Antimicrobial Activity of Methanolic Root Extracts of *Euphorbia Condylocarpa* against Pathogenic Bacteria

Sepideh Mohammadi¹, Vahid Asgary²*, Seyed Ataollah Sadat Shandiz³, Erfan Heidari⁴, Hamidreza Jozaghkar⁵, Reza Ahangari Cohan⁶ and Amir Mirzaie³

¹Department of Microbiology
Iranian Azad University, Pharmaceutical Sciences Branch
Tehran, Iran

²Department of Immunology
School of Medicine, Tehran University of Medical Sciences
Tehran, Iran

³Corresponding author

³Department of Biology
Science and Research Branch, Iranian Azad University
Tehran, Iran

⁴Department of Biology
Iranian Azad University, Varamin Pishva Branch
Varamin, Iran

⁵Department of Biology
East Tehran Branch, Iranian Azad University
Tehran, Iran

⁶Department of Rabies
Virology research group, Pasteur Institute of Iran
Tehran, Iran

Copyright © 2014 Sepideh Mohammadi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Abstract

In the present study, the antibacterial activity effect of methanolic root extracts of *Euphorbia Condylecorpa* was evaluated against five Gram positive, three Gram negative bacterial strains. The antibacterial activity was determined by agar disk diffusion method. The most susceptible bacteria to *Euphorbia Condylecorpa* root extracts were *Bacillus subtillis*, *B. pumilis* and *Staphylococcus epidermidis* and displayed larger inhibition zones than Gram negative bacteria. Minimum inhibition concentrations (MIC) of extract against these bacteria strains were determined. The lowest MIC values were obtained with *B. pumilis*, *B. subtillis* and *S. epidermidis* (1.87 mg/mL), followed by *Staphylococcus aureus* and *Escherichia coli* (7.50 mg/mL) and the highest were obtained with *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis* (15.00 mg/mL). The cell proliferation and standard cytotoxicity assays of *Euphorbia Condylecorpa* root extract were revealed that there is no toxic against L929 normal cell line. Based on the current investigation, it is potentially suggested that methanolic *Euphorbia Condylecorpa* root extract can be proposed as an antibacterial agent and it can be further assessed to discover bioactive natural products involved in its activity.

Keywords: *Euphorbia Condylecorpa*, Pathogenic Bacteria, Antimicrobial Activit

Introduction

Despite of enormous advances in human medicines, infectious disease caused by bacteria, fungi, parasites and viruses are still an important threat to public health. In recent years, due to increased and indiscriminate use of commercial antibiotics used in treatment of such diseases, multiple drug resistance (MDR) in human pathogenic microorganisms has been developed a great deal in developing countries [1]. Hence, there is an urgent need to research on new antimicrobial agents with promising biological activities has provided an alternative to overcome the mentioned obstacles [2] [3] More so, herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environment friendly. They are also cheap, easily available and affordable [4]. In recent years, the natural product research has focused the work on isolation and identification of compounds for application mostly in pharmaceutical area[5] Many plants in different locations around the world have been discovered and estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological properties [6]. Plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism. There are numerous published reports in describing the antimicrobial properties of extracts obtained from Medicinal plants [7].

The genus *Euphorbia* is the largest in the plant family Euphorbiaceae, comprising
Antimicrobial activity of methanolic root extracts

about 2000 known species and ranging from annuals to trees [8]. Euphorbiaceae is the largest family of angiosperm having 300 genera and 5000 species. The antibacterial activity of several Euphorbia species has been evaluated in different occasions. Moreover, the extracts of Euphorbia species have been found to have significant anti-inflammatory, analgesic, haemostatic (stop bleeding) and wound healing properties [9].

The aim of the present study was to evaluate the antibacterial activity of methanolic root extracts of *Euphorbia Condylocarpa* against pathogenic microorganisms.

**Materials and Methods**

*Preparation of Methanolic extraction*

The *Euphorbia Condylocarpa* was collected from agricultural field located at sanandaj, Iran. The taxonomic position of the plant was confirmed and authenticated. The fresh plant was harvested in a large quantity and washed thoroughly in distilled water. Plant tubers were air dried under shade for 15 days, powdered and stored in an airtight bottle until needed for extraction purposes. 50 g of dried and powdered plant materials were subjected to extraction with 150 ml methanol (Merck, Germany) by aid of Soxhelt apparatus at 60 °C for 12 h. The resulted extract was filtered and concentrated under vacuum at 40 °C by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany). The extract was stored in refrigerator for further antimicrobial study.

**Test organisms**

The bacteria strains were used include; *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 465), *Bacillus pumilis* (PTCC 1274), *Staphylococcus aureus* (ATCC25923), *Staphylococcus epidermidis* (ATCC12228), *Klebsiella pneumoniae* (ATCC 10031), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 85327) and L929 normal cell line (Pasteur Institute of Iran, Tehran).

