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Antibacterial Properties of Essential Oil of *Heracleum persicum* (Golpar) and Foodborne Pathogens

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Abstract

Background: Antimicrobial resistance is a serious health threat to human society. Antibiotics and preservatives are also the most common modalities to increase the shelf life of foods.

Objectives: Antibacterial activity of essential oil of *Heracleum persicum* (Golpar) fruit against some of the main foodborne bacteria was determined.

Materials and Methods: Antibacterial activity of essential oil was evaluated against the bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera*, and *Yersinia enterocolitica*) using disc diffusion method. Broth micro-dilution method was used to determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The data was expressed as mean and standard deviation and analyzed using analysis of variance (ANOVA) in SPSS software ($P<0.05$).

Results: Comparing with *S. typhi* strains (inhibition zone = 18 ± 0.46 mm), essential oil was found to be more effective against *V. cholera* strains (inhibition zone = 34 ± 0.2 mm). The MIC value (%) of the essential oil against *V. cholera* and *S. typhi* were 8 and 32 respectively. Essential oil of *H. persicum* (Golpar) showed a good antimicrobial activity against foodborne pathogens.

Conclusion: The results revealed that the essential oil of *H. persicum* can be used in food preservation systems to inhibit the growth of *V. cholera* strains and improve food quality and safety.

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Background

Foodborne pathogens belong to a group of micro-organisms that cause foodborne diseases and it is essential to find effective drugs against the infection.[1] Prevalence of foodborne diseases has increased worldwide and become a major cause of mortality in individuals with impaired immune systems in the developing countries.[2] There is an urgent need to monitor antimicrobial resistance by improving the ways the antibiotics may be used and reduce hospital cross-infection. New antibiotics need to be developed continuously to maintain the effectiveness of antimicrobial therapy.[3-5] The recent decades have demonstrated an increase in interests in the use of herbal products.[6] Plants play an important role in human health. It is estimated that 25% of modern medicines are derived directly or indirectly from herbs.[7,8] Many plant compounds have been proven to be effective insecticidal, antifungal, anti-bacterial, anti-viral, anti-parasitic, and anti-oxidant agents.[3,9] Aromatic essential oils are often used in food industry to add flavor and prevent growth of microbial and mold contaminants.[10] *Heracleum*, which is used for medicinal purposes, is an annual herbaceous plant. It is a genus of about 60 species, which mainly grows in Iran and Europe.[11,12] *Heracleum persicum* is known as *Heracleum* in Farsi and as tromso palm in Norwegian. Fruits of this plant are used as spices and as anti-infection and analgesic in traditional medicine and the stems are used in pickles.[13] Many studies have been conducted on the antibacterial effect of *Heracleum* and most of them have been on the leaves and stems; still, there has been no study on antibacterial effect of essential oil of its fruits. Literature review showed a paucity of studies on the antibacterial effects of chemical composition of the oil of *H. persicum* fruit on foodborne pathogens.

Objectives

This study aimed at determining the antibacterial effects of essential oil of *Heracleum* fruit on some of the

foodborne pathogens.

Materials and Methods

Plant Materials

Heracleum fruits were collected in May 2013 from the heights in Shemiranat-Tehran and then identified and confirmed in Herbarium Center of School of Pharmacy, Tehran University of Medical Sciences. The collected plants were dried in the shade and then the fruits were separated from the stems and ground by mechanical means.

Chemicals and Reagents

All chemicals and standards were obtained from Merck (Darmstadt, Germany).

