CHEMICAL COMPOSITION, ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITY OF ALLSPICE (*PIMENTA DIOICA* (L.) MERR.) ESSENTIAL OIL AND EXTRACT

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Allspice (*Pimenta dioica* (L.) Merr.) fruit was used for the essential oil and ethanolic extract isolation in the present study. The essential oil was obtained by Clevenger-type hydrodistillation from allspice fructus with hydromodule 1:10 m/v during 180 minutes, while the ethanolic extract was obtained by reflux extraction at the boiling temperature with solvmodule 1:10 m/v during 120 minutes. The qualitative and quantitative composition of the essential oil was determined by GC/MS and GC/FID methods, respectively. The total phenolic and total flavonoid content in the extract was determined according to the Folin-Ciocalteu and aluminium chloride methods, respectively. The antioxidant activity of allspice fructus essential oil and the extract was determined spectrophotometrically by DPPH assay and the antimicrobial activity using the micro-dilution method on the following microorganisms: two types of bacteria (*Bacillus cereus*, *Salmonella* spp.) and one yeast (*Candida albicans*), pairs of reference strains and their isolates. The obtained results proved the presence of 20 components in the essential oil, mainly oxygenated monoterpenes. The extract and essential oil have shown the best antioxidant activities after 20 and 60 minutes of incubation with EC₅₀ values of 0.011 mg/cm³ and 0.023 mg/cm³, respectively. Both the essential oil and the extract have shown an inhibitory effect on all investigated microorganisms. Allspice ethanolic extract showed a better inhibitory effect on *B. cereus* and *S. enterica* (isolate and ATCC strains) than the essential oil. On the other side, allspice essential oil showed a better inhibitory effect on *C. albicans* strains. The results obtained indicate that both the essential oil and the extract isolated from allspice fructus are a good source of natural antioxidants and antimicrobial agents with potential applications in food and pharmaceutical industries as a safer alternative to the synthetic additives.

Keywords: Allspice, Essential oil, Extract, Antioxidant activity, Antimicrobial activity

Introduction

Pimenta is a genus of the flowering plants in the Myrtaceae family, mostly found in the Caribbean region of America, and has about 15 species. Allspice, *Pimenta dioica* (L.) Merr.) is commonly used for culinary and medicinal purposes, because of the high content of phenolic compounds and various biological activities [1]. Allspice, well known for its fruits called pimento, has been used as an important spice in the food industry, as an ingredient in cosmetic products, as well as a therapeutic agent for diverse diseases. It is also known as allspice due to its intricate aroma which is a medley of aroma from spices such as clove, nutmeg and cinnamon. In India, the leaves of pimenta are used to flavor rice giving it a typical aroma [2,3].

A systematic research of aromatic constituents of allspice unripe berries and leaves have resulted in a discovery of different metabolites such as phenylpropanoids, galloylglucosides [4], flavonoids and tannins [5], that show

numerous (antibacterial, hypotensive, anti-neuralgic and analgesic) properties. Many already known compounds with antioxidant properties are isolated from leaves and berries of allspice, such as quercetin [6], eugenol [7], gallic acid [8] and others. Some studies have shown that eugenol and gallic acid have selective antiproliferative and anti-tumor properties on human cancer cells and their animal models. A compound such as ericifolin from the aqueous extract of allspice berries shows potent anti-prostate cancer and anti-breast cancer properties that could be verified *in vitro* and *in vivo*. Considering the phytochemical profile and availability at a low cost of organically-grown berries, allspice may have an additional space in the kitchen and alternative medicine [9,10].

Essential oils are complex mixtures of low molecular weight compounds extracted most commonly by hydrodistillation, than by steam distillation or solvent extraction [11]. The essential oil of allspice can be obtained

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from fructus or leaves with a yield of up to 5%. The major compound of the allspice oil is eugenol (fructus 60–90%, leaves >90%) [12]. In the essential oils isolated from allspice leaves 1,8-cineole, α -humulene, β -caryophyllene and cadinene-derivatives were found as further important constituents in higher concentrations (up to 10% for single compounds) in comparison to the oil isolated from fructus [13].

In general, the spices belonging to the Myrtaceae family are rich in polyphenols which are responsible for the antioxidant activity. These secondary metabolites support mainly the natural defense system, depending on the development stage of plants [14].

