Study of antimicrobial activity of selected plant extracts against bacterial food contaminants

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Summary

The microbial characteristics have a high influence on the quality and durability of food products. Rosemary, sage, ginkgo, cinnamon and evening primrose, which are rich in antioxidants, belong to widely used food ingredients. Antioxidants present in the mentioned plants possess both antioxidant and antimicrobial activities. These substances were tested for their activity against common microbial contaminants of food and cosmetics products (*Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*). Their antimicrobial activity was tested by the dilution method and using the description of growth curves of the tested microorganisms. The antimicrobial effect of plant extracts were correlated to their concentration of 0.1% in the following sequence: rosemary, sage, ginkgo, evening primrose and cinnamon. These results confirm the hypothesis that the extracts act in food not only as antioxidants but also as antimicrobial agents.

Keywords

antimicrobial activity; rosemary; sage; ginkgo; evening primrose; cinnamon

The spoilage and poisoning of foods by microorganisms is a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available. On the other hand, consumers are increasingly avoiding foods prepared with preservatives of chemical origin and natural alternatives are therefore sought for to achieve the sufficiently long shelf life of foods together with a high degree of safety with respect to foodborne pathogenic microorganisms. In nature, a large number of different types of antimicrobial compounds are available (e.g. phytoalexins, such as flavonoids) that play an important role in the natural defence of various kinds of living organisms. Flavonoids constitute a large group of secondary plant metabolites that are ubiquitous among higher plants. These are polyphenolic compounds that generally occur as glycosylated derivatives. As dietary compounds, they are known as antioxidants that inhibit the oxidation of low-density lipoproteins and reduce thrombotic tendencies [1].

Ginkgoaceae, Ginkgoales have been known since the Triassic period, and the Eastern chinese tree, *Ginkgo biloba* L., is the only living species. Leaves of this tree contain various antioxidant compounds. Three such compounds have been isolated, namely, kaempferol, quercetin and isoharmentin. The antioxidant activity of ginkgo extract is determined mainly by flavonoids, which are known to scavenge and destroy free radicals and the reactive forms of oxygen [2].

Rosemary (*Rosmarinus officinalis* L., *Lamiaceae*) is an aromatic evergreen shrubby herb widespread in the Mediterranean region. It is a well-known and greatly valued medicinal herb that is widely used in pharmaceutical products and folk medicine as a digestive, tonic, astringent, diuretic, diaphoretic and useful for urinary aliments [3].

Cinnamon (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, belonging to the family *Lauraceae*, native to Sri Lanka and South India. Its bark is widely used as a spice due to its distinct odour. In India, it is also known as "Daalchini". In medicine, its volatile oils once had a reputation as a cure for colds. It has also been used to treat diarrhea and other problems of the digestive system. Cinnamon is known to possess a high antioxidant activity. The essential oil of cinnamon has also antimicrobial properties, which can aid in the preservation of certain foods. "Cinnamon" has also been reported to have

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remarkable pharmacological effects in the treatment of the type II diabetes. However, the plant material used in the study was actually cassia, as opposed to true cinnamon. Cinnamon has traditionally been used to treat toothache and to fight bad breath. Its regular use is also believed to stave off common cold and aid digestion [4].

Sage (*Salvia officinalis*) is a small evergreen subshrub with woody stems, grayish leaves and blue to purplish flowers. It is native to southern Europe and the Mediterranean region. The Latin name for sage, salvia, means "to heal". Although the effectiveness of "common sage" is often open to debate, it has been recommended at one time or another for virtually every ailment. The modern evidence supports its effects as an antihydrotic, antibiotic, antifungal, astringent, antispasmodic, estrogenic, hypoglycemic and tonic. In a double blind, randomized and a placebo-controlled trial, sage was found to be effective in the management of mild to moderate Alzheimer's disease [5].

Oenothera biennis (Common evening primrose or Evening star) is native to the eastern and central North America, and widely naturalized elsewhere in temperate and subtropical regions. The mature seeds contain approximately 7–10% γ-linolenic acid, a rare essential fatty acid. The *O. biennis* seed oil is used to reduce the pains of a premenstrual stress syndrome and is beneficial to the skin of the face. Also, poultices containing *O. biennis* were at one time used to ease bruises and speed wound healing [6].

Plants, including herbs and spices, contain many phytochemicals which are potential sources of natural antioxidants, e.g. phenolic diterpenes, flavonoids, tannins and phenolic acids. These compounds possess antioxidant, anti-inflammatory and anticancer activities. Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol in the phospholipids bilayer of lipoprotein particles back to its active antioxidant form. Sage and rosemary have similar patterns of phenolic compounds and their antioxidant activity was attributed mainly to the contained carnosic acid, carnosol and rosemary acid [7].

