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Efficacy of Aqueous and Methanol Extracts of *Euphorbia helioscopia* for Potential Antibacterial Activity

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Abstract

Medicinal plants are used for treatment of many diseases all around the world. Besides, some of medicines are expensive or not readily available. These situations urgently forced scientists for searching new inexpensive drugs with prolonged periods before resistance set in. Because of the side effects and the resistance that pathogenic microorganisms build against the common antibiotics, much recent attention has been m to extracts and biologically active compounds which are isolated from plants in herbal medicine. This study has carried out in order to clarify antibacterial efficacy of *Euphorbia helioscopia* in Sari, Iran in 2016. Gram negative (*Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae*) and Gram positive (*Staphylococcus aureus, Bacillus cereus*) bacteria were used in this study. The results showed that the ethanolic extract of *Euphorbia helioscopia* had a promising antibacterial effect. In the present study, the best antibacterial efficacy of *Euphorbia helioscopia* was on *Staphylococcus aureus*.

Keywords: Methanol; Aqueous; Extract; *Euphorbia helioscopia*; Antibacterial

Introduction

Infectious diseases are the major public health concern. They are responsible for considerable mortality and morbidity worldwide. It has reported that enteric diseases cause 5.8 million deaths in infants and children below 5 years all around the world [1]. Chemotherapy is the main way for treatment of bacterial infections, but bacterial resistance to antibiotics leads to failure [2]. Other problems relate to the antibiotics such as toxicity, low efficacy and high cost leads to research for new alternatives. These days, modern medicine appreciates traditional pharmacology and search for potential medicinal plants. This can be a new approach for treatment of many infectious diseases [3]. There is a vast traditional usage of herbal medicine in many parts of the world. In developing countries including Iran, it is estimated that about 80% of the population using traditional medicine for their primary health care [4]. *Euphorbia* (*Euphorbiaceae*) with about 2000 species in the world and 100 species in Iran is a cosmopolitan genus and considerable distribution in tropical regions [5]. Species of this genus are usually herbaceous and mainly distributed in temperate areas of the Northern Hemisphere. With the exception of *E. helioscopia*, a widespread herb in temperate regions worldwide, the remaining species occur in the Alborz, Zagros and northwestern regions of Iran [6,7]. Traditionally, the plant is used in skin eruption, cholera, glactagogue and the leaves are useful as laxative [8]. Antibacterial activity of *E. helioscopia* extract on some bacteria as *Eschericha coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureu, Pseudomonas aeruginosa* and *Salmonella typhi* in several studies has been evaluated although the results were controversial. Anti-viral cytotoxicity, anti- fungal, anthelminthic, antioxidant and vasodepressor activities, related to having terpenoids, glycosides and amino acids, make this plant interesting for studying [9] Many plants have been used in antimicrobial traits, because they have compounds synthesized in the secondary metabolism of plants. Therefore, more studies for using plants as therapeutic agents should design, especially those related to the control of antibiotic resistant microbes [10]. The objective of this study was to evaluate the potential of plant extracts and phytochemicals on standard microorganism strains as well as multi-drug resistant bacteria.

Materials and Methods

Study area

Sari is a city in Mazandaran province, Iran. It is located in 36.56 latitude and in 53.06 longitude, also it is situated at elevation of 43 meters above the sea level.

Plant collection

Plants were gathered from Sari city in March 2016. Collected fresh plants were examined and the old, verminous and fungus-infected leaves were removed. Leaves were dried at room temperature (25°C) for one week in a purpose-built drying machine until the leaves were brittle enough to break easily. The dried plants were ground to a fine powder (diameter c. 0.1 mm) by using a laboratory grinding mill (Telemecanique/MACSALAB model 200 LAB) and stored in airtight bottles in the dark until extraction.

Extraction procedure

Separated aliquots of finely ground plant materials (50 g) were extracted with 120 ml of solvents of acetic acid and ethanol (technical grade, Merck) in conical flask for 4 h, while shaking every 3 to 5 min on a Labotec model 20.2 shaking machine. The process continued with covering the flask and incubating at 37°C for 48 h. The solution was subsequently shaken and filtered using Whatman filter paper. The filtrate was evaporated to dryness using a rotary evaporator (Model type Laborota 4010, Germany) and finally ethyl acetate and ethanolic extracts were achieved. Then the extracts stored below ambient temperature.