The first step of this study was the isolation of the essential oil from allspice fructus by Clevenger hydrodistillation and the total extractive matter extraction from the allspice fructus by reflux extraction with 96% v/v ethanol. The second step was to determine the chemical composition of the isolated essential oil and the ethanolic extract, as well as their antioxidant activity using DPPH assay, and the antimicrobial activity using a microdilution method to contribute to a greater use of allspice in pharmaceutical and food industry in southeast Serbia.

Experimental

Reagents and chemicals

Ethanol, 96%, Folin-Ciocalteu reagent, gallic acid, sodium-carbonate (Na_2CO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH radical), rutin (Merck, WGK Germany), aluminum(III)-chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$), potassium-acetate (CH_3COOK), (Sigma Chemical Company, St. Louis, USA), BHT (butylated hydroxytoluene) (Sigma-Aldrich), dimethyl sulfoxide (DMSO). All other chemicals are of analytical grade (p.a.).

Plant material

The commercial sample of allspice fructus (*P. dioica* (L.) Merr., country of origin: Mexico, packs Gyros Thessaloniki and top spices; Ugrinovski put, part 25-No.32, Zemun, Altina.) was used for investigations. Right before the analysis, the plant material was ground in an electric mill (laboratory electric mill "BRAUN AROMATIC KSM2").

Essential oil isolation

The essential oil was obtained by Clevenger hydrodistillation, with hydromodule 1:10 m/v for 180 minutes. The isolated oil was separated from the measuring tube after distillation, dried over anhydrous sodium sulfate and stored in the dark vials in a refrigerator at +4 °C.

Gas chromatography/mass spectrometry (GC/MS) and gas chromatography/flame ionization detection (GC/FID) analysis of essential oil

GC/MS analysis of the essential oil obtained from allspice fructus was performed on Agilent Technologies 7890 B gas chromatograph, equipped with weakly polar, silica capillary column, HP-5MS (5% diphenyl- and 95%

dimethyl-polysiloxane, 30 m × 0.25 mm, 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA) and coupled with inert, selective 5977 A mass detector of the same company. Three μl of the sample dissolved in diethyl ether in the concentration of 1000 ppm was injected in 5:1 split mode. Helium was used as the carrier gas, at a constant flow rate of 1 ml/min. The oven temperature was programmed from 50 °C for 2.25 minutes and then increased to 290 °C at the rate of 4 °C/min. The temperatures of the MSD transfer line, ion source and quadrupole mass analyzer were set at 300 °C, 230 °C and 150 °C, respectively. The ionization voltage was 70 eV and mass range m/z 35–650. GC/FID analysis was carried out under identical experimental conditions as GC/MS. The temperature of the FID detector was set at 300 °C. Data processing was performed using MSD Chem-Station, MassHunter Qualitative Analysis and AMDIS_32 softwares (Agilent Technologies, Santa Clara, CA, USA). Retention indices of the components from the analyzed samples were experimentally determined using a homologous series of n-alkanes from C_8 – C_{20} as standards. The percentage composition of a particular component in the essential oil was determined based on the area percent report (uncalibrated calculation procedure) generated by Agilent ChemStation software.

Reflux extraction

The homogenized plant material (2.5 g of ground allspice fructus) was extracted with 96% ethanol by the reflux extraction for 120 minutes with solvomodule 1:10 m/v at the boiling point. After the extraction, the plant material was separated from the liquid extract by vacuum filtration on a Büchner funnel. The solvent was removed by evaporation on a rotary evaporator at 50 °C. The resulting extract was dried in a vacuum oven at 40 °C to constant weight and stored in the refrigerator at +4 °C until analysis. The yield of total extractive matter (TEM, dry extract) was determined by drying the liquid extract in an oven at 105 °C to constant weight.

