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Comparison of Antioxidant Activity of *Dracocephalum polychaetum* Bornm and *Nepeta cataria* L. and Their Effect on Probiotic Bacteriae in a Simulated Gastrointestinal Environment

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ABSTRACT

Introduction: *Dracocephalum polychaetum* Bornm and *Nepeta cataria* L. are two plants from the Lamiaceae family with antibacterial, antifungal, and antiviral properties. This study evaluated the phenolic compounds, antioxidant activity, and effect of aqueous extracts on the survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* in a simulated gastrointestinal environment. **Method**: The aerial parts of plants were collected at the vegetative growth stage from the Hanza-Kuh's highlands in the Bahr Asman region of Jiroft city, Iran, in spring 2018. The total phenolic content of plants and antioxidant activity were measured using Folin–Ciocalteau and DPPH (2, 2-diphenyl-1-picrylhydrazyl) methods, respectively. For investigating the survival of probiotic bacteria in a simulated gastrointestinal environment, bacterial suspension was inserted into tubes containing 0, 100, 250, 500, and 1000 ppm of extracts and then incubated in a simulated gastrointestinal environment. The probiotic bacteria were counted using an MRS agar medium at various incubation times. **Results**: The results showed that the amount of total phenolic compounds in the *D. polychaetum* Bornm extract (44.55 mg/g dry matter) was higher than that of *N. cataria* L. (18.37 mg/g dry matter). With increased extracts concentrations, the percentage of DPPH-free radicals increased, and *D. polychaetum* Bornm extract in all concentrations showed higher DPPH free radical inhibitory content compared to the *N. cataria* L. extract. The viability results in the same gastrointestinal environment showed that samples containing *N. cataria* extract had a more remarkable survival rate than the controls and *D. polychaetum* Bornm extract. **Conclusion**: Using less than 500 ppm of *D. polychaetum* Bornm and *N. cataria* L. aqueous extracts can increase probiotic bacteria growth and viability.

INTRODUCTION

Essential oils and extracts of plants are a rich source of medicinal compounds and are used in preservatives and antioxidants in food, pharmaceutical, and cosmetic products [8, 9]. *Nepeta cataria* L. plant belongs to the Lamiaceae family, which grows in central, western, southwestern Asia, the Himalayas, North Africa, and North America. In Iran, it occurs in the north, northwest, center, and south [3]. The essential oil of *N. cataria* has biological and pharmacological properties. Due to its nepetalactone, *N. cataria* essential oil has antibacterial, antifungal, and antiviral properties and is effective against some molds, e.g., *Microsporum*, *Aspergillus*, and *Penicillium* species [1]. A study reported lutein, epinephrine, and their glycosides, phenolic components (caffeic acid, rosmarinic acid, p coumaric acid), and terpenoids (ursolic acid, citral, nerol, and geraniol) in the methanolic extract of this plant [24].

The genus *Dracocephalum* comprises other members of the Lamiaceae family and has 50 species worldwide, of which eight herbaceous species grow in Iran. *Dracocephalum polychaetum* Bornm is one of the eight species of the *Dracocephalum* genus that grows only in Iran's Kerman province, is used as a local spice in dairy products [29], and has antioxidant activity [14]. *Dracocephalum* species contain flavonoids, diterpenes, tannins, and phenolic acids [17]. The main components in *D. polychaetum* Bornm essential oil include geraniol, geraniol acetate, nerol, and phenolic acids (rosmarinic

acid and caffeic acid), cinnamic acid derivatives, tannins, di, and triterpenes such as oleanolic acid and acacetin [24]. The flavonoid, apigenin, and luteolin compounds are the main components in the plant's methanolic extract [22]. Phenolic compounds in *D. polychaetum* Bornm extract include rosmarinic acid, naringin, apigenin, thymol, carvacrol, quercetin, limonene, and rutin [34]. Studies have shown that limonene, which is one of the main components of this plant, is an antibacterial [26], antiviral, antifungal compound, and peril aldehyde, which makes up more than 50% of the essential oil, has bactericidal, antimicrobial, and antifungal effects [39].

