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Effects of Ethanolic *Ferolagu angulata* Extract on Pathogenic Gastrointestinal Bacteria and Probiotic Bacteria in Skimmed Milk Medium

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ABSTRACT

***Background*:** Due to excessive consumption of synthetic drugs, drug resistance rate of pathogenic bacteria is increasing and there is an ever-increasing need to find new safe compounds to tackle this problem. This study was conducted to investigate the consequences of chavill extract on the growth and viability of gastrointestinal pathogenic bacterium and probiotics bacteria.

***Methods*:** The experiment contained three levels of the chavill extract concentrations (0, 1 and 3%) which were added to the milk free fat in accompany with three probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* and *lactobacillus plantaram)* and a pathogenic gastrointestinal bacterium (*Salmonella typhimurium*). Bacterial inoculums (1×10^7 CFU/ml) with different concentrations of chavill extract were added to skimmed milk medium and bacteria growth were enumerated.

***Results*:** The concentration of 1% chavill extract significantly increased the total count of probiotic bacteria compared to the control group, while the number of pathogenic bacteria was decreased. At 3% chavill extract the growth of *Lactobacillus acidophilus* and *Lactobacillus plantaram* were increased. On the other hand, it prevented the growth of *Salmonella typhimurium*

***Conclusion*:** Chavill extracts would play as an alternative to antibiotics in pharmacological studies to decreases harmful bacteria and increase probiotic bacteria.

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Introduction

There are many pathogenic and non-pathogenic bacteria in gastro intestinal tract that may cause variety of diseases. In order to prevent the growth of pathogenic microbes in humans, the gastrointestinal tract needs to function healthy. A negligible damage to gastrointestinal tract, because of pathogenic microbe effects, could reduce the feed efficiency and growth rate in animals and human being. One way to prevent gastro intestinal damage is likely to use synthetic/natural antibiotics. *Ferolagu angulata* (locally referred to as Chavill) is a perennial shrub with the height 60-150 cm. The *F. angulata* has two subspecies: subsp. *angulata* (Schlecht) that is widely distributed in Turkey, Iraq and Iran, and *Carduchorum* subsp. *Ferulago* species which are used in traditional/folk medicine for their sedative, tonic, digestive and anti-parasitic effects (1). Therefore, it is thought, chavill could increase the count of useful bacteria and reduce the count of harmful bacteria (*in vitro* conditions). Probiotics are living microorganisms that provide beneficial outcomes for their host. Chavill extracts have a wide range of compounds with different interaction on bacteria (2). To best of our knowledge, there are small number of studies undertaken to find the effects of chavill extract on acidity, counts of harmful bacteria and important probiotics bacteria; this study was conducted to investigate the effects of chavill extract on the viability, proliferation and mortality of three probiotics and the harmful endemic gastrointestinal bacteria adopting *in vitro* conditions.

The chavill plant was collected in Dena Mountain, Kohgiluyeh and Boyerahmad province, Iran in spring season. The plants were milled (Retsch mill, Germany) and extracted using of hydro alcohol technique. Briefly; 20 gram of powdered plant material was put in 200 ml conical flask. Then around 100 ml of ethanol was added and kept in a reciprocating shaker for 24 hours. Extraction process was carried out by Whatman no: 1 filter paper. The solvent from the extract was removed using rotary vacuum evaporator (Hahnshin, Korea) with the water bath temperature of 50 °C (3, 4). Lyophilized bacteria were prepared from Central Bacteria and Fungi Collection (Tehran, Iran) adopting culture base of company directions. In order to secure ourselves in terms of lack of secondary pollution in chavill extract, 100 µl of diluted extract was cultured on Nutrient Agar medium (Merk, Germany) and incubated at 37 °C for 3 days. This study was done in the context of completely randomized design with three probiotic bacteria and one pathogenic gastrointestinal bacterium. Experimental treatments were prepared by combination of 0, 1 and 3 percent of chavill extracts with four bacteria [*Lactobacillus casei* (PTCC: 16081), *Lactobacillus plantarum* (PTCC: 1058), *Lactobacillus acidophilus* (PTCC: 1643) and *Salmonella typhimurium*]. These bacteria were purchased from local collection center of industrial fungus and bacteria in Iran and cultured according to this protocol [(liquid Deman, Rogosa and sharp medium for probiotic ones (MRS) and Mueller Hinton Broth medium for *Salmonella typhimurium*]. Both media were incubated in 37 °C for about 48 hours (5, 6). In the first step, the numbers of lived bacteria were counted by lived pure plate count method. Additional 0.5 % sterilized cysteine hydrochloride Merk, Germany) was added to the medium of *Lactobacillus acidophilus*. Cultured plates were incubated (Helmert, Korea) at 37 °C for 24 hours and colonies were counted with colony counter. This was followed by addition of 7.5 ml fat-free sterilized skimmed milk to each tube containing in triplicates. . After which 7.5 ml of sterilized saline or 7.5 ml of 1% extract or 7.5 ml of 3% extract was added in to tubes of 1 to 3, respectively. For the final step, 100 µl of bacteria (including 1.5×10^9 CFU/ml) was added to each tube with the final count of 1×10^7 CFU/ml. Tubes were horizontally incubated at 37 °C for 24 hours (figure 1). The pH of media and number of bacteria were measured (7, 8). The experiment was undertaken in the framework of Completely Randomized design (CRD). The data were analyzed using general

linear model (GLM), SAS (SAS Inc. Version 9.1, 2004, USA).

