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Bactericidal and Fungicidal Activity of Methanolic Extracts of *Heracleum persicum* Desf. ex Fischer against Some Aquatic and Terrestrial Animal Pathogens

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Abstract: The herb Persian hogweed (*Heracleum persicum* Desf. Ex Fischer) has been used in folkloric and traditional medicine especially in Asia and Middle East to increasing immunity and resistance to some diseases. The aim of the present study was screening antibacterial and antifungal activity of the methanolic extract of mixed leaves and flowers of *Heracleum persicum* collected from Mazandaran province Kelardasht area was conducted by the disc diffusion method. The methanolic extracts of this plant extracts were tested at concentration of 15 mg per disc and showed antimicrobial impact against selected pathogenic bacteria tested such as *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Streptococcus iniae* (zoonotic agent), *Bacillus cereus*, *Bacillus subtilis* *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Klebsiella oxytoca* and *Escherichia coli* and fungi such *Aspergillus niger* and *Candida albicans*. The result showed that, leaves and flowers extracts of *H. persicum* have antimicrobial activity over 4 Gram-negative, 4 Gram-positive bacteria and antifungal effect over 2-fungi's, upon increasing of concentrations of extracts the antimicrobial activities in all of microorganisms also increases. Fungi are more sensitive than bacteria. This species has a huge distribution around the world especially in Europe and United state, achieve to this question that whether those herbs have similar effects at different climate, merits further investigation.

Key words: Bactericidal and fungicidal activity, methanolic extract, *Heracleum persicum*

INTRODUCTION

The genus *Heracleum* includes approximately 10 indigenous species in northern part of Iran in mountainous area over 1500 m and all around the world specially in Europe (called Tromsø Palme in Norway) which most of them are traditionally utilized as remedies for some diseases (Nielsen *et al.*, 2005; Jahodova *et al.*, 2007). Fruits of *H. persicum* (Apiaceae) are used as pain killer in Persian folkloric medicine (Hajhashemi *et al.*, 2009). Several researches have been investigated the chemical components of *Heracleum* sp. and its medicinal activities.

The amount of 56.5% hexyl butyrate, 16.5% octyl acetate, 5.2% hexyl 2-methyl butanoate and 3.4% hexyl isobutyrate in the essential oil of *H. persicum* with anti-inflammatory and analgesic effects identified by Hajhashemi *et al.* (2009). Immuno-modulatory activity of aqueous extract of *H. persicum* evaluated at three doses of 50, 100 and 200 mg kg⁻¹ body weight of female mice by Sharififar *et al.* (2009) with a significant stimulatory effect in all doses.

In a study carried out by Nazemi *et al.* (2005) demonstrated antibacterial effect of methanolic extract of *H. persicum* on 5 bacteria species (*Bacillus polymyxa*, *Bacillus subtilis*, *Enterococcus faecalis*, *Nocardia* and *Staphylococcus aureus*), respectively. At previous study showed by Kuljanabhadgavad *et al.* (2010) identified 25 components in essential oil of *Heracleum* sp. along with bactericidal and fungicidal activity against five bacterial and two fungal strains, respectively (*Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Streptococcus faecalis*, *Streptococcus aureus*, *Microsporum gypseum*). In a study on antibacterial effects, Ahmadian-Attari *et al.* (2009) demonstrated an antibacterial effect on Gram-negative and Gram-positives bacteria. Anti-tumor activity along with antibacterial effect of *H. persicum* also showed by Noudeh *et al.* (2010). In a study on chemical composition and antibacterial activity, Habibi *et al.* (2010) showed the maximum inhibitory activity of *H. rechingeri* species against Gram-positive bacteria especially *B. subtilis*. Previous study carried out on anti-fungal effect of *H. persicum* and 220 other plants as well (Bonjar *et al.*, 2004).

