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Antifungal Effect of the Extract of the Plants Against *Candida albicans*

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Abstract

Background: Medicinal plants are widely used throughout the world. Since these plants are known to have minimal side effects, many people embrace them. This study aims to investigate the antifungal effects of the extracts of the plants against *Candida albicans*.

Methods: The extracts of *Echinophora platyloba*, *Peganum harmala*, *Heracleum persicum* and *Rosmarinus officinalis* were prepared using a rotary device. The inhibitory concentration against Candida albicans was determined using incubation in media.

Results: The results show that highest MIC of *R. officinalis* against *C. albicans* was 100 ppm and low MIC was 12.5 ppm against *C. albicans* (Table 1) and the highest MIC of *E. platyloba* against *C. albicans* was 150 ppm and low MIC was 12.5 ppm against *C. albicans*. The results of this study showed that by increasing the concentration of plant extracts inhibited by the fungus has also been increased. Futhermore, *Heracleum persicum* extract concentration inhibited growth of fungal ppm 25 while *Peganum harmala* extract concentration inhibited growth ppm 50.

Conclusions: The results showed good antifungal activity *Peganum harmala*, *Echinophora platyloba*, *Rosmarinus officinalis* and *Heracleum persicum* has expressed Feb plants can be used to treat infections caused by these.

Keywords: Antifungal, *Echinophora platyloba*, *Rosmarinus officinalis*, *Peganum harmala*, *Heracleum persicum*, *Candida albicans*

1. Background

Iran, with its five major climates, has been a unique land for growing more than 7,500 plant species, many of them are categorized as herbal medicine (1). Herbal medicines, as a basement of treatment of various diseases and ailments, have been described by ancient well known Persian medical scholars including Rhazes, Heravi, Avicenna and many others (2).

Peganum harmala L. (*Zygophyllaceae*), which is also known as *Harmal*, Suryin Rue, is a perennial, bushy, and wild-growing flowering plant with a short creeping root, which may grow to 30 - 100 cm high (3, 4), is known as "Espand" in Iran and also known as *Harmal* in North Africa and African Rue, Mexican Rue, Syrian Rue or Turkish Rue in the United States (5). *P. harmala* traditionally has been used in Iran as an antiseptic and disinfectant agent by burning its seeds (6, 7). This plant has been considered for the treatment of a variety of human ailments, such as lumbago, asthma, colic, jaundice and as a stimulant emmenagogue (8).

The genus *Heracleum* is one of the largest genera of *Umbelliferae* (Apiaceae) and there are almost 125 *Heracleum* species in the world. This genus is widely distributed in Asia1 and represented by 10 species in the flora of Iran (9). Umbelliferous plants have been used not only as foodstuff and spice, but also as a traditional folk medicine. In Iran *H. persicum* (Golpar) fruits are used commonly as spices, while the fruits and stems are used as a flavoring agent for making pickles. The fruits and leaves of this genus are also used as antiseptic, carminative, digestive and analgesic in traditional Iranian medicine (10). *Candida albicans* is the major fungal pathogen in humans, particularly in immunocompromised patients. Candidiasis can take many forms, ranging from mucosal candidiasis to disseminated disease, often with multiple organ involvement, depending on the underlying host defect. Host defense mechanisms against *C. albicans* infections are highly complex and several studies have demonstrated an essential role for phagocytic cells, cell mediated immunity and even the humoral immune responses in the resolution of candidiasis.

Rosemary (*Rosmarinus officinalis* L.), belonging to the *Lamiaceae* family, is a pleasant- smelling perennial shrub that grows in several regions all over the world. It is a well-known valuable medicinal herb that is widely used in pharmaceutical products and traditional medicine as a diges-

tive, tonic, astringent, diuretic, diaphoretic and is also useful for urinary ailments. *Echinophora* is a ten-species genus of Apiaceae that contains four species and is native to Iran, including *E. orientalis*, *E. sibthorpiana*, *E. cinerea* and *E. platyloba*, which are called "Khousharizeh" or "Tigh Touragh" in Persian. *E. platyloba* is widely used in western and central Iran as a food seasoning and edible vegetable. Local people add the plant to pickles and tomato pastes as an antifungal and antimicrobial preservative.

2. Objectives

This study aims to investigate the antifungal effects of the extracts of the *Echinophora platyloba*, *Peganum harmala*, *Heracleum persicum* and *Rosmarinus officinalis* on *Candida albicans*.