Phytochemical studies

Preparation of plant extracts for preliminary phytochemical studies: The grinded tuber materials of 5 g were weighed separately using an electronic balance and crushed in 25 ml of sterile water, boiled at 50-600°C for 30 minutes on water bath and it was filtered through

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Whatman No.1 filter paper. Then filtrated fluid was centrifuged at 2500 rpm for 15 minutes and stored in sterile bottles at 50°C for further use [11].

Gas chromatography-mass spectrometry analysis: Gas Chromatography-Mass Spectrometry (GC-MS) was performed on Hewlett Packard 6890 series, using a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 µm)) which was programmed as follows: 70°C for 5min and then up to 280°C at 4°C/min. The carrier gas was helium at a flow rate of 1 mL/min; split ratio, 1: 40; ionization energy, 70 eV; scan time, 1 s; acquisition mass range, 40-400(m/z). The components of the oil were identified by their retention time, retention indices relative to C_9-C_{28} n-alkanes, computer matching with the WILEY275.L library and as well as by comparison of their mass spectra with those of authentic samples or with data already available in the literature [12].

Selected test microorganisms

Pathogenic microorganisms selected for study include five bacteria, (*Staphylococcus aureus) S. aureus* (PTCC 1431), (*Escherichia coli) E. coli* (PTCC 1330), (*Bacillus cereus) B. cereus* (PTCC 1154), (*Pseudomonas aeroginosa*) *P. aeroginosa* (PTCC 1074) and (*Klebsiella pneumoniae) K. pneumonia* (PPTCC 1053). Selected microorganisms were procured

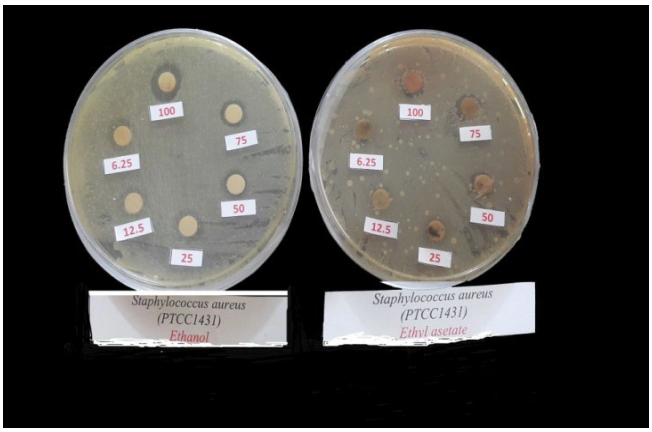

Figure 1: Disc diffusion assay showing inhibition zone induced by several concentrations of ethanolic/ethyl acetate extracts of *E. helioscopia* against *S. aureus*.

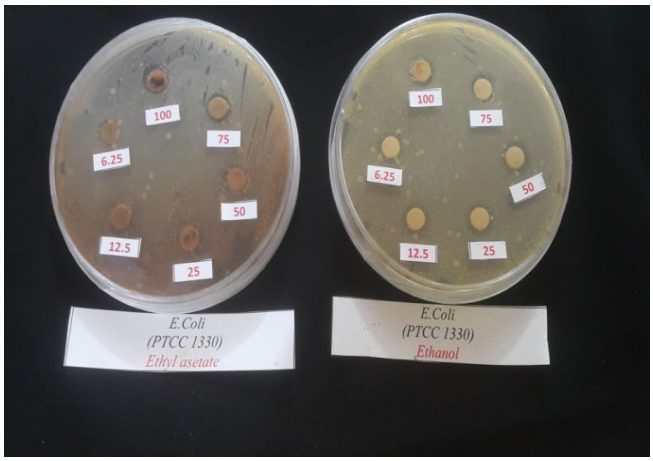

Figure 2: Disc diffusion assay showing inhibition zone induced by several concentrations of ethanolic/ethyl acetate extracts of *E. helioscopia* against *E. coli*.

