Antifungal and Antibacterial Properties of Grapevine (Vitis vinifera L.) Leaves Methanolic Extract from Iran - *in vitro* Study

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Abstract: In this study leaves of grapevine (*Vitis vinifera* L.) were shade dried, powdered and extracts were made by using methanol. The antimicrobial activity of methanolic extract (50, 100, 150, 200, 250, 300, 400 and 500 µg/mL) was evaluated against pathogenic organisms of two fungi (*Candida albicans* and *Aspergillus niger*), two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The antimicrobial activity was determined by both the disc diffusion method and the microbroth dilution method. Result showed at 250, 300, 400 and 500 µg/mL of the extract assayed concentrations, significantly inhibited the growth of *A. niger* and *C. albicans* (*P* < 0.05). MIC for *A. niger* and *C. albicans* were 75.4 and 80.9 µg/mL of extract, respectively. Results showed that the maximum inhibition zones at concentration of 500 µg/mL on the growth of two Gram-positive bacteria, *S. aureus* and *B. subtilis*. The plant extracts no significantly inhibited the growth of Gram-negative bacteria, *P. aeruginosa* and *K. pneumoniae* (*P*<0.05). All bacteria, were only marginally inhibited in the disc diffusion assay and showed no activity in the broth dilution assay (MIC > 100 µg/mL). And so, *V. vinifera* extract has antifungal and antibacterial properties, corroborating the traditional therapeutic uses of this plant and can be used in the therapy of infectious diseases as well as an antimicrobial supplement in foods.

Key words: Grapevine • Disc diffusion method • Microbroth dilution method • Minimum inhibitory concentration (MIC) • Inhibition zone

INTRODUCTION

The emergence of antibiotic resistant strains of bacteria decreases the number of antibiotics available to cure clinical infections caused by this pathogen [1, 2]. Today, natural products have played an important role in development and drug discovery particularly for agents against infectious diseases [3-6]. Plants are very significant natural source due to production of complex molecular substances. The plant produce structures such as secondary metabolites (such as phenolic compounds, alkaloids, flavonoids, tannins, cumarins, glycosides, terpenes and isoflavonoids) and their derivatives have antifungal and antimicrobial properties [7-11].

Despite plants are extremely exploited in traditional healing systems, only in some cases their curative potential in human has been substantiated [12]. The need
of herb-based medicines, food supplements, cosmetics, pharmaceuticals and health products is successively increasing all over the world, since in some cases, natural products i) are non or low toxic, ii) have low side effects and iii) are available at affordable costs [13].

Grapevine (Vitis vinifera L.) belongs to family Vitaceae. V. vinifera is grown in the worldwide. It is a deciduous woody climber with coiled climbing tendrils and wide leaf. In Iran, V. vinifera leaves are used in a vine leaf dolma (a traditional food) and for treatment of bleeding and diarrhea.

The present study has been concentrated on antifungal and antibacterial properties of V. vinifera leaf methanolic extract against two fungi (Candida albicans and Aspergillus niger), two gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two gram-negative bacteria (Klebsiella pneumoniae and Pseudomonas aeruginosa).

MATERIALS AND METHODS

Plant Materials and Organic Leaf Extracts:
The Grapevine (Vitis vinifera L. cv. ‘Yahghoti’) leaves (Fig. 1) were collected in June 2014 from Zabol region (Coordinates: 31° 1' 43" N, 61° 30' 4" E), in Sistan and Baluchestan Provinces of Iran. Plant parts were air-dried in the shade at ambient temperature (18–25 °C) for 5 days. The leaves of plants were powdered in a knife mill. Ground sample (50 g) was mixed with 200 ml of 85% methanol using a shaking water bath for 24 h at room temperature. The extract was separated from the solid concentrate by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 30°C using a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany).

Antifungal and Antibacterial Assays: The leaf extracts were assayed against two fungi (Aspergillus niger ATCC 9142 and Candida albicans ATCC 10231) and two gram-positive bacteria (Bacillus subtilis ATCC 6633 and Staphylococcus aureus ATCC 6538) and two gram-negative bacteria (Pseudomonas aeruginosa ATCC 9027 and Klebsiella pneumoniae ATCC 10031). The fungi and bacteria were cultured for 14–24 hour at 37°C and the densities were adjusted to 0.5 McFarland standards at 530 nm.

The antibacterial tests were carried out by the disc diffusion method [14]. Of the 100 µL microbial suspensions was spread on nutrient agar (Merck, Germany) plates (100 mm × 15 mm). Discs (6 mm diameter) were impregnated with 100 µL of different concentrations of extract (50,100, 150, 200, 250, 300, 400, 500 µg/mL) and placed on the inoculated agar. All the inoculated plates were incubated for 24 h at 37°C. We used positive control discs included ketoconazole, gentamicin and ampicillin (10 mg/disc) for fungi, gram-negative and gram-positive bacteria, respectively. Also, we used dimethyl sulfoxide (DMSO) as the negative control. Antimicrobial activity was appraised by measuring the zone of inhibition.

