



Research Article

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Comparison of Antimicrobial activity of *Cichorium intybus*, *Dorema aucheri* and *Prangos ferulacea* extracts against some food borne pathogens

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ABSTRACT

In response to the propagation of bacteria resistant to many antibiotics, the discovery of new and more efficient antibacterial agents is primordial. The present study was designed to evaluate the antimicrobial activity of three extracts of medical plants, *Cichorium intybus*, *Dorema aucheri* and *Prangos ferulacea* against some food born pathogens. These extracts were first prepared with methanol. The antimicrobial activity of the extracts was then assessed using the agar-well diffusion method and microtiter broth dilution method against nine bacterial strains i.e. *Staphaureus*, *Bacillus subtilis*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Citrobacter freundii*, *Salmonella enteritidis*, *Enterococcus aeroguns* and *Klebsiella pneumoniae*. The studied extracts displayed various degrees of antibacterial activities. The extracts of *Dorema aucheri* showed the best spectra of activity. In conclusion, we can use these extracts as food additives and antibacterial preservatives after improvement at food safety studies.

Key words: *Cichorium intybus*, *Dorema aucheri*, *Prangos ferulacea*, Antimicrobial activities, Food born pathogens

INTRODUCTION

Nature has provided a complete store of remedies to cure all ailments of human. The natural or herbal remedies are still the backbone of medicines. Phytotherapy is a medicinal practice based on the use of herbal plants and their extracts[1]. Single and poly herbal preparations have been used numerously throughout history for the treatment of various diseases [2]. The increased demand for safe and natural food, without chemical preservatives, provokes many researchers to investigate the antimicrobial effects of herbal compounds [3]. A large number of diverse types of plants grow wild in different parts of Iran. The use of different parts of several medicinal plants to cure specific diseases has been in vogue from ancient times in Iran. Some of these plants are; *Cichorium intybus*, *Dorema aucheri* and *Prangos ferulacea*.

Cichorium intybus L. (Chicory) belongs to the family Asteraceae and it is a small aromatic biennial or perennial herb. The whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds (monoterpenes and sesquiterpenes), coumarins, flavonoids and vitamins [4]. In traditional medicine, all parts of the plant specially root and leaves are used as diuretic, laxative, antibilious, antipyretic, blood purification and strengthen of the stomach. It is also used as an appetizer as well as in the treatment of hepatic failure, jaundice, intermittent fever and mild states of chronic skin diseases [5]. Leaves of chicory are good sources of phenols, vitamins A and C as well as potassium, calcium, and phosphorus [6]. Furthermore, chicory is rich in cichoric acid may

stimulate the immune system as well as prevents inflammation and bacterial infections to a limited extent [7]. A careful review of literature has shown that little data are available on the antimicrobial properties of chicory leaves extracts.

Dorema aucheri is one of the endemic plant species with economic and ecological values, growing in central Zagros mountains of the southwest of Iran. Increasing anthropogenic pressures, including deforestation, re-forestation, intensification of agriculture, drainage of wetland, have already had a great impact on the growth, survival and distribution of native species in Iran, especially the rare and endemic species [8]. This plant grows well in subhumid to humid climate. Various members of *Dorema*, are effective antispasmodic, expectorant diuretic, carminative, diaphoretic, vasodilator [9, 10], antimicrobial and antifungal [11,12], hepatoprotector[13] and are intensively used as a green vegetable or as a popular medicine for treatment of many human diseases [14]. Crude extract of the plant that demonstrated antioxidant activity and anti-lipidemic effects [15]; the resin that exudes from punctures in the stem caused by insect attack has similar impacts.

The genus *Prangos* (Jashir in Persian), which belongs to the Umbelliferae family, consists of about 30 species [16]. Some of *Prangos* species are used in traditional medicine as emollient, carminative, tonic, anti-flatulent, anthelmintic, antifungal and antibacterial agents. *Prangos ferulacea* leaves have been used to treat digestive disease and shows no toxicity [17]. In addition, the leaf of *Prangos ferulacea* is consumed for gastrointestinal disorders in traditional medicine [18]. In some study has been shown that the *Prangos ferulacea* is rich sources of antioxidants, including coumarines, flavonoids, alkaloids, umbelliferon, monoterpenes[19].

Nowadays, the world market for functional foods and nutraceuticals is large and growing. The characteristic of these three herbal in terms of bioactive compounds and characteristic flavor was considered to have a potential for use as functional food and value added processed products. In the present study, extracts of *Cichorium intybus*, *Dorema aucheri* and *Prangos ferulacea* evaluated for their antimicrobial activities against some food born pathogens.