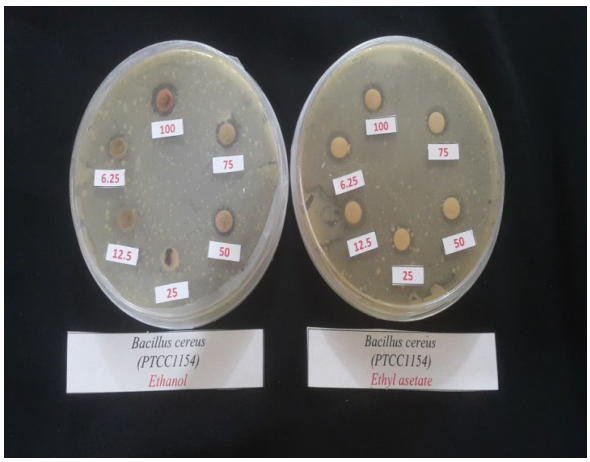

Figure 3: Disc diffusion assay showing inhibition zone induced by several concentrations of ethanolic/ethyl acetate extracts of *E. helioscopia* against *B. cereus*.

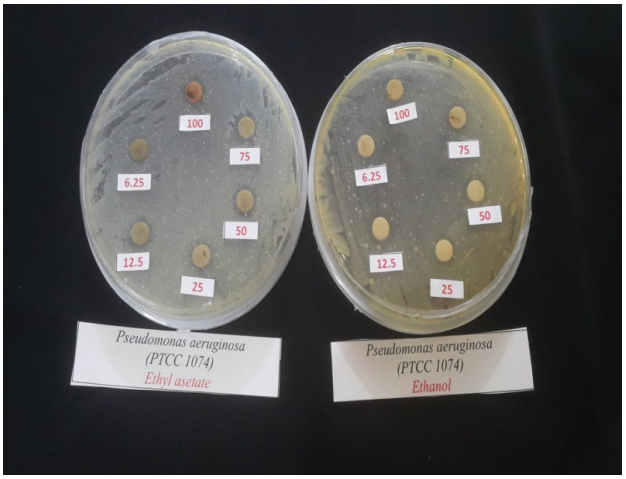

Figure 4: Disc diffusion assay showing inhibition zone induced by several concentrations of ethanolic/ethyl acetate extracts of *E. helioscopia* against *P. aeruginosa*.

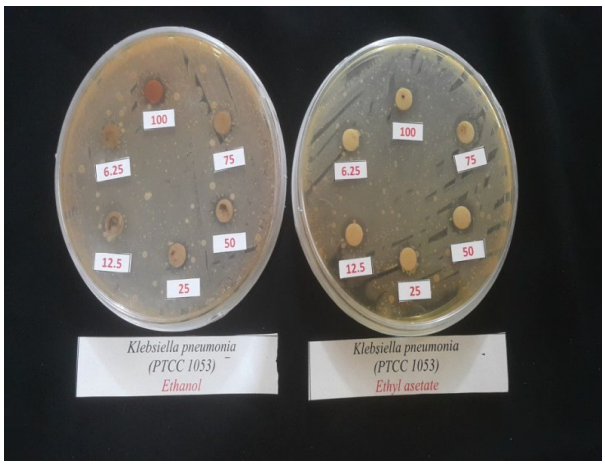

Figure 5: Disc diffusion assay showing inhibition zone induced by several concentrations of ethanolic/ethyl acetate extracts of *E. helioscopia* against *K. pneumonia*.

Figure 6: Disc diffusion assay showing inhibition zone induced by Gentamicin (control) against tested microorganisms.

