

Evaluation of Anti-Bacterial Properties of *Euphorbia Condylcarpa* Methanol Extract

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Abstract

Objective: Infections disease caused by antibiotic resistant bacteria leads to increasingly difficult therapeutic problems. In present study, the antibacterial effect of the *Euphorbia Condylcarpa* methanol extract against eight pathogenic bacterial strains including: *Staphylococcus aureus* ATCC 29737, *Enterococcus faecalis* ATCC 11700, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae* ATCC 10031 were evaluated.

Methods: The total root methanol extract of the *Euphorbia condylocarpa* was prepared by percolation method. The antibacterial effect was primarily investigated by agar diffusion method followed by determination of the MIC and MBC using broth Macro dilution method. In Addition, the cytotoxicity effect of the extract was evaluated by MTT test using HEK293 cell line.

Results: The highest antibacterial activity of extract against Gram positive bacteria was measured against *Staphylococcus epidermidis* (22.67 ±0.58mm). The extract had the highest antibacterial effect against *Pseudomonas aeruginosa* (MIC 62.50 mg/ml), While, it possess the lowest antibacterial activity against *E. coli* (MIC 250 mg/ml). The highest MBC values were finding against *S. epidermidis*, *E.coli*, *E. faecalis*, *Salmonella typhimurium*, and *K. pneumonia* equal to 500mg/ml. The standard cytotoxicity assays of *E. condylocarpa* extract showed no toxic effect against HEK293 normal cell line in concentration up to 10 mg/ml.

Keywords: Antibacterial Properties; *Euphorbia Condylocarpa*; Cytotoxic; Pathogenic Bacteria

1. Introduction

Infections disease caused by antibiotic resistant bacteria leads to increasingly difficult therapeutic problems [1]. Therefore, there is an urgent need to intensive research efforts on discovering new antimicrobial agents and development of alternative antimicrobial agents to combat these obstacles [2,3]. In last decade, herbal medicines have been used as a valuable origin of natural products for being safe, less bio hazard and environment friendly [4]. Majority of plants species in different locations around the world have been examined for their pharmacological properties and they are distinguish to possess numerous secondary metabolites such as flavonoids, steroids, tannins, tirpenes, saponins [5-7]. Medicinal plants have been employed as traditional treatment for various human disease and different investigations have been published on describing the pharmacological properties of Medicinal plant extracts [8]. The family Euphorbiaceae is the largest family of angiosperm including 300 genera and 5000 specie. The genus *Euphorbia* is the well-known in the plant family Euphorbiaceae, including about 2000 species and ranging from annuals to trees [9]. The antibacterial and cytotoxic activities of several *Euphorbia* species have been reported in different studies. Furthermore, studies revealed that *Euphorbia* species extracts possess significant anti-inflammatory, analgesic, haemostatic and wound healing properties [10]. Employing plants for medicinal purposes is a famous part of culture and tradition in Iran. Therefore, in this study the antibacterial effect of the total methanol extract of *Euphorbia Condylocarpa* against 8 bacterial strains including: *Staphylococcus aureus* ATCC29737, *Enterococcus faecalis* ATCC11700, *Bacillus subtilis* ATCC6633, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC8739, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae* ATCC 10031 were investigated. Cytotoxicity effect of extract was also determined by MTT test using HEK293 normal cell line.

2. Material and Methods

2.1. Plant Material

The fresh root of *Euphorbia Condylocarpa* was harvested from agricultural field located at Sanandaj (Kurdistan Province, Iran). The plant was identified by Mr. M. Kamalinejad in the Department of Pharmacognosy, Faculty of

Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Voucher specimens No. 2396).

2.2. Preparation of The Methanolic Extract

The plant roots were washed thoroughly for 3 times with distilled water and shade dried for 14 days. The dried plant was milled to a fine powder (Sieve no80). Approximately 250 grams of powdered root plant materials were subjected to extraction with 500 ml of methanol (Merck, Germany) by percolation apparatus at 60°C for 72hours. The extract was evaporated and concentrated under reduced pressure at a 49°C in Buchi rotavapour (Switzerland) and the dried extract was stored in refrigerator at 4°C for further studies.

2.3. Bacterial Strains and Cell Line

Gram-negative bacteria including *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10031), and *Salmonella typhimurium* (ATCC 14028), and Gram-positive bacteria including *Staphylococcus aureus* (ATCC29737), *Enterococcus faecalis* (ATCC 11700), *Bacillus subtilis* (ATCC 6633), and *Staphylococcus epidermidis* (ATCC 12228) were purchased from Iranian Research Organization for Science and Technology, Persian Type Culture Collection (PTCC). The normal cell line (HEK293) was obtained from the National Cell Bank of Iran (NCBI), Pasteur institute of Iran, Tehran.