Probiotics are helpful microorganisms that can affect the host digestive tract microbial flora once consumed by humans or animals. Probiotics stimulate the growth of beneficial intestines bacteria or reduce the pathogenesis of harmful microbes, and their mechanism of action depends on their survival in the digestive system [4].

Considering that plants are sources of antioxidants and protect cells from oxidative damage, research in this field has received much attention [19, 20]. Since probiotics are often used in dairy products, the interaction of the medicinal plant extracts with with these bacteria is essential due to their antimicrobial properties.

This study investigates the antioxidant compounds in the aqueous extract of *D. polychaetum* Bornm and *N. cataria* L. from Jiroft city and their effect on the survival of probiotic bacteria *Lactobacillus acidophilus* and *Bifidobacterium animalis* in a simulated gastrointestinal environment.

MATERIALS AND METHODS

The chemicals used in the study, including methanol 80%, folin-Ciocalteu reagent, sodium carbonate, DPPH reagent, hydrochloric acid, sodium chloride, dihydrogen phosphate, were purchased from the Merck and Sigma Company with the highest purity.

Collection of vegetable samples. The aerial parts of *D. polychaetum* Bornm and *N. cataria* L. were collected from the heights of Hanza-Kuh Bahrasman Mountain (2100 m above sea level) of Jiroft city in the spring 2019 and dried in the shade. The dried plants were powdered with an electric mill and stored in plastic bags at 5℃ until used.

Preparation of plant extract. Amounts of 10 g of the plant powders were mixed with 100 ml of distilled water and placed on a stirrer at ambient temperature for 12 h. The resulting suspensions were passed through a Whatman No. 1 filter paper, transferred into closed-door jars and wrapped in aluminum foil, and kept at 5℃ until use [34].

Measurement of phenolic compounds. The folin-Ciocalteu method was used to measure total phenolic compounds. 20 µl of the prepared extracts were mixed with 1.16 ml of distilled water and 100 µl of folin reagent. After 5 min, 300 µl of sodium carbonate (20%) was added to the solution, and the samples were stored at 40°C for 30 min. The absorption of the samples was read with a UV-visible spectrophotometer at 760 nm. Gallic acid was used as a standard for the calibration curve [6].

Measurement of antioxidant activity by radical DPPH trapping. 2 and 2-diphenyl1-Picrylhydrazyl (DPPH) is a fat-friendly radical that has a maximum absorption at 517 nm. The ability of extracts to absorb DPPH radicals was determined, as described by others [5]. Various concentrations of extracts [0 (control), 50, 100, 250, 500, and 1000 ppm] were prepared.

Briefly, 1 mL methanolic solution of DPPH (1 mM) was mixed with 3 mL of extract solution in methanol (containing 50-400 µg of dried extract). The mixture was then homogenized vigorously and left for 30 min in a dark place at room temperature. Its absorbance was measured at 517 nm, and activity was expressed as a percentage of DPPH scavenging relative to the control using the following equation:

DPPH scavenging activity (%) = Absorbance of control- Absorbance of sample/ Absorbance of control×100

Activation of probiotic bacteria. Pure probiotic culture of *L. acidophilus* and *B. animalis* subs *animalis*, purchased from "Persian Type Culture Collection, Iranian Research Organization for Science and Technology", were inoculated into MRS-broth medium and incubated at 37˚C for 24 h under anaerobic conditions. The probiotic biomass in the late-log phase was collected by centrifugation at 10,000 rpm for 10 min and washed twice in sterile saline. Viable probiotic concentrations were measured by pour plate counting on MRS agar. One gram of suspension was added to 99 mL of sterile Ringer's solution. Then, 0.1 ml of the resulting solution was transferred to MRS agar medium and incubated at 37˚C for 72h. The number of viable cells was obtained by multiplying the colony number in the inverse of the dilution factor [12].