3. Methods

3.1. Plant Preparation

After collecting the plants, they are rinsed with water and chopped for microbial tests. Afterwards they are dried for preparation of the plant extract in the shadow.

3.2. Extract Preparation

Peganum harmala, *Echinophora platyoba*, *Rosmarinus officinalis* and *Heracleum persicum* were collected from the mountainous regions of Iran (Zabol, Kerman) and then chopped. For the extract preparation, 10 g of dry powder of the plant was placed in a half-liter of erlens containing 100 mL of methanol. The content of erlens was mixed at room temperature for 24 hours by a shaker device with 130 rpm speed and was then filtered using Whatman paper No. 2. The solvent was then separated from the extract by a rotary device and by a vacuum pump (vacuum distillation). The obtained extract was weighted and then dissolved in DMSO solvent and it was maintained in the refrigerator at 4°C for use.

3.3. Isolates of Candida albicans

After sampling the vaginal using the sterile swap and Falcon tube by the gynecological specialists, 30 samples were isolated and transferred to the laboratory and cultivated on agar dextrose saburo and broth dextrose saburo according to the manufacturer's instructions. After the growth of each sample, lam was prepared and the candidate samples were identified.

Colonies of *Candida albicans* were prepared in the media of agar dextrose saburo at 37°C in homogenous suspension sterile physiology serum and the rate of the light passing of the suspension was measured using the spectrophotometry device with 530 nm.

The rate of the passing light of 90% is necessary for preparing a suspension with nearly 10^6 fungi cells per mL. For determining the inhibitory concentration of the extracts, incubation in media was used (the concentration of 25, 50 and 100 ppm were used). Finally, they were placed in the incubator and the samples were analyzed after 24 - 48 hours.

4. Results

The results show that the highest MIC of *R. officinalis* against *C. albicans* was 100 ppm and low MIC was 12.5 ppm against *C. albicans* (Table 1) and the that highest MIC of *E. platyloba* against *C. albicans* was 150 ppm and low MIC was 12.5 ppm against *C. albicans*. The results of this study showed that by increasing the concentration of plant extracts which inhibited the fungus has also been increased. Due to this, the *heracleum persicum* extract concentration inhibited growth of fungal ppm 25 while *Peganum harmala* extract concentration inhibited a growth ppm of 50.

5. Discussion

The seeds of *Peganum harmala* (*P. harmala*) contain several alkaloids (11) and other phytochemicals that attribute various medicinal properties to this multipurpose plant (11). The seeds of this plant possess antibacterial activity against drug-resistant bacteria, smoke from the seeds kills algae, bacteria, intestinal parasites and mold and the roots are used to kill lice and insects (11). Cytotoxic activity against the tissues of liver and kidney at very high dose of 150 g/kg of body weight in rats has been reported (12). However, at a dose range of 75 - 100 g/kg body weight in rats, moderate liver and kidney toxicity was observed (12). *P. harmala* extract and powdered seeds have been used in folk medicine of different parts of the world to treat colic in man and animals due to their antispasmodic effect by blocking different types of intestinal calcium channels.

The study of Darabpour MIC and MBC values for both seed and root extract against MRSA and seed extract against *E. coli* and *S. typhi* were the same (0.625 mg/mL) (13), it is generally held that for bactericidal agents, the MIC and MBC are often near or equal values, which can conclude that these extracts of P. harmala have a bactericidal effect on the mentioned bacteria (14).

The study of Ali showed that water extract of *Syzygium aromaticum* L. (Myrtaceae) buds, methanol extracts of *Ficus carica* L. (Moraceae) and *Olea europaea* L. (Oleaceae) leaves and *Peganum harmala* L. (Nitrariaceae) seeds had MIC ranges of 31.25 - 250 μg/mL. *S. aromaticum* inhibited growth of *Staphylococcus aureus*, *Staphylococcus epider-*

Table 1. Minimum Concentration Inhibitory Plant Extract Against *Candida albicans* (PPM)

Code	MIC *H. persicum*	MIC *P. harmala*	MIC *E. platyloba*	MIC *R. officinalis*
1	50	50	50	25
2	100	100	50	25
3	25	50	12.5	12.5
4	25	100	12.5	50
5	100	100	50	12.5
6	50	50	100	100
7	25	50	150	50
8	50	100	50	50
9	25	50	50	25

midis, *Streptococcus pyogenes*, *Salmonella enterica* serovar *Typhi* and *Pseudomonas aeruginosa*. *F. carica* and *O. europaea* inhibited growth of *S. aureus*, *S. epidermidis* and *S. pyogenes* whereas *P. harmala* was effective against *S. aureus*, *Acinetobacter calcoaceticus* and *Candida albicans*. Ampicillin, velosef, sulfamethoxazole, tetracycline and ceftazidime, cefotaxime, cefepime, which are used as control, had MIC $\geq$ 50 and 1.5 μg/mL, respectively, for organisms sensitive to extracts (15).

