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Phytochemical Screening and Antibacterial Activity of Bitter Leaf (Vernonia amygdalina)

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ABSTRACT

Herbal medicine is the oldest form of healthcare known to mankind. The study was aimed to determine the phytochemical constituents and antibacterial activity of Vernonia amygdalina leaf extracts against some bacteria associated with gastroenteritis. Five (5) different bacterial isolates associated with gastroenteritis namely; Klebsiella pneumoniae, Salmonella typhi, Shigella sp, Escherichia coli, and Staphylococcus aureus were obtained from Pathology Laboratory of Murtala Muhammad Hospital Kano. Phytochemical screening of the leaf extract was conducted to ascertain the presence and amount of bioactive components present in the leaves. The antimicrobial assay of the leaves extracts was performed by disc diffusion method. The qualitative and quantitative phytochemical screening of the extract indicated the presence of Alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin, with flavonoid being the most abundant constituent making about 12.2% followed by steroid, alkaloid and phenol constituting 4.8, 4.6 and 3.6% respectively. The result shows that the extracts were active against the microorganisms. The Methanol extract showed higher activity (12.2 mm) against the bacterial isolates than aqueous extracts (10.2 mm). Statistical analysis of the result shows no significant different on the activity of the extract against the test isolates at p<0.05. It is concluded that the application of V. amygdalina leaf in ethno medicine is justified.

Keywords: Antibacterial activity, bacteria, gastroenteritis, phytochemical, Vernonia amygdalina

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 [1]. The World Health Organization (WHO) estimates that approximately 80% of the world’s inhabitants rely on traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments [2,3]. The rediscovery of the connection between plants and health is responsible for the launching of a new generation of multi-component botanical drugs, dietary supplements and plant produced recombinant proteins [3]. However, the increasing problems of multi-drug resistant (MDR) bacteria is of great concern to both the clinicians and pharmaceutical industries and this has made it significant to search for newer drugs that are highly effective, affordable, acceptable and available[4].

Vernonia amygdalina commonly called bitter leaf because of its bitter taste is a member of the Asteraceae family. Ethno-medically, it is consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for the treatment of various diseases [5]. V. amygdalina is a medicinal plant use in folk medicine to manage several ailments [6]. The leaves are characteristically bitter although the bitterness can be abated by boiling or by soaking in several changes of clean water [7]. The bitter taste had been associated with the presence of saponins, alkaloids, tannins, and glycosides. These made them act as a bittering agent and a hop substitute used for controlling microbial contamination in beer brewing without reducing the quality of malt [8]. The roots and leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and
stomach discomfort among several other uses [7]. Both aqueous and alcoholic stem, root and leaf extracts are reported to be extensively used as purgative and anti-malarial as well as in the treatment of eczema [9]. The plant has acquired special relevance recently, having been shown in human medicine to possess potent antitumorigenic properties [10]. Nutritionally, *V. amygdaлина* is used mainly in soup making in the tropics and also as an appetizer and febrifuge [11] and has successful been used as a supplement in weaning foods [12].

Pharmacological studies have shown that the leaf extract of the plant has both hypoglycemic and hypolipidaemic properties in experimental animals and so could be potentially useful in the management of diabetes mellitus [13]. Several studies carried out on this plant had suggested that it contains different bioactive compounds, including, flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones [14]. These bioactive compounds made them possess different pharmacological properties like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, anthelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others [15].

The aqueous and ethanol extracts of *V. amygdaлина* leaves had shown antimicrobial effects against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Candida albicans* with the MIC values ranged between 12.5 and 50 mg/ml [16]. In another study, ethanolic and aqueous extracts of *V. amygdaлина* leaves had shown a higher value of MIC inhibitions on *Streptococcus mutans* at 25 and 55 mg/ml respectively [17,18]. The study was aimed to determine the phytochemical constituents and antibacterial activity of *Vernonia amygdaлина* leaf extracts against some bacteria associated with gastroenteritis.

**MATERIAL AND METHODS**

**Ethical Approval**

An approval (MOH/off/797/T.I/50) for the study was obtained from Research and Ethic Committee Kano State Ministry of Health. The approval was based on the consent of the Hospitals Ethical Committees of Murtala Mohammed Specialists Hospital (MMSH), Kano.

**Bacterial Isolates**

Five (5) different bacterial isolates associated with gastroenteritis namely; *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella* sp, *Escherichia coli*, and *Staphylococcus aureus* were obtained from Pathology Laboratory of Murtala Muhammad Hospital Kano. The isolates were characterized to specific level at Microbiology of Kano University of Science and Technology Wudil. The isolates were characterized using different procedures including Gram's stain, cultural characterization and Biochemical tests (Indole, Methyl red, Voges Proskauer, Catalase, Citrate utilization and coagulase tests) as described by Holt *et al.* [19]. The isolates were maintained on Nutrient agar slants for further use.