Number	Component	RT	Area%	KI
1	Methyl 5-methylfuryl sulfide	11.025	3.23	1046
2	Ammelide N-METHYL-PARABANIC ACID	12.551	1.24	1090
3	Benzeneethanol	13.629	0.24	1121
4	2,3-Dihydro-3,5-dihydroxy-6-methyl -4H-pyran-4-one	14.834	1.19	1156
5	3-Methylacetophenone	15.968	0.18	1188
6	Benzoic acid	16.29	0.47	1197
7	1,2-Benzenediol	17.655	8.35	1237
		17.858		1243
8	Phenol, 4-ethenyl-2-methoxy	20.484	0.26	1321
9	Tetradecane	23.033	0.21	1400
10	Dihydroactinidiolide	27.22	0.35	1537
11	Hexadecane	29.062	0.22	1600
12	Benzophenone	30.014	0.19	1634
13	Quinic acid	31.855	2.54	1701
14	Tetradecanoic acid	33.865	0.77	1776
15	Loliolide	34.131	1.27	1786
16	Neophytadiene	35.524	2.01	1841
		36.155		1866
		36.603		1884
17	Methyl heptyl ketone	36.631	0.35	1895
18	Phthalic acid	36.358	0.37	1874
19	Xycaine	37.471	0.84	1919
20	1,2-Benzenedicarboxylic acid	38.668	0.32	1969
21	Hexadecanoic acid	38.99	28.02	1982
		39.369		1998
22		49.08		2566
23	2-Hexadecen-1-ol, 3,7,11,15-tetram ethyl-, [R-[R*, R*- (E)]]- (CAS) Phytol	42.687	9.7	2119
24	Linolenic acid	43.934	7.65	2158
25	Melezitose	46.511	0.35	2276
26	Kasuminl	--	--	--
27	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester	48.828	0.67	2530
28	1-Docosanol	50.145	0.77	2700
29	Ethyl 2-isothiocyanatobenzoate	50.467	4.09	2699
30	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- Squalene	51.321	1.72	2698
31	2,7-Anhydro-7-[(diphenyloxyphosphoryl)amino]-4,5,7-trideoxy-1,3-di-O-methyl-	51.902	1.01	2696
32	1-Hexacosene	52.042	1.2	2696
33	4,6-Dimethoxy-7-formyl-2,3-diphenylindole	53.064	0.88	2694
34	Vitamin E	55.522	0.93	2689
35	Bicyclo[4.3.0]nonane, 3-buty l-4-hexyl-	59.191	1.57	2682
36	Stigmast-5-en-3-ol, (3.beta., 24S)-	59.513	8.22	2681
37	Olean-12-ene	59.688	4.58	2681
38	Lanosterol	59.926	3.03	2680

Table 1: Quantitative analysis of *Euphorbia helioscopia* extract.

Extracts and concentrations (mg/ml)		*Staphylococcus aureus* IZ (mm)	*Pseudomonas aeruginosa* IZ (mm)	*Klebsiella pneumonia* IZ (mm)	*Bacillus pereus* IZ (mm)	*Escherichia coli* IZ (mm)
Ethyl acetate	100	12	9.5	11	12	10
	75	11.8	8.2	10	9.9	11
	50	8.2	7.8	8.6	9	6.3
	25	7.8	7	6.1	8.3	6.1
	12.5	6.5	6.5	6	8.1	6
	6.25	6.2	6.2	5.7	8	5.8
Ethanolic	100	14	10.2	12	14	12
	75	11	9.8	8	12	9
	50	10.2	8.5	7.5	9.9	8.1
	25	9.8	8	6.6	8	6.3
	12.5	6	7	6.3	7.7	6.1
	6.25	5.5	6	5.5	6.1	6
Control*		28	28	27.5	30	31

*=Gentamicin (Standard disc)
IZ=Inhibition Zone in mm (mean value; include 6 mm diameter of disc)

Table 2: Zone of inhibition of *Euphorbia helioscopia* extracts (ethil acetate and ethanolic) on test organisms.

from Iran Scientific and Industrial Research, Tehran, Iran. Bacterial strains were grown and maintained on Muller- Hinton Agar Medium (Oxoid, UK).

