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Antibacterial activity of crude extracts and pure compounds isolated from Vernonia galamensis leaves

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The aim of this study was to test the antibacterial property of the extract of the leaves and isolated compounds of Vernonia galamensis that is traditionally claimed to have diverse medicinal use. The disk diffusion method was used to test the successively extracted dried leaves of V. galamensis on Staphylococcus aureus, Escherichia coli, Salmonella typhi and Shigella boydii. Further fractionation of the acetone extract by a combination of column chromatography, gel filtration using Sephadex LH-20 and Prep-TLC afforded two compounds. The results showed that Vernonia Acetone Extract (VAE) of the leaves of V. galamensis showed weak to moderate antibacterial growth inhibition on the test bacteria. Two active compounds; C-I (vernolide) and C-II (vernonioside) were isolated that were not reported from V. galamensis before. C-I (0.6 mg/disc) showed antibacterial activity on all bacteria except E. coli with minimum inhibitory concentration (MIC) value of 2.5 mg/mL and C-II (0.48 mg/disk) showed growth inhibition only against S. boydii and S. typhi with MIC value of 1 mg/mL. In conclusion, V. galamensis leaves have been proved to possess antibacterial chemicals. The plant can possibly be exploited as a source of lead compounds for antibacterial drug development.

Key words: Antibacterial, Vernonia galamensis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella boydii, vernolide, vernonioside.

INTRODUCTION

Plants synthesize secondary metabolites as defences against plant pathogens. The genus Vernonia, Family Asteraceae comprises about 1000 species of herbs and shrubs. Most members of the genus are well known for their bioactivities. V. galamensis is widely distributed in the tropics. The ethnobotanical importance has been...
reported before by Teklehaymanot and Giday (2010). In Ethiopia, it is distributed in the south and some parts of the north of the country (Baye and Becker 2005). In vitro studies done previously have shown that *V. galamensis* possess anti diabetic, sedative and analgesic properties (Johri et al., 1995; Adetutu et al., 2011). Many Vernonia species are reported to have antimicrobial properties. Worth mentioning are *V. amygdalina* (Erasto et al., 2006; Oboh and Masodje, 2009; Sharma and Sharma, 2010; Adetutu et al., 2011), *V. auriculifera* (Hamill et al., 2003), *V. cinerea* (Yoga-Latha et al., 2009), *V. colorata* (Kelmanson et al., 2000) and *V. leopoldii* (Mothana et al., 2009). *V. hymenolepis* has shown antibacterial activity against Gram-negative multidrug-resistant bacteria (Noumedem et al., 2013). *V. galamensis* demonstrated antimicrobial activity against *E. coli*, *B. subtilis* and *S. aureus*, and the fungi *S. cerevisiae*, *Microsporum gypseum* and *Trichophyton mentagrophytes* (Sobrinho et al., 2015). Furthermore, the antibacterial activity and mode of action of *V. Adoensis* extracts against *S. aureus* and *P. aeruginosa* was reported (Moizirandi and Mukanganyana, 2017).

Therefore, the main aim of this study was to assess the antibacterial activity of the crude extract and isolated pure compounds from the leaves of *V. galamensis* on some pathogenic bacteria.

**MATERIALS AND METHODS**

**Plant material**

Fresh leaves of *V. galamensis* were collected from Bule-Hora area about 450 km south of Addis Ababa, Ethiopia, in November 2010. The plant was identified by a botanist and a specimen was kept in the National Herbarium of the Addis Ababa University with voucher number GT/005. Leaves were dried under shade to avoid any contamination and then ground with a blender to an appropriate size (about 0.5 mm) for extraction. The ground plant material was kept in closed container until used.

**Extraction and isolation of pure compounds**

The powdered leaves of *V. galamensis* (700 g) were macerated in *n*-hexane, acetone, ethanol and methanol successively in each solvent at a ratio of 1:7 (w/v) with some minor modifications as previously described by Pino-Rodriguez et al. (2003) for 24 h. The filtration was done using a double layer filter paper (Whatmann No.1) giving filtrate and residue in which the latter was subjected to the next maceration stage. Solvent was made to evaporate using Rotavapor R-210 with Vacuum Pump V-700 (Buchi) to collect crude extracts which were then weighed and kept at 4°C until further use. Preliminary antibacterial tests were conducted for each crude extract and the extract with the best activity was selected for further investigation. The extract Vernonia Acetone Extract (VAE) was then subjected to bioactivity-guided fractionation through flash column chromatography using the following solvent systems: pure *n*-hexane: chloroform, chloroform: methanol with increasing polarity. Different fractions (about 50mL each) were collected which were then combined based on their thin layer chromatography (TLC) profiles.

Further fractionation was done by gel-filtration using Sephadex LH-20 eluting with 1:1 chloroform/methanol. Non UV active compounds developed on TLC plate were visualized using vanillin sulfuric acid spray. Fractions with similar TLC profiles were combined and Prep-TLC was done for each combined fraction being eluted with ethyl acetate: chloroform (7:3). Bands made on Prep-TLC plate were scrapped off independently resulting in solid mixture of compound and silica. The obtained mixture was dissolved in chloroform and filtered out with filter paper (Whatmann No.1. 9cm) to separate the target compound from silica. Each compound was then poured in small container and kept in a hood being left open until the solvent completely evaporated. After completely dried, each pure compound was subjected to 1D (1H and 13C) and 2D (DEPT and HMBC) NMR for identification.