**Collection and identification of *V. amygdaлина* leaves**

The plant’s leaves used in this study (*Vernonia amygdaлина*) were obtained from Sabon-gari Market in Kano metropolis in Kano, Nigeria. Identification and authentication of the leaves were conducted at Herbarium in the Department of Plant science, Bayero University Kano with the following voucher number BUKHAN 0235 and voucher specimen were deposited in the herbarium for references. The leaves were air dried for 2 weeks, then ground into fine powder under laboratory condition using sterile pestle and mortar and stored in air tight container for further use.

**Extraction of *V. amygdaлина* Leaf**

The leaf extracts were prepared according to the method adopted by Ali *et al.* [20]. Fifty grams of powdered sample of the plant leaf were extracted exhaustively (cold maceration) using distilled water and methanol for 3 seven days. The extracts were filtered using Whatman No. 2 filter paper and concentrated at to powder form in water bath and rotary evaporator for aqueous and methanol extract respectively. The samples were kept in the refrigerator at 4°C until use. The dried powdered leaf was measured into McCartney bottles and appropriate volume of Dimethylsulphoxide (DMSO) was added to make a stock solution of 100mg/ml. Sterilization of the extract was done using 0.65 membrane filter by suction pump. The sterilized extract was stored in sterile McCartney bottle and kept in the refrigerator at 4°C before use.

**Phytochemical screening**

Phytochemical screening of the leaf extract was conducted to ascertain the presence of bioactive components present in the leaves. Presence of
Phytochemical Screening and Antibacterial Activity of Bitter Leaf (*Vernonia amygdalina*)

alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin were determined using procedure described by Sofowora [2] and Trease and Evans [21].

**Quantitative Phytochemical Analysis**

Different methods were used in evaluating the quantity of phytochemical constituents of the plant materials used. Spectrophotometric method was used to determine terpenoid, tannin and steroids. Folin-Ciocalteu procedure was used to determine phenol content. Flavonoids, alkaloids and saponins were determined by the methods described by Adeniyi *et al.*[22].

**Standardization of Bacterial Isolates**

Standardization of bacterial inoculum was done by picking a loop full colonies of each organism into nutrient broth and incubated at 37°C for 24 hours. Turbidity produced by the isolates was adjusted to match 0.5 McFarland standards [20].

**Antibacterial Activity of the Extracts**

The antimicrobial assay of *V. amygdalina* leaves extracts was performed by disc diffusion method as described by Kirby-Bauer [23]. The Mueller Hinton agar plates were inoculated separately with of each of the test bacterial strain culture (Equivalent to 0.5 Mac Farland) and evenly spread on entire surface of each plate. The sterile discs (6 mm diameter), were dipped aseptically in different extracts (25, 50, 75 and 100 mg/ml) for one minute and placed over the Mueller Hinton agar plates already seeded with bacterial culture. The plates were left at ambient temperature for 30 minutes and then incubated at 37°C for 24 hours and observed for zone of inhibition [24]. Commercially available Ciprofloxacin discs (10 μg, Oxoid, UK) were used as control. The diameter of inhibition zones was measured in millimeters. The experiment was performed in triplicate and the resulting zones of inhibition were recorded as mean ± standard error.

**Statistical Analysis**

The data of average zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at \( p < 0.05 \).

**RESULTS**

**Phytochemical screening**

The qualitative and quantitative phytochemical screening of *V. amygdalina* leaf extract is presented in Table 1. The result of qualitative phytochemical screening indicated the presence of Alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin. Quantitatively, flavonoid was found to be the abundant constituent in *V. amygdalina* leaf making about 12.2% followed by steroid, alkaloid and phenol constituting 4.8, 4.6 and 3.6% respectively.

Table 1. Qualitative and quantitative phytochemical screening of *V. amygdalina* leaf extract

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical</th>
<th>Qualitative analysis</th>
<th>Quantitative analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>4.60±0.23</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoid</td>
<td>+</td>
<td>12.20±1.30</td>
</tr>
<tr>
<td>3.</td>
<td>Saponin</td>
<td>+</td>
<td>2.70±0.50</td>
</tr>
<tr>
<td>4.</td>
<td>Steroids</td>
<td>+</td>
<td>4.80±0.25</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenoid</td>
<td>+</td>
<td>1.70±0.04</td>
</tr>
<tr>
<td>6.</td>
<td>Phenol</td>
<td>+</td>
<td>3.60±0.20</td>
</tr>
<tr>
<td>7.</td>
<td>Tannin</td>
<td>+</td>
<td>1.20±0.03</td>
</tr>
</tbody>
</table>

Key: + = Presence of phytochemical, - = Absence of phytochemical.