2.4. Anti-Bacterial Assay

The agar diffusion method was used for preliminary evaluation of the antibacterial activity of the methanolic extract against eight bacterial strains [10]. The total methanol extract were serially diluted by Tween20 (20% v/v) from 31.25 to 1000 mg/ml. The sterile Muller Hinton Agar(MHA) medium (Merck, Germany) was poured into the sterile Petri dish and was streaked with the microorganism's saline suspension from overnight bacterial agar culture with a turbidity equivalent to a 0.5 Mc Farland. Wells with 6 mm diameter were punched in the agar and filled with 100 µl of the different concentration of the extract. The Plates were incubated at 37°C for overnight. Tween20 (20% v/v) was also added to one cup as a control which did not reveal any inhibition. The produced inhibition Zone of the each dilution of extract was measured in mm after 24 hours. These tests were carried out 3 times and the results were presented as mean ± SD.

2.5. Determination of The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Following the confirmation of the extract antibacterial activity the MIC and MBC values of the extract were determine by broth macrodilution method using Muller-Hinton Broth (MHB) medium(Merck, Germany) against the test organisms as recommended by the Clinical and Laboratory Standards Institute (CLSI) [11,12]. Briefly the methanol extract was serially diluted ranging from 7.812, 500 mg/mL and the dilutions were added to each of the tubes containing Muller Hinton Broth (Merck, Germany) with the final inoculums count of 5×10^5 CFU/ml. After 24 hours incubation at 37 °C, possible growth in the tubes was evaluated. The lowest concentration which inhibits the visible growth of the bacteria in liquid medium was defined as the MIC. In order to confirm MICs and establish the MBCs, 10 µl of each well with no visible growth was inoculated in MHA plates. After 24 hours of aerobic

incubation at 37°C MBC was defined as the lowest extract concentration at which no growth of bacteria was seen. The experiments were carried out in triplicate [11].

2.6. *In Vitro* Toxicity Assay

2.6.1. Cell Culture

The HEK293 cell line was cultured in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1X Pen-Strep (All from Gibco, Scotland). The cells were counted and distributed into 96-well microplate with 10^4 cells in each well. The plates were incubated with 37°C, in 5% CO₂ for 24-48 hours to allow the cells attaches to the bottom of the well.

2.6.2. MTT Assay

The cell viability was evaluated by using methyl thiazolyl tetrazolium bromide (Sigma-Aldrich, Germany) assay. Briefly, HEK-293 cell line were cultured in a 96-well plate (10.000 cells/well) (Nunc, USA) and incubated overnight at 37°C and 5 % CO₂. Different concentrations of *E.Condylocarpa* (10^{-2} , 10^{-1} , 1 and 10 mg/ml) were added to each well and incubated for 24 hours at 37°C. 100 µl (0.05 mg/well) of MTT was added to each well and then incubated at 37°C for 4 hours. The supernatants were removed and 100 µl DMSO (Sigma, USA) was added to each well and the reaction read at 570 nm. Finally, the percentage of cell viability was calculated by using equation 1. Viability (%) = (Absorbance of test samples /Absorbance of control samples) × 100 (Equation 1).

2.7. Statistical Data

Statistical data analyses were expressed as mean±SEM and p<0.05 were considered to be statistically significant. For quantitative data analysis paired, t-test and One Way analysis of variance (ANOVA) in case of cluster comparison, were applied by using SPSS version 18.

3. Results

3.1. Evaluation of antibacterial effect by the Agar well Diffusion Method

For agar well diffusion test, the bacterial cultures in plates were checked for growth inhibition zone of microorganisms after 24hours. The diameter of inhibition zones made by the total root methanolic extract of *E.Condylocarpa* is shown in Table 1. Tween 20 (20% V/V), as negative control, showed no inhibitory effect against the bacterial strains. As shown in Table 1, the total methanol extract showed antibacterial activity against all of the tested microorganisms (diameters of zone of inhibition ranging between 12.33 to 27 mm). Among Gram negative bacteria, the extract revealed the highest antibacterial activity towards *P. aeruginosa* (27.00±1 mm). While, the lowest antibacterial activity (14.00±1 mm) was recorded by the *E.Condylocarpa* extract against *E.coli*. Among gram positive bacteria, the extract exhibited the lowest antibacterial activity (15.67±0.58 mm) towards *S. aureus*, While, the highest antibacterial activity of extract against Gram positive bacteria was measured against *Staphylococcus epidermidis* (22.67 ±0.58 mm) and similarly on it towards *Bacillus subtilis*.