The study of Nenaah (16), the β-carboline alkaloids of *Peganum harmala* L were extracted through a bioassay-guided fractionation and their antimicrobial activities were investigated. Results revealed significant differences ($P > 0.05$) between compounds depending on the microorganism tested and the application method. When examined individually, *harmine* was the most effective against Proteus vulgaris and *Bacillus subtilis* and *Candida albicans* where inhibition zones ranged between 21.2 and 24.7 mm. Potentiality, the alkaloids increased when applied as binary mixtures suggesting a kind of synergistic interaction with inhibition zones reaching 31.5mm with the total alkaloidal extract.

The aim of the study done by Shahverdi et al. (17) was to determine the antimicrobial activity of two smoke condensates from *Peganum harmala* seeds. Furthermore the composition of smoke preparations was studied using gas chromatography and mass spectroscopy analysis. The most prevalent compound detected in a dichloromethane extract was harmine. Standard harmine as well as the dichloromethane extract showed antimicrobial activity against all test strains.

The results of the study done by Saeidi et al. (18) showed that in the disk diffusion method, 80 samples of *E. coli* produced ESBLs. In the PCR method, the TEM gene distribution in the isolated ESBL-producing organisms was 50 (41.6%). Amikacin was the most effective anti-bacterial agent and ciprofloxacin was the least effective against *E. coli* isolates. All the natural plant extracts mentioned above, especially *P. harmala*, were effective against the selected isolates of ESBL-producing *E. coli*. The most frequent ESBL rate producing *E. coli* isolates (32 out of 50) had MIC of 2.5 mg/mL in ethanol extract of *P. harmala*.

The study done by Amin et al. (19) evaluates in vivo and in vitro anti-*Helicobacter pylori* (*H. pylori*) efficacy of silver nanoparticles (Ag-NPs) prepared via a cost-effective green chemistry route where in the *Peganum harmala* L. seeds the extract was used as a reducing and capping agent. The result showed a considerable anti-*H. pylori* activity. In case of in vivo trial against *H. pylori* induced gastritis, after oral administration of 16 mg/kg body weight of S5 for seven days; a complete clearance was recorded in male albino rates. In comparative time-killing kinetics, S5 exhibited dose and time dependent anti-H. pylori activity, which was almost similar to tetracycline and clarithromycin, less than amoxicillin, but higher than metronidazole.

The study of Shokri et al. (20) stated that the mean values of inhibition zones were found to be more than 60mm for *T. copticum*, 56.7 mm for *Z. multiflora*, 40.8mm for N. sativa, 33.7 mm for *Z. clinopodiodes* and 18.7 mm for *H. persicum*. In GC/MS analysis, thymol (63.4%), carvacrol (61%), trans anthol (39%), pulegone (37%) and hexyl butyrate (30.2%) were found to be the major components of *T. copticum*, *Z. multiflora*, *N. sativa*, *Z. clinopodiodes* and *H. persicum*, respectively.

The study of Miladinovic et al. (21) stated that the composition and antimicrobial activity of the essential oil of *Heracleum sibiricum* L. (Apiaceae) was studied. The aerial part of the plant was hydro-distilled and chemical composition of the essential oil was analyzed by GC and GC-MS. Forty-six compounds, corresponding to 95.12% of the total oil were identified. Esters represented the major chemical class (69.55%) while the main constituents were octyl butanoate (36.82%), hexyl butanoate (16.08%), 1-octanol (13.62%) and octyl hexanoate (8.10%). Antibacterial activity of the essential oil and reference antibiotics against nine bacterial strains was tested by the broth microdilution method. The results of the bioassays showed that the essential oil had slight antimicrobial activities

against all tested microorganisms (MIC and MBC values were in the range of 2431.2 to 9724.8 microg/mL). Reference antibiotics were active in concentrations between 0.5 and 16.0 microg/mL.

