PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF BITTER LEAF (VERNONIA AMYGDALINA) COLLECTED FROM LAPAI, NIGER STATE, NIGERIA ON SOME SELECTED PATHOGENIC MICROORGANISMS

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ABSTRACT

Vernonia amygdalina called ‘Shuwaka’ in Hausa is commonly used to treat stomach-ache and gastrointestinal troubles in Northern Nigeria ethno-medicine. This study was aimed to verify the claimed antimicrobial activity of V. amygdalina collected from Lapai, Niger State, Nigeria and analyse its phytochemicals. Phytochemical investigation and studies on antimicrobial activity of stem barks and roots extract of V. amygdalina were carried out on Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger using agar-well diffusion assay. Preliminary phytochemical screening revealed the presence of alkaloids, steroids, glycosides, flavonoids, tannins, saponins, phlobatannins, and phenolics in the stem bark and roots extract. The largest diameter of inhibition zone was observed on the acetone extract of stem bark against S. aureus (16 mm) and P. aeruginosa (16 mm). All the tested pathogens were resistant to the water extract of stem bark and root, except for S. aureus which showed inhibitory activity with 7 mm diameter of inhibition zone on stem bark extract. The outcomes of this work suggest the presence of pharmacologically active compounds in the extracts with antimicrobial activity against the pathogenic strains which justifies its ethno-medicinal use.

Keywords: Vernonia amygdalina, phytochemical, antimicrobial activity, pathogens

INTRODUCTION

With the recent trend of high percentage resistance of multiple drug-resistant microbial strains, efforts have been intensified by researchers to search for possible alternatives (Adetunji et al., 2013). Medicinal plants and traditional preparation with antimicrobial activities have been used extensively in the West African regions. These plants of medicinal importance have been proven to be very effective even where treatments with antibiotics failed (Oshim et al., 2016). Vernonia amygdalina commonly called bitter leaf (English), Shuwaka (Hausa), Ewuro (Yoruba), Oriwo (Edo), and Obulu (Igbo) is a perennial shrub belonging to the family Asteraceae (Ghamba et al., 2014; Gashe & Zeleke, 2017). V. amygdalina is a shrub that can grows to 10 m tall with petiole leaf of about 6 mm in diameter and elliptic in shape and grows throughout tropical Africa and has been domesticated in various parts of West Africa including Nigeria, where it is locally used as vegetable in soups (Elm et al., 2012; Habtamu & Melaku, 2018). In some African countries including Nigeria, this plant species is traditionally used to treat many ailments including diabetes (Akah & Okafo, 1992), malaria, helminth infections, fever, (Magadula & Erasto, 2009), promote wound healing (Adetutu et al., 2011) and to treat microbial infections (Noumedem et al., 2013). Also, the Hausa tribe of the northern part of Nigeria used the root and twig of V. amygdalina to treat stomach-ache and gastrointestinal troubles (Akinpelu, 1999). It is also prescribed to nursing mother as it improves lactation (Anibijuwon et al., 2012).

The pharmacological study of V. amygdalina have been reported to demonstrated Antihelmitic and Antimalarial properties (Abosi & Rasero, 2003), antitumorigenic properties (Izebogie et al., 2004), analgesic and antipyretic activities (Tijjani et al., 2017), hypoglycemic and hypolipidaemic effect in experimental animals (Nwanjo, 2005). The reported biologically active phytoconstituents from V. amygdalina are alkaloids, flavonoids, terpenes, saponins, coumarins, xanthones, phenolic acids, lignans, steroids, anthraquinones (Tona et al., 2004), edotides (Izebogie, 2003), and sesquiterpene lactone (Kupchan et al., 1969). Despite the vast traditional used of V. amygdalina, it is still considered among the under exploited crops of economic significance. Thus, this study is aimed at determining the antimicrobial activity and the phytochemical constituents of V. amygdalina collected from Lapai, Niger State, Nigeria.

MATERIALS AND METHODS

Collection and preparation of plant materials

Stem barks and roots of V. amygdalina plant were collected in a sterile polythene bags from the Federal Low-cost, Lapai, Niger State, Nigeria. The samples were taken to the Department of Biology, Faculty of Natural Science, Ibrahim Badamasi Babangida University, Lapai, Niger state, Nigeria for proper identification and authentication. The samples were washed with distilled water chopped into pieces and air-dried for two weeks after which they were grounded with mortar and pestle.

Sample extraction

Aqueous Extract

Ten grams each of the grounded stem barks and roots was added
to 100 mL of sterile distilled hot water in order to obtain aqueous extracts (100 mg/mL) and mixed thoroughly after which the plant residue was filtered through a muslin cloth and the obtained filtrate was further sterilised by filtration through membrane filter HC type. The sterile extract obtained was then transferred into sterile capped McCartney bottles and refrigerated at 4°C until further use.

