Comparative evaluation of the antimicrobial properties of plant extracts

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Abstract. The spectrum of antimicrobial agents used to combat the microorganism of spoilage of food products, in connection with the developed problem of antibiotic resistance, is steadily narrowing. Plant polyphenols exert a variety of biological effects on numerous cellular systems both in vitro and in vivo. For many flavonoids, antioxidant, antimicrobial, anti-inflammatory, anti-cancer effects have been established, which led to the widespread use of flavonoid-containing plant materials for the production of therapeutic and prophylactic agents. For research, we used water extracts of fennel fruit (Foeniculum), medicinal sage (Salvia officinalis), chaga (Inonotus) (10g / 100ml of water) and CO2 extract of bay leaves and CO2 extract of allspice. Samples were tested against native microflora of chicken paste and chilled fish. All these objects showed antimicrobial activity against microflora of food products. The most pronounced antimicrobial activity was observed in fennel, CO2 extract of bay leaves and CO2 extract of allspice. This study shows the possibility of using aqueous infusions of medicinal plants and extracts of spices as an additive to a food product to extend its shelf life.

1. Introduction
The selectivity of antimicrobial components has an interesting effect on various microbiota. For example, one and the same substance may not have any effect on a certain type of microorganism, at the same time, on another type it may have an inhibitory effect on growth, and it kills still others. The same with antibiotics, in micro doses they can either suppress or kill bacterial cultures [1].

The mechanism of the antimicrobial effect is the transformation that is caused in the energy, structure and metabolism of the microbe, which subsequently lead to the killing of the microbe, a decrease in the microbial population, as well as their suppression of growth, development and reproduction [2, 3].

The selectivity of antibacterial substances depends directly on its potential access to the target in the damaged microbial cell. Spore-forming forms of bacteria are resistant to substances that can cause the death of asporogenic forms or inhibit their growth and development [3, 4].

Therapeutic plants are used as popular antimicrobial agents, usually they have been used by local healers and in traditional medicines [5, 6]. Therapeutic plants are an excellent source of components that act as antimicrobial agents [7, 8]. Many parts of plants that are used as medicines comprise the stem, fruits, leaves, flowers, bark, root, seeds [9–11].

Of course, that safety for human life and health is the main measure for evaluating antibacterial substances in the process of food production in enterprises. Along with this, there are many variations in the study of the properties of antibacterial substances and the establishment of an alleged negative effect on macroorganism [12].
The aim of this work is to evaluate the antibacterial activity of CO\textsubscript{2}-extracts and extracts of medicinal plants against food spoilage microorganisms.

2. Materials and methods
In the work, 3 types of official medicinal plants in dry dried form, purchased in the pharmacy network, and 2 names of CO\textsubscript{2}-extract were used (table 1).

Table 1. Samples of plant materials included in the study.

| №  | Type name                          | Manufacturer                          |
|----|-----------------------------------|---------------------------------------|
| 1  | Fennel fruit (Foeniculum)         | PKF LLC «Fitofarm», Russia            |
| 2  | Salvia officinalis(Salvia officinalis) | PKF LLC «Fitofarm», Russia           |
| 3  | Chaga (Inonotus)                   | PKF LLC «Fitofarm», Russia            |
| 4  | CO\textsubscript{2} extract of bay leaf | LLC «CompanyCaravan», Russia         |
| 5  | CO\textsubscript{2} extract of Allspice | LLC «CompanyCaravan», Russia         |

2.1 Preparation of solutions of plant extracts
The extracts were prepared by adding sterile distilled water to dry plant material, brought to a boil, and boiled for 5 min. After which the mixture was sedimented, filtered, and then filtered through a sterilizing filter CHROMAFIL Xtra pa-20/25. Extracts were prepared in the following concentrations:
- extracts with a concentration of plant materials of 5 g / dm\textsuperscript{3};
- extracts with a concentration of 20 g / dm\textsuperscript{3};
- extracts with a concentration of 200 g / dm\textsuperscript{3}.

