharm. 45:123-128.
- Oboh FOJ, Masodje HI (2009). Nutritional and antimicrobial properties of *Vernonia amygdalina* leaves. Int. J. Biomed. Health Sci. 5:51-57.
- Ogbeibu AE (2005). Biostatistics: A practical approach to research and data handling. Mindex publishing company, Ltd. Ugbowo, Benin City, Nigeria. pp. 75-87.
- Okoh IA, Babalola GO, Ilori MO (1995). Effect of methanol extract of *Vernonia amygdalina* on malting and brewing properties of sorghum. Tech. Q. Master Brew. Assoc. Am. 32(1):11-14.
- Osman OF, Mansour IS, El-Hakim S (2003). Honey compound for wound care: a preliminary report. Ann. Burns Fire Disaster 16(3).
- Oteo J, Campos J, Baquero F (2002). Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European antimicrobial resistance surveillance system. J. Antimicrob. Chemother. 50:945-952.
- Sofowora A (1993). Standardization of Herbal Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Lagos-Nigeria. pp. 51-61.
- Subrahmanyam M, Archan HS, Pawar G (2001). Antibacterial activity of honey on bacteria isolated from wounds. Ann. Burns Fire Disaster 16(1).
- Suree N, Pana L (2005): Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other Enterobacteriaceae. KMITL Sci. Tech. J. 5(3):527-538.
- Taiwo O, Xu H, Lee SF (1999). Antibacterial activities of extracts from Nigerian chewing sticks. Phytother. Res. 13(8):675-679.
- Tula MY, Iruolaje FO, Toy B (2011). *In vitro* antimicrobial activity and preliminary screening of the leaf extracts of *Vernonia amygdalina*. Yank. J. 7:73-77.
- Uzoigwe CI, Agwa OK (2011). Antimicrobial activity of *Vernonia amygdalina* on selected urinary tract pathogens. Afr. J. Microbiol. Res. 5(12):1467-1472.
- Yedjou CG, Rogers C, Brown E, Tchounwou PB (2008). Differential effect of ascorbic acid and N-acetyl 1-cysteine on arsenic trioxide mediated oxidative stress in human leukemia (HL-60) cells. J. Biochem. Mol. Toxicol. 22:85-92.