Viability of probiotic bacteria in simulated gastrointestinal environments. One gram of activated probiotic bacteria suspension containing 10^{10} bacterial cells was transferred into the tubes containing 9 ml of gastric fluid (0.8 M hydrochloric acid containing 0.2% sodium chloride with pH: 1.55 and no pepsin). Various concentrations, 0, 100, 250, 500, and 1000 ppm of aqueous extract of *N. cataria* L. and *D. polychaetum* Bornm was added to the tubes and kept at 37ºC. At 0, 30, 60, 90, and 120 min of incubation, 1 ml of bacterial suspension containing plant extracts were transferred into 9 ml of intestinal fluid (potassium dihydrogen phosphate 0.05 M, Ph:7.43 with 0.6% of the biles salt) and incubated for 150 min at 37ºC. Each medium was diluted with peptone water (0.1%) and incubated at 37ºC for 72 h in the MRS agar medium [18].

Statistical analysis of data: Results were expressed as mean ± SD values, which were the average of

triplicate experiments. Significant differences between the results were calculated by analysis of variance (ANOVA) using SPSS:23 software. Differences at $P<0.05$ were considered to be significant.

RESULTS

Total phenolic compounds in the aqueous plant extracts

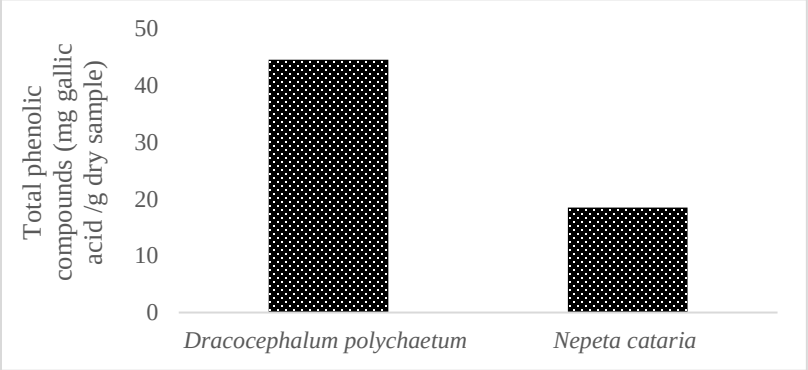

Fig. 1. Total phenolic compounds in the aqueous extract of *N. cataria* L. and *D. polychaetum Bornm*.

As shown in Fig. 1, the value of the total phenolic compounds in the aqueous extract of *D. polychaetum* Bornm (44.55 mg of gallic acid/g of dry matter) was significantly higher than the total phenolic compounds in *N. cataria* L extract (187.37 mg gallic acid/ g dry matter) ($P<0.05$).

Antioxidant activity of two studied plants extracts. As shown in Fig. 2, the free radical elimination percentage of DPPH was highest in the concentration of 1000 ppm and decreased with the dilution. The 50 ppm concentration showed the lowest percentage of the DPPH free radical inhibitor in both plant extracts. The *D. polychaetum* Bornm extract had a higher percentage of DPPH radical scavenging than *N. cataria* L extracts.