**Antibacterial test**

Four pathogenic standard bacterial strains namely, *S. aureus* (ATCC25223), *E. coli* (ATCC25922), *S. typhi* (ATCC1331) and *S. boydii* (ATCC9207), obtained from Ethiopian Health and Nutrition Research Institute (EHNRI) were used for the antibacterial tests. These bacterial strains were first grown on their own selective media prior to the test by incubating them at 37°C for 24 h. Each was incubated in nutrient broth and their turbidity was compared with McFarland (0.5 standard) before spreading them on the plates. Antibacterial tests were performed for the VAE crude extract and two major isolated compounds using the disk diffusion method (Tadeg et al., 2005). The four bacterial strains were streaked on different (independent) plates made of Muller-Hinton agar using sterilized swaps. VAE at 100, 200 mg and 300 mg and compound I (C-I) and compound II (C-II) dissolved in 1 mL of 3% Tween 80 at 20 and 16 mg respectively were used for the test.

Standard drugs were selected on the susceptibility of the bacteria to the drugs. Each drug at a concentration of 2.5 mg/mL in distilled water was prepared. Sterilized paper disks (6 mm each) were impregnated with 30 µL of each of 100, 200, 300, giving 3, 6 and 9 mg per disk of VAE; 0.6 mg/disk of C-I and 0.48 mg/disk of C-II. Similarly, four paper disks were impregnated with the standard drugs (75 µg/disk each): ampicillin, chloramphenicol, ciprofloxin and erythromycin. The same amount of 3% Tween 80 was loaded on different disks and all of them were left to dry. The paper disks were then kept on the allotted place of each plate that was partitioned externally into compartments. All plates were kept in incubator at 37°C for 24 h. After 24 h, zone of inhibition were measured by ruler and recorded in mm. The antibacterial tests were done in triplicate for the crude extract and each compound.

**Minimum inhibitory concentration**

Minimum inhibition concentration (MIC) was conducted for C-I and C-II according to the methods in Taiwo et al. (1999) and Adebolu and Oladimeji (2005). C-I at concentrations of 20, 10, 5 mg/mL, 2.5, 1.25 and 0.65 mg/mL dissolved each in 3% Tween 80 and C-II at 16, 8, 4, 2, 1, 0.5 and 0.25 mg/mL dissolved each in 3 % Tween 80 were prepared. The same procedure used for the antibacterial tests was applied. The MIC of each pure compound was performed only on those bacterial strains that gave positive results.
Figure 1. Structure of C-I, vernolide [2-Propenoic acid, 2-methyl-, (1aR,4Z,5aR,8aR,9S,10aR,11R)-1a,2,5a,7,8a,9,10-octahydro-11-hydroxy-8-methylene-7-oxo-3H-4,10a-(methanoxy)methano(5,6) cyclodeca (1,2-b)furan-9-yl ester]. PubChem CID: 6436299.
Figure 2. Structure of C-II, vernonioside [(2R,3S,5R)-hexahydro-6-((16R)-2,3,4,5,6,10,12,13,14,15,16,17-dodecahydro-3,16-dihydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-3,5-dihydroxy-3-isopropyl-2-methylfuro[3,2-b]furan-2-yl acetate]; PubChem CID: 6324766.

Table 1. Level of inhibition of Vernonia Acetone Extract (VAE), C-I (vernolide), and C-II (vernonioside) on the test bacteria as compared to the controls.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Stock Conc. (mg/mL)</th>
<th>Load/disk</th>
<th>Effect of inhibition Level</th>
<th>Test organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Drug</td>
<td>2.5</td>
<td>75 µg</td>
<td>+++</td>
<td>S. typhi</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3 mg</td>
<td>+</td>
<td>E. coli</td>
</tr>
<tr>
<td>VAE</td>
<td>200</td>
<td>6 mg</td>
<td>++</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>9 mg</td>
<td>+++</td>
<td>S. boydii</td>
</tr>
<tr>
<td>C-I</td>
<td>20</td>
<td>0.6 mg</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>C-II</td>
<td>16</td>
<td>0.48 mg</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

St. Drug = Standard Drug: Chloramphenicol (for S. typhi), ampicillin (for E. coli), erythromycin (for S. aureus) and ciprofloxacin (for S. boydii); VAE = Vernonia leaf Acetone Extract; - = no effect; + = weak effect; ++ = moderate effect; +++/++++ = strong effect.