MATERIALS AND METHODS

Preparation of the Spices Extracts

Aerial parts of *Cichorium intybus*, *Dorema aucheri* and *Prangos ferulaceae* collected from Dena Mountain area in Southwestern of Iran in May 2013 and identified at Faculty of Pharmacy, Shiraz University Medical Sciences. The alcoholic extracts were prepared by double maceration in 95% methanol for 4 days. Solvent was removed from filtered extract under reduced pressure to produce a thick dark green extract. The resulting extract was kept in a dark and cold place in sterile vials for further tests.

Bacterial strain, culture conditions, and preparation of inocula

Nine standard bacterial strains were used as follow, 4 Gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 15561, *Bacillus cereus* ATCC 14579 and *Listeria monocytogenes* ATCC 19115), 5 Gram negative bacteria (*Escherichia coli* O₁₅₇: H₇ ATCC 43895, *Citrobacter freundii* ATCC 43864, *Salmonella enteritidis* ATCC 13076, *Enterococcus aerogenes* ATCC 13048 and *Klebsiella pneumoniae* ATCC 3583). A pure culture of the strains were maintained at -20 °C in Brain Heart Infusion broth (Merck) containing 25% glycerol, which was thawed before use.

To prepare bacterial inocula, 0.1 ml of the thawed cultures was transferred to 10 ml of sterile Tryptic Soy Broth (TSB, Merck) and was incubated overnight at 37 °C. A Tryptic Soy Agar (TSA, Merck) plates were streaked from these broths and incubated overnight at 37 °C.

To establish a correlation between the colony forming unit (CFU) ml⁻¹ and absorbance of the dilutions at 600 nm, a standard curve was prepared. A single colony from the TSA plates was inoculated into 10 ml of TSB and was incubated over night at 37 °C. A volume of 0.1 ml of the suspensions was transferred to 10 ml of TSB and was incubated at 37 °C for about 18 h. The cultures were harvested at the midlog phase, three times pelleted by centrifugation at 3000g for 20 min and washed in 0.1% peptone–water. Final cell pellets were resuspended in 10 ml of 0.1% peptone–water. Five twofold serial dilutions were made and their absorbances were measured at 600nm. To determine the log₁₀CFUml⁻¹ corresponding to the absorbance of that same dilution, viable cell counts were performed in duplicate by plating serial dilutions onto TSA and incubating the plates at 37 °C for 24 h.

Antimicrobial assay using Disc diffusion method

The antimicrobial assay of spices was performed by disc diffusion method as described by Kirby-Bauer [20]. All the experiments were performed under sterile conditions. The Tryptic Soy Agar (TSA) plates were inoculated separately with 10⁷ CFU of each test bacterial strain culture and evenly spread by sterile L shaped glass rod on entire surface of

each plate. The sterile discs (5 mm diameter) were dipped aseptically in different extracts for one minute and placed over nutrient agar plates seeded with bacterial culture. The plates were left at ambient temperature for 15 minutes and then incubated at 37°C for 16 hours and observed for zone of inhibition. The antimicrobial activities of discs were determined by measuring the zone of inhibition expressed in millimeter. Antimicrobial assay was performed in triplicate with each bacterial strain.

Microtiter broth dilution method

Microtiter broth dilution method was performed as described previously [21]. Briefly, overnight cultures of bacteria in TSB were sub-cultured and grown for 24 hours. The cells were diluted with TSB to 1×10^6 colony-forming units per ml (CFU/ml). 100 μ l of TSB was added into each of the wells of the first column of a microtiter plate. 100 μ l of each filter-sterilized extract was added to the first well of the microtiter plate and then serially diluted (2-fold) to the last well of the plate (or in a horizontal manner to the last well of the plate). Finally each well received 100 μ l of 1×10^6 (CFU/ml) of selected bacteria. The plate was sealed and incubated 24 hours at 37 °C. Then, their absorbance read at 600 nm by microplate reader (BMG Labtech, Germany). Growth inhibition for each extract was calculated based on the following relationship (or equation):

$$\text{Inhibition growth (\%)} = \frac{\text{Fraction absorbance} - \text{control negative absorbance}}{\text{Control positive absorbance} - \text{control negative absorbance}} \times 100$$

Statistical analysis

All the experiments were performed in triplicate. Data were analyzed using One-way Analysis of Variance (ANOVA) by the SPSS software (version 21). Duncan's multiple range test was used as a post hoc test. Correlation between the results of two methods was determined. $P < 0.05$ was considered as a level of significance.

