Antibacterial Activity of the Eerial Axtracts from *Xanthium brasilicum*

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Abstract

Antibacterial activity of the aerial extracts from *Xanthium brasilicum* prepared in methanol, diethyl ether, petroleum benzene and an equal mixture of the three solvents were studied against bacterial laboratory standards and clinical isolates using the disk diffusion method. The best antibacterial results were obtained when methanol or the solvent mixture was used. The crude extract with the highest antibacterial activity was fractionated by silica gel chromatography and the biologically active fractions were subjected to thin layer chromatography. All bands were separated and tested for antibacterial activity and the compounds of the active (TLC) bands were identified by \(^1\)HNMR spectroscopy. The results showed the presence of two substances, a xanthanolide and a flavonoid.

Keywords: *Xanthium brasilicum*; Antibacterial activity.

Introduction

*Xanthium brasilicum* Velloso (Asteraceae) is one of the four species of *Xanthium* growing in Iran. The plant is generally found in Asia particularly in Iran, Afghanistan, India, Japan and parts of the Mediterrania (1, 2). *Xanthium brasilicum* is an annual weed with arrow shaped dentated leaves. The fruit is oblong and 1-1.5 cm long. Studies have shown that *X. strumarium* leaves and fruits have medicinal uses including antimicrobial activity and contain linoleic acid, vitamin C and a glucoside, xanthostrumarin (3, 4). However, no such information exists on *Xanthium brasilicum*.

We studied the antibacterial activity of *X. brasilicum* aerial crude extracts prepared in methanol, diethyl ether, petroleum benzene and a mixture of the three solvents. The biologically active extract was then partially purified using silica gel chromatography and fractions showing antibacterial activity were further fractionated by thin layer chromatography. The individual bands with antibacterial activity were then subjected to \(^1\)HNMR spectroscopy to identify the active compounds.

Experimental

Plant material

The aerial parts of the plant were collected from Chitgar Park area in Tehran in June 2003. The plants were dried in the shade and identified at the Herbarium of the Biology Department, Shaheed Beheshti University (Tehran, Iran).

Extract preparation from the aerial parts of *Xanthium brasilicum*

The air dried aerial parts of *Xanthium*...
brasilicum were soaked in methanol, diethyl ether, petroleum benzene or an equal mixture of the three, (MEP) at room temperature (10% w/v). The mixtures were filtered after 24 h and the filtrates were concentrated using a rotavapor (Eyela) and placed at -15°C to remove heavy hydrocarbons and lipids. The extracts were then diluted with methanol, filtered and evaporated to complete dryness. Crude extracts were reconstituted in methanol (10% w/v) before use. The aqueous extracts were prepared by adding boiling water to the dried plant (10% w/v), placing the mixture in a boiling water bath for 1 h followed by filtration and drying over water bath. The dried pellets were reconstituted to 10% (w/v) in sterile distilled water before use.

**Fractionation of the crude extracts**

The pellet from the crude extract in MEP (100 ml) was dissolved in chloroform and mixed with silica gel powder (70-230 mesh ASTM, Merck) until well adsorbed. The mixture was fractionated on a silica gel column (6 x 40 cm) previously equilibrated with petroleum benzene. Gradient elution was carried out using 150 ml of petroleum benzene, petroleum benzene and diethyl ether at different ratios, diethyl ether, diethyl ether and methanol at different ratios and finally methanol alone. Twenty six fractions (80 ml each) were collected and each fraction was tested for antibacterial activity by the disk diffusion method. The fractions with good antibacterial activity were subjected to thin layer chromatography (TLC, 20 x 20 cm containing silica gel 60 F254, Merck). Several bands were observed under UV lamp (254 and 365 nm) which were cut out, eluted using methanol and further concentrated before testing for antibacterial activity. One band with biological activity common to all active fractions was subjected to 1H NMR spectroscopy to identify the active components.

**Bacterial strains**

Twelve bacterial strains were used which included; Bacillus subtilis (ATCC 465), two Enterococcus faecalis (ATCC 29737 and a clinical isolate), three Staphylococcus aureus (ATCC 25923, ATCC 29737 and a clinical isolate), three Escherichia coli (ATCC 25922, ATCC 10536 and a clinical isolate) and three Pseudomonas aeruginosa (ATCC 85327, ATCC 9027 and a clinical isolate) strains. The organisms were cultured overnight on Mueller Hinton Agar (MHA) plates from frozen stocks before each experiment.