Extraction of Essential Oil

The essential oils of the *Heracleum* were obtained using hydro distillation and a Clevenger apparatus; 300 g of fruits was placed in the Clevenger with sufficient distilled water to cover the material. Extraction continued for 3 consecutive hours when the water started to boil.[14]

Antimicrobial Activity Test

Disk Diffusion Test

The essential oils were tested against *Staphylococcus aureus* ATCC 25913, *Escherichia coli* ATCC 8739, *Salmonella enterica* PTCC 1709, *Vibrio cholera* PTCC 1611 and *Yersinia enterocolitica* PTCC 1477. The microorganisms were cultured in BHI (brain heart infusion) for 18 hours at 37°C, compared with 0.5 McFarland standard (5×10^8 CFU/mL), and inoculated directly in boards with Müller-Hinton agar (Merck). After inoculation of each microorganism, the diffusion was carried out; so that 10 µL of the essential oil was placed on paper discs (6 mm of diameter) at 37°C for 24 hours. Afterward, the zone of inhibition was measured.[3]

Minimum Inhibitory Concentration and Minimum Bactericide Concentration

To obtain minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC), quantitative tests were performed using 1%-9% concentrations of the essential oil in Müller-Hinton broth medium. The concentrations were added by 1×10^6 CFU/mL of the bacteria. Positive control (cultured along with the bacterium without the essential oil) and negative control (cultured without the bacterium) were also prepared. Then, the samples were placed in an incubator (37°C, 24 hours). Before reading the results, the last concentration without opaqueness (no growth) was adopted as MIC for each sample. The samples without turbidity were cultured in Müller-Hinton broth medium. After incubation (24 hours, 37°C), the last concentration suitable enough to be used as bactericide was adopted as MBC.

Statistical Analysis

Each experiment, from sample preparation to analysis, was repeated for three times, and the data were analyzed using one-way analysis of variance (ANOVA) and Tukey tests in SPSS (v. 16).

Results

The results of disc diffusion method showed antimicrobial activity of essential oil of *H. persicum* against all of the bacterial strains; including *S. aureus* (inhibitory zone: 32.16 ± 0.76 mm), *S. enterica* (inhibitory zone: 18 ± 0.46 mm), *E. coli* (inhibitory zone: 19 ± 1.32 mm), *V. cholera* (inhibitory zone: 34 ± 2 mm), and *Y. enterocolitica* (inhibitory zone: 22.16 ± 0.76 mm) (Table 1).

The results showed that yield of essential oil of *Heracleum* fruit was about 11%. Impact of the essential oil on each bacterium (*Staphylococcus*, *E. coli*, *Yersinia*, *Vibrio*, and *Salmonella*) is listed in Table1. The highest and lowest zones of growth inhibition at concentration of 100% were observed in *Vibrio* and in *Salmonella* respectively. In addition, minimum and maximum MIC against bacteria were in *Vibrio* and *Yersinia* respectively. As listed in Table 2, *Yersinia* and *Salmonella* (64%) and *Staphylococcus* (16%) have the highest and lowest values of MBC. There was no significant difference ($P<0.05$) between the bacterial strains in terms of inhibitory halo diameter except for *E. coli* and *Salmonella*.

Discussion

Many antimicrobial drugs have been found and there are still more drugs in the nature not found yet. In light of this, the past decade has witnessed an increase in research on plants as medicine source.[15,16]

Essential oils are potential sources of novel antimicrobial compounds especially against bacterial pathogens. Essential oil plants may also be applied for the treatment of infectious diseases, thanks to its antimicrobial activity.[15,16] Results from in vitro studies in this paper showed that the essential oils can inhibit bacterial growth; although, their effectiveness varied.

Nazemi et al indicated that aqueous extract of *Heracleum* did not have any antibacterial effect; however, *Heracleum* methanol extract had significant effect on the bacteria *Bacillus polymyxa*, *Bacillus subtilis*, *Enterococcus faecalis, Nocardia*, and *S. aureus*.[17,18]

Kousha and Bayat indicated that extracts of leaves and

Table 1. Average Inhibitory Halo Diameter (mm) of Various Bacterial Strains for *Heracleum persicum* Essential Oil

Bacterial Strain	Samples	Range	Mean ± SD
Staphylococcus aureus ATCC 25913	3	32.5-33	32.16 ± 0.76[a]
Escherichia coli ATCC 8739	3	17.5-20	19 ± 1.32[b]
Salmonella enterica PTCC 1709	3	18-19.5	18 ± 0.46[b]
Vibrio cholera PTCC 1611	3	32.5-34	34 ± 2[a]
Yersinia enterocolitica PTCC 1477	3	22-22.5	22.16 ± 0.76[a]