In control group, *Salmonella typhimurium* numbers were the maximum (10.33±0.18 CFU/ml). Application of 1% chavill extract, significantly increased count of *Lactobacillus acidophilus* (10.21±0.15 CFU/ml), *Lactobacillus casei* (9.89±0.23 CFU/ml) and *Lactobacillus plantarum* (9.49±1.10 CFU/ml (table 1). The highest bacterial count was seen for *Salmonella typhimurium*. In 3% chavill extract, the bacterial count of *Lactobacillus acidophilus* (10.85±0.009 CFU/ml) and *Lactobacillus plantarum* (10.75±0.48 CFU/ml) increased, but the bacterial count of *Lactobacillus casei* (8.71±0.18 CFU/ml) decreased. Despite the *Lactobacillus acidophilus*, the count of the bacterial strains were different between 1 and 3% of chavill extracts (table 2).The lowest acidity was observed in *Lactobacillus acidophilus* (pH 4.17±31) and *Lactobacillus plantarum* bacteria (pH 4.30±03) that had 1% chavill extract (table 3). Mechanistic effects of phenolic compounds on increasing the number of probiotic bacteria are not yet well known. Chavill contains phenolic compounds such as arvacrol, thymol, coumarin and it is seems that may have effects on the number of lactobacillus. Carvacrol destroys cell membrane and it may affect intracellular pH homeostasis. This might impact on inorganic ions and in this way; it could prevent the proliferation of pathogenic bacteria (9, 10).

Table 1. The effect of experimental treatments of bacteria count (CFU/ml).

Experimental treatments	Bacterial count
1. *Lactobacillus acidophilus* (%0)	4.85 ± 0.45^{f}
2. *Lactobacillus acidophilus* (%1)	10.21 ± 0.15^{abc}
3. *Lactobacillus acidophilus*(%3)	10.85 ± 0.009^{a}
4. *Lactobacillus casei* (%0)	5.5 ± 0.16^{f}
5. *Lactobacillus casei* (%1)	9.89 ± 0.23^{bcd}
6. *Lactobacillus casei* (%3)	8.71 ± 0.18^{e}
7. *Lactobacillus plantarum* (%0)	5.32 ± 0.13^{f}
8. *Lactobacillus plantarum* (%1)	9.49 ± 1.10^{d}
9. *Lactobacillus plantarum* (%3)	10.75 ± 0.48^{a}
10. *Salmonella typhimorium* (%0)	10.33 ± 0.18^{ab}
11. *Salmonella typhimorium* (%1)	9.51 ± 0.09^{dc}
12. *Salmonella typhimorium* (%3)	0^{g}
±SEM	0.22

Table 2. The effect of experimental treatments on pH Experimental treatments. Different characters show significant difference at 0.05 probabilities.

Table2: The effect of experimental treatments on pH Experimental treatments	pH
1. *Lactobacillus acidophilus* (%0)	5.36 ± 0.51^{b}
2. *Lactobacillus acidophilus* (%1)	4.17 ± 0.01^{d}
3. *Lactobacillus acidophilus* (%3)	4.04 ± 0.04^{d}
4. *Lactobacillus casei* (%0)	5.56 ± 0.10^{b}
5. *Lactobacillus casei* (%1)	4.81 ± 0.01^{c}
6. *Lactobacillus casei* (%3)	5.01 ± 0.02^{c}
7. *Lactobacillus plantarum* (%0)	5.35 ± 0.32^{b}
8. *Lactobacillus plantarum* (%1)	4.36 ± 0.03^{d}
9. *Lactobacillus plantarum* (%3)	4.34 ± 0.02^{d}
10. *Salmonella typhimorium* (%0)	6.03 ± 0.04^{a}
11. *Salmonella typhimorium* (%1)	5.57 ± 0.07^{b}
12. *Salmonella typhimorium* (%3)	5.49 ± 0.02^{b}
±SEM	0.104

Different effects of chavill extract on *Lactobacillus* and *Salmonella typhimurium* bacteria could be the result of different cell wall of these bacteria. It is thought that this different could increase the effect of chavill on *Lactobacillus acidophilus, Lactobacillus casei* and *Lactobacillus planetarium* and decrease the effect on *Salmonella typhimurium.* It seems that viability of these bacteria will be increased in low pH values.

Conclusion

Use of this medicinal plant as an alternative to antibiotics after pharmacological studies is recommended.

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Conflict of interests

No conflict of interests is declared.

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