The study done by Torbati et al. (22) stated that Myristicin was the dominant component in both EOs. It was identified as 96.87% and 95.15% of the essential oil composition of *H. transcaucasicum* and *H. anisactis* roots, respectively. The TLC-bioautography showed antioxidant spots in both EOs and IC_{50} of *H. anisactis* and *H. transcaucasicum* EO was found to be 54 $\mu g \times mL$ (-1) and 77 $\mu g \times mL$ (-1), respectively. Regarding the antimicrobial assay, *H. anisactis* EO exhibited weak to moderate antibacterial activity against Gram-positive bacteria and also *Escherichia coli*, whereas the essential oil from *H. transcaucasicum* was inactive.

The study of Sadeghi Nejad et al. (23) was done to investigate the in vitro anti-*Candida* activity of the hydroalcoholic extracts of *Heracleum persicum* fruit. The minimum inhibitory concentration (MIC) values at 24 and 48 hours were 0.625 - 20 $\mu g/\mu L$ for *C. albicans*, 0.625 - 40 $\mu g/\mu L$ for *C. glabrata*, and 5.0 - 20 $\mu g/\mu L$ for *C. tropicalis*.

The study of Jagannath et al. (24) stated that the essential oil was extracted from the seeds of *Heracleum rigens* by hydrodistillation and a total of twenty compounds accounting for 98.5% of the total oil composition were identified. Physicochemical properties and chemical composition of the oil was determined by a combination GC/FID and GC/MS analysis. The major compounds identified were bornyl acetate (51.2%), alpha-pinene (22.6%), limonene (9.62%), octyl acetate (3.94%), rho-cymene (2.85%) and gamma-terpinene (1.93%). The antimicrobial activity of the oil was screened by the disc diffusion method against nine pathogenic bacterial strains. Maximum antimicrobial activity was noted against *Klebsiella pneumonia* and *Bacillus subtillis*.

The study of Guleria Volatile et al. (25) oil composition of hydro-distilled (HD) and supercritical carbon dioxide (SC-CO(2)) essential oil of freshly collected aerial parts of *Heracleum thomsonii* (Umbeliferae) from the western Himalayas was studied by GC-FID and GC-MS. Results revealed qualitative and quantitative dissimilarity in the composition of hydro-distilled and SC-CO(2) extracted oils. Nineteen constituents, which accounted for 89.32% of total constituents in HD oil, represented by limonene (4.31%), (Z)-β-ocimene (3.69%), terpinolene (22.24%), neryl acetate (36.19%), nerol (9.51%) and p-cymene-8-ol (2.61%) were identified. In SC-CO (2) extracted oil, 24 constituents representing 89.95% of the total constituents were identified. Terpinolene (5.08%), germacrene D (2.17%), neryl acetate (51.62%), nerol (9.78%), geranyl acetate (2.06%), α-bisabolol (2.48%) and 1-nonadecanol (4.96%) were the dominating constituents. In vitro antimicrobial activity of hydro-distilled oil was conducted against microrobial strains using the broth microdilution method including two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and five Gram-negative (*Burkholderia cepacia*, *Escherichia coli*, *Enterobacter cloacae*, *Klebseilla pneumoniae* and *Pseudomonas aeruginosa)* bacteria as well as seven fungi *(Candida albicans, Issatchenkia orientalis, Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Aspergillus sydowii* and *Trichophyton rubrum*). The results of the bioassay showed that the oil exhibited moderate to high antimicrobial activity against fungi *C. albicans* (MIC 625 μg mL (-1)), *A. parasiticus* (MIC 312.5 μg mL (-1)), *A. sydowii* (MIC 312.5 μg mL (-1)), *T. rubrum* (MIC 625 μg mL(-1)), Gram-positive bacteria *B. subtilis* (MIC 625 μg mL (-1)) and Gram-negative bacteria *P. aeruginosa* (MIC 312.5 μg mL (-1)).

The study of Khajeh et al. (26) showed the effect of *E. platyloba* extract on expression of CDR1 and CDR2 genes in fluconazole-resistant clinical isolates of *C. albicans* using real-time PCR. The results show that twenty of the 148 total isolates were resistant to fluconazole. The MIC and MFC for the alcoholic extract of *E. Platyloba* were 64 mg/ml and 128 mg/mL, respectively. Real-time PCR results revealed that the mRNA levels of CDR1 and CDR2 genes significantly declined after incubation with *E. Platyloba* (both $P < 0.001$).