Table 2. MIC and MBC Value (%) for *Heracleum persicum* Essential Oil Against Pathogenic Bacterial Strains

	Staphylococcus aureus	*Vibrio cholera*	*Yersinia enterocolitica*	*Escherichia coli*	*Salmonella*
MIC	11	8	18	30	32
MBC	16	16	64	32	64

flowers of *H. persicum* had antimicrobial activity against 4 gram-negative and 4 gram-positive bacteria.[19]

Manikandan et al[20] reported that the methanolic extract of leaves of different plants were more active than the aqueous extract in presence of *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus* and *S. aureus*.

Habibi et al[21] showed that the highest inhibitory effect of *H. rechingeri* species was against gram-positive bacteria and *B. subtilis* in particular. Consistent results were obtained at concentration level of 50% ($P < 0.05$).

Hemati et al indicated that essential oil and hydro-alcoholic extract of *H. persicum* had analgesic, anti-inflammatory, and antioxidant effects.[22]

Inhibition effects of *H. persicum* (57.16%) on *Agrobacterium tumefaciens* was reported by Dehghan Noudeh et al.[23] They reported that *H. persicum* (57.16%) had inhibiting effect on all tested gram-positive and gram-negative strains. Our results were consistent with the results of that study.

Frey and Meyers[24] indicated that the traditional medicines were capable of creating inhibition zone against the bacteria *S. typhimurium, S. aurous, E. coli* and *Streptococcus lactis*. Moreover, their data supported the hypothesis that the selection and use of these plants for disease treatment was not random.

Comparison of our results with those obtained by Dehghan Nodeh et al indicated that 4 fractions from *Heracleum* had antimicrobial activity against *A .tumefaciens, E. coli, S. aureus, B. subtilis* and *P. aeruginosa* and the highest and lowest MIC were observed in *E. coli* and *B. subtilis* respectively.[23]

Several studies have been conducted on antibacterial activity of herbs, which are rich in monoterpenoids. Using drop diffusion method, these studies have shown significant inhibition zones for all microorganisms under study such as gram-positive bacteria (*S. aureus*) and fungus (*Candida albicans*).[25,26] Results of the present study were consistent with other studies as to gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli, S. enterica, V. cholera,* and *Y. enterocolitica*).

Inconsistencies between the present study and other works might be because of different genotypes of *H. persicum* utilized, growth ecosystem (habitat, temperature, height), and setting of the experiments (pH and temperature). Several factors influence growth and performance of the plants in an ecosystem including type, habitat, soil, height, and geographical position. Some of these factors have notable effects on the results quantitatively and qualitatively.

Medical herbs as immunostimulants have been widely used as food additives for years and the procedures have been recorded by several research works. Moreover, in vivo, in vitro, and ex vivo surveys of the effects of *Heracleum* sp. used as an immunostimulant agent in terrestrial and aquatic animals or human require deeper investigations. In addition, more studies are needed on antiviral effects of this family.

Given the information obtained from these studies and the present study, anti-bacterial properties of *Heracleum* against the pathogens transmitted by food is supported.

Conclusion

Notable antimicrobial activity against foodborne pathogenic bacteria was demonstrated by the essential oil of *Heracleum*. The essential oil may be effective on other gram-positive and gram-negative bacteria. The practical application of essential oils is to increase shelf life of food. Therefore, it can be a good alternative for artificial preservatives used in the food industry today.

Authors' Contributions

This article was extracted from the MSc project of IM where NS supervised these project and suggested the problem; TM, AA and MS participated in determination of sample points, sample preparation procedure and extraction of essential oil; MR wrote and edited the manuscript. All authors read and approved the final manuscript.

Ethical Approval

This study was approved by Ethics Committee of Tehran University of Medical Sciences.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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