CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF FLAVONOIDS IN VERNONIA AMYGDALINA: A COMMON CHEWING STICK IN SOUTH-WESTERN NIGERIA

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In South-Western Nigeria, local people chew the stems of the plant Vernonia amygdalina (chewing-stick) to inhibit the negative effects of oral microbial activity. The use of chewing-stick is increasing; this could be due to financial constraints and/or belief in the cleansing ability of chewing stick. To investigate the bioactive compounds of Vernonia amygdalina, the phytochemical and antimicrobial screening was carried out using agar disc diffusion method. The highest zone of inhibition of 22.5 mm was recorded for P. aeruginosa and lowest zone of inhibition of 10.5 mm for A. flavus. The phytochemical analysis identified important secondary metabolites such as anthraquinones, flavonoids, steroids and saponins. The presence of the phytochemicals (flavonoids) could be responsible for the observed antifungal and antibacterial activities on the susceptible organisms studied.

Key words: Microbes, Chewing stick, Vernonia amygdalina, Flavonoids, Antimicrobial activities.

INTRODUCTION
The use of herbal medicine for the treatment of diseases caused by bacteria and fungi gave rise to the development of natural antibiotics (Akinyemi, 2000). Plants are important sources of medicines and play a key role in world health. Almost all cultures from ancient times to today have used plants as medicine. Medicinal plants are important to the global economy (Adekunle and Odukoya, 2006), as approximately 85% of traditional medicine preparations involve the use of plants or plant extracts (Vieira and Skorupa, 1993).

The use of plant-based drugs world-wide is increasing. Through recent researches on herbal plants, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine (Shrestha et al 2016; Gajendiran et al 2016; Agarwal et al 2015; Avula et al 2015; Srividya et al 2012; Dahiya, 2008; 2007; Dahiya and Kumar, 2008). Herbal plant products are emerging all over the world due to the belief that many herbal medicines are free from health and environmental effects. The widespread fear of the side-effects of synthetic drugs often accompanies their single or multiple health benefits (Al Sadhan and Almas, 1999).

In South-Western Nigeria, local people chew the stems of the plant Vernonia amygdalina (chewing-stick) (Figure 1) for oral hygiene. Recent interest in chewing-sticks and their extracts has focused on their effects on organisms that are involved in oral infections. Africans who use chewing-sticks have fewer carious lesions than those who use toothbrushes and chewing-stick usage has been encouraged by World Health Organization (Ndrukwe et al 2005).
In some countries, however, the National Health Agencies have restricted and discouraged the people from the use of chewing-sticks from plants because of a lack of studies to analyze and characterize the chemical composition of the plant raw material and ensure the effectiveness and safety (Chopra et al 1956). Therefore, there is a need to examine the bioactive components present in Vernonia amygdalina.

### MATERIALS AND METHODS

#### Chemicals

Chemicals were from Sigma unless otherwise stated. All chemicals and solvents used were of analytical grade.

#### Sample collection and preparation

The plant materials were collected from a farm in Ogbomoso and identified by the herbarium unit of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso Oyo State, Nigeria. The samples were air-dried for 25 days, pulverized and sieved through a mesh size of 20 mm.

#### Extraction

Soxlet apparatus was used for the extraction according to a developed method (Adelowo and Oladeji, 2016) with a little modification in the solvents ethanol and methanol.

#### Concentration

The extracts were concentrated using a Rotary Evaporator. Three concentrated extracts were obtained using ethanol, ethyl acetate and chloroform as solvents.

#### Clean up

The clean-up method was carried out based on the method reported in the literature (Adelowo and Oladeji, 2016).

### Thin-layer chromatography analysis of phenolic compounds

Thin-layer chromatography was performed using pre-coated silica plates. The solvent system used was petroleum ether : ethanol : acetone (5:3:1 vol: 18 ml) as the mobile phase.

### FT-IR spectroscopic analysis of phenolic compounds

The spectrophotometric analysis was carried out according to the reported method in the literature (Adelowo and Oladeji, 2016).

### Phytochemical screening

Qualitative screening of the phytochemical components of the chewing sticks was carried out to detect the presence of alkaloids, saponins, tannins, flavonoids, anthraquinone, steroids, terpenoids, reducing sugars and phlobatannins (Owoyale et al 2005).

### Screening for antimicrobial activity

#### Preparation of solutions of the fractions

Stock solutions of extracts at 25 mg/ml were prepared by dissolving 0.05 µg of each extract in 20 ml of de-ionized water. Other concentrations of the extracts were prepared by serial dilution of the stock solution.

#### Preparation of fungus and bacteria culture

The fungi were cultured on Potato Dextrose Agar (PDA) and the bacteria on Nutrient Agar in accordance to the reported method (Owoyale et al 2005). The isolates were identified at the microbiological laboratory of the Department of Pure and Applied Biology, LAUTECH, Ogbomosho, Nigeria.

#### Antifungal and antibacterial screening

The antifungal activity of the fraction(s) was determined after incubating at 30°C for 3 days. The antibacterial activity of the fraction(s) was determined after incubating at 37°C for 24 h.

#### Antibiotic susceptibility test on the collected bacteria and fungi

Streptomycin and Gentamicin were used as positive controls for gram-negative and gram-positive bacteria respectively while Nystatin was used as positive control for the fungi tested.

### Statistical analysis

The Statistical Package for Social Scientists (SPSS, version 19.0) and two way ANOVA test were used to determine the level of significance.
of the crude extracts at different concentrations.