**Ethanol extract**

Ten grams each of the ground stem barks and roots was separately added to 100 mL of 70 % ethanol or acetone in order to obtain ethanol and acetone extracts (100 mg/mL). The extraction was done for 72 h (Newton et al., 2002). The muslin cloth was used to filter the plant residue and the obtained filtrate was further sterilised by filtration through membrane filter HC type. The sterile extract obtained was then transferred into sterile capped McCartney bottles and refrigerated at 4°C until further use.

**Phytochemical Screening of the Stem Barks and Roots of V. amygdalina**

Phytochemical screening of the V. amygdalina stem bark and root extracts for major constituents including tannins, alkaloids, saponins, steroids, phenols, phlobatannins, flavonoids, and glycosides was carried out using standard qualitative method as previously described by Trease and Evans (1989).

**Sterility test of the plant extracts**

Each of the extract (aqueous, ethanol, and acetone extract) was tested for sterility after sterilization by inoculating 1 mL of each extract on sterile nutrient agar and incubated at 37°C for 24 h. The plates were observed for growth. No growth in the extracts after incubation indicates that the extracts were sterile.

**Test organisms**

The test organisms employed in this study were medical isolates of Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger. They were obtained from Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai.

**Standardization of the bacterial cell suspension**

Few colonies of each tested organism were picked into sterile test-tube containing sterile nutrient broth and incubated at 37°C for 24 h. The turbidity of the suspensions was adjusted to 0.5 turbidity McFarland standard.

**Determination of Antimicrobial Activity of Various Crude Extracts**

The antimicrobial activity of the crude extracts was done using agar-well diffusion method described by (Gashe & Zeleke, 2017) with few modifications. Briefly, 0.2 mL of the adjusted suspensions were separately inoculated using a sterile wire loop on the solidified media plates (Nutrient Agar (NA) for bacteria and Potato Dextrose Agar plate (PDA) for fungi) and spread uniformly using a sterile glass rod. The agar medium was punched out using a sterile hole cork-borer of 8 mm diameter and cut agar discs were aseptically and carefully removed with sterile forceps. A sterile Pasteur pipette was used to introduce 0.5 mL of the crude plant extract samples into the 8 mm holes bored on the surface of the agar media containing the cultures. The plates were allowed to stand for one hour at room temperature to allow diffusion of the substrates to proceed before the growth of the organisms commenced. The plates were finally incubated at 37°C for 24 h for bacteria and at room temperature for fungi. The presence of zone of inhibition around the holes containing the extracts indicates the antimicrobial activity against the test organisms. Antimicrobial activity was expressed in terms of diameter of zones of inhibition (mm).

**RESULTS**

The root and stem bark of the plant were found to be rich in alkaloids, steroids, glycosides, flavonoids, saponins and Phlobatannins, though tannins and phenols were only present in the stem bark as shown in table 1 below.

**Table 1:** Phytochemical Constituents of Root and Stem bark of V. amygdalina

<table>
<thead>
<tr>
<th>Phytochemical Compounds</th>
<th>Root</th>
<th>Stem bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ + +</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+ + +</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ +</td>
<td>+ + +</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>++</td>
<td>+ +</td>
</tr>
<tr>
<td>Phenolics</td>
<td>-</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

Key: +: present, - : Absent, + +: moderately present, + + +: Highly present

Table 2 below shows the zone of inhibition of ethanol extract of Vernonia amygdalina stem bark and root against different pathogenic organisms. When tested with the extract of stem bark, the sensitivity of the tested organisms showed that S. aureus, C. albicans, and A. niger produced the same diameter of inhibition zone of 8 mm (38.10%). P. aeruginosa produced the lowest diameter of inhibition zone of 5 mm (17.24%). For the ethanol extract of root, A. niger produced the largest diameter of inhibition zone with 8 mm (47.06%), followed by S. aureus with 6 mm (35.29%) and C. albican 2 mm (11.77%) diameter of inhibition zone.

