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Antibacterial effect of thyme, peppermint, sage, black pepper and garlic hydrosols against *Bacillus subtilis* and *Salmonella enteritidis*

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Abstract

The antibacterial effect of thyme, peppermint, sage, black pepper and garlic hydrosols, widely used in food products as drinks and food additives, were tested for their inhibitory effects against *Bacillus subtilis* and *Salmonella enteritidis*. Single and combined hydrosols of previous common herbs were used to evaluate *in vitro* the antibacterial activity against these pathogenic bacteria. The obtained data showed that all of the tested herbal hydrosols demonstrated antibacterial activities against all of tested bacteria. Garlic hydrosols demonstrated stronger antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with thyme, peppermint, sage and black pepper. Combined extracts of thyme, mint and sage (1: 1 mixing ratio) had slightly higher antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with single plant hydrosols. Thyme hydrosols demonstrated higher antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with sage, peppermint and black pepper. Generally, single or combined common herbal hydrosols had inhibitory effects against the tested pathogenic bacteria. Therefore, these hydrosols are considered natural food and/or feed additives to improve the gut health of humans and animals.

Key words: Gut health, pathogenic bacteria, food and/or feed additives, spices, herbs, medicinal value.

Introduction

Spices and herbs have been used for centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have demonstrated the antimicrobial properties of some spices, herbs and their components [12-14].

Recently, there has been an increasing interest in the discovery of new natural antimicrobials, because of an increasing risk in the rate of infection with antibiotic-resistant microorganisms and also due to side and residual effects of antibiotics. The leafy part of different common herbs, such as thyme, peppermint, sage, black pepper and garlic, has been added to food and food products to improve the flavour. Certain spices and essential oils prolong the storage life of foods by their antimicrobial activity. Being natural foodstuffs, they appeal to consumers who tend to question the safety of synthetic additives [4].

Hydrosols, also known as floral water, distillate water or aromatic water, are the co-products or the by-products of hydro- and steam distillation of plant material. Hydrosols are quite complex mixtures containing traces of the essential oils and, of course, several water-soluble components. They have practically been used as beverages for a long time in the Middle East countries. Thyme, peppermint, sage, black pepper and garlic have commonly been used in foods mainly for their flavour, aromas and preservation, herbal tea, alternative medicines and natural therapies [1, 5].

A few different methods can be used to make water extractions of herbs. These include tea, the infusion and the decoction. The methods differ according to the part of the plant used and in the length of steeping time. Leaves, flowers and seeds require a shorter steep, while roots and barks need a longer one. Herbs contain a wide array of essential vitamins and minerals, as well as other beneficial compounds. The appropriate steeping time ensures adequate extraction of herbal constituents [6].

In this study, thyme, peppermint, sage, black pepper and garlic hydrosols were examined for their potential to inhibit common bacteria encountered in foods as pathogens, namely *Bacillus subtilis* and *Salmonella enteritidis*. Therefore, these hydrosols are considered natural food and/or feed additives to improve the gut health of humans and animals.

Material and Methods

Tested bacteria: The tested bacteria in this study were *Bacillus subtilis* and *Salmonella enteritidis* which were isolated and identified at the Laboratory of Food and Veterinary Microbiology, College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, Saudi Arabia, and stored at -70°C. Tested strains from stock cultures kept in the refrigerator were inoculated into sterile nutrient broth (Oxoid Ltd, England) and incubated at 37°C for 24 h. Then, *Bacillus subtilis* and *Salmonella enteritidis* were activated in nutrient broth for pouring into sterile 9 cm Petri dishes.

Plant samples and preparation of hydrosols: The plants used in this study are given in Table 1. Thyme, peppermint, sage, black pepper and garlic were collected from local market, Qassim, Saudi Arabia.

Table 1. Plants used in the experiments.

English name	Botanical name	Family	Part
Garlic	*Allium tuberosum*	Alliaceae	Cloves
Thyme	*Thymbra spicata*	Labiataea	Leaves
Sage	*Salvia fruticosa* subsp. *hirtum*	Labiataea	Leaves
Peppermint	*Mentha piperita* L	Labiataea	Leaves
Black pepper	*Piper nigrum* L.	Piperaceae	Fruits

Hydrosols of thyme, peppermint, sage, black pepper and milled garlic cloves were prepared by the hydro-distillation [2, 3]. Plant materials (about 100 g), cut into small pieces, were placed in a flask (2 litres) with 1000 ml of double distilled water and hydro-distilled for 1 h. After hydro-distillation, the mixture with trace essential oil in the flask was identified as hydrosol. The amount of obtained hydrosols were approximately 200 ml. The hydrosol was then filtered and preserved in sterile dark bottles (500 ml) in a cool environment (4°C) until further use.

Antibacterial activity of plant hydrosols using agar disk diffusion method: The thyme, peppermint, sage, black pepper and milled garlic cloves hydrosols were tested for antibacterial activity by the inhibition zone using the disc diffusion method [7]. *Salmonella enteritidis* and *Bacillus subtilis* were propagated in nutrient broth medium, shaked gently at 37°C for exactly 12-18 hours. Nutrient agar (15 ml) was inoculated with 150 µl of fresh culture (containing 10^9-10^{10} cfu/ml) and poured in sterile 9 cm Petri dishes. After solidification of agar in laminar flow (LABCONCO Laminar Flow Poducts, USA), the discs were drilled on the agar surface and inoculated with test bacteria. Hundred microlitres of thyme, peppermint, sage, black pepper and milled garlic cloves hydrosols or sterilized water (control) were applied to a sterilized disc gabbing (6 mm in diameter) followed by incubation at 37°C for 18 h. The inhibition zones were recorded in mm [1, 7]. All experiments were conducted in duplicate and the results are expressed as average values of inhibition.