Antimicrobial screening of extracts

Disc diffusion assay (DDA) was performed for antimicrobial screening. MH (Muller-Hinton) agar base plates were seeded with the standard inoculums size of bacteria (1×10^8 CFU/ml). Sterile filter paper discs (6 mm in diameter) were impregnated with 100 µl of each concentration of both aqueous and methanol extracts (100, 75, 50, 25, 12.5, 6.25 mg/mL), left to dry to remove residual solvent, which might interfere with the determination. Extract discs were then placed on the seeded agar plates. Each concentration of extracts was tested in triplicate along with Gentamicin (1 mg/disc) as a control. The plates were kept 1h at 4°C for diffusion of extracts, there-after were incubated at 37 ± 2°C for 24 h. Finally, the zone of inhibition (IZ) or depressed growth of microorganisms in several concentrations of both ethyl acetate and ethanolic extract were measured (Figures 1-6).

Statistical analysis

The data obtained from the study were analyzed statistically using the analysis of variance (*ANOVA*) and GLM Univariate. Also Dancan's multiple range test was used to separate the means [13].

Results

Phytochemical screening of extract

Quantitative analysis of *Euphorbia helioscopia* is shown in Table 1.

The main components including:

1. Hexadecanoic acid (28.02%).
2. 2-Hexadecen-1-ol,3,7,11,15-tetram ethyl-, [R- [R*, R*-(E)]] -(CAS) Phytol (9.7%).
3. 1,2- Benzenediol (8.35%).
4. Stigmast-5-en-3-ol, (3. beta.,24S) (8.22%).
5. Linolenic acid (7.65%).

Antibacterial activity of *Euphorbia helioscopia*

Disc diffusion assay: The observed antimicrobial activity of *E. helioscopia* at several concentrations (6.25-100 mg/mL) expressed as zone of inhibition (mm) is shown in Table 2. The results show that increase in concentration of extracts can increase the inhibition zone of growth in some of the microorganisms. Ethanolic extract of *E. helioscopia* (100 mg/mL) displayed good antibacterial activity against Gram negative (*P. aeroginosa*) bacteria (Fig 4) while ethyl acetate extract did not revealed the same efficacy. Also, ethanolic extract of *E. helioscopia* (100 mg/mL) showed a good inhibitory effect on *S. aureus* and in contrast, ethanolic extract did not show favorable efficacy on this bacteria (Fig 1). The same results by ethanolic extract for *B. cereus* (Figure 3), *K. pneumonia* and (Figure 5), *E. coli* (Figure 2) were achieved. Altogether, ethanolic extract seems to be more efficient in destroying both Gram positive and Gram negative bacteria while ethyl acetate extract displayed a weak inhibitory effect in all cases (Figures 1-5). The best antibacterial efficacy of *Euphorbia helioscopia* was on *Staphylococcus aureus* which revealed with the biggest zoon around the bacteria. Also our control, Gentamicin showed highest inhibitory effect on all bacteria with lowest efficacy on *P. aeroginosa* (Figure 6).

Discussion

For many years people around the world have healed the sick with herbal derived remedies and handed down through generations. Traditional medicine has an old history in health maintenance, as well as to prevent, diagnose, improve or treat physical and mental illness [14]. Medicinal plants has a great economic value in Iran and several types of traditional medicines are using as complementary or alternative medicine [15]. *Euphorbia helioscopia* (L.) (*Euphorbiaceae*) typically best-known as "Sun spurge " often grow in cultivated fields, pastures, rangelands and cultivated fields. It is a promising green fleshy annual herb up to 50 cm tall [8]. Some of *Euphorbia* spp are invasive and noxious weeds in many regions of the world [16] As toxic plants, they threat grazing livestock and even humans when accidentally consume mixed with forage and harvested products. For example, *Euphorbia* seeds in grain crops are one of the suspected reasons for the high rate of cancers in northern Iran [17]. In present study we evaluated antibacterial efficacy of ethyl acetate and ethanolic extracts *E. helioscopia* cultured in Mazandaran province, Iran on Gram negative and Gram positive pathogenic bacteria. Our results indicated that ethanolic extract has stronger antibacterial efficacy than ethyl acetate although Gentamicin as a control has better antibacterial effect than our extracts (Figures 1-6). Interestingly ethanolic extract of *E. helioscopia* showed good antibacterial efficacy on *S. aureus, K. pneumonia, B. cereus* and *E. coli* and the lowest efficacy on *P. aeruginosa*. The phytochemical constituents existing in the plant declared in a study carried out in 2013 were

