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Short communication

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AND EXTRACTS FROM BLACK PEPPER, CUMIN, CORIANDER AND CARDAMOM AGAINST SOME PATHOGENIC AND SAPROPHYTIC MICROORGANISMS

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Summary

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The antimicrobial activity of essential oils and extracts of fruits of black pepper (*Piper nigrum* L.), cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.) and cardamom (*Elettaria cardamomum*) against pathogenic (*Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* G) and saprophytic (*Bacillus cereus* ATCC 11778, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger*, *Saccharomyces cerevisiae*) microorganisms was examined by disc-diffusion and well-diffusion methods. The essential oils and the extracts inhibited the growth of pathogenic and saprophytic microorganisms causing gastrointestinal diseases. Gram-positive bacteria were more sensitive to the extracts and oils (inhibition zones between 9.5 and 20 mm) and the minimum inhibitory concentration was more than 600 ppm. The tested Gram-negative bacteria were less sensitive (zones of inhibition between 8 and 12 mm) with a minimum bactericidal concentration more than 600 ppm. The obtained essential oils and extracts are suitable for use as biopreservative agents.

Key words: antimicrobial activity, black pepper, cardamom, coriander, cumin, essential oil

Antimicrobial properties of the herbs are due to various chemical compounds including volatile oils, alkaloids and tannins that are presented in their tissue. Plant metabolites are a promising alternative to antifungal treatment and control because plants generate a wide variety of compounds, either as part of their development or in response to stress or pathogen attack (Bakkali *et al.*, 2008; Rajkovic *et al.*, 2015). Preliminary clinical trials have documented their therapeutic use for the treatment of variety of diseases and conditions that include diarrhoea, asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, influenza and dental caries (Gilani *et al.*, 2001; Ali & Blunden, 2003). Numerous studies have demonstrated the antimicrobial activity of essential oils and extracts obtained from the fruits of black pepper, cumin, coriander and cardamom (Hajlaoui *et al.*, 2010). Essential oils are one of the most promising groups of natural compounds for the development of safer antifungal agents (Moghaddam *et al.*, 2015). The pronounced antibacterial and antioxidant properties of oils and extracts turn them into natural preservatives, helping to increase the safety of food. They are also applied in perfumery and cosmetics industry and in medicine. The antimicrobial activity of spices and their effect in food products is determined by several factors: type, composition and quantity of spices; microbial species and the extent of contamination; composition of the medium; conditions of the technological process and storage of food products (Dobreva *et al.*, 2009). As a result of the inherent secondary metabolism of plants a number of substances are synthesised: essential oils, alkaloids, flavonoids, tannins, glycosides and other compounds that explain the antimicrobial properties of the products, derived from them (Iacobellis *et al.*, 2005; Souza *et al.*, 2005).

The aim of the present research was to determine the antimicrobial activity of essential oils and extracts from fruits of black pepper, cumin, coriander and cardamom against pathogenic and saprophytic microorganisms for their application as natural preservatives that would improve food safety.

Essential oils were extracted by water distillation in a modification of a laboratory glass apparatus of the British Pharmacopoeia (Balinova & Dyakov, 1974).

Minced spice fruits were processed at process parameters with respect to the ratio of material:water = 1:20; ratio raw material:volume of the flask = 1:100, 6% rate and duration of 300 min. Distillation ended when two consecutive measurements within 30 min did not show an increase in the amount of essential oil. The extraction was carried out in the laboratory extractor with a volume of 1 dm^3 with the following parameters: temperature 25–30 °C, pressure 0.7–0.8 MPa and continuous perculation for 30 min. Freon 134a (1,1,1,2 tetrafluoroethane) was used as extragen, authorised for food. The solvent had the following indicators: relative density at 20 °C and 100 kHz – 1.013, dipole moment – 2.060 Debay, polarisation 13.8 cm^3/mol, dynamic viscosity at 20 °C – 198 µPa.s, surface tension at 20 °C – 8 mN/m (Georgiev & Stoyanova, 1997).

Test microorganisms for determining the antimicrobial activity were as followed: *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* G, *Bacillus cereus* ATCC 11778, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger*, *Saccharomyces cerevisiae*. All strains have been deposited in the culture collection of the Department of Microbiology at University of Food Technology, Plovdiv.

The antimicrobial activity was studied by two methods:

1) Agar disc diffusion method using 6 mm paper discs and pipetting 6 µL of the sample;

2) Agar serial tube dilution method with results expressed as minimum inhibitory concentration (MIC).