**Antimicrobial screening by the disk diffusion method**

The antibacterial activity of the extracts and fractions were determined by the disk diffusion method (5). Concentrations of 2.5 mg/disk were used for assaying crude extracts against all test bacteria. In addition, a range of concentrations (7, 3.5, 2.5, 1.75 and 0.875 mg/disk) were also tested against the ATCC laboratory standards. Four to six colonies from overnight grown MHA plates were resuspended in tubes containing 5 ml of Mueller Hinton Broth (MHB) and placed at 37°C for 4 h before adjusting the turbidity to MacFarland standard 0.5. MHA plates were seeded using sterile cotton tip swabs in 3 planes before placing 6 mm sterile disks containing the appropriate amount of each extract. The plates were incubated at 37°C for 18-24 h at which time the zones of inhibition were measured and reported in mm. Triplicate tests were carried out for each extract.

**Minimum inhibitory concentrations (MICs)**

MICs of the crude extracts were determined against E. coli and S. aureus strains using a tube doubling dilution assay. Serial two fold dilutions of MEP or methanol extracts were made in MHB within the range of 2000-3.75 µg/ml. Fresh bacterial suspensions were prepared in the same medium and 10⁶ bacteria were added/ml before incubating the tubes at 37°C for 18-24 h. The first dilution with no bacterial growth was recorded as MIC.

**Results and Discussion**

The disk sensitivity results for crude extracts showed that methanol, MEP and water extracts contained antibacterial activity. Extracts prepared in other solvents showed poor or no antibacterial activity and the results are not included. Table 1 shows the results of the antibacterial activity of the extracts prepared in methanol, MEP or water.
Twelve bacterial strains were used which included; Bacillus subtilis (ATCC 465), Escherichia coli (ATCC 25922, ATCC 29737 and a clinical isolate), three Staphylococcus aureus isolates (ATCC 25923, ATCC 29737 and a clinical isolate), three Pseudomonas aeruginosa isolates (ATCC 85327, ATCC 9027 and a clinical isolate), and four E. faecalis isolates (ATCC 10536, ATCC 27058, a clinical isolate, and an E. faecalis strain with HNMR spectroscopy to identify the active components.

Isolated cultures of B. subtilis, E. faecalis, S. aureus, and E. coli were subjected to antibacterial activity. One band with biological activity and the results are not included. Table 1 shows that methanol, MEP and water extracts contained antibacterial activity. Extracts prepared in other solvents showed poor or no antibacterial activity and the results are not included.

Table 1. Antibacterial activity of Xanthium brasilicum extracts.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Crude Extracts</th>
<th>Silica Gel Fraction No.</th>
<th>TLC</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Methanol</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>26(1)</td>
</tr>
<tr>
<td>B. subtilis ATCC 465</td>
<td>22</td>
<td>20</td>
<td>12</td>
<td>NT</td>
</tr>
<tr>
<td>E. faecalis ATCC 29737</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>26</td>
<td>22</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 85327</td>
<td>18</td>
<td>12</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>ATCC 9027</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Clinical</td>
<td>18</td>
<td>12</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Including disk diameter (6mm).
<sup>b</sup> Methanol:Dichloromethane:Petroleum Benzene (1:1:1).
<sup>c</sup> G10, Gentamycin (10µg), E10, Erythromycin (10µg). NT, not tested.

Franciation of the crude MEP extract on silica gel column was followed by TLC and further concentrated before testing for antibacterial activity. One band with antibacterial activity was subjected to thin layer chromatography (TLC, 20 x 20 cm containing silica gel 60 F 254, Merck). Several bands were obtained even with 0.875 mg of extract/disk. MIC results agreed with the disk sensitivity profile and were 125 µg/ml for MEP and 15.5 µg/ml for methanol extracts against S. aureus isolates. As expected, E. coli strains were more resistant and the MIC for both extracts against all 3 isolates was 500 µg/ml. However, these values are significant since only a fraction of the crude extract has been responsible for antibacterial activity. Unfortunately, because of the large volumes required for measuring MICs, purified fractions were not used.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>7 mg</th>
<th>3.5 mg</th>
<th>2.5 mg</th>
<th>1.75 mg</th>
<th>0.875 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis ATCC 29212</td>
<td>21</td>
<td>13</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>40</td>
<td>27</td>
<td>26</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>22</td>
<td>16</td>
<td>14</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 85327</td>
<td>20.5</td>
<td>18</td>
<td>18</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Methanol:Dichloromethane:Petroleum Benzene (1:1:1).
<sup>b</sup>Including disk diameter (6mm).
from several bands observed under UV, only one band showed strong antibacterial activity (26/1, Table 1). Finally, when band 26/1 from the TLC plate was subjected to 1H NMR spectroscopy two compounds were identified, a xanthanolide and a flavonoid.

Flavonoids have been shown to have antibacterial and antiviral activities by several investigators (6-9). There are also reports on the antibacterial activity of xanthanolides isolated mostly from Xanthium strumarium (3, 10-14) and Xanthium spinosum (15). This is the first report on the antibacterial activity of Xanthium brasilicum. Further work is needed to determine the ethnomedicinal potential uses of this plant.

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References


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