**Antimicrobial assay**

*Antibacterial sensitivity testing using disc diffusion method*

The agar disc diffusion method was adopted [10] to determine the antimicrobial activities of the methanolic extracts of the plants against representatives of gram-positive bacteria (*Enterococcus faecalis*, *Bacillus subtilis*, *B.pumilis*, *S aureus* and *S. epidermidis*) and Gram-negative bacteria (*K. pneumoniae*, *E. coli* and *P. aeruginosa*).

The Muller Hinton Agar (MHA, Difco) was weighed and dissolved in 100 ml of distilled water in a sterile conical flask. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into the sterile Petri plate. 100 µl of 18 h culture of bacteria adjusted to $1.5 \times 10^8$ cfu/ml was spread into a sterile plate so as to achieve a confluent growth. Circular disc of 6 mm diameter were impregnated with 10 µl of plant extract and followed by placing
on the medium plate swabbed with the culture of microorganisms. Plates were incubated at 37°C for 24 to 48 hr. After incubation, the inhibition zones were measured (in mm diameter) and diameters less than 5 mm indicated no effect. The presence or absence of growth inhibition zone around each disc was compared with the standard antibiotic disc (Ampicillin Disk, Merck, Germany).

**Antibacterial activity by microdilution MIC assay method**

The minimum inhibitory concentration (MIC) is the most basic laboratory measurement of the activity of an antimicrobial agent against microorganisms [10]. The MIC value of plant extract was determined by comparing the various concentrations of extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition [11]. In the test, a 96 well microplate (U bottom) was used. For performing MIC of *Euphorbia Condyllocarpa* extract, 12 washed tubes were taken. Each test tube was filled with 100 μl of culture media (i.e. Muller Hinton broth) for bacteria. For each culture test tube, a control test tube was also adopted. In the 1<sup>st</sup> test tube of cultured test tubes, 50 μl of extract was added and mixed properly. The test tubes were then serially diluted till the 11<sup>th</sup> test tube. Then, 100 μl of an 18 h old culture of each of the bacteria earlier adjusted at 1.5×10<sup>8</sup> CFU (Colony Forming Unit) was put into each tube except the 11<sup>th</sup> test tubes in each line and thoroughly mixed. (The 11<sup>th</sup> tube in each line was a blank –having only extract and medium). Finally, the test tubes with culture were prepared in triplicates and incubated at 37 °C for 24 h and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

**Cytotoxicity Assay**

**Cell culture**

L929 normal cell line was purchased from National Cell Bank of Iran (NCBI). The cell line was grown in DMEM medium supplemented with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% heat inactivated fetal calf serum (FCS) (All from Gibco, Scotland) at 37 °C in a 5% CO2 atmosphere. After 24-48 h, the attached cells were harvested with 0.25% trypsin (Sigma, USA), counted and distributed into 96-well plates with 10000 cells in each well. The plates were incubated 24 h under a humid atmosphere (37°C, 5% CO2) to permit the cells attaches to the bottom of the well. Then 100 μl of different concentrations of synthesized *Euphorbia Condyllocarpa* root extract (10, 5, 2.5 and 1.25 mg/ml) were treated into grown cell (1×10<sup>4</sup> cells/well) and the plates were incubated for 24 h in order to further analysis.

**Cell viability assay**

The cell viability was evaluated by using methyl thiazolyl tetrazolium bromide (MTT, Sigma, USA) assay [12]. Briefly, 100 μl of the MTT [3-(4, 5-dimethylthiazol-2, 5 diphenyl tetrazolium bromide] solution (5 mg/ml in PBS) was added to each well. The plates were incubated for 4 h at 37 °C, for reduction
of MTT by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan solubilized the MTT crystals by adding and quantified by spectrophotometric mean and then the supernatants were removed. For solubilization of the MTT crystals, 100 μL of DMSO was added to the wells. The plates were placed on a shaker for 15 min to complete solubilization of crystals and then the optical density of each well was determined against blank reagent with a multi-well scanning spectrophotometer (ELISA reader, Organon Teknika, Netherlands) at a wavelength of 570 nm. Each experiment was done in duplicate. The relative cell viability (%) related to control wells was then calculated by equation 1.

Relative Cell Viability (%) = \[ \frac{[A]_{\text{test}}}{[A]_{\text{control}}} \times 100 \]  

(Equation 1)

Where \([A]_{\text{test}}\) and \([A]_{\text{control}}\) are the absorbance of test and control, respectively [12]. In addition, optical density (OD) value was subjected to percentage of viability by using equation 2.

Viability (%) = (OD value of test samples / OD value of test controls) \times 100

(Equation 2).

Results

In this study, the methanolic root extracts of *Euphorbia Condylocarpa* were tested against eight Gram positive and negative bacteria and MIC was determined for each bacterium. MIC values of the extract tested against clinical isolates were summarized in Table 2. The lowest MIC values were obtained for *B. pumilis*, *B. subtilis* and *S. epidermidis* (1.87 mg/mL), followed by *S. aureus* and *E. coli* (7.50 mg/mL) and highest MIC value were obtained with *P. aeruginosa*, *K. pneumoniae* and *E. faecalis* (15.00 mg/mL).