including: terpenoids (euphornin L,euphoscopin F, epieuphoscopin B, euphoscopin B, euphoscopin C Euphoheliosnoid D, helioscopinolide A,Euphornin N, Euphoheliosnoids A, Euphoheliosnoids B, Euphoheliosnoids C), glycosides (Quercetin-3-β-glucoside, quercetin-3-β-galactoside,quercetin-3-β-galactoside-2"-gallate,3β,7β,15β-trihydroxy-14-oxolathyra-5E,12E-dienyl-16-O-β-d glucopyranoside) and aminoacids. Also Pharmacological studies reported anti-viral, Cytotoxic, anti-fungal, anti-bacterial, anti-tumor, wound healing affect, vassodepressor and Phytodermatitis properties of *E. helioscopia* [8]. Similar to our results, researchers in 2013 indicated that methanolic extracts of *E. helioscopia* showed antibacterial efficacy on *S. aureus, K. pneumoniae, P. multocida* and *E. coli* [18]. We observed that the best antibacterial efficacy of *Euphorbia helioscopia* was on *Staphylococcus aureus*. In other study carried out in 2009, Dichloromethane extract of *E. helioscopia* exhibited significant activity with 90% Inhibition against *Fusarium solani*, whereas no significant activity against *Salmonella typhi* and *Bacillus subtilis* observed. These findings were in contrast to our results that ethyl acetate and ethanolic extracts of *E. helioscopia* showed antibacterial efficacy on *P. aeroginosa* and *B. cereus* [19]. Similar to our findings, in other study carried out in 2010, antimicrobial and anti fungal activities of ethanol extract of *E. hirta* L. leaves on *S. aureus*, *B. ceresus, S. typhi*, *K. pneumoniae*, *P. aeuroginosa* and fungal species namely *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Rhizopus oryzae* demonstrated. It is clear that compounds in family *Euphorbiaceae* is responsible for antibacterial activity. Phytochemical studies on leaves of *E. hirta* L. revealed the presence of tannins, flavonoids alkaloids, glycosides, proteins, sterols and saponins [20]. The antimicrobial activity of *E. hirta* L. may be due to one/more group of earlier mentioned phytoconstituent(s) Results from a study carried out in 2005 on *Euphorbia hirta* L. and *Euphorbia tirucalli* L. indicated that aqueous extract of *Euphorbia hirta* L. was inactive against both the Gram-positive bacteria, i.e. *B. subtilis* and *S. epidermidis*, while that of *Euphorbia tirucalli* L. was active only against *P. pseudoalcaligenes*. Also results showed that (methanol) extracts revealed more consistent antimicrobial activity compared to those extracted in water [21]. Another researchers in 2010 demonstrated that ethanol and methanol extracts of *Euphorbia hirta* L. were more effective in inhibition the growth of the pathogenic bacteria including *B. Subtilis, S. aureus, E. coli, K. pneumoniae* and *P. vulgaris* than aqueous and chloroform extracts [22]. *Euphorbia helioscopia L.* has been employed as a conventional therapy for cancer in China. Euphornin is one of the main bioactive components of *Euphorbia helioscopia L.* It is believed that Euphornin is responsible for cytotoxicity and has assessed by mice lung denocarcinoma cells (LA795) [23].

Conclusion

Our study compiled all the findings about the ethanolic and ethyl acetate extracts of *Euphorbia helioscopia*. The aim of present study was evaluation of antibacterial efficacy of these extracts. As the plant has been in use in traditional medicine, for long time in Asian subcontinent, it has many therapeutic claims. In order to verify those claims various researchers have undertaken, various experimental research are needed. In the present study we have tried to reveal the antibacterial results, which will act as single point information source about the plant for further use by scientific community.

Conflict of Interest

The authors would like to declare that there are no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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