No.	Bacteria	Concentration (mg/ml)					
		1000	500	250	125	62.5	31.25
1	<i>E. coli</i>	14.00±1	12.67±0.58	-	-	-	-
2	<i>Pseudomonas aeruginosa</i>	27.00±1	26.00±1	18.67±0.58	17.33±0.58	15.00±1	12.33±0.58
3	<i>Salmonella typhimurium</i>	16.67±0.58	14.33±0.58	-	-	-	-
4	<i>Klebsiella pneumoniae</i>	17.67±0.58	17.33±0.58	-	-	-	-
5	<i>Staphylococcus aureus</i>	15.67±0.58	14.67±0.58	13.33±0.58	-	-	-
6	<i>Staphylococcus epidermidis</i>	22.67±0.58	21.33±0.53	-	-	-	-
7	<i>Enterococcus faecalis</i>	17.33±0.58	16.67±0.58	-	-	-	-
8	<i>Bacillus subtilis</i>	22.67±0.58	22.33±0.58	20.67±1.15	14.67±0.58	13.67±1.15	-

Table1: Mean diameter of growth inhibition zone (mm) of the total methanol extract of *E. Condylocarpa*.

3.2. Determination of MIC and MBC

The MIC and MBC values of total methanol extract of *E. Condylocarpa* against clinical isolates were described in Table 2 and Figure 1. The lowest MIC values were observed for *P. aeruginosa*, (62.50 mg/mL), followed by *B. subtilis* and *S. aureus* (125 mg/mL) and highest MIC value were obtained with *Salmonella typhimurium*, *K. pneumoniae*, *E. faecalis* and *S. epidermidis* (250 mg/mL). The MBC values obtained in this study (Table 2) ranged from 125 to 500 mg/ml. The lowest MBC value for *P. aeruginosa* were 125 mg/mL followed by *B. subtilis* and *S. aureus* (250mg/mL) and highest MBC value for the *E. Condylocarpa* against *S. epidermidis*, *E. coli*, *E. faecalis*, *Salmonella typhimurium*, and *K. pneumonia* (500 mg/mL).

Number	Bacteria	MBC	MIC
1	<i>Escherichia coli</i>	500	250
2	<i>Pseudomonas aeruginosa</i>	125	62.5

3	<i>Salmonella typhimurium</i>	500	250
4	<i>Klebsiella pneumoniae</i>	500	250
5	<i>Staphylococcus aureus</i>	250	125
6	<i>Staphylococcus epidermidis</i>	500	250
7	<i>Enterococcus faecallis</i>	500	250
8	<i>Bacillus subtilis</i>	250	125

Table2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of total extract of *Euphorbia Condylocarpa* against eight bacterial strain

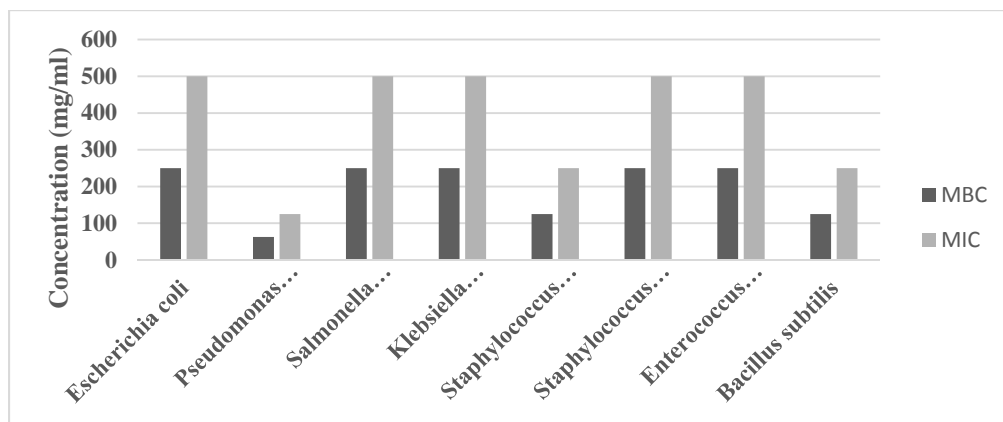


Figure 1: MIC and MBC values of *Euphorbia Condylocarpa* extract on some test bacteria

3.3. MTT assay

The *in vitro* toxicity assay using various concentration of *E. Condylocarpa* ranging from 10^{-2} , 10^{-1} , 1 and 10 mg/ml against HEK293 cell line demonstrated that the extract are not toxic (Figure 2).