In a study on antioxidant activity of some furanocoumarins isolated from *H. persicum*, Souri and Farsam (2004) reported that the antioxidant activity of crude ethyl acetate extract was stronger than single isolated constituents. The anti-oxidant activity of *H. persicum* and 3 other species from Apiaceae family evaluated by Coruh *et al.* (2007). Antioxidative activities (IC₅₀) of ethanol extracts reported by Nickavar and Abolhasani (2009). Antitumor activity also reported by Noudeh *et al.* (2010). The acetone extract of *H. persicum* seed reported by previous study which showed anticonvulsant activity of those herbs (Sayyah *et al.*, 2005). Xanthotoxin (8-Methoxypsoralen) from the fruits of *H. persicum* isolated by Sajjadi and Noroozi (2007) to treatment of Psoriasis disease that causes skin chronic autoimmune disease.

Fumigant toxicity effect of essential oils using as a major insect pest of stored-grain legumes in agriculture showed by Manzoomi *et al.* (2010). Moshafi *et al.* (2009) studied on cytotoxicity effect regards to octyl-acetate component for more studies in apoptosis activity and cancer research.

There is no way to predict the next important new pathogens especially zoonotic will emerge or what its ultimate importance as geographically epidemic (Zowghi *et al.*, 2008). Food and Agricultural Organization (FAO) reported 16 and 30% economical damages caused by microbial infections in developed and developing countries over their whole livestock productions, respectively (Tavakoli *et al.*, 2008).

In the Persian traditional folk medicine the *H. persicum* using for an anti anorexia supplement, food digestion, anti dropsy and remedy of tonsillitis; also in both human and dairy cattle's using the herb after pregnancy to increase the mother's milk as well. Other traditional uses of this herb are: Angina (chest pain) relief and care of hiccup, increasing sweat secretion and disposal poison in body, prevents the stomach from bloating and nervous tension. *H. persicum* fruits extracts can be use for cure of inflammatory and painful conditions (Asgarpanah *et al.*, 2012).

Behdadipour *et al.* (2007) studied on effect of *H. persicum* extract on rabbit coroner vessels fed with high cholesterol, results showed that *H. persicum* can decrease plasma cholesterol level in rabbit based on atherosclerosis disease. Bazzaz and Haririzadeh (2003) studied on 306 plants consist of *H. persicum* and reported no anti-microbial effect of the leaf of this herb.

The objectives of this study were to examine the antimicrobial effect of the methanolic extract of leaves and flowers of *H. persicum* on some bacteria strains like; *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*,

Staphylococcus aureus, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Klebsiella oxytoca* and *Escherichia coli* and two fungus including; *Aspergillus niger* and *Candida albicans*.

MATERIALS AND METHODS

Herbs material: Leaves and flowers of *H. persicum* were collected on mountains of Mazandaran province Kelardasht region (Kooohpar village) in northern part of Iran in 2010 and identified at department of plant and agriculture in Islamic Azad University.

Extracts: Leaves and flowers of the herb were collected and dried in shadow separately and mixed together. Then they were grinded powdered mechanically. The amount of 100 g of each powder was extracted by 80% methanol in the room temperature for 24 h. Then filtered by Whatman paper filter number 1 then dried in room temperature for evaporation of methanol solutions and sterilized by flashing of UV in the end of experiment and store in room temperature for further analysis. Related to the mass of dry matter (mg), the concentrations of the extracts for antibacterial investigation (ml) were calculated according to previous studies (Nazemi *et al.*, 2005; Manikandan *et al.*, 2009).

Antibacterial and antifungal activity assay: The disc-diffusion assay (Agar Diffusion Test) was used to determine the bactericidal and fungicidal activity of the plant extracts according to method of Bansod and Rai (2008).