This work is focused on the antibacterial effects of the extracts of the five mentioned plants.

MATERIALS AND METHODS

Preparation of ethanolic plant extracts

Ginkgo biloba leaves were collected in Bratislava in August 2006; *Oenothera biennis* seeds were collected in the Martin region in September 2006; *Salvia officinalis*, *Cinnamomum verum*, synonym *C. zeylanicum*, *Rosmarinus officinalis* L. (Fytopharma, Malacky, Slovakia) extracts were prepared according to Zahradníková et al. [8].

The plant extracts were dissolved in dimethyl sulfoxide (DMSO, Sigma Aldrich, Steinheim, Germany) and then in Brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) to the final concentration of 0.1%, 0.05%, 0.02%, 0.01% (by weight). The final concentration of DMSO never exceeded 1% (v/v) in either control or treated samples [9].

Bacterial strains

Strains *Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 3955, *Staphylococcus aureus* CCM 3953 (Czech Collection of Microorganisms, Brno, Czech Republic), *Listeria mocytogenes* NCTC 4886 (National Collection of Type Cultures, London, United Kingdom), *Bacillus cereus* (isolated from milk by Department of Food Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia) were cultured in BHI broth.

Antibacterial assay

The antibacterial effect was assayed by a microdilution method in 96-well microtitration plates. The culture was grown at (37 ± 1) °C under aerobic conditions for 16–18 h. Freshly prepared bacterial cultures in BHI broth were used for experiments. The initial density of bacterial cultures was approx. 10^7 CFU.cm^{-3}. This bacterial suspension (180 μl) was added to 20 μl of the tested plant extract solution and statically cultured for 12 h in a thermostat at (37 ± 1) °C. The time course of absorbance (A_{630}) was determined in triplicates by Humareader (Human, Wiesbaden, Germany). To compare the antibacterial activity, gentamicin (Zentiva, Hlohovec, Slovakia) at concentrations of 0.1%, 0.05%, 0.02%, 0.01% (by weight) was used as a standard. The antibacterial effect was characterized by IC_{50} values, i.e. the minimal concentration of a substance which inhibited bacterial growth by 50% relative to the control (without tested plant extracts). The IC_{50} values were determined from the toxicity curves [9]. The growth parameters were estimated by fitting to the modified Gompertz equation [10].

RESULTS AND DISCUSSION

The plants under study have been used for long time in traditional medicine and, thanks to their unique properties (antioxidant, antimicrobial), their use has also been extended to other fields.

Tab. 1. Antibacterial activity of plant extracts.

Plant extracts	*S. aureus*	*B. cereus*	*P. aeruginosa*	*E. coli*	*L. monocytogenes*
			IC_{50} [%]		
sage	0.05	0.02	0.02	0.05	0.05
rosemary	0.01–0.02	0.01-0.02	0.01	0.01	0.01–0.02
evening primrose	> 0.1	> 0.1	> 0.1	0.1	> 0.1
ginkgo	0.05	0.1	0.1	0.1	0.1
cinnamon	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1
gentamicin	0.02–0.05	0.1	0.02	0.02–0.05	0.02–0.05

In recent years, economical preparation of extracts from these plants has been described, which enabled their use in food industry mainly in the field of fatty products, to increase stability of oils. In this study, we aimed at testing the antimicrobial effects of plant extracts against microbial food contaminants.

Results of the antimicrobial effects, evaluated on the base of IC_{50} values, are summarized in Tab. 1. We chose five species of G+ and G– bacteria as the representatives of common contaminants in the food industry.

Extract from rosemary proved to be the most effective with all the tested microorganisms. The rosemary extract even in the concentration of 0.01% inhibited *P. aeruginosa* and *E. coli* (Fig. 1). The higher extract concentration was effective with all other tested microorganisms. The rosemary extract was more effective than gentamicine in the same concentration with all tested bacteria. The sage extract at a concentration of 0.02% was the most effective against *B. cereus* (Fig. 2) and *P. aeruginosa*. *L. monocytogenes*, *S. aureus*, *E. coli* were inhibited by using the concentration even up to 0.05% of the sage extract. The extract from ginkgo leaves at a concentration of 0.05% was able to inhibit *S. aureus* (Fig. 3). Extracts from evening primrose and cinnamon were less effective against foodborne pathogens (Fig. 4, Fig. 5). They inhibited all tested strains only at a concentration higher than 0.1%. However, this concentration is not too high and several natural antioxidants are applied to food at this concentration.