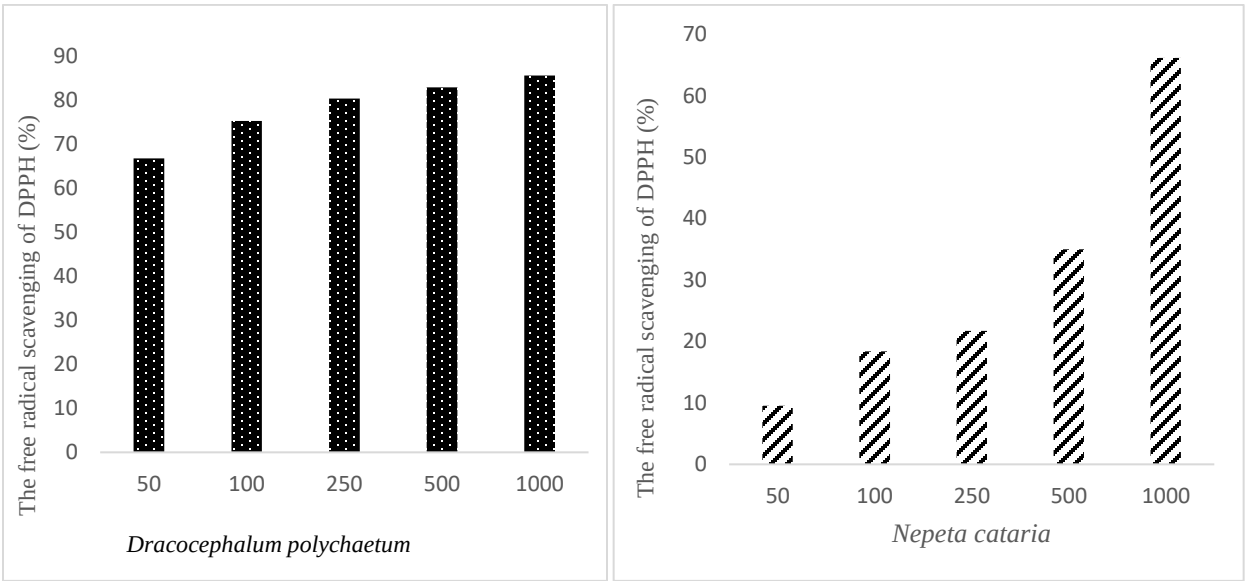

Fig. 2. The free radical scavenging of DPPH (%) for different concentrations on *N. cataria* L. and *D. polychaetum* Bornm

The survival rate of probiotic bacteria in simulated gastrointestinal environments. The effect of aqueous extract of the two plants during 120 min of incubation in simulated gastrointestinal environments was remarkable (Table 1).

Table 1. Viability of probiotic bacteria (CFU/ml) in simulated gastrointestinal environments

Extract (ppm)	Bacterial type	1 min	30 min	60 min	90 min	120 min
Control	*L. acidophilus*	$5.2\pm 0.4\times 10^{9}$	$7.3\pm 0.8\times 10^{7}$	$4.1\pm 0.7\times 10^{6}$	$<10^{6}$	$<10^{6}$
(Without extract)	*Bifidobacterium animalis*	$2.5\pm 0.1\times 10^{9}$	$4.4\pm 0.3\times 10^{6}$	$<10^{6}$	$<10^{6}$	$<10^{6}$
100 ppm *N. cataria*	*L. acidophilus*	$8.5\pm 0.4\times 10^{9}$	$6.1\pm 0.2\times 10^{8}$	$7.7\pm 0.9\times 10^{7}$	$5.3\pm 0.2\times 10^{6}$	$5.1\pm 0.1\times 10^{6}$
	B. animalis	$5.1\pm 0.9\times 10^{9}$	$6.7\pm 0.1\times 10^{7}$	$4.1\pm 0.3\times 10^{7}$	$3.7\pm 0.6\times 10^{7}$	$5.2\pm 0.1\times 10^{6}$
500 ppm *N. cataria*	*L. acidophilus*	$7.8\pm 0.7\times 10^{10}$	$4.7\pm 0.1\times 10^{9}$	$4.5\pm 0.2\times 10^{8}$	$6.1\pm 0.5\times 10^{7}$	$8.2\pm 0.3\times 10^{7}$
	B. animalis	$4.3\pm 0.4\times 10^{10}$	$5.2\pm 0.2\times 10^{8}$	$8.1\pm 0.6\times 10^{7}$	$4.5\pm 0.7\times 10^{7}$	$5.4\pm 0.6\times 10^{6}$
1000 ppm *N. cataria*	*L. acidophilus*	$6.5\pm 0.3\times 10^{9}$	$8.5\pm 0.2\times 10^{7}$	$5.7\pm 0.1\times 10^{6}$	$<10^{6}$	$<10^{6}$
	B. animalis	$3.4\pm 0.5\times 10^{9}$	$3.3\pm 0.6\times 10^{6}$	$1.3\pm 0.8\times 10^{6}$	$<10^{6}$	$<10^{6}$
100 ppm *D. polychaetum*	*L. acidophilus*	$6.1\pm 0.7\times 10^{9}$	$3.6\pm 0.7\times 10^{8}$	$5.4\pm 0.2\times 10^{6}$	$<10^{6}$	$<10^{6}$
	B. animalis	$3.3\pm 0.6\times 10^{9}$	$5.1\pm 0.3\times 10^{7}$	$1.4\pm 0.1\times 10^{6}$	$<10^{6}$	$<10^{6}$
500 ppm *D. polychaetum*	*L. acidophilus*	$4.1\pm 0.7\times 10^{10}$	$3.7\pm 0.3\times 10^{9}$	$8.5\pm 0.3\times 10^{8}$	$4.1\pm 0.7\times 10^{8}$	$7.4\pm 0.2\times 10^{6}$
	B. animalis	$3.2\pm 0.2\times 10^{10}$	$6.1\pm 0.9\times 10^{8}$	$3.8\pm 0.6\times 10^{8}$	$4.2\pm 0.3\times 10^{7}$	$1.2\pm 0.5\times 10^{7}$
1000 ppm *D. polychaetum*	*L. acidophilus*	$6.1\pm 0.7\times 10^{9}$	$3.6\pm 0.7\times 10^{8}$	$5.4\pm 0.2\times 10^{6}$	$<10^{6}$	$<10^{6}$
	B. animalis	$3.3\pm 0.6\times 10^{9}$	$5.1\pm 0.3\times 10^{7}$	$1.4\pm 0.1\times 10^{9}$	$<10^{6}$	$<10^{6}$