RESULT AND DISCUSSION

The results of antibacterial activity of methanolic extracts of selected plant extracts against 9 laboratory strains of microorganisms, including 4 Gram positive and 5 Gram negative bacteria, by the agar diffusion method and Microtiter broth dilution method are presented in tables 1 & 2.

The zone of inhibition varied from 5 to 16 mm. *Salmonella enteritidis* and *Citrobacter freundii* were the most sensitive to all extracts. The antibacterial effect of *Dorema aucheri* and *Prangos ferulacea* were more than *Cichorium intybus* for *Salmonella enteritidis* and *Enterococcus aerogenes*. *Cichorium intybus* and *Prangos ferulacea* showed more antibacterial effect than *Dorema aucheri* for *E. coli*, *Staph. aureus* and *Listeria monocytogenes*. *Cichorium intybus* and *Prangos ferulacea* produced higher zone of inhibition than *Dorema aucheri* for *Klebsiella pneumonia* and *Cichorium intybus* showed the most antibacterial effect on *Bacillus subtilis*.

The results of Micro titer broth dilution method were in agreement to the antimicrobial assay using Disc diffusion method. The Pearson correlation coefficient between two methods was 0.87 ($p < 0.01$).

Table 1: Antibacterial activity of plant extracts against the bacteria by disc diffusion assay

Bacteria Herbal extract	Sal	Kleb	Ent	Citro	E.coli	Sta	Bac.c	Bac.s	Lis
<i>Cichorium. i</i>	10.62±0.33a	12.62±1.44b	6.81± 0.63a	14.84±1.42a	10.28±1.30b	9.86±0.65b	9.66±0.62a	8.32±0.14c	10.22±1.12b
<i>Dorema. a</i>	14.3±1.36b	8.48± 0.58a	12.6±0.84b	12.11±1.22a	12.35±1.27b	8.79± 0.53b	10.32±0.84a	7.76± 0.31b	12.14±0.96b
<i>Prangos. f</i>	12.42±1.31b	11.06±1.50b	10.82±0.98b	12.76±1.53a	7.92± 0.88a	7.81± 0.26a	9.48±0.41a	6.67± 0.17a	8.32±0.64a

Means within a column with different letters denote significant differences ($P < 0.05$)

Table 2: Inhibition growth (%) of plant extracts against the bacteria by Microtiter broth dilution method after 24 hours

Bacteria Herbal extract	Sal	kleb	Ent	Cit	E. coli	Sta	Bac.c	Bac.s	Lis
<i>Cichorium. i</i>	95.8± 3.52a	97.6±1.18b	71.3±2.42a	99.3±0.38a	93.1±5.28b	91.3±2.38b	91.6±2.88a	86.8±1.78c	94.3±1.74b
<i>Dorema. a</i>	99.1± 0.76b	88±2.26a	93±1.08b	97.9±1.42a	97.5±1.64b	90.9±3.58b	93.6±1.14a	80.6±2.32b	97.9±2.12b
<i>Prangos. f</i>	99± 0.48b	94.3±3.78b	89.2±4.39b	97.5±1.94a	82.2±2.96a	81.4±1.86a	91.4±3.06a	69.7±4.66a	90.6±1.08a

Means within a column with different letters denote significant differences ($P < 0.05$)

Medicinal plants and their extracts were used as first medicines since ancient times. Plants may be an important source of potentially useful structures for the development of new chemotherapeutic agents [22]. The first step towards this goal is the in vitro screening of plant extracts for their bioactivity.

The increase in the number of antibiotic resistant bacteria is no longer matched by expansion in the arsenal of agents available to treat infections. Plants may provide natural sources of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally [23].

Each of the extract tested in the present study displayed antibacterial activity on the all bacterial strains. However differences were observed between antibacterial activities of the extracts. Chicory shows significant antibacterial activity that may be due to the presence of many potent compounds such as inulin, bitter sesquiterpene lactones, coumarins, flavonoids, etc. The methanolic extracts of chicory showed a good inhibitory effect against the studied bacteria except *Enterococcus aerogenes*. In general, gram- negative bacteria were more sensitive than gram-positive bacteria. *Dorema aucheri* have some anti-bacterial component such as terpene, flavonoids and phenolic compounds [24].Antimicrobial activity of the *Dorema aucheri* extract can be attributed to the high amounts of phenolic compounds, such as p-coumaric and caffeic acid [25].Phytochemical examinations of *Prangos ferulacea* have led to identifying and extracting some coumarines, alkaloids, flavonoids, terpenoids, α -pinen, monoterpenes, sesquiterpenes, and coumarines have been detected [26]. These components have some anti-bacterial effect on gram negative and gram positive bacteria.