Bacterial and fungal strain: The examined bacterial strains were Gram-negative bacteria consist of: *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and Gram-positive strains including: *Streptococcus iniae*, *Micrococcus luteus*, *Staphylococcus aureus* (Sittiwet *et al.*, 2008; Sittiwet and Puangpronpitag, 2008; Abdul *et al.*, 2008), *Bacillus subtilis* (Al-Howiriny, 2002; Abdul *et al.*, 2008; Al-Saghir, 2009) and *Bacillus cereus*. The two tested fungal strains were *Aspergillus niger* and *Candida albicans* (Al-Howiriny, 2002; Abdul *et al.*, 2008). All bacteria and fungal strains were obtained from the stock cultures of the microbiology department of Islamic Azad University.

Evaluation of antibacterial activity: These were maintained at 4°C on nutrient agar plates. Petri plates were prepared by pouring 10 mL of Mueller Hinton agar and

allowed to solidify, 0.1 mL of standardized inoculums suspension was added and spread uniformly. The discs were then applied and plates were incubated at 37°C for 24 h. The inhibition zone was measured from the edge of the disc to the inner margin of bacterial colony. DMSO used as a negative control and gentamicin and nystatin used as a positive control. The experiment was done in triplicate. Minimum Inhibitory Concentration (MIC) of the herb extract was tested in Mueller Hinton broth by the two-fold serial dilution method. The culture tubes were incubated in incubators at 37°C for 24 h. The lowest concentration, which did not show any growth of tested organism after microscopic evaluation was determined as minimum inhibitory concentration.

Statistical analysis: Statistical analysis of the results was performed using Excel software (version 12). Numerical results were compiled into Means±SD. The product-moment correlation coefficient was calculated and tested for significance against the no correlation zero hypotheses through the procedure correlation. Data performed to analysis of variance (ANOVA) test to observed variance in a particular variable. The significance level was chosen at p<0.05.

RESULTS AND DISCUSSION

The results revealed that the *H. persicum* extract showed significantly the antibacterial and antifungal effects (p<0.05). The result indicated there is a direct relation between enhancements of antimicrobial activities with concentration of herbal extract (6.25-50%). The results of diameter zone of inhibition and Minimum Inhibitory Concentration (MIC) are shows in Table 1. According to Table 1 the maximum diameter zones of inhibition for the microorganisms and the amount of MIC

were in range of 2.26-51.01 mm and 128-512 mg mL⁻¹, respectively. *Aspergillus niger* and *Micrococcus luteus* showed highest and lowest effect at all ranges, respectively.

The antibacterial activity may be due to several agents such as presence of oil, alkaloids, flavonoids and tannin, reported by Brantner *et al.* (1996) or the different solvent extract. Nazemi *et al.* (2005) carried out a study on antibacterial effect of methanol and aquatic extract of *H. persicum* and published those results in Persian language, the antibacterial activity of extracts in that research showed that the aquatic extract of *H. persicum* had no effect but the methanolic extract had a significant effect on 5 species of *Bacillus polymyxa*, *Bacillus subtilis*, *Enterococcus faecalis*, *Nocardia* and *Staphylococcus aureus*, respectively (Nazemi *et al.*, 2005).

The results of the present survey showed a significant effect (significantly differentiation at p<0.05) of *H. persicum* extract on different bacterial and fungal strains (Table 1). Bazzaz and Haririzadeh (2003) reported no anti-microbial effect of the leaf of this herb but the present study and unpublished results of inhibitory effect of aquatic extract of Tromsø Palme (*H. persicum*) in the Arctic area by author, showed some inhibitory effects using *in vitro* growth inhibition test in all parts of herb.

Some of the other researchers showed that the methanolic extract of leaves of different plants has more active than the aqueous extracts against *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus* and *Staphylococcus aureus* (Manikandan *et al.*, 2009), the present study showed significant effects at 50% concentration of all strains especially on *Staphylococcus aureus* but two strains of *Aeromonas hydrophila* and *Streptococcus iniae* showed significant effect at 25 and 12.5% concentrations, respectively.