Adding 100 and 500 ppm of the two plant extracts improved the survival of the two probiotic bacteria in the simulated gastrointestinal conditions and increased their concentration to more than 10^6 CFU/ml after 120 min. The 1000 ppm concentration of both plant extracts reduced probiotic bacteria survival, which is likely due to the higher concentration of the antibacterial materials, especially the phenolic compounds in this concentration. According to table 1, *L. acidophilus* was more viable than *B. animalis*. The samples containing *N. cataria* L. extract showed more viability than the control and *D. polychaetum* Bornm extract**.**

DISCUSSION

According to the study results, the total phenolic compounds observed in *D. polychaetum* Bornm and *N. cataria* L. plant extract were 44.55 and 18.37 gallic acid per gram of dry matter, respectively. In a similar study, the phenolic compounds of the alcoholic extract of *Dracocephalum moldavica* were equal to 63.38 mg of gallic acid per gram of dry matter [34]. The methanolic, ethanolic, and ethanolic/methanolic extracts of this plant contained 3.04, 1.56, and 2.308 mg of gallic acid per 100 grams of sample, respectively. The percentage of free radical inhibition in the mentioned extracts was 87.77, 60.88, and 25.96%, respectively [7]. In another research amount of the phenolic compounds and the antioxidant activity of the aqueous extracts of *Nepeta cadmea* were more than those of ethanolic, methanolic, and acetone extracts [16]. The observed differences between the phenolic compounds in different studies may be due to various factors such as growth conditions, ripening stages, season, geographic area, type of fertilizer used, type of soil, sunlight, the extraction conditions and method, and especially the solvent used for extraction of phenolic compounds [8]. Water as the extraction solvent makes a completely polar environment in which some phenolic compounds are extracted at the lower polarity resulting in reduced phenolic compounds compared to other solvents. The addition of water to organic solvents results in making a relatively polar medium, ensuring the extraction of more significant amounts and types of phenolic compounds. The aqueous extract contains large amounts of impurities, such as organic acids, proteins, and soluble sugars, which can interfere with the detection and determination of phenolic compound amounts [13]. The DPPH free radical scavenging method is widely used to evaluate the ability of various natural products to suppress free radicals and has been accepted as a model compound for free radicals containing initiating lipids [27]. In the present study, *D. polychaetum* Bornm plant extract at all concentrations contained the highest percentage of the free radical inhibition of DPPH compared to the *N. cataria* L. extract. This plant extract also has the highest concentrations of phenolic compounds, which have the highest free radical scavenging activity. The *N. cataria* L. plant extract had a lower percentage of total phenolic compounds and had a lower free radical removal of DPPH. There is a significant relationship between radical scavenging and plants' phenolic compounds [35, 38]. Other studies also show that high phenolic compounds are the main reason for the high antioxidant activity of some extracts, including polar extracts [15]. Increasing the concentration of phenolic compounds enhances the ability of different extracts to inhibit free radicals directly. At higher concentrations of phenolic compounds, due to the increased hydroxyl groups in the reaction medium, the possibility of hydrogen donation to free radicals and consequently the inhibitory power of the extracts increases [6, 36]. A study reported 85.30% free radical inhibition of DPPH at a concentration of 20 mg/ml of methanolic extract of the *D. polychaetum* Bornm plant [28]. In another study, the free radical inhibition activity of the aqueous extract of *N. cataria* L. was more than its methanolic and acetone extracts [24]. *N. cataria* L. has a relatively large amount of essential oil. Extracts and essential oil include Neurol, geraniol, citral, and ursolic acid, and polyphenols (flavonoids, phenolic acids) with antioxidant activity;

