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Inhibition of Growth of Some Food Borne Bacteria by *Falcaria vulgaris* Extract

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Aerial parts of *Falcaria vulgaris* were successively extracted with methanol using a Soxhlet extractor, then the crude extract was screened for antibacterial activities. Extracts of *Falcaria vulgaris* were tested for their antibacterial activity in agar disk diffusion assays, whereas the minimum inhibitory concentrations (MIC) of single compounds were determined by the microbroth dilution method. Significant antibacterial activities were found against various strains, in particular *Acidovorax facilis, Bacillus cereus, Bacillus dipsauri, Bacillus lentimorbus, Bacillus* spp., *Bacillus subtilis, Brevibacillus agri, Brevibacillus brevis, Corynebacterium ammoniagenes, Flavimonas oryzihabitans, Kocuria kristinae, Kocuria rosea, Micrococcus lylae, Paenibacillus apiarius, Paenibacillus macerans and Pseudomonas syringae syringae.* These results support the ethnomedicinal use of *Falcaria vulgaris* both for treatment of infectious diseases and use as preservative in traditional herby cheese. This result may also suggest that the methanol extracts of *Falcaria vulgaris* possess compounds with antibacterial properties and thus can be used as a natural preservative ingredient in food and pharmaceutical industry.

Key Words: Antibacterial screening, Crude extract, *Falcaria vulgaris*, Herby cheese.

INTRODUCTION

Microbial activity is a primary mode of deterioration of many foods and is often responsible for the loss of quality and safety. Concern over pathogenic and spoilage microorganisms in foods is increasing due to the increase in outbreaks of food borne disease[1]. Currently there is a growing interest to use natural antibacterial compounds, like plant extracts of herbs and spices for the preservation of foods, as these possess a characteristic flavour and sometimes show antioxidant activity as well as antimicrobial activity[2]. For centuries, indigenous

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plants have been used in herbal medicine for curing various diseases[3]. Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to available antibiotics have led the authors to investigate the antimicrobial activity of medicinal plants[4, 5].

With its richness in genetic diversity, Turkey has a unique position. Two important gene centres (Near East and Mediterranean) described by Vavilov are located in the country. Besides these two gene centres, Turkey also includes diversity centres for many wild, transitional and cultivated forms of annual and perennial, herbaceous and woody plants The rich Turkish flora include more than 9000 varieties of plants. About 3000 are endemic to Turkey and grow in nature nowhere else in the world[6].

Falcaria vulgaris is an edible medicinal plant, which is widely distributed in Anatolia, and locally named as 'Kazayagi'. Leaves, flowers and stem of *Falcaria vulgaris* are frequently used as wild vegetable or additive in foods to offer aroma and flavour in Turkey. In particular, in the eastern part of Turkey, it is added in a special cheese, namely 'herby cheese'. To make herby cheese, sheep milk is first filtered immediately after milking and then coagulated with calf rennet at the milking temperature. After cutting the coagulum, the whey is removed and previously prepared herbs are added into the curd. About 25 kinds of herbs can be used to make herby cheese, *e.g., Falcaria vulgaris, Allium* spp., *Thyrnus* spp., *Ferula* spp., *Anthriscus nemorosa,* etc. From these herbs, a single herb or a mixture of some herbs can be added. The rate of addition of herbs changes between 0.5–2 kg per curd obtained from 100 L of milk[7].

Falcaria vulgaris has also been used as a folk remedy to treat various ailments such as muscle pains, indigestion, diarrhea and infectious diseases in Turkey[8]. However, so far there have been no attempts to study the potential of *Falcaria vulgaris* antibacterial activity against a wide range of food-associated micro-organisms such as bacteria.

In the present work, the investigation on antibacterial activities of *Falcaria vulgaris* to exploit its potential as a natural preservative is reported.

EXPERIMENTAL

The plants (aerial parts) used for the present study were collected locally in Erzurum region of Turkey. Plant materials were further identified by the senior taxonomist, Avni Ozturk, Department of Botany, Yuzuncu Yil University, Van, Turkey and voucher specimen was deposited in the herbarium of the Horticulture Department of Agricultural Faculty, Ataturk University, Erzurum, Turkey. The dried and powdered plant materials (400 g) were extracted successively in a Soxhlet with methanol (MeOH) at 72 h at a temperature not exceeding the boiling point of the solvent[9]. The extracts were filtered using Whatmann filter paper No. 1 and then concentrated *in vacuo* at 40°C using a rotary evaporator. The residues obtained were stored in a freezer at –80°C until further tests.