The study of Ranjbar and Babaie (27) was done to investigate the effects of *Echinophora platyloba* extract on inhibiting the growth of *Salmonella typhi*, *Salmonella enteritidis*, and *Salmonella choleraesuis*. The results indicated that, in 250 mg/mL of extracts discs, the largest growth inhibition zones were formed and they were 26.11 ± 1.16, 21.23 ± 0.89 and 19.65 ± 0.60 in *S. enteritidis*, *S. typhi* and *S. choleraesuis* groups, respectively. The statistical results indicated that in each type of bacteria, there was a statistical difference ($P < 0.01$) between the various concentrations of the extracts and the chloramphenicol discs. Furthermore, it was indicated that this extract at a concentration of 150 mg/ml had a germicidal effect on *S. enteritidis* and *S. typhi* bacteria and that 250 mg/mL had a bactericidal effect on *S. choleraesuis*.

The study of Jaarar was done to determine the antimicrobial activity of rosemary (*Rosmarinus officinalis* L.) and to investigate the synergistic effects of this extract combined with ceforuxime against methicillin-resistant *Staphylococcus aureus* (MRSA).

The results show that the minimum inhibitory concentrations (MICs) of the ethanol extract of rosemary were in the range of 0.39 - 3.13 mg/mL. The minimum bactericidal concentrations (MBCs) were usually equal to or double MICs. The antimicrobial activity of combinations of the ethanol extract of rosemary and cefuroxime indicated their synergistic effects against all MRSAs (28).

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Footnotes

Authors' Contribution: All authors had an equal role in design, work, statistical analysis and manuscript writing.