2.2 Preparation of CO\textsubscript{2}-extract solutions
We used 1% aqueous solutions of CO\textsubscript{2} extracts. Under aseptic conditions, 9.9 g of sterile distilled water or sterile vegetable oil was added to a sterile tube, and 0.1 g of CO\textsubscript{2}-extracts were aseptically added to it. The finished solution was thoroughly mixed.

The concentrations of extracts used in the research process are presented in table 2.

Table 2. Concentrations of Plant Extracts Used.

| The investigated object                        | Concentration |
|-----------------------------------------------|---------------|
| Fennel extract                                | 5 g / dm\textsuperscript{3} |
|                                               | 20 g / dm\textsuperscript{3} |
|                                               | 200 g / dm\textsuperscript{3} |
| Salvia extract                                | 5 g / dm\textsuperscript{3} |
|                                               | 20 g / dm\textsuperscript{3} |
|                                               | 200 g / dm\textsuperscript{3} |
| Chaga extract                                 | 100 g / dm\textsuperscript{3} |
|                                               | 200 g / dm\textsuperscript{3} |
| CO\textsubscript{2} extract of bay leaf       | 1 g / dm\textsuperscript{3} |
| CO\textsubscript{2} extract of Allspice       | 1 g / dm\textsuperscript{3} |

Isolates of S. aureus, V. parahaemolyticus, Enterococcus spp., Proteus spp, that are typical spoilage microorganisms of food.

The determination of the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) of plant extracts and CO\textsubscript{2} extracts was carried out by microdilution in 96-well plates. Muller-Hinton broth prepared twice serial dilutions of extracts from 1:10, 1:40, 1: 400 (initial concentration 1: 5, 1:10, 1: 200 diluted 2 times) to 1: 960 each. Bacterial suspensions with an optical density of 0.5 according to MacFarland (10\textsuperscript{8} CFU / ml) were prepared from diurnal cultures of
microorganisms previously obtained by spoiling food products isolated, identified and grown on agar in a sterile isotonic solution. 1.5 μl of the resulting suspension was added to the plate wells containing 150 μl of serial dilutions of plant extracts, the final concentration of microorganisms was $10^6$ CFU / ml.

The plates were incubated in an incubator for 18 hours at 35–37 °C.

The presence of microorganism growth in the broth (turbidity of the broth) or on the surface of the agar indicates that this concentration of the extract is insufficient to suppress its viability. Therefore, MIC experimental results were compared with uninoculated culture medium. To determine MBC, 10 μl of the contents of each well was plated on a solid medium plate (GRM agar). After a 24-hour incubation at 35–37 °C, the growth of microorganisms on time agar was evaluated, the minimum concentration preventing the growth of microorganisms was designated as MBC.

3. Results and discussion

The pronounced antibacterial activity (BMD ≤ 1 mg / ml) in relation to isolates (S. aureus and Proteus spp) was detected for the chaga extract at a concentration of 200 g / dm³ (table 3).

Extracts of fennel and sage at a concentration of 200 g / dm³ showed pronounced antibacterial activity against isolates of V. parahaemolyticus (IPC 0.71–0.74 mg / ml) as well as isolates of Enterococcus spp (IPC 0.87–0.93 mg / ml) CO₂-extracts of bay leaf and black pepper had high antibacterial activity against all isolates included in the study (MPC 0.62–0.87 mg / ml).

Table 3. Minimum inhibitory concentrations (MIC) of aquatic plant extracts in relation to spoilage microorganisms, mg / ml.