Bacterial strains

Total 100 bacterial strains belonging to 52 bacteria species which are listed in

Table-1 were used in this study. The bacteria, maintained on nutrient agar (Merck, Germany) were supplied by Microbiology Laboratory of Agricultural Faculty of Ataturk University, Erzurum, Turkey. The food-associated bacteria were selected because they are frequently reported in foods. Identity of the bacteria used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Ataturk University.

Antibacterial activity test

The antibacterial activity of the extracts was carried out by disc diffusion test[10] using 100 μL of suspension containing 10^8 CFU/mL of bacteria spread on nutrient agar (NA) medium. Sterile 6 mm diameter filter paper discs were impregnated with 300 μg of the sterile test material and placed on to nutrient agar. Negative controls were prepared using the same solvents as employed to dissolve the plant extracts. Ofloxacin (5 μg/disc), sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc) and/or netilmicin (30 μg/disc) were used as positive reference standards to determine the sensitivity of one strain in each bacterial species tested. The inoculated plates with food-associated bacteria were incubated at 27°C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimetres as its antibacterial activity. Five discs per plate and three plates were used and each test was run in triplicate[11].

Microdilution assays

The minimal inhibition concentration (MIC) values were also studied for the bacteria which were determined as sensitive to the extracts in disc diffusion assay. The inocula of bacteria were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. *Falcaria vulgaris* extracts dissolved in 0.5% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (500 μg/mL) to be tested, and then serial two-fold dilutions were made in a concentration range from 7.80–500 μg/mL in 10 mL sterile test tubes containing nutrient broth. MIC values of radish extracts against bacterial strains were determined based on a micro-well dilution method[12]. The 96-well plates were prepared by dispensing into each well 95 μL of nutrient broth and 5 μL of the inoculum. 100 μL from the *Falcaria vulgaris* extracts initially prepared at the concentration of 500 μg/mL was added into the first wells. Then 100 μL from their serial dilutions was transferred into six consecutive wells. The last well containing 195 μL of nutrient broth without compound and 5 μL of the inoculum on each strip was used as negative control. The final volume in each well was 200 μL. Maxipime (Bristol-Myers Squibb) at the concentration range of 500–7.8 μg /mL was prepared in nutrient broth and used as standard drug for positive control. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, USA) and confirmed by plating 5 μL samples from clear wells on nutrient agar medium. The extract tested in this study was screened two times against each organism. The MIC of each extract was taken as the lowest concentration that showed no growth.

RESULTS AND DISCUSSION

The antibacterial activity of crude methanol extracts of *Falcaria vulgaris* against 52 food-associated bacteria including 100 strains are described. The results of the antibacterial activity of the investigated extracts are shown in Tables 1 and 2.

The most pronounced activity with inhibition zones of more than 14 mm was shown by the methanolic extracts of *Falcaria vulgaris* against *Acidovorax facilis, Bacillus cereus, Bacillus dipsauri, Bacillus lentimorbus, Bacillus* spp., *Bacillus subtilis, Brevibacillus agri, Brevibacillus brevis, Corynebacterium ammoniagenes, Flavimonas oryzihabitans, Kocuria kristinae, Kocuria rosea, Micrococcus lylae, Paenibacillus apiarius, Paenibacillus macerans* and *Pseudomonas syringae syringae* (Table-1).

The extract of the investigated plants exhibited only low activity against *Arthrobacter atrocyaneus, Arthrobacter ilicis, Chryseomonas luteola, Citrobacter amalonaticus, Corynebacterium cystitidis, Corynebacterium flavescens, Enterobacter hormaechei, Enterobacter intermedius, Enterobacter sakazakii, Erwinia carotovora, Erwinia chrysanthemi, Exiguobacterium acetylicum, Moraxella catarrhali, Pantoea agglomerans, Proteus vulgaris, Psychrobacter immobilis, Serratia liquefaciens, Staphylococcus cohnii-cohnii and Xanthotnonas arb. Corylina* (Table-1).