**Table 2:** Sensitivity Patterns of Some Pathogenic Organism to Ethanol Extract as Measured by Zone of Inhibition.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Stem bark</th>
<th>Percentage (%)</th>
<th>Root</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>8 mm</td>
<td>38.10</td>
<td>6 mm</td>
<td>35.29</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>5 mm</td>
<td>17.24</td>
<td>1 mm</td>
<td>5.86</td>
</tr>
<tr>
<td>C. albicans</td>
<td>8 mm</td>
<td>38.10</td>
<td>2 mm</td>
<td>11.77</td>
</tr>
<tr>
<td>A. niger</td>
<td>8 mm</td>
<td>38.10</td>
<td>8 mm</td>
<td>47.06</td>
</tr>
</tbody>
</table>

The sensitive pattern of the tested pathogenic organism towards
acetone extract of Vernonia amygdalina stem bark and root and their percentage zone of inhibition are presented in Table 3 below. It was shown that S. aureus and P. aeruginosa produced the largest diameter of inhibition zones of 16 mm (37.21%) when tested with acetone stem bark extract of Vernonia amygdalina, followed by C. albicans with diameter of inhibition zones of 7 mm (16.28%). A. niger produced the lowest diameter of inhibition zones with 4 mm (9.30%). Interestingly, A. niger produced the largest diameter of inhibition zones of 8 mm (30.77%) when exposed to acetone root extract of Vernonia amygdalina, while S. aureus and C. albicans produced the same size of diameter of inhibition zones of 6 mm (23.08%).

**Table 3:** Sensitivity Patterns of Some Pathogenic Organism to Acetone Extract as Measured by Zone of Inhibition

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Stem bark</th>
<th>Percentage (%)</th>
<th>Root</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>16 mm</td>
<td>37.21</td>
<td>6 mm</td>
<td>23.08</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>16 mm</td>
<td>37.21</td>
<td>2 mm</td>
<td>7.56</td>
</tr>
<tr>
<td>C. albicans</td>
<td>7 mm</td>
<td>19.28</td>
<td>6 mm</td>
<td>23.08</td>
</tr>
<tr>
<td>A. niger</td>
<td>4 mm</td>
<td>9.30</td>
<td>8 mm</td>
<td>30.77</td>
</tr>
</tbody>
</table>

The result of the hot aqueous extract showed that except for S. aureus which exhibited activity with the diameter of inhibition zone of 7 mm, all other organism were resistant to the extract (Table 4).

**Table 4:** Sensitivity patterns of some pathogenic organism to hot aqueous extract as measured by zone of inhibition

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Stem bark</th>
<th>Percentage (%)</th>
<th>Root</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>7 mm</td>
<td>19.28</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>A. niger</td>
<td>R</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It has been revealed that all the tested plant extracts possesses antimicrobial properties. S. aureus (gram positive) was more susceptible to the various extracts; ethanol, acetone, and aqueous extracts and thus the higher size of diameter of inhibition zones. It was previous reported that plant extracts displayed stronger antimicrobial effect on the gram positive strains than on their gram- negative counterparts (Desta, 1993; Okiob & Omomari, 2008; Chekole et al., 2016). P. aeruginosa, C. albicans and A. niger were inhibited by ethanolic and acetone extracts but not inhibited by the aqueous hot extracts as shown in (Table 2, 3 and 4). This is supported by the previous studies by Ibekeke et al. (2001); Adetunji et al. (2013); Adetutu et al. (2011); Koduru et al. (2006), and Moreno et al. (2006) which showed that the ethanolic and acetone extracts possess more antimicrobial activity than aqueous extract. In this study, the hot water decoction was used to mimic the traditional way of preparation. The extracts obtained from water decoction in this study displayed weak or no antimicrobial activity, which is contrary to the anticipation. Water is used as a solvent in traditional medicinal probable because it is more convenient to prepare in a household environment using either decoction or infusion. Earlier, Elloff (1998) reported that due to the inability of water to extract nonpolar compounds, the water preparation is usually not suitable for antimicrobial discovery. Another reason for organic extract to be more active than water extract is due to the better solubility of the active components in organic solvents (Boer et al., 2005; Doughari et al., 2007). The presence of alkaloids, steroids, Glycosides, flavonoids, tannins, saponins, phlobatannins, and phenolics in the extracts of V. amygdalina may explain the reason for its antimicrobial actions since the antimicrobial properties of most of these phytoconstituents have previously been documented (Taleb-contini et al., 2003; Mandalari et al., 2007; Nenaar, 2013; Jasim et al., 2015; Al-Harbi et al., 2017; Jin et al., 2017).

**Conclusion**

This study highlighted the antimicrobial effects of V. amygdalina extracts on some human pathogens thereby verifying the traditional healer’s claim. Sooner than expect, broad-spectrum drug which might be able to cure various human ailments could be developed from V. amygdalina. Further work should be carried out in order to isolate the active compounds for further antimicrobial, pharmacological and clinical testing.

**REFERENCES**


**Vol 3** (No 3) 2018

www.scienceworldjournal.org

ISSN 1597-6343

Published by Faculty of Science, Kaduna State University.