Total phenolics content (TPC) in the ethanolic extract

The total phenolics content in the extract was determined spectrophotometrically according to the Folin–Ciocalteu method [15] with certain modifications, using gallic acid as a standard (the concentration range: 0.00625 to 0.2 mg/ cm^3). To 0.5 cm^3 of ethanolic extract, 4.5 cm^3 of distilled water, 0.5 cm^3 Folin–Ciocalteu's reagent and 5 cm^3 of 7% Na_2CO_3 were added. After 90 minutes of incubation at room temperature, the absorbance was determined spectrophotometrically at 765 nm. Total phenolics are expressed as gallic acid equivalents per gram of the dry extract or as gallic acid equivalents per gram of the plant material (mgGAE/g d.e. or mgGAE/g p.m.) according to the Equation 1:

$$A_{765\text{nm}} = 0.01582 + 5.04832 \cdot \text{CGA}, R^2 = 0.9991 \dots \dots \dots (1)$$

Total flavonoids content (TFC) in the ethanolic extract

The total flavonoids content was determined according to the aluminum chloride colorimetric method [16-18] with certain modifications, using routine as a standard (the concentration range: 0.005 to 0.100 mg/cm³). The reaction mixture was prepared by mixing 2 cm³ of the ethanolic extract with 0.1 cm³ of 10% AlCl₃·6H₂O, 0.1 cm³ of 1 M CH₃COOK and 2.8 cm³ of distilled water. After 40 minutes of incubation at the room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. The results are expressed as routine equivalents per gram of the dry extract or as routine equivalents per gram of the plant material (mgRE/g d.e. or mgRE/g p.m.) according to the Equation 2 [19]:

$$A_{415} = 14.171 \cdot CR + 4.61 \cdot 10^{-2}, R^2 = 0.9991 \dots \dots \dots (2)$$

Antioxidant activity (DPPH assay)

The extract and essential oil of allspice were diluted with ethanol, and a series of solutions of different concentrations were made (0.00732-0.938 mg/cm³ for the essential oil and 0.00197-0.5 mg/cm³ for the extract). The ethanol solution of DPPH radical (1 cm³, 3×10⁻⁴ mol/dm³) was added to 2.5 cm³ of the prepared samples. For the extract, the procedure was performed in two samples. For one sample the absorbance at 517 nm was immediately measured, and another sample was incubated at room temperature and the absorbance was measured after 20 minutes (A_s). This procedure was performed in four tests for the essential oil. In one sample the absorbance was immediately measured at 517 nm and other samples were incubated at room temperature for 20, 40 and 60 minutes in the dark and after that the absorbance was measured (A_s). The absorbance at 517 nm was determined for the ethanolic solution of DPPH radical which was diluted in the same ratio (1 cm³ of the DPPH radical solution with 2.5 cm³ of ethanol, A_c), as well as for the extract or the essential oil before the treatment with DPPH radical (2.5 cm³ of extract/essential oil diluted with 1 cm³ of ethanol, A_b). Free radical scavenging activity was calculated according to the formula [20]:

$$\text{DPPH radical scavenging capacity (\%)} = 100 - \left[(A_s - A_b) \cdot \frac{100}{A_c} \right] \dots \dots \dots (3)$$

where A_s is the absorbance of the sample, A_b is the absorbance of the blank and A_c is the absorbance of the control at 517 nm.

DPPH radical scavenging capacity was also determined for butylated hydroxytoluene (BHT). The extract or the essential oil concentration needed for the neutralization of 50% of the initial DPPH radical concentration is called EC₅₀ value. All experiments were performed in triplicate and the absorbances were measured on Cole Parmer Spectrophotometer.

Antimicrobial activity

In order to investigate the antimicrobial activity for the obtained essential oil and the extract, the microdilution method was used [21]. The minimum inhibitory concen-

tration was determined by the serial dilution method in 96 well microtiter plates. The bacterial strains tested in this method were grown for 18 h at 37 °C on Miller Hinton agar (Muller Hinton agar, MHA), while the yeast was grown on Sabouraud Dextrose agar, SDA. The cell suspension was made at sterile Miller Hinton Broth (Muller Hinton Broth, MHB) and Sabouraud Dextrose Broth, SDB. Cell turbidity was adjusted to 0.5 McFarland (turbidity corresponding to the bacterial concentration of 10⁸ cells/cm³) using a densitometer (DEN-1 McFarland Densitometer, Biosan). The final inoculum density was 10⁶ CFU/cm³ (colony forming units). Stocks of the essential oil solutions were made in dimethyl sulfoxide (DMSO) and diluted in sterile MHB. Serial dilutions of the samples (dilution factor 2x) were tested, the final concentration range in the medium was 0.02-50 mg/cm³. After the serial dilution of the test oil was prepared, the inoculum was added to the wells and the plates were incubated for 24 h at 37 °C. The highest solvent concentration of DMSO was 5% (previously confirmed not to affect cell viability). Antibiotic chloramphenicol for bacteria and antifungal agent nystatin for the yeast were used as a positive control. An uninoculated well with medium without an agent was also left to test its sterility.