These antibacterial components have shown significant antibacterial activity against both gram negative and gram positive effect. Antimicrobial activity of flavonoids results from the ability to form complexes with bacterial cell wall, preventing the growth of microorganisms. To apply their antimicrobial activity, phenolic compounds inhibit enzyme activity through reacting with their sulfhydryl groups or through nonspecific interactions with protein. With respect to antibacterial mechanism of plant extracts, it is shown that polyphenols are capable of forming heavy soluble complexes with proteins, thus binding to bacteria and destroying bacterial cell surface acceptors [25].

These differences could be due to the differences in the chemical composition of these extracts as the secondary metabolites of plants have many effects including antibacterial and antiviral properties [27, 28].

This research indicates that *Dorema aucheri* have antibacterial effects more than *Cichorium intybus* and *Prangos ferulacea* against a number of food spoilage and pathogenic bacteria. Previously, the chemical compounds in the essential oil of *Prangos ferulacea* were identified and its antimicrobial properties have been shown[29].In total, the bacteria isolate *Bacillus subtilis* showed the lowest sensitivity to all of the plant extract. Comparison of findings of different studies seems complicated because the results are affected by many factors such as incubation temperature and time, pH and type of culture medium, microbial growth phase, and volume of medium. Also, chemical composition, type, and mechanism of action of phenolic compounds of each extract are among factors which make difference in the results of the antimicrobial activity of the solvents [30].

CONCLUSION

According to the result, using natural products as antibacterial substances is a proper method for controlling pathogenic bacteria and increasing food shelf life. The results of the present study support the traditional use of the studied plants in the treatment of bacterial infections. Their antibacterial properties indicate that they can be introduced as a replacement antibiotic to treatment and complementary therapy.

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REFERENCES

- [1] Asif, M., Antimicrobial potential of *Azadirachta indica* against pathogenic bacteria and fungi, *Journal of Pharmacognosy and Phytochemistry*, **2012**, 1(4), 78-83.
- [2] Abu-Shanab, B., Adwan, G., Abu-Safiya, D., Jarrar, N., Adwan, K., Antibacterial activities of some plant extracts utilized in popular medicine in Palestine, *Turkish Journal of Biology*, **2004**, 28 (2), 99-102.
- [3] Gheisari, H. R., Habibi, H., Antimicrobial and antifungal effects of *Echinacea purpurea* and *Thymus vulgaris* extracts on food pathogens, *Online Journal of Pharmacology (and Pharmacokinetics)*, **2013**, 9, 1-7.
- [4] Nandagopal, S., Ranjitha Kumari, B. D., Phytochemical and Antibacterial Studies of Chicory (*Cichorium intybus* L.) - A Multipurpose Medicinal Plant, *Advances in Biological Research*, **2007**, 1 (1-2), 17-21.
- [5] Ghaderi, R., Zardast, M., Hosseini, M., Delgir, B., Hassanpour, M., Comparison of Antibacterial Effect of *Cichorium intybus* L. with Vancomycin, Ceftriaxone, Ciprofloxacin and Penicillin (In Vitro), *Clinical and Experimental Pharmacology*, **2012**, 2(2), 1-3.