The essential oils or extracts were diluted and the experiments were conducted with dilutions 1×, 10× and 100× in order to determine the MIC. Paper discs soaked in distilled water were used as controls. The results were recorded as diameters of the clear zones around the paper discs, in millimeters, after 24–48 hours of incubation of the Petri dishes

Table 1. Antimicrobial activity of extracts from fruits of black pepper, cumin, coriander and cardamom

Spices	Black pepper		Cumin		Coriander		Cardamom	
Test-micro-organisms	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
Bacillus cereus ATCC 11778	10±0.48	60	12±0.47	>600	10±0.48	60	18±0.47	>600
Penicillium sp.	9±0.47	>600	9.5±0.4	60	9.5±0.4	60	8±0.47	600
Rhizopus sp.	8±0.47	600	8±0.47	600	9±0.47	60	8±0.47	600
Aspergillus niger	8.5±0.4	>600	10±0.47	60	8±0.47	60	8±0.47	60
Saccharomyces cerevisiae	9.5±0.4	60	9±0.47	60	9±0.47	6	8±0.47	600
Staph. aureus ATCC 6538	–	–	8±0.47	600	9±0.4	>600	9±0.47	>600
Proteus vulgaris G	–	–	9.5±0.4	60	9±0.47	60	9±0.48	6
Salmonella sp.	–	–	8±0.47	600	8±0.48	600	9±0.40	6
E. coli ATCC 8739	9±0.48	6	9.5±0.48	60	10±0.47	60	9±0.48	6
E. coli ATCC 25922	–	–	9.5±0.47	60	8.5±0.65	60	9±0.47	6

Table 2. Antimicrobial activity of oils from fruits of black pepper, cumin, coriander and cardamom

Spices	Black pepper		Cumin		Coriander		Cardamom	
Test-micro-organisms	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
Bacillus cereus ATCC 11778	9.5±0.48	60	11±0.47	>600	9±0.45	>600	9.5±0.47	>600
Penicillium sp.	8±0.4	600	25±0.81	>600	8±0.40	60	10±0.4	60
Rhizopus sp.	9±0.47	6	8±0.47	600	9±0.48	60	8±0.47	600
Aspergillus niger	9±0.48	60	25±0.94	>600	8±0.47	6	8±0.4	600
Saccharomyces cerevisiae	9±0.47	60	9±0.47	60	8±0.47	600	9±0.48	60
Staph. aureus ATCC 6538	10±0.47	6	20±0.94	>60	10±0.48	60	10±0.48	60
Proteus vulgaris G	9±0.47	6	8±0.47	600	8±0.47	600	9±0.48	60
Salmonella sp.	9±0.48	6	9±0.48	>600	8±0.4	600	12±0.4	6
E. coli ATCC 8739	9±0.47	6	9±0.48	60	8±0.47	600	8±0.47	600
E. coli ATCC 25922	9±0.48	6	8±0.4	600	8±0.4	600	8±0.47	600

at optimal temperature for the growth of the corresponding test-microorganism (at 30 °C for the saprophytic microorganisms and at 37°C for the pathogenic microorganisms) (Jirovetz *et al.*, 2006). The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth (Randrianarivelo *et al.*, 2009).

The experiments were performed in quadruplicate. The mean values and the standard deviations were calculated using MS Office Excel 2010. The MICs, in ppm, were calculated on the basis of the obtained results.

The essential oils and the extracts of black pepper, cumin, coriander and cardamom inhibited the growth of pathogenic and saprophytic microorganisms causing gastrointestinal diseases. The method used to assess the antimicrobial activity, and the choice of the test microorganisms vary between publications. The permeability of the bacterial membranes, the presence of porin proteins in Gram-negative bacteria and the intracellular distribution of the oil constituents are key elements that influence the diffusion and the action of the essential oil into the cell. In most literature reports, Gram-positive organisms appear to be more sensitive than Gram-negative to essential oils. However, other studies do not confirm this observation, as Gram-positive bacteria have been found to be less or equally sensitive to Gram-negative bacteria as well (Burt, 2004; Randrianarivelo *et al.*, 2009).

In the present study Gram-positive bacteria were more sensitive to the extracts and oils (inhibition zones between 9.5 and 20 mm) and the minimum inhibitory concentration was more than 600 ppm. The tested Gram-negative bacteria were less sensitive (zones of inhibition between 8 and 12 mm) with the minimum bactericidal concentration exceeded 600 ppm (Tables 1 and 2). This was due to the difference in the structure and composition of the cell walls of the two groups of bacteria. The presence of an outer membrane in Gram-negative bacteria hindered the diffusion of the extracts through the membrane to the protoplasm of the cell, making them more stable under the effect of the tested extracts. The black pepper oil demonstrated antimicrobial activity against all tested test microorganisms as compared to the extract. This was due to the difference in the chemical composition of the oil and the extract. Strong antimicrobial activity against fungi and yeasts was observed: the respective inhibition zones attained up to 25 mm, and the MIC was more than 600 ppm.

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