hence this plant can be used as an antioxidant [23]. The antimicrobial properties of essential oils and extracts were proved against a wide range of microbes, including bacteria, yeasts, and molds. However, Simsek *et al*. [31] reported that mint, garlic, and thyme essential oils effect on lactic acid bacteria was not significant compared to the control sample. Also, it was observed that the number of lactic acid bacteria in all cheese samples containing different essential oils (peppermint, lemon) did not change significantly during the ripening period [2]. In comparison, Shahdadi *et al*. [33] reported that *Ziziphora* and Peppermint essences increased the probiotic bacteria growth in drinking yogurt compared to samples without essential oil. Sarabi-Jamab and Niazmand [30] investigated the effects of *Mentha piperita* and *Ziziphora clinopodioides* essential oils on *L. acidophilus* and showed no significant difference in bacteria survival among samples containing various concentrations of *M. piperita*, *Z. clinopodioides* essential oils, and the control. Laboratory studies showed that spices at low concentrations increased probiotics growth significantly while preventing pathogens [10]. The positive effect of spices and vegetable extracts on bacteria growth and survival is likely due to high amounts of metal ions, especially magnesium and manganese, in these substances [20]. The reason for more survival of probiotic bacteria in aqueous extract of the *N. cataria* L. plant can be attributed to its lower phenolic compounds or possibly its fewer antimicrobial components. Among all identified substances in plant tissues, phenolic compounds, or nitrogen-free secondary compounds, are the most abundant and the most important substances that have antimicrobial effects [11]. In total, plants with higher phenolic compounds have more antibacterial activity [4]. Low concentrations of plant extracts contribute to the growth of probiotic bacteria due to the antioxidant content and vitamins. More concentrations of extracts may result in antimicrobial properties due to the existence of high phenolic compounds [32]. The phenolic compounds in plant extracts play an important role and improve the yogurt starter bacteria growth [25] and probiotic bacteria [21]. However, with the increased concentration of extracts, the number of the probiotic bacteria decreased, and this may be due to the inhibitory effect of some compounds such as thymol, carvacrol, beta-caryophyllene, and di-germacrene, which have significant antibacterial effects) [37]. Our results indicated that the viability of *L. acidophilus* in simulated gastric fluid conditions was higher than the *B. animalis*, which may be due to the higher overall resistance of *Lactobacillus* to acidic conditions compared to the *Bifidobacterium* [18].

In general, the results of the present study showed that the aqueous extract of two plants, *D. polychaetum* Bornm and *N. cataria* L. improved the viability of the probiotic bacteria in simulated gastrointestinal environments, Therefore, using concentrations of less than 500 ppm of these plant extracts in probiotic food products is recommended to increase the survival of probiotic bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests associated with this manuscript.

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