- [6] Mulabagal, V., Wang, H., Ngouajio, M., Nair, M. G., Characterization and quantification of health beneficial anthocyanins in leaf chicory (*Cichorium intybus*) varieties, *European Food Research and Technology*, **2009**, 230, 47–53.
- [7] Nayeemunnisa, A., Alloxan diabetes-induced oxidative stress and impairment of oxidative defense system in rat brain: neuroprotective effects of *Cichorium intybus*, *International Journal of Diabetes and Metabolism*, **2009**, 17, 105–109.
- [8] Mirinejad, S., Salahi Ardakani, A., Phenology, distribution and cultivation of endangered medicinal plant species (*Dorema aucheri* Boiss), in Kohgiluyeh-va-Boyer-Ahmad Province, Iran, *Journal of Biology and Environmental Science*, **2014**, 5(5), 177–182.
- [9] Azarniushan, F., Khatamsaz, S., Sadeghi, H., The effect of Hydroalcoholic Extract of *Dorema aucherion* Blood concentration of Gonadotropin and Androgen Hormones in Adult Male Rats, *Armaghan Danesh*, **2009**, 14(3), 63–70.
- [10] Ghollassi, M. S., A contribution to some ethnobotanical aspects of Birjand Flora (Iran), *Pakistan Journal of Botany*, **2008**, 40(4), 1783–1791.
- [11] Mokhtari, M., Shariati, M., Niknam, H., Effects of Anti-inflammatory and analgesic aqueous-alcoholic *Dorema aucheri* using Karzhynan model formalin test in rats, *Journal of medical sciences Rafsanjan*, **2008**, 37, 165–172.
- [12] Shahidi, G. H., Moein, M. R., Foroumadi, A. R., Rohbakhsh-Zamin, F., Cytotoxic activity of medicinal plants used in Iranian traditional medicine on two strains of *Saccharomyces cerevisiae*, *DARU Journal of Pharmaceutical Science*, **2002**, 10, 162–164.
- [13] Govind, P., Medicinal plants against liver diseases, *International Research Journal of Pharmacy*, **2011**, 2(5), 115–121.
- [14] Ibadullayeva, S., Movsumova, N., Gasymov, H., Mamedli, T., Protection of some rare and endangered vegetable plants in the flora of the Nakhichevan AR, *International Journal of Biodiversity and Conservation*, **2011**, 3(6), 224–229.
- [15] Dehghan, G., Fatholahi, G., Sheikhzadeh, N., Ahmadiasl, N., Hypocholesteremic and antioxidant effects of *Dorema aucherion* extract in rats fed high cholesterol diet, *Journal of the Iranian Chemical Society*, **2009**, 6, 115–143.
- [16] Sajjadi, S. E., Shokohinia, Y., Gholamzadeh, S., Chemical composition of essential oil of *Prangos ferulacea* (L.) Lindl. Roots, *Chemija*, **2011**, 22 (3), 178–180.
- [17] Wadood, N., Wadood, A., Nisar, M., Effect of *Ficus religiosa* on blood glucose and total lipid levels of normal and alloxan diabetic rabbits, *Journal of Ayub Medical College Abbottabad*, **2003**, 15(4), 40–42.
- [18] Mohammadi, J., Zare, T., Hypoglycemic action of *Prangos ferulacea* in normal and streptozotocin induced diabetic Wistar rats, *Journal of American Science*, **2013**, 9(10), 51–54.
- [19] Coruh, N., Celep, A. G., Ozgokce, F., Antioxidant properties of *Prangos ferulacea* (L.) Lindl., *Chaerophyllum macropodium* Boiss. and *Heracleum persicum* Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase, *Food Chemistry*, **2007**, 100, 1237–1242.
- [20] Kirby – Bauer, Antimicrobial sensitivity testing by agar diffusion method, *African Journal of Clinical Pathology*, **1996**, 44, 493.
- [21] Holowachuk, S. A., Bal'a, M. F., Buddington, R. K., A kinetic microplate method for quantifying the antibacterial properties of biological fluids, *Journal of Microbiological Methods*, **2003**, 55(2), 441–446.
- [22] Mahesh, B., Satish, S., Antimicrobial activity of some important medicinal plant against plant and human pathogens, *World Journal of Agricultural Sciences*, **2008**, 4, 839–843.
- [23] Akinpelu, D. A., Onakoya, T. M., Antimicrobial activities of medicinal plants used in folklore remedies in south-western, *African Journal of Biotechnology*, **2006**, 5, 1078–1081.
- [24] Khanahmadi, M., Miraghaee, S., Shand Karimi, I., Evaluation of the Antioxidant and antimicrobial Properties of *Dorema aucheri* plant, *Iranian Red Crescent Medical Journal*, **2012**, 14(10), 684–685.
- [25] Mianabadi, M., Hoshani, M., Salmanian, S., Antimicrobial and Anti-oxidative Effects of Methanolic Extract of *Dorema aucheri* Boiss., *Journal of Agricultural Science and Technology*, **2015**, 17, 623–634.
- [26] Kafash-Farkhad, N., Asadi-Samani, M., Rafieian-Kopaei, M., A review on phytochemistry and pharmacological effects of *Prangos ferulacea* (L.) Lindl., *Life Science Journal*, **2013**, 10(8), 360–367.
- [27] Cowan, M. M., Plant products as antimicrobial agents, *Clinical Microbiology Reviews*, **1999**, 12, 564–582.
- [28] Noumedem, J., Mihasan, M., Lacmata, S. T., Stefan, M., Kuate, J., Kuete, V., Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria, *BMC Complementary and Alternative Medicine*, **2013**, 13, 190.
- [29] Razavi, S. M., Nazemiyeh, H., Zarrini, G., et al., Chemical composition and antimicrobial activity of essential oil of *Prangos ferulaceae* (L.) Lindl from Iran, *Natural Product Research*, **2010**, 24(6), 530–533.
- [30] Wen, A., Delaquis, P., Stanich, K., Toivonen, P., Antilisterial Activity of Selected Phenolic Acids, *Food Microbiology*, **2003**, 56, 305–311.