Statistical analysis: Analysis of variance (t-test) of inhibition zone diameters was conducted as described by Miller and Miller [8].

Results and Discussion

The antibacterial activity of thyme, peppermint, sage, black pepper and garlic hydrosols against *Bacillus subtilis* and *Salmonella enteritidis* are shown in Tables 2 and 3. All herbal hydrosols showed inhibitory effect (inhibition diameter 10-30 mm) against tested bacteria. As expected, the control treatment had no inhibitory effect on any of the test bacteria. Single and combined hydrosols showed inhibitory effect on all of the tested pathogenic bacteria. Garlic hydrosols showed the highest antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis*. The mean values of the inhibition zone diameter were 30 and 25 mm for *Bacillus subtilis* and *Salmonella enteritidis* respectively.

Black pepper and peppermint hydrosols exhibited the lowest antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis*. The mean values of the inhibition zone diameter with black pepper were 10 for *Bacillus subtilis* (Table 2) and 11 mm for *Salmonella enteritidis* (Table 3). The mean values of the inhibition zone diameter with peppermint were 13 and 12 mm for *Bacillus subtilis* and *Salmonella enteritidis* respectively (Tables 2 and 3).Combined extracts of thyme, mint and sage (1:1 mixing) had slightly higher antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with single plant hydrosols. The present data revealed that thyme hydrosols demonstrated higher antibacterial activity compared to other hydrosols.

Table 2. Antibacterial properties of spice hydrosols against *Bacillus subtilis* (inhibition zone diameter in mm; diameter of deep disc, 6 mm, included).

Tested hydrosol	Min	Max	Mean	SD	CV%
	Diameter in mm				
Control	0	0	0	0	0
Garlic	25	30	30	3.53	11.79
Thyme	18	22	20	2.82	14.14
Sage	13	17	15	2.82	18.86
Peppermint	10	16	13	4.24	32.64
Black pepper	8	12	10	2.82	28.28
Mixture (thyme, sage and peppermint)	18	23	20.5	2.53	17.25

Min Minimum values (mm), Max Maximum values (mm), SD Standard deviation, C.V. Coefficient of variation.

Table 3. Antibacterial properties of spice hydrosols against *Salmonella enteritidis* (inhibition zone diameter in mm; diameter of deep disc, 6 mm, included).

Tested hydrosol	Min	Max	Mean	SD	CV%
	Diameter in mm				
Control	0	0	0	0	0
Garlic	20	30	25	7.07	28.28
Thyme	13	17	15	2.82	18.86
Sage	11	15	13	2.82	21.76
Peppermint	10	14	12	2.82	23.57
Black pepper	9	13	11	2.82	25.71
Mixture (thyme, sage and peppermint)	14	18	16	2.82	17.68

Min Minimum values (mm), Max Maximum values (mm), SD Standard deviation, C.V. Coefficient of variation.

The obtained data is in agreement with the findings of Sagdt [10] who used 50 µl from spice hydrosols such as thyme and found that there was an inhibitory effect against *E. coli* O157:H7, *Bacillus subtilis, S. aureus* and *Y. enterocolitica* (inhibition zone diameter ≥ 12-18 mm). Aktu and Karapinar [11] tested the inhibitory effects of various concentrations of thyme, mint and bay leaves and their alcohol extracts against the growth of three food poisoning bacteria, *Salmonella typhimurium*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*. They found that of the three spices studied, thyme inhibited the growth of the tested bacteria most effectively. In each growth medium containing spice extracts, *V. parahaemolyticus* was found to be the most sensitive bacteria; growth was inhibited by the addition of 1000, 5000 and 6000 ppm of thyme, bay leaves and mint respectively [11].

Our findings also are in agreement with those of Sagdic *et al*. [9], who studied the antibacterial effects of single or combined extracts of plants such as black thyme, fennel, sage, wild tea and wild mint against common pathogenic bacteria. They reported that the antibacterial effects of combined plant extracts (1: 1 mixing ratio) were similar to single plant extracts against pathogenic bacteria. Among the plant extracts, black thyme had the strongest antibacterial activity, and this was followed by sage, wild tea and wild mint in a descending order. The combined plant extracts with black thyme had strong antibacterial activity against pathogenic bacteria. However, combined plant extracts without black thyme had only slight antibacterial effect on all bacteria.

From the foregoing considerations it can be concluded that common herbal hydrosols of thyme, mint, sage, black pepper and garlic, used as single or combined extracts, have antibacterial activities against *Bacillus subtilis* and *Salmonella enteritidis*. Further studies to evaluate these extracts *in vivo* are under investigation.

Conclusions

The study dealed with the antibacterial activity of hydrosols of some common herbs, such as thyme, mint, sage, black pepper and garlic, against *Bacillus subtilis* and *Salmonella enteritidis.* The obtained data showed that all of the used aqueous extracts demonstrated antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis.* Garlic hydrosols demonstrated stronger antibacterial activity compared with thyme, mint, sage and black pepper. Thyme hydrosols demonstrated stronger antibacterial activity against *Bacillus subtilis* and *Salmonella enteritidis* compared with mint, sage and black pepper. Generally single or combined hydrosols of used common herbs had inhibitory effects against pathogenic bacteria. Therefore, these hydrosols could be considered natural food and/or feed additives to improve the gut health of human and animal. Herbal teas are one of the easiest and most enjoyable ways to support the body and warm up in the winter.

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