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References

1. Geographical and Cartographical Institute . A Cosmological Atlas of Iran's Provinces. Iran: Geographical and Cartographical Institute; 2004.
2. Adhami HR, Mesgarpour B, Farsam H. Herbal Medicine in Iran. *Herbal Gram.* 2007;**74**:34–43.
3. Shamsa F, Monsef HR, Ghamooghi R, Verdian Rizi MR. Spectrophotometric determination of total alkaloids in Peganum harmala L. using bromocresol green. *Res J Phytochem.* 2007;**1**:79–82. doi: 10.3923/rjphyto.2007.79.82.
4. Goel N, Singh N, Saini R. Efficient in vitro multiplication of Syrian Rue (Peganum harmala L.) using 6-benzylaminopurine pre-conditioned seedling explants. *Nat Sci.* 2009;**7**(129-34).
5. Mahmoodian M, Jalilpour H, Salehian P. Toxicity of Peganum harmala: Review and a case report. *Iran J Pharmacol Ther.* 2002;**1**:1–4.
6. Fathiazada F, Azarmi Y, Khodaie L. Pharmacological effects of Peganum harmala seeds extract on isolated rat uterus. *Iranian J Pharmaceut Sci.* 2006;**2**:81–6.
7. Arshad N, Neubauer C, Hasnain S, Hess M. Peganum harmala can minimize Escherichia coli infection in poultry, but long-term feeding may induce side effects. *Poult Sci.* 2008;**87**(2):240–9. doi: 10.3382/ps.2007-00341. [PubMed: 18212366].
8. Bukhari N, Choi JH, Jeon CW, Park HW, Kim WH, Khan MA. Phytochemical studies of the alkaloids from Peganum harmala. *Appl Chem.* 2008;**12**:101–4.
9. Pimenov MG, Leonov MV. The Asian umbelliferae Biodiversity Database(ASIUM) with particular reference to south- west Asian Taxa. *Turk J Bot.* 2004;**28**:139–45.
10. Amin G. Popular medicinal plants of Iran. Tehran, Iran: Tehran university of medical sciences press; 2008.
11. Harsh ML, Nag TN. Antimicrobial principles from in vitro tissue culture of Peganum harmala. *J Nat Prod.* 1984;**47**(2):365–7. doi: 10.1021/np50032a022. [PubMed: 6736972].
12. Mohamed AHS, AL-Jammali SMJ, Naki ZJ. Effect of repeated administration of Peganum harmala alcoholic extract on the liver and kidney in Albino mice: a histo-pathological study. *J Sci Innov Res.* 2013;**2**(3):585–97.
13. Darabpour E, Poshtkouhian Bavi A, Motamedi H, Seyyed Nejad SM. Antibacterial activity of different parts of Peganum harmala growing in iran against multi drug resistant bacterial. *Experiment Clin Sci J.* 2011;**10**:252–63.
14. Reuben KD, Abdulrahman FI, Akan JC, Usman H, Sodipo OA, Egwu GO. Phytochemical screening and in vitro antimicrobial investigation of the methanolic extract of Croton zambesicus Muell ARG stem bark. *Eur J Scientific Res.* 2008;**23**:134–40.
15. Ali NH, Faizi S, Kazmi SU. Antibacterial activity in spices and local medicinal plants against clinical isolates of Karachi, Pakistan. *Pharm Biol.* 2011;**49**(8):833–9. doi: 10.3109/13880209.2010.551136. [PubMed: 21501041].
16. Nenaah G. Antibacterial and antifungal activities of (beta)-carboline alkaloids of Peganum harmala (L) seeds and their combination effects. *Fitoterapia.* 2010;**81**(7):779–82. doi: 10.1016/j.fitote.2010.04.004.
17. Shahverdi AR, Monsef-Esfahani HR, Nickavar B, Bitarafan L, Khodaee S, Khoshakhlagh N. Antimicrobial activity and main chemical composition of two smoke condensates from Peganum harmala seeds. *Z Naturforsch C.* 2005;**60**(9-10):707–10. [PubMed: 16320612].
18. Saeidi S, Amini Boroujeni N, Ahmadi H, Hassanshahian M. Antibacterial Activity of Some Plant Extracts Against Extended- Spectrum Beta-Lactamase Producing Escherichia coli Isolates. *Jundishapur J Microbiol.* 2015;**8**(2):15434. doi: 10.5812/jjm.15434. [PubMed: 25793093].
19. Amin M, Hameed S, Ali A, Anwar F, Shahid SA, Shakir I, et al. Green Synthesis of Silver Nanoparticles: Structural Features and In Vivo and In Vitro Therapeutic Effects against Helicobacter pylori Induced Gastritis. *Bioinorg Chem Appl.* 2014;**2014**:135824. doi: 10.1155/2014/135824. [PubMed: 25214825].
20. Shokri H, Sharifzadeh A, Ashrafi Tamai I. Anti-Candida zeylanoides activity of some Iranian plants used in traditional medicine. *J Mycol Med.* 2012;**22**(3):211–6. doi: 10.1016/j.mycmed.2012.04.006. [PubMed: 23518076].
21. Miladinovic DL, Ilic BS, Mihajilov-Krstev TM, Nikolic DM, Cvetkovic OG, Markovic MS, et al. Antibacterial activity of the essential oil of Heracleum sibiricum. *Nat Prod Commun.* 2013;**8**(9):1309–11. [PubMed: 24273873].
22. Torbati M, Nazemiyeh H, Lotfipour F, Nemati M, Asnaashari S, Fathiazad F. Chemical composition and in vitro antioxidant and antibacterial activity of Heracleum transcaucasicum and Heracleum anisactis roots essential oil. *Bioimpacts.* 2014;**4**(2):69–74. doi: 10.5681/bi.2014.004.
23. Sadeghi Nejad B, Rajabi M, Zarei Mamoudabadi A, Zarrin M. In Vitro Anti-Candida Activity of the Hydroalcoholic Extracts of Heracleum persicum Fruit Against Phatogenic Candida Species. *Jundishapur J Microbiol.* 2014;**7**(1):8703. doi: 10.5812/jjm.8703.
24. Jagannath N, Ramakrishnaiah H, Krishna V, Gowda PJ. Chemical composition and antimicrobial activity of essential oil of Heracleum rigens. *Nat Prod Commun.* 2012;**7**(7):943–6. [PubMed: 22908589].
25. Guleria S, Saini R, Jaitak V, Kaul VK, Lal B, Rahi P, et al. Composition and antimicrobial activity of the essential oil of Heracleum thomsonii (Clarke) from the cold desert of the western Himalayas. *Nat Prod Res.* 2011;**25**(13):1250–60. doi: 10.1080/14786419.2011.557375. [PubMed: 21854172].
26. Khajeh E, Hosseini Shokouh SJ, Rajabibazl M, Roudbary M, Rafiei S, Aslani P, et al. Antifungal effect of Echinophora platyloba on expression of CDR1 and CDR2 genes in fluconazole-resistant Candida albicans. *Br J Biomed Sci.* 2016;**73**(1):44–8. doi: 10.1080/09674845.2016.1155269. [PubMed: 27182677].
27. Ranjbar R, Babaie S. Evaluation the antibacterial effects of Echinophora platyloba extracts against some Salmonella species. *Electron Physician.* 2016;**8**(2):1943–8. doi: 10.19082/1943.
28. Jarrar N, Abu-Hijleh A, Adwan K. Antibacterial activity of Rosmarinus officinalis L. alone and in combination with cefuroxime against methicillin-resistant Staphylococcus aureus. *Asian Pacific JTropical Med.* 2010;**3**(2):121–3. doi: 10.1016/S1995-7645(10)60049-1.