Minimum inhibitory concentration (MIC) was determined using serial dilutions of the extract (0–500 µg/mL) using microdilution assay approved by Clinical and Laboratory Standards Institute [15]. We were suspended the bacteria and fungi in Luria-Bertani media and the densities were regulated to 0.5 McFarland standards at A530 nm (10^6 CFU/mL).

The extract (100 µL) and the bacteria and fungi suspensions (100 µL) were added to microtiter plates and incubated at 37°C for 24 h. In this study, medium without bacteria and fungi was as sterility control and medium with bacteria and fungi but without extract was as growth control. The growth in each well with that of the growth in the control well was compared. The Minimum inhibitory concentration were visually detected in comparison with the growth in the control well and delineated as the lowest concentration of the components with >95% growth inhibition.

Statistical Analysis: The plant leaves extract was prepared in triplicate for antifungal and antibacterial assays. Data were subjected to analysis of variance following a completely random design to determine the least significant difference (LSD) at P<0.05 using SPSS v. 11.5.
RESULTS AND DISCUSSION

Results of antifungal and antibacterial of disc diffusion test are shown in Table 1. The extract showed significantly inhibited the growth at 250, 300, 400 and 500 µg/mL assay concentration of the growth of A. niger and C. albicans at P< 0.05. MIC for A. niger and C. albicans were 75.4 and 80.9 µg/mL of extract, respectively. The plant extract showed the maximum inhibition zones at concentration of 500 µg/mL on the growth of two Gram-positive bacteria, S. aureus and B. subtilis. All concentrations of plant extracts showed significantly inhibited the growth of S. aureus compare than controls except two concentrations of 300 and 400 µg/mL (P<0.05). Also all concentrations of plant extracts showed significantly inhibited the growth of B. subtilis compared to controls except two concentrations of 50 and 100 µg/mL (P<0.05). In addition, plant extracts no significantly inhibited the growth of Gram-negative bacteria, P. aeruginosa and K. pneumoniae (P<0.05). All bacteria, were only marginally inhibited in the disc diffusion assay and showed no activity in the broth dilution assay (MIC > 100 µg/mL).

Schnee et al. [16] studied Vitis vinifera canes, antifungal compounds against Plasmopara viticola, Erysiphe necator and Botrytis cinerea. They reported that methanolic and ethanolic crude extracts of Vitis vinifera canes exhibited significant antifungal activity against the three major fungal pathogens affecting grapevines, Plasmopara viticola, Erysiphe necator and Botrytis cinerea. Also they identified six compounds (ampelopsin A, hopeaphenol, trans-resveratrol, ampelopsin H, e-viniferin and E-vitisin B) presented antifungal activities against P. viticola. e-Viniferin also exhibited a low antifungal activity against B. cinerea. None of the identified compounds inhibited the germination of E. necator. The antifungal activity results in our study also showed significant inhibition in some assayed concentrations of the growth of A. niger and C. albicans. These results may be related to recognized compounds in this extract such as hopeaphenol, ampelopsin A, ampelopsin H, trans-resveratrol, E-vitisin B and e-viniferin.

Ahmad et al. [17] investigated the antibacterial activity of Vitis vinifera leaf extracts against some pathogenic bacterial strains. Sample consisting of fresh healthy leaf that were tested for their properties to inhibit growth of four species of bacteria, namely, Staphylococcus aureus Pseudomonas aeruginosa, Enterococcus faecalis and Escherichia coli. The inhibition zones against the tested bacteria were ranging from 23.7 to 30 mm. The highest zone of inhibition produced by hot water extract for S. aureus, E. faecalis, E. coli and P. aeruginosa were 30, 28.9, 28 and 23.7mm, respectively.

Ceyhan et al. [18] studied in-vitro antimicrobial activities of different extracts of Grapevine Leaves (Vitis vinifera L.) from West Anatolia against some pathogenic microorganisms. They reported that Ethanolic extracts of grapevine leaves showed various antimicrobial activity (0-25 20µL⁻¹ inhibition zone) to the microorganisms assayed. The methanolic extracts showed antmicrobial activity (0-16 20µL⁻¹ inhibition zone) to the microorganisms tested. The aqueous extracts showed no inhibition zone five out of ten microorganisms. The Ethanolic extract displayed the best activity.