**Aqueous Extract**

The average antibacterial activity of aqueous *V. amygdalina* leaf extract is presented in Table 2. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *Shigella* sp (14.3mm) at 100mg/ml. The zone of inhibition of the control (Ciprofloxacin 10 μg) ranges from to 19-23 mm

Table 2. Average Antibacterial Activity of aqueous *V. amygdalina* leaf extract

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Concentration (mg/ml)/zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>
Phytochemical Screening and Antibacterial Activity of Bitter Leaf (Vernonia amygdalina)

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella typhi</td>
<td>8.3±0.12</td>
<td>10.4±0.16</td>
<td>12.4±0.19</td>
<td>13.7±0.20</td>
<td>21</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.00±0.00</td>
<td>9.2±0.25</td>
<td>11.2±0.26</td>
<td>11.8±0.31</td>
<td>20</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>8.9±0.14</td>
<td>10.5±0.23</td>
<td>12.5±0.19</td>
<td>13.6±0.18</td>
<td>22</td>
</tr>
<tr>
<td>Shigella sp</td>
<td>9.3±0.10</td>
<td>11.8±0.18</td>
<td>13.8±0.34</td>
<td>14.3±0.21</td>
<td>23</td>
</tr>
</tbody>
</table>

Methanol extract

The average antibacterial activity of Methanol V. amygdalina leaf extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by E. coli (15.2 mm) at 100mg/ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-23mm

**Table 3. Average Antibacterial Activity of MethanolV. amygdalina leaf extract**

<table>
<thead>
<tr>
<th>Concentration (mg/ml)/zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates</td>
</tr>
<tr>
<td>Escherichia coli</td>
</tr>
<tr>
<td>Salmonella typhi</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
</tr>
<tr>
<td>Shigella sp</td>
</tr>
</tbody>
</table>

DISCUSSION

The phytochemical screening of the leaves extracts of V. amygdalina revealed the presence of Alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin. These phytochemicals exhibit various pharmacological and biochemical actions and found to be beneficial to human health as well as possessing antioxidant activity [25]. Several studies had been conducted in isolating and characterizing some bioactive compounds from V. amygdalina leaf extracts [8,26,27,28]. The phytochemical studies had resulted in the isolation of flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones [14,29]. The finding of the study was in conformity with the finding of Atangwho et al. [30] reported V. amygdalina leaves contain 0.87% flavonoid, 0.37% tannins, 2.15% saponins and 2.13% alkaloids while Ndukwe et al. [31] reported 0.47% flavonoids, 0.74% Alkaloids, 0.64% saponinsand 0.74 tannins. Alkaloids are known to play some metabolic roles and control development in living system [32]. It also interferes with cell division, hence the presence of alkaloids in V. amygdalina could account for their use as antimicrobial agents. Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group agents its antimicrobial activity [33]. Flavonoids have also been implicated as antioxidants both in physiological and diseased states. For instance tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides [34]. Flavonoids are also expressed in plants in response to microbial infection suggesting their antimicrobial activity [35]. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability [36]. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have incivility effect on inflammation [37,38]. Present of saponin in V. amygdalina leaves supports the usefulness of the plant in managing inflammation. Steroids are important in pharmacy as they possess compounds like sex hormones and can be used for drug production [39]. Terpenoid have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoid are also known to possess antimicrobial, antifungal, anti-parasitic, antiviral, anti-allergenic, antispasmodic, anti-inflammatory and immunomodulatory properties [40]. It have been reported that phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities and they might induce mechanism that affect cancer cells and inhibit tumor invasion [41]. They also lower the risk of heart disease and provide anti-inflammatory activities attributable to their ability to neutralize and quench free radicals [42]. Tannin is known to have potentials antiviral activity [43] as well as potential prophylactic and therapeutic effect against cancer cells [44].

The present study showed that the leaves of V. amygdalina possess antimicrobial potential against some bacterial isolates associated with...
gastroenteritis. Several studies were conducted on antibacterial activity of *V. amygdalina* leaf extracts [45,46]. Their results show that there was variation in the degree of antibacterial activities of the extracts. Based on the findings of this study, methanol extracts showed more activity (12.2 mm) against the bacterial isolates than aqueous extracts (10.2 mm). This may be due to the higher volatility of the methanol which tends to extracts more active compounds from the samples than water. The result of this study was in conformity with that of Ogundare [47] who found ethanol extract of *V. amygdalina* leaf effective against *S. aureus* and *Shigella*. This result also justifies the finding of Zubairu et al. [48] who found *V. amygdalina* leaf extract effective against *E. coli*, *S. aureus* and *S. typhi*. On the other hand, the result contradicts the finding of Ogundare [47] who found no activity of *V. amygdalina* leaf extract against *E. coli* and *Salmonella*.

The *V. amygdalina* leaf extracts has demonstrated considerable activities against both Gram-positive and Gram negative bacteria. There was however, more activity against the Gram negative ( *E. coli*, *Shigella*, *Klebsiella* and *S. typhi*) organism than the Gram positive. Pelczar et al. [49] suggested that the difference in susceptibility of Gram positive and Gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls. For example, 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 marked difference in the effects of the extracts on the organism therefore, is suggestive of the activity against cell wall components of the organism. The antimicrobial substance appears to exert antimicrobial activity by inhibiting the growth of and by killing the sensitive bacteria.

**CONCLUSION**

From the findings of this study, it can be concluded that the extracts of *V. amygdalina* exhibits antibacterial activity against both gram positive and gram negative bacteria with methanol extract being the most effective. The activity of the extracts against the isolates is due to present of bioactive compounds in the extracts such as alkaloids, saponin, tannin and flavonoid. As result, the extracts can be used to develop new herbal formulation for preventing bacterial infection.

**REFERENCES**


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