VAE showed moderate (S. typhi, E.coli) to strong (S. aureus, S. boydii) dose dependent antibacterial activity at the dose of 6 and 9 mg/disc respectively. Table 2 shows C-I at the dose of 0.6 mg/disc showed mean growth inhibition of 18.7±0.6, 18±1.0 and 17.7±1.5 mm on S. typhi, S. boydii and S. aureus respectively. C-II is significantly different in inhibiting the growth of the test bacteria from the negative control (Tween 80) as well as the respective standard drugs in all tests (P = 0.00, Table 2). C-I showed antibacterial activity on all bacteria except E. coli with MIC value of 2.5 mg/mL (Table 3). Vernolide isolated from V. amygadalina was previously reported to have antibacterial activity with MIC of 0.5 mg/mL (Erasto et al., 2006). The difference in MIC values could be explained partly due to the species difference.

C-II at a dose of 0.48 mg/disc showed strong antibacterial activity only against S. boydii and S. typhi with mean inhibition of 28.7±1.5 and 25.7±5.9 mm respectively, while the standard drugs ciprofloxacin (S. boydii) and chloramphenicol (S. typhi) inhibited the bacteria at mean inhibition of 30.0± 1.0 and 30.3 ±1.5 mm respectively (Table 2). The MIC value for C-II was 1
Table 2. Inhibitory activity of the Vernonia Acetone Extract (VAE), compound I (vernolide) and compound II (vernonioside) on the test bacteria.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Stock Conc. (load/disk)</th>
<th>Zone of Inhibition in mm (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Drug</td>
<td>2.5 mg/mL (75 µg)</td>
<td>S. typhi: 30.3±1.5, E. coli: 25±1.0, S. aureus: 25±1.0, S. boydii: 30±1.0</td>
</tr>
<tr>
<td>VAE</td>
<td>300 mg/mL (9 mg)</td>
<td>S. typhi: 22±1.0, E. coli: 0.00, S. aureus: 0±0.0, S. boydii: 24±0.6</td>
</tr>
<tr>
<td>C-I</td>
<td>20 mg/mL (0.6mg)</td>
<td>S. typhi: 18.7±0.6, E. coli: 0±0.0, S. aureus: 17.7±1.5, S. boydii: 18±1.0</td>
</tr>
<tr>
<td>C-II</td>
<td>16 mg/mL (0.48mg)</td>
<td>S. typhi: 25.7±5.9, E. coli: 0±0.0, S. aureus: 0±0.0, S. boydii: 28.7±1.5</td>
</tr>
</tbody>
</table>

VAE = Vernonia leaf Acetone Extract; St. Drugs (Standard Drug): chloramphenicol (for S. typhi), ampicillin (for E. coli), erythromycin (for S. aureus), and ciprofloxacin (for S. boydii). P-value represents a comparison of the effect of the extract or pure compounds with the control drugs.

Table 3. Minimum inhibition concentration (MIC) of compound I (vernolide).

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Activity (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>++</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>++</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT = Not Tested; because this compound lacked activity against this bacterium.

Table 4. Minimum inhibition concentration (MIC) of compound II (vernonioside).

<table>
<thead>
<tr>
<th>Test bacteria</th>
<th>Activity (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>++</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>NT</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT = Not Tested; because this compound lacked activity against this bacterium.

mg/mL (Table 4). Vernonioside, isolated from V. guineensis was reported to have antifungal activity with MIC value of 7.9 µg/mL (Donfack et al., 2012). Furthermore vernonioside B2 from V. condensate is reported to have analgesic and anti-inflammatory property (Valverde et al., 2001).

Previous work on V. amygda lina using disk diffusion method showed activity against some bacterial strains (Jisaka et al., 1993; Taiwo et al., 1999; Cos et al., 2002). Work on other species of Vernonia; namely Vernonia hymenolepsis (Noumedem et al., 2013), V. galamensis (Sobrinho et al., 2015) and V. adoensis (Mozirandi and Mukanganyana, 2017) have substantiated the antibacterial properties of the plant.

There was lack of activity against some tested bacteria by the pure compounds in the present work while the crude extract showed activity which might suggest the presence of synergism of compounds in the later. As reported by Erasto et al. (2006), vernolide is also inactive against some Gram negative bacteria, which might be supportive to the lack of activity by C-I and C-II against E. coli in the present study.

The results of the present study showed that V. galamensis contains phytochemicals that can control the
growth of some pathogenic bacteria especially those affecting the gastrointestinal tract normal function. Although vernolide was isolated from other Vernonia spp. it is the first report from V. galamensis. It is also the first report for vernonioside with aglycone from this plant. The effect of the crude extract and C-I against S. aureus can support the traditional use of this plant in wound healing treatments. The inhibitory activity of C-II against S. typhi and S. boydii might suggest that this plant can be a potential candidate for antibacterial drug development.

Conclusion

In conclusion, V. galamensis leaves have constituents that have inhibited the growth of pathogenic bacteria. C-II showed significant inhibition on the growth of S. typhi and S. boydii. Further study is recommended to fully exploit the medicinal importance of the plant.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abbreviations: VHE, Vernonia Hexane Extract; VAE, Vernonia Acetone Extract; VEE, Vernonia Ethanol Extract; VME, Vernonia Methanol Extract; C-I, compound I, vernolide; C-II, compound II, vernonioside.

REFERENCES


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