<table>
<thead>
<tr>
<th>Extract concentrations (µg/mL)</th>
<th>C. albicans</th>
<th>A. niger</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>14.3±0.0  f</td>
<td>16.5±0.3  f</td>
<td>15.6±0.5  h</td>
<td>18.1±0.2  g</td>
<td>3±0.0  g</td>
<td>3.5±0.1  e</td>
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<tr>
<td>100</td>
<td>14.5±0.1  f</td>
<td>17.9±0.5  f</td>
<td>18.5±0.2  g</td>
<td>19.5±0.3  g</td>
<td>4.6±0.1  f</td>
<td>4.4±0.2  d</td>
</tr>
<tr>
<td>150</td>
<td>25.0±0.5  e</td>
<td>31.7±0.0  c</td>
<td>25.6±0.7  e</td>
<td>24.6±0.1  f</td>
<td>5.9±0.0  e</td>
<td>4.5±0.1  d</td>
</tr>
<tr>
<td>200</td>
<td>26.7±0.2  e</td>
<td>32.2±1.1  e</td>
<td>39.9±0.5  d</td>
<td>38.9±0.5  e</td>
<td>6.7±0.5  d</td>
<td>5±0.0  c</td>
</tr>
<tr>
<td>250</td>
<td>34.5±0.0  d</td>
<td>38.9±0.0  d</td>
<td>44.2±0.2  c</td>
<td>46.5±0.1  d</td>
<td>6.5±0.3  d</td>
<td>5.3±0.1  c</td>
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<td>300</td>
<td>39.8±0.4  c</td>
<td>41.6±0.2  c</td>
<td>59.5±0.5  b</td>
<td>54.7±0.9  c</td>
<td>9.4±0.0  e</td>
<td>6.1±0.2  b</td>
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<td>400</td>
<td>59.9±0.5  b</td>
<td>64.9±0.1  b</td>
<td>59.1±0.9  b</td>
<td>65.5±0.2  b</td>
<td>10.2±0.0  b</td>
<td>6.5±0.0  b</td>
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<td>500</td>
<td>95.6±0.8  a</td>
<td>100.7±0.0  a</td>
<td>62.5±0.5  a</td>
<td>79.3±0.1  a</td>
<td>10.4±0.3  b</td>
<td>6.5±0.1  b</td>
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<tr>
<td>DMSO</td>
<td>5±0.0  h</td>
<td>5.5±0.0  b</td>
<td>2.5±0.0  i</td>
<td>2.2±0.0  i</td>
<td>3.1±0.0  g</td>
<td>1±0.0  f</td>
</tr>
<tr>
<td>Ketoconazole (µg/mL)</td>
<td>8.2±0.1  g</td>
<td>9.9±0.0  g</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>Ampicillin</td>
<td>-</td>
<td>-</td>
<td>20.5±0.2  f</td>
<td>14.5±0.3  h</td>
<td>-</td>
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<tr>
<td>Gentamicin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.2±0.0  a</td>
<td>13.5±0.3  a</td>
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<tr>
<td>MIC</td>
<td>80.9±0.9  a</td>
<td>75.4±0.5  &gt;100</td>
<td>100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD of inhibition zone diameter (mm) for different concentration of leaves extract, controls and minimum inhibitory concentration (MIC) (µg/mL); DMSO: Dimethyl sulfoxide (Negative control). The values with different letters within a column are significantly different (P < 0.05; LSD).
(MIC 6.25 µg/ml) against S. typhimurium CCM 583. Other microorganisms (S. aureus ATCC 6538/P, P. aeruginosa ATCC 27853, K. pneumoniae CCM 2318, C. albicans ATCC 10239) were showed between MIC 12.5-200 µg/ml. The methanolic and aqueous extracts were between MIC 50-400 µg/ml and between MIC 100-400< µg/ml, respectively. They illustrated the in vitro study indicate that grapevine leaf extracts, particularly ethanolic, could be used as natural antimicrobial agents in the food preservation and human health for microorganisms.

In conclusion, the results of this study there are hopes that methanolic extract of Vitis vinifera leaf may be used for treatment of some resistant types of microorganisms such as against two fungi (Aspergillus niger ATCC 9142 and Candida albicans ATCC 10231) and two gram-positive bacteria (Bacillus subtilis ATCC 6633 and Staphylococcus aureus ATCC 6538) and two gram-negative bacteria (Pseudomonas aeruginosa ATCC 9027 and Klebsiella pneumoniae ATCC 10031). In addition, it can be used as food supplement and material for food in food industries. In addition, screening for bioactive phytochemicals from extracts of Vitis vinifera leaf should be carried out in search of novel chemotherapeutic agents.

Conflict of Interest: The authors declare no financial or other conflicts of interest.

REFERENCES