The extracts did not display any antibacterial activity against *Arthrobacter agilis, Arthrobacter protophormiae, Bacillus lichemiformis, Bacillus marinus, Bacillus megaterium, Bacillus psychrosaccharolyticus, Bacillus pumilus, Bacillus sphaericus, Bacillus* spp., *Brevibacillus linens, Enterococcus faecalis, Micrococcus luteus, Neisseria subflava, Paenibacillus polymyxa, Pseudomonas putida, Salmonaella typhimuirum and Shigella dysenteriae* (Table-1).

According to literature searched, nothing is known about the antibacterial properties and uses of *Falcaria vulgaris* against a number of food-borne bacteria The results obtained in the course of the present study are in agreement to a certain degree with the traditional uses of *Falcaria vulgaris* evaluated. *Falcaria vulgaris* seems to be a valuable source for antibacterial drugs, especially against *Bacillus cereus, Bacillus* spp., *Bacillus subtilis, Corynebacterium ammoniagenes* and *Micrococcus lylae*. The bioassay-guided fractionation procedure to characterize and isolate the antibacterial active constituents is under way in our laboratory. In addition, this plant is currently being investigated for other pharmacological activities. With phytochemical studies, *Falcaria vulgaris* was found to be rich in particular for sesquiterpene[13] and the plant also reported strong antifungal agent[14]. Based on these results, it is possible to conclude that aerial parts of *Falcaria vulgaris* has stronger and broader spectrum of antibacterial activity against many food-borne bacteria. This is the first study to provide information that the extracts of *Falcaria vulgaris* evaluated against a wide range of bacteria possess potential antibacterial activities.

TABLE-1
ANTIBACTERIAL ACTIVITY OF *FALCARIA VULGARIS* EXTRACTS AGAINST THE BACTERIA

Bacterial species	Number of strains/ origins	Inhibition zone in diameter (mm/sensitive strains)		Positive controls (mm)† Standard antibiotic disc
		Falcaria vulgaris extracts (300 µg/disc)	Negative control MeOH	
Acidovorax facilis	1/food	13	—	28 (OFX)
Arthrobacter agilis	2/food	—	—	31 (SCF)
Arthrobacter atrocyaneus	1/food	8	—	15 (OFX)
Arthrobacter ilicis	1/food	7	—	20 (OFX)
Arthrobacter protophormiae	2/food	—	—	21 (NET)
Bacillus cereus	1/food	17	—	21 (OFX)
Bacillus dipsauri	1/food	15	—	26 (OFX)
Bacillus flexus	1/food	12	—	27 (SCF)
Bacillus lentimorbus	1/food	15	—	30 (OFX)
Bacillus lichemiformis	4/food	—	—	29 (OFX)
Bacillus marinus	4/fbod	—	—	14 (OFX)
Bacillus megaterium	3/food	—	—	26 (OFX)
Bacillus psychrosaccharolyticus	6/food	—	—	15 (OFX)
Bacillus pumilus	7/food	—	—	24 (SCF)
Bacillus sphaericus	2/food	—	—	21 (OFX)
Bacillus spp.	1/food	17	—	20 (SCF)
Bacillus subtilis	3/food	17	—	29 (OFX)
Brevibacillus agri	1/food	13	—	27 (OFX)
Brevibacillus brevis	4/food	14	—	32 (NET)
Brevibacterium linen	3/food	—	—	22 (SCF)
Chryseomonas luteola	1/food	7	—	30 (OFX)
Citrobacter amalonaticus	1/food	8	—	23 (NET)
Corynebacterium ammoniagenes	1/food	18	—	20 (OFX)
Corynebacterium cystitidis	1/food	8	—	18 (OFX)
Corynebacterium flavescens	1/food	10	—	24 (OFX)
Enterococcus faecalis	5/food	—	—	10 (SCF)