Table 1: Extract of *H. persicum* leaves and flowers bactericidal and fungicidal activity against different strains

Strains	Zone of inhibition (mm)				DMSO	Gentamicin	Nystatin	MIC (mg mL ⁻¹)
	6.25%	12.50%	25%	50%				
----- <i>H. persicum</i> -----								
<i>Aeromonas salmonicida</i>	11.03±0.40	13.33±1.15	17.56±0.46	22.15±1.04	-	29		127
<i>Aeromonas hydrophila</i>	21.52±1.65	13.53±0.53	51.01±0.24	34.65±1.42	-		35	511
<i>Streptococcus iniae</i>	16.00±0.09	17.38±1.05	15.03±0.82	16.00±1.50	-			512
<i>Micrococcus luteus</i>	2.56±0.67	5.00±1.02	8.86±0.20	11.75±0.80	-	33		256
<i>Klebsiella oxytoca</i>	2.26±1.40	11.46±0.60	14.57±3.15	16.35±0.33	-	34		256
<i>Klebsiella pneumoniae</i>	12.55±2.03	17.56±0.55	20.00±1.02	23.05±0.18	-	29		128
<i>Pseudomonas aeruginosa</i>	12.04±1.00	14.36±1.88	16.67±1.20	18.54±0.16	-	6		256
<i>Escherichia coli</i>	10.93±0.60	16.33±1.09	20.86±0.56	23.75±1.34	-	31		256
<i>Aspergillus niger</i>	22.83±0.65	23.63±0.87	34.00±1.24	36.67±1.83	-		35	512
<i>Candida albicans</i>	14.50±2.19	15.28±0.15	18.43±1.88	20.00±3.84	-		30	512
<i>Staphylococcus aureus</i>	5.06±0.50	08.00±1.20	11.23±1.50	15.46±2.88	-	35		128
<i>Bacillus subtilis</i>	11.04±2.05	17.56±1.00	20.96±3.10	24.05±0.04	-	34		256
<i>Bacillus cereus</i>	15.00±1.67	19.00±1.58	22.00±1.65	23.34±0.02	-	32		512

Values (Mean±SD) are significantly differentiation at (p<0.05)

Habibi *et al.* (2010) revealed the maximum inhibitory activity of *H. rechingeri* species against Gram-positive bacteria especially *B. subtilis*, at the present study we achieve to same results at 50% concentration with highly significant ($p < 0.05$).

Noudeh *et al.* (2010) has also recorded the effect of *H. persicum* (57.16%) inhibition effects on *Agrobacterium tumefaciens* also inhibited the growth of all tested Gram-positive and Gram-negative strains respectively, present study showed the same effect in different strains and concentrations.

There are numerous studies about of antibacterial activity in some herbs species have high antibacterial activity due to presence of monoterpenoids by the drop diffusion method, showing highly significant inhibition zones for all microorganisms tested, contained Gram-positive bacteria (*Staphylococcus aureus*) and fungus (*Candida albicans*) (Oliveira *et al.*, 2007; Kuljanabhadgavad *et al.*, 2010), the results of present study showed the same significant results ($p < 0.05$) for Gram-positive bacteria (*Staphylococcus aureus*) and fungus (*Candida albicans*), respectively.

Using medicinal herbs as an immunostimulants and acute stress response have been used as feed additives for several years and well documented by several authors (Askarian and Kousha, 2008; Kousha and Askarian, 2008; Faghani *et al.*, 2008; Ringo *et al.*, 2012). Furthermore *in vivo*, *in vitro* and *ex vivo* surveys on effect of *Heracleum* sp. as an immunostimulant agent, acute and chronic stress response on terrestrial and aquatic animals or human health also need further investigation. Otherwise, antiviral activities of this family merit further investigation.

CONCLUSION

It is concluded that, leaves and flowers extracts of *H. persicum* have some antimicrobial activity over 4 Gram-negative, 4 Gram-positive bacteria and antifungal effect over 2-fungi's. Several species or subspecies belong to this family have a huge distribution and well growth around the world specially in Europe and USA as an exotic herbs in different climate, so investigation on similar effects at different regions and climate merit further study.

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