RESULTS AND DISCUSSION

**Thin-layer chromatographic analysis of flavonoids**

The retardation factors of *V. amygdalina* were analyzed on a pre-coated silica gel plate. The *R* \(_f\) values obtained were compared with the standard (**Table 1**). The qualitative analyses of the chloroform, ethyl acetate and ethanolic fractions of *V. amygdalina* stem were carried out using Quercetin as the standard for flavonoid content. The mean *R* \(_f\) values show proximity to that of Quercetin which indicates the presence of some content of flavonoids. The detected spots exhibited light yellow appearance on silica gel plate, indicating the presence of flavonoids.

**Table 1.** The *R* \(_f\) values and their mean values of the *Vernonia amygdalina* extracts spotted

<table>
<thead>
<tr>
<th>Sample spotted</th>
<th><em>R</em> (_f) values</th>
<th>Mean <em>R</em> (_f) values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>0.68, 0.71, 0.74</td>
<td>0.71</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.64, 0.67, 0.72</td>
<td>0.68</td>
</tr>
<tr>
<td>Ethanolic leaf</td>
<td>0.64, 0.72, 0.75</td>
<td>0.70</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0.66, 0.71, 0.73</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**FT-IR spectroscopic analysis of flavonol in *Vernonia amygdalina***

The FT-IR spectrum of *Vernonia amygdalina* showing the important functional bands and peaks (**Figure 2**). Flavonol is one of the most important phytochemical compounds present in *Vernonia amygdalina*. Flavonols are known to possess a characteristic structure containing an aromatic ring cross-linked together (Adelowo and Oladeji, 2016). The O-H bond indicated the presence of hydrogen bonding. The O-H functional group of the flavonol absorbed at 3500 cm\(^{-1}\) which could be due to the mesomeric effect with the electron withdrawing group such as C=O. The weak band of 2972.31 cm\(^{-1}\) and 2924.09 cm\(^{-1}\) indicated the presence of aryl C-H stretching of the flavonols aromatic ring. The sharp band at 1545.33 cm\(^{-1}\) indicated the presence of C-O stretching of ketone on carbon one (C=O stretch) of ester (**Table 2**).

**Table 2.** The bands and the corresponding functional groups obtained from the spectrum

<table>
<thead>
<tr>
<th>Band (cm(^{-1}))</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500.00</td>
<td>O-H stretch</td>
</tr>
<tr>
<td>2972.31</td>
<td>C-H stretch</td>
</tr>
<tr>
<td>2924.09</td>
<td>C-H stretch</td>
</tr>
<tr>
<td>1923.03</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1649.14</td>
<td>C=O stretch</td>
</tr>
<tr>
<td>1454.33</td>
<td>C=O stretch</td>
</tr>
</tbody>
</table>

**Phytochemical screening**

The important phytochemicals present in the ethanol, ethyl acetate and chloroform extracts of *V. amygdalina* are presented in **Table 3**. The preliminary phytochemical screening revealed the presence of anthraquinones, tannins, steroids, flavonoids, terpenoids, saponins and alkaloids.

![Fig. 2-IR spectrum of *Vernonia amygdalina*](image)

**Antimicrobial activity of *Vernonia amygdalina***

The ethanol extract showed high activity against all the test organisms with the exemption of *A. flavus*. The highest zone of inhibition (ZOI) was observed at C\(_1\) (500 mg/ml). The zone of inhibition of 10.5 mm at 500 mg/ml was observed in *A. flavus*. The zone of inhibition of 20.0 mm was observed at 500 mg/ml for ethyl acetate extract against *A. niger* and 9.5 mm at 500 mg/ml for *A. flavus*. The ZOI of 18.5 mm at 500 mg/ml was observed in *P. aeruginosa* and *Klebsiella* sp and the ZOI of 11.0 mm at 500 mg/ml for *A. flavus* and *Candida* sp (**Figure 3**).
**Table 3.** Phytochemical components of ethanol, ethyl acetate and chloroform extracts of *V. amygdalina*

<table>
<thead>
<tr>
<th>Active compound</th>
<th>Ethanol extract</th>
<th>Ethyl acetate extract</th>
<th>Chloroform extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthraquinone</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Keys: + = present, - = absent

The believe of local people in the use of chewing stick (*V. amygdalina*) as cleansing and antimicrobial in nature proved productive; since both gram-negative and gram-positive bacteria and fungi were sensitive to the extracts. The highest zone of inhibition of 22.5 mm was observed at 500 mg/ml for ethanolic extract against *P. aeruginosa*, followed by zone of inhibition of 20.0 mm of ethyl acetate extract against *A. niger* and the least ZOI of 18.5 mm was observed in chloroform extract against *P. aeruginosa* and *Klebsiella* sp. The activity shown in ethanolic extract could be due to the polarity of the solvent. Ethanol has proved to be an important solvent in phytochemical extraction (extracts more active compound) (Ibekwe et al 2000). The ethanolic extract compared statistically with the standard antibiotics (Streptomycin and Gentamicin); the inhibition observed in *Klebsiella* sp, *E. coli* and *P. aeruginosa* were significantly higher than Streptomycin. The ethanolic extract showed significant activity against *Trichoderma* sp. when compared to Nystatin.

**CONCLUSION**

Extracts of the stem of *V. amygdalina* (chewing stick) proved effective in inhibiting both bacterial and fungal infections. The antimicrobial activity could be due to the presence of the phytochemicals such as flavonoids, saponins, tannins. The results suggested that the ethanol extract of the chewing stick showed higher inhibitory effects on most of the test organisms when compared with chloroform and ethyl acetate extracts, hence appraising ethanol as a better solvent for extraction.

**REFERENCES**


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https://www.prota4u.org/database/protav8.asp?g=pe&p=Vernonia+amygdalina+Delile

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