Bacterial species	Number of strains/ origins	Inhibition zone in diameter (mm/sensitive strains)		Positive controls (mm)† Standard antibiotic disc
		Falcaria vulgaris extracts (300 µg/disc)	Negative control MeOH	
Enterobacter intermedius	2/food	7–8	—	16 (SCF)
Enterobacter sakazakii	1/food	11	—	21 (NET)
Erwinia carotovora	1/food	11	—	20 (NET)
Erwinia chrysanthemi	1/food	7	—	17 (SCF)
Exiguobacterium acetylicum	1/food	9	—	20 (OFX)
Flavimonas oryzihabitans	1/food	14	—	30 (OFX)
Kocuria kristinae	1/food	16	—	24 (NET)
Kocuria rosea	1/food	13	—	15 (OFX)
Micrococcus luteus	5/food	—	—	28 (OFX)
Micrococcus lylae	1/food	19	—	30 (OFX)
Moraxella catarrhali	1/food	10	—	18 (OFX)
Neisseria subflava	1/food	—	—	24 (OFX)
Paenibacillus apiarius	1/food	13	—	30 (OFX)
Paenibacillus macerans	1/food	16	—	30 (OFX)
Paenibacillus polymyxa	2/food	—	—	10 (OFX)
Pantoea agglamerans	1/food	8	—	30 (OFX)
Proteus vulgaris	1/food	10	—	20 (OFX)
Pseudomonas putida	3/food	—	—	17 (OFX)
Pseudomonas syringae syringae	2/food	9–14	—	15 (OFX)
Psychrobacter immobilis	1/food	9	—	20 (OFX)
Salmonella typhimuirum	5/food	—	—	28 (OFX)
Serratia liquefaciens	1/food	9	—	30 (OFX)
Shigella dysenteriae	3/food	—	—	21 (NET)
Staphylococcus cohnii–cohnii	1/food	8	—	12 (OFX)
Xanthomonas arb. corylina	1/food	11	—	22 (OFX)
Total 52 bacterial species	102 strains			

†OFX, ofloxacin (5 µg/disc); SCF, sulbactam (30 µg) + cefoperazona (75 µg) (105 µg/disc); NET, netilmicin (30 µg/disc) were used as positive reference standard antibiotic discs (oxoid).

TABLE-2
MIC VALUES (μg/mL) OF *FALCARIA VULGARIS* EXTRACTS AGAINST BACTERIA TESTED IN MICRODILUTION ASSAY

Bacterial species	Number of strains/origin	*Falcaria vulgaris* extracts (μg/mL)	Standard drug (Maxipime) (μg/mL)
Acidovorax facilis	1/food	> 500	250
Arthrobacter atrocyaneus	1/food	250	62.50
Arthrobacter ilicis	1/food	250	7.81
Bacillus cereus	1/food	> 500	7.81
Bacillus dipsauri	1/food	250	250
Bacillus flexus	1/food	> 500	125
Bacillus lentimorbus	1/food	250	7.81
Bacillus spp.	1/food	62.50	7.81
Bacillus subtilis	1/food	62.50	7.81
Brevibacillus agri	1/food	62.50	> 500
Brevibacillus brevis	1/food	250	7.81
Chryseomonas luteola	1/food	> 500	31.25
Citrobacter amalonaticus	1/food	250	7.81–15.60
Corynebacterium ammoniagenes	1/food	250	7.81
Corynebacterium cystitidis	1/food	> 500	7.81
Corynebacterium flavescens	1/food	250	7.81
Enterobacter hormaechei	2/food	62.50–125	7.81
Enterobacter intermedius	1/food	250	31.25
Enterobacter sakazakii	1/food	> 500	7.81
Erwinia carotovora	1/food	> 500	31.20
Erwinia chrysanthemi	1/food	> 500	15.60
Exiguobacterium acetylicum	1/food	250	7.81
Flavimonas oryzihabitans	3/food	250	15.60
Kocuria kristinae	1/food	250	15.60
Kocuria rosea	1/food	62.50	31.25
Moraxella catarrhalis	1/food	62.50	7.81
Micrococcus lylae	1/food	250	7.81
Paenibacillus apiarius	1/food	> 500	62.50
Paenibacillus macerans	1/food	> 500	> 500
Pantoea agglomerans	1/food	> 500	7.81
Proteus vulgaris	1/food	> 500	62.50
Psychrobacter immobilis	1/food	250	7.81
Pseudomonas syringae syringae	2/food	125–250	250
Serratia liquefaciens	1/food	250	62.50
Staphylococcus cohnii cohnii	1/food	62.50	7.81
Shigella dysenteriae	1/food	> 500	15.60
Xanthomonas arboricola corylina	1/food	250	> 500

OFX, ofloxacin (5 μg/disc); SCF, sulbactam (30 μg) + cefoperazona (75 μg) (105 μg/disc); NET, netilmicin (30 μg/disc) were used as positive reference standard antibiotic discs (oxoid).

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