Besides IC_{50}, specific growth rates were also used to describe the antimicrobial effect of plant extracts. Results are summarized in Tab. 2. The values of the specific growth rate were indirectly proportional to the increasing concentrations of individual extracts. This means that the higher the extract concentration, the lower the growth rate was, which confirmed the antimicrobial properties of the tested plant extracts.

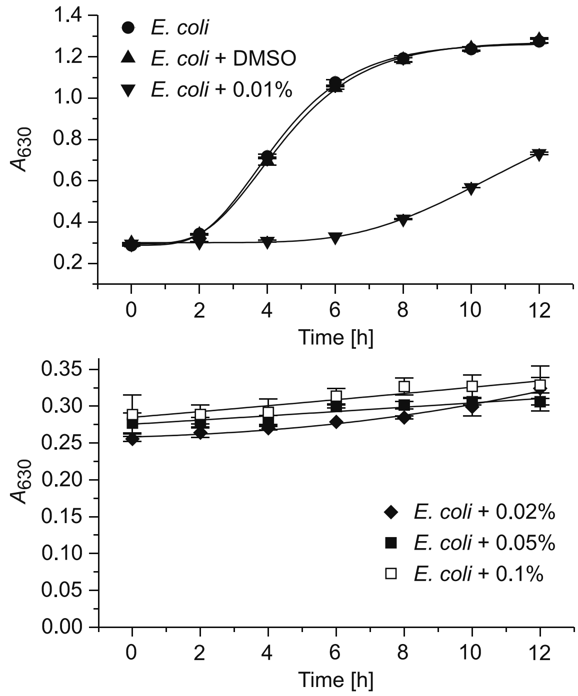

Fig. 1. Growth curves of *E. coli* with addition of rosemary extract at concentrations of 0.01–0.1%.

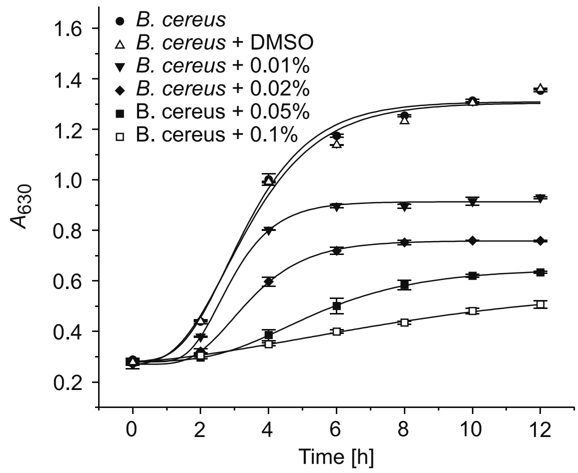

Fig. 2. Growth curves of *B. cereus* with addition of sage extract at concentrations of 0.01–0.1%.

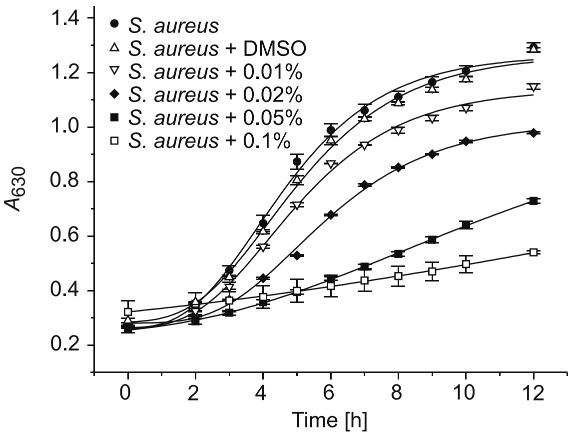

Fig. 3. Growth curves of *S. aureus* with addition of ginkgo extract at concentrations of 0.01–0.1%.

From our results it can be stated that the most antimicrobially effective of the five tested extracts was the rosemary extract, which conforms to literature data [7]. It is known that mainly polyphenolic agents are responsible for the antimicrobial effects of the tested plant extracts. The contents of carnosoic acid, carnosole and rosemary acid is in rosemary and sage, which are believed to be responsible for both antioxidant and antimicrobial properties, are approximately the same [7]. SHAN et al. [11] have also proved rosemary's, sage's and cinnamon's high antimicrobial effect against foodborne pathogens (G+ and G– bacteria). Of these three tested plants, cinnamon showed the highest antimicrobial activity in testing. The difference in our results might be caused by the different method of determination of the antimicrobial activity, and obviously also by the different representation of agents responsible for the antimicrobial effects. The biological activity of natural extracts depends on their chemical composition which is determined by the plant genotype and greatly influenced by several factors, namely, geographical origin, environmental and agronomic conditions [12].

Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne pathogens [13, 14]. However, the results reported are difficult to be compared directly, usually because of the low number of plant samples tested, different test methods and diverse bacterial strains and sources of the used antimicrobial samples. Many studies have reported that phenolic compounds in spices and herbs significantly contribute to their antioxidant and pharmaceutical proper-

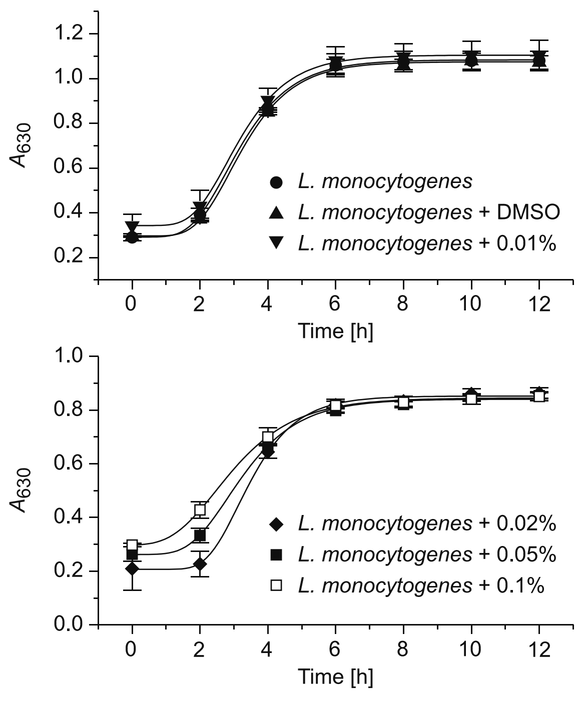

Fig. 4. Growth curves of *L. monocytogenes* with addition of cinnamon extract at concentrations of 0.01–0.1%.

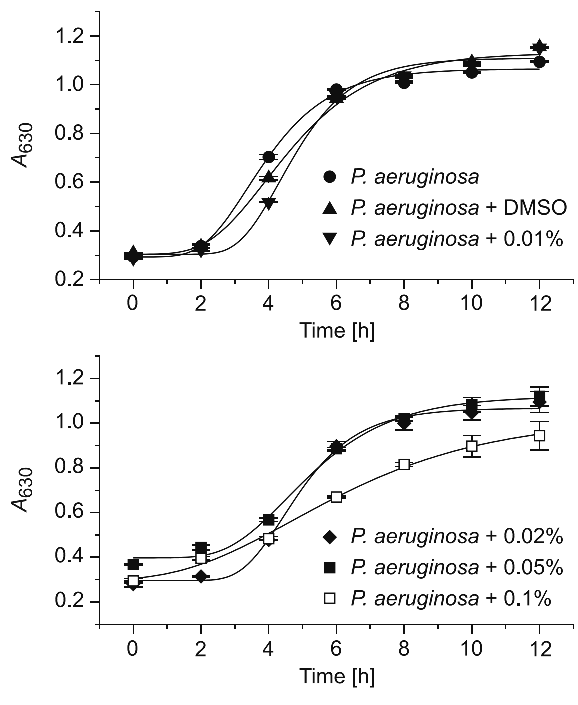

Fig. 5. Growth curves of *P. aeruginosa* with addition of evening primrose extract at concentrations of 0.01–0.1%.

Tab. 2. Specific growth rate [h^{-1}].

Microorganism	Tested extract	Pure culture	DMSO	Addition of tested extracts [%]			
				0.1	0.05	0.02	0.01
S. aureus	ginkgo	0.181	0.164	0.023	0.050	0.119	0.149
B. cereus	sage	0.280	0.257	0.024	0.063	0.153	0.253
P. aeruginosa	evening primrose	0.214	0.178	0.083	0.152	0.234	0.250
E. coli	rosemary	0.228	0.217	0.0056	0.0099	0.013	0.084
L. monocytogenes	cinnamon	0.287	0.281	0.156	0.187	0.253	0.276

ties. Some studies claim that phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects [14–16].

CONCLUSION

In this work, it is confirmed that the plant extracts tested act in food products not only as antioxidants but also as antimicrobial substances. The extract from rosemary was antimicrobially the most effective of the five tested plant extracts. Numerous studies prove that the antimicrobial effects of plant extracts depend on a number of factors (natural conditions, process of treating plants, part of a plant that was used for extraction as well as the process used for preparation of individual extracts). The antioxidant effects of extracts from evening primrose are well described in literature, yet its antimicrobial effects have not been studied so far. The future work will investigate both the antioxidant and antimicrobial effectiveness of the fractions obtained from an extract of evening primrose meal.

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