| Extract name | Concentration | S. aureus | V. parahaemolyticus | Enterococcusspp | Proteus spp |
|--------------|---------------|-----------|----------------------|-----------------|-------------|
| Fennel extract | 5 g / dm³ | > 5 | > 5 | > 5 | > 5 |
| | 20 g / dm³ | > 5 | > 5 | > 5 | 4.3 |
| | 200 g / dm³ | 1,25 | 0,74 | 0,93 | 2,5 |
| Salvia extract | 5 g / dm³ | > 5 | > 5 | 10 | 7 |
| | 20 g / dm³ | > 5 | 1,25 | 1,75 | 2,5 |
| | 200 g / dm³ | 1,03 | 0,71 | 0,87 | > 5 |
| Chaga extract | 100 g / dm³ | 4,5 | 12 | 5 | 2,1 |
| | 200 g / dm³ | 0,98 | 7 | 1,01 | 0,92 |
| CO₂-extract of bay leaf | 1 g / dm³ | 0,78 | 2,5 | 0,62 | 0,87 |
| CO₂-extract of Allspice | 1 g / dm³ | 0,71 | 1,25 | 0,67 | 1,5 |

The minimum bactericidal concentrations for most plant extracts were equal to MPC or differed from it by no more than 1 dilution (table 4). This may indicate a predominantly bactericidal effect of substances of plant origin on the microbial cell.

Table 4. Minimum bactericidal concentrations (MBC) of aquatic plant extracts in relation to spoilage microorganisms, mg / ml.

| Extract name | Concentration | S. aureus | V. parahaemolyticus | Enterococcusspp | Proteus spp |
|--------------|---------------|-----------|----------------------|-----------------|-------------|
| Fennel extract | 5 g / dm³ | > 12 | > 10 | > 15 | > 15 |
| | 20 g / dm³ | > 15 | > 15 | > 5 | > 10 |
| | 200 g / dm³ | 5 | 3 | 0,93 | > 5 |
| Salvia extract | 5 g / dm³ | > 15 | > 15 | > 12 | > 15 |
| | 20 g / dm³ | > 15 | > 10 | > 15 | 12 |
| | 200 g / dm³ | 1,03 | 0,91 | 5 | > 5 |
| | 100 g / dm³ | > 10 | 12 | > 15 | > 10 |
Chaga extract | 200 g /dm³ | 3,5 | 7 | 5 | 0,92
---|---|---|---|---|---
CO₂-extract of bay leaf | 1 g /dm³ | 0,78 | 5 | 0,74 | 0,87
CO₂-extract of allspice | 1 g /dm³ | 0,71 | 3,5 | 0,67 | 1,5

No significant antibacterial activity was detected in relation to all studied cultures of microorganisms for all plant extracts of fennel and sage with a concentration of 5 g /dm³, 20 g /dm³.

4. Conclusion
The study found that some plant species that are widely used in traditional medicine exhibit a pronounced bactericidal effect against isolates of both gram-positive and gram-negative bacteria. The minimum inhibitory concentrations of aqueous infusions from plant materials are tens and hundreds of times higher than the IPC of antimicrobial chemotherapy for antibiotic-sensitive microorganisms. A question for further study is the prospect of using plant extracts to prolong the shelf life of fermented milk products.

References
[1] Nemeshchina O N et al. 2012 *Successes in modern natural science* 54–58
[2] Tapalsky D V and Tapalsky F D 2018 *Kursk Scientific and Practical Bulletin “Man and His Health”* 78–83
[3] Simakova I, Volf E et al. 2019 *Agronomy Research* 17 (4) 1761–1768
[4] Tereshchuk L V and Starovoitova K V 2013 *Foods and Raw materials* 1 (2) 67–75
[5] Elango G et al. 2016 *Journal of Molecular Liquids* 1249–1255
[6] Fowsiya J et al. 2016 *J. Photochem. Photobiol. B: Biol* 395–401
[7] Gurusamy S et al. 2019 *J. Photochem. Photobiol. B Biol* 118–130
[8] Elango G S et al. 2016 *J. Photochem. Photobiol. B: Biol*. 162–167
[9] Mahmood A, Mahmood A and Qureshi R A 2012 *Pak. J. Pharm. Sci.* 203–206
[10] Kannan B K 2015 *South Ind. J. Biol. Sci*. 52–59
[11] Park C H et al. 2016 *Molecules* p 157
[12] Krasulya O N et al. 2015 Modeling of food recipes and technologies of their production: theory and practice: study guide *GIORD* St. Petersburg p 320 (in Russian)