For disk diffusion test, the bacterial cultures in Petri plates were incubated along with test compounds, which were checked for growth inhibition zone of organisms after 24h. The antibacterial activity of root extracts of *Euphorbia Condylocarpa* was studied and listed in Table 1 and Figure 1. The most susceptible microbes to *Euphorbia Condylocarpa* root extracts were *B. subtilis*, *B. pumilis* and *S. epidermidis* displayed larger inhibition zones against Gram positive bacteria than Gram negative bacteria. The *in vitro* cytotoxicity assay (Figure 2) using different concentrations of *Euphorbia Condylocarpa* root extracts (10, 5, 2.5 and 1.25 mg/ml) against L929 cell line showed that the extract are not toxic.

Discussion

Plants and their products have been used in wide range of biologically active molecules making them a rich source for different medicines which play a dominant role in the maintenance of human health since time immemorial [13]. During the past investigations, it was revealed that the root extract of *Euphorbia condylocarpa* (*Euphorbiaceae*) has significance applications in folk medicine to cure skin diseases, gonorrhea, costiveness, and migraine[14] Furthermore, Anti-
bacterial activity has been reported for various Euphorbia species[15, 16] Similar studies illuminate the antibacterial properties of ethanolic extract of E. fusiformis, E. hirta and E. tirucalli against S. epidermidis and S. aureus strains [17,18] At the present investigation, it was found that Euphorbia Condylocarpa was effective against all tested bacterial strains. Amir Muhammad Khan et al. (2008) determined the antibacterial properties of Euphorbia hirta plant extracts against all the enteric bacteria. Similar results were found by Abubakar (2009) that methanolic extract of E. hirta could be beneficial in treating enteric infections caused by E. coli [19].

According to Muhammad Khan et al. (2011), the crude extract of Euphorbia hirta effectively exhibits higher antibacterial activity against all the tested bacterial strains and minimum inhibitory concentration (MIC) values was recorded in 1 mg/ml against Euphorbia. faecalis and Euphorbia. Coccus [20]

Other studies show that extracts of Euphorbia plants inhibit the growth of various microorganisms at different concentrations [21-24]. The antibacterial potential test of E. Condylocarpa revealed that methanolic root extract of this plant was able to inhibit the growth of gram negative and positive bacterial indicated by the presence of pure inhibition zone around paper disc. Also, the cell proliferation and standard cytotoxicity assays of Euphorbia Condylocarpa root extract were revealed that there is no toxic against L929 normal cell line. The formed inhibition zone is the ability measure of antimicrobial compounds against target bacteria. Inhibition around paper disc is depended on diffusion of antibacterial compounds used. Finally, the results therefore shows the plant is proven very harmless to consume and useful in the treatment against infectious diseases caused by pathogens. This report reveals the safe use of Euphorbia Condylocarpa in ethnomedicine for treating several infectious diseases.

**Conclusion**

The results obtained herein on antibacterial activity of Euphorbia Condylocarpa root extract support the traditional use of this plant and provide grounds for further establishing its use in medicinal chemistry. However, further studies are needed to better evaluate the potential effectiveness of the crude extract as the antibacterial agents.

**References**

Antimicrobial activity of methanolic root extracts


Antimicrobial activity of methanolic root extracts


Figures:

Figure 1. Antibacterial activity of methanolic extract of Euphorbia Condylocarpa.

Figure 2. Evaluation of Euphorbia condylocarpa inhibitory effects on cell proliferation by MTT assay.
Table 1. Antibacterial activity (Zone of inhibition) of methanolic extract of *Euphorbia Condylocarpa*.

<table>
<thead>
<tr>
<th></th>
<th>E.F</th>
<th>B.S</th>
<th>B.P</th>
<th>S.A</th>
<th>S.E</th>
<th>K.P</th>
<th>E.C</th>
<th>P.A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>12</td>
<td>25</td>
<td>27</td>
<td>19</td>
<td>24</td>
<td>14</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>11±0.3</td>
<td>14±0.2</td>
<td>15±0.3</td>
<td>13±0.3</td>
<td>19±0.5</td>
<td>-</td>
<td>12±0.5</td>
<td>10±0.3</td>
</tr>
</tbody>
</table>

*Enterococcus faecalis E.F.; Bacillus subtillis, B.S.; B. pumilis, B.P.; Staphylococcus aureus S.A.; Staphylococcus epidermidis S.E.; Klebsiella pneumoniae, K.P.; Escherichia coli, E.C.; Pseudomonas aeruginosa P.A.*

Table 2. Minimum Inhibitory Concentration (MIC) of *Euphorbia Condylocarpa* extracts and standard antibiotic against the test organisms.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.F</td>
</tr>
<tr>
<td>Extract</td>
<td>15</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>14.3</td>
</tr>
</tbody>
</table>

*Key: Enterococcus faecalis E.F.; Bacillus subtillis, B.S.; B. pumilis, B.P.; Staphylococcus aureus S.A.; Staphylococcus epidermidis S.E.; Klebsiella pneumoniae, K.P.; Escherichia coli, E.C.; Pseudomonas aeruginosa P.A.*

Received: October 15, 2014; Published: December 11, 2014