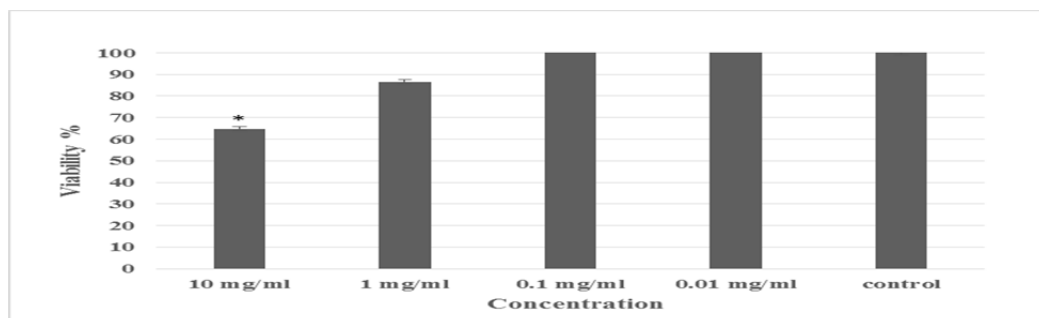


Figure 2: Cell viability assay of HEK293 cells after treatment with *Euphorbia condylocarpa* at 24 hours. Results were statistically analyzed with a Student's *t*-test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

4. Discussion

Plant extracts and their products can be of great source for different medicines which play a significant role in therapeutic treatment [13]. The root extract of *Euphorbiaceae* plant family have traditionally provided important application in folk medicine to cure gonorrhea, skin diseases, migraines and intestinal parasites [14]. Therefore, in this study, the anti-bacterial effects of the plant *Euphorbia condylocarpa* by Iranian Kurdish name 'Shoaleh Koleh' were evaluated. *Euphorbia condylocarpa* is known as a medicinal plant and distributed over Turkey, Iraq and west of Iran. The total methanol extract of the *Euphorbia condylocarpa* was prepared by percolation method. Primary antibacterial properties of methanol extract were investigated by agar well diffusion followed by determination of MIC and MBC methods using macro dilution method against eight bacterial strains. Several studies demonstrated that *Euphorbia* plant extracts can be inhibiting the growth of different microorganisms [15, 16]. Moreover, antimicrobial activity has been reported for numerous *Euphorbia* species [16-18]. Similar studies demonstrated the antibacterial activity of *Euphorbia hirta* extract [19].

Abubakar et al. (2009) have reported the inactivity of the *E. hirta* against *E.coli*, *K. pneumonia*, *Sh. dysenteriae*, *S. typhi*, and *P. mirabilis*. The water extract are more effective than hexane and methanol extracts. All the bacteria used in their study are susceptible to *E.hirta* extracts through to varying degree [19]. The antibacterial properties of *Euphorbia hirta* plant extracts using three solvents methanol, hexane and distilled water against a group of gram-negative bacteria that frequently cause enteric infection in humans[19]. Muhammad Khan et al. (2011) reported that crude extract of *Euphorbia hirta* effectively exhibits higher antibacterial activity against all the pathogenic bacterial strains [20]. In our study, the antibacterial potential test of *E. Condylocarpa* percolation extract revealed that it can be able to inhibit the growth of gram negative and positive bacterial because of the presence of pure inhibition zone around paper disc. In previous study, we reported that the most susceptible bacteria to methanolic *Euphorbia Condylocarpa* soxhlet extracts were *B. pumilis*, *B. subtilis*, and *S. epidermidis*. With larger inhibition zone of Gram positive bacteria in comparison to Gram negative bacteria [9]. While, during present study, we found that *Euphorbia Condylocarpa* percolation extract was effective against *p.auroginosa*, as a gram negative bacteria, as well as *B. subtilis* and *S. epidermidis*. Generally, in this study, Gram negative bacteria were more resistant to the total extract. *Euphorbia Condylocarpa* showed the minimum value of the MIC and MBC against *Pseudomonas auroginosa* at the concentration of 125, 62.50 mg/ml. Also, the standard cytotoxicity assay of *Euphorbia Condylocarpa* was showed that there is no toxic against normal HEK293 cells. Therefore, we are trying to done a more comprehensive study for effective anti-bacterial activity of Iran's native plants. There is a little study on the potential effects of the *Euphorbia Condylocarpa* plants extract. Finally, the results in the present study demonstrated that the *Euphorbia Condylocarpa* root plant extract is proven great useful in the treatment against pathogenic diseases and may be of great use for development of pharmaceutical industries as a therapy against several infectious diseases. This report supports the safe use of *Euphorbia Condylocarpa* in development of antibacterial in medicine.

5. Conclusion

The results of current study support the antibacterial activity of *Euphorbia Condyllocarpa* extract against some gram negative and positive pathogenic bacteria. Further works are needed to describe the potential antibacterial activity of the crude extract in more detail.

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