The bacterial growth after incubation of the plate was detected by the addition of 20 µl of 0.5% aqueous triphenyltetrazolium chloride (TTC), and the plate was incubated for 30 min. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibits the visible growth (red-colored precipitate at the bottom of the well after the addition of TTC). The minimum microbicidal (bactericidal/fungicidal) concentration (MMK) was defined as the lowest concentration that kills 99.9% of bacteria/fungi. To determine MMK, 10 µl of broth from each well without the visible growth was seeded on nutrient/SDA after which the plates were incubated for 24 h at 37 °C. All experiments were performed in triplicate.

Results and discussion

Qualitative and quantitative essential oil composition

Figure 1 shows the influence of hydrodistillation time on the essential oil yield. The maximal essential oil yield of 1.59 ± 0.01 cm³/100g of the plant material was achieved after 180 minutes. Other studies have found similar data for the allspice essential oil yield [7,22,23]. Usually, essential oil percentage yields from allspice are minimalistic, irrespective of the processing technology and the plant part utilized. The yield of the essential oil isolated from fructus is between 1.0 and 4.0% [13,24,25]. The hydrodistillation curve shows that there are two different periods of hydrodistillation (Fig. 1). Namely, in the first hydrodistillation period (the fast oil hydrodistillation) the essential oil was evaporated out from the surface of the plant material cells. In the second, the slow oil hydrodistillation period, a slow molecular diffusion of the essential oil from the internal part of the plant material cells occurred [26].

Figure 1. Hydrodistillation kinetics of the allspice essential oil

Components identification in the essential oil was performed by comparing their retention indices (RI^{exp}) with the literary (RI^{lit}) available values; by comparing their mass spectra with the spectra from the Willey, NIST, and RTLPEST libraries (MS) and by co-injecting an identical standard (Co-I) (Table 1) [27]. The percentage content of each component in the essential oil was determined based on the automatic integration of the peak area of the GC/FID signal.

The chemical composition of the allspice essential oil is presented in Table 1, the GC/FID chromatogram is given in Figure 2, and the structures of the major components present in the allspice essential oil are given in Figure 3.

GC/MS analysis of the allspice essential oil resulted in identifying 20 compounds, representing 97% of the total oil composition (Table 1). The major compounds were eugenol (48.5 %) and methyl-eugenol (35.0 %), followed by myrcene (4.5 %), (*E*)-caryophyllene (2.5 %), 1,8-cineol (1.8 %), and the others in much smaller percentages. In one study, the chemical composition of the allspice essential oil was analyzed by GC and GC/MS analysis and 45 components were identified, with eugenol (74.71%), methyl-eugenol (4.08%) and (*E*)-caryophyllene (4.9%) being the main components [7]. Dharmadasa and co-workers identified the components in the allspice essential oil by the following order of their abundance: eugenol ($85.33 \pm 2.0\%$), β -caryophyllene ($4.36 \pm 0.3\%$), 1,8-cineole ($4.19 \pm 0.3\%$), linalool ($0.83 \pm 0.11\%$) and α -humulene ($0.76 \pm 0.12\%$) [28]. One review paper reported that this oil contains eugenol (68.6-87.0%), methyl eugenol (2.9-13.0%), β -caryophyllene (2.5-5.4%) and 1,8-cineole (2.3-3.3%) as main constituents [29]. The high content of eugenol in the essential oil shows a strong antimicrobial activity [30-33]. This phenolic (phenylpropanoid) compound can denature proteins and react with the cell membrane of microorganisms by altering their permeability [34-39]. Regarding the composition of the essential oil of allspice, the pattern that eugenol

is present in the highest percentage is usually repeated, followed by methyl-eugenol etc. [7,13,40].

Table 1. Chemical composition of the allspice (*P. dioica* (L.) Merr.) essential oil

No.	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of identification	Content, %
1	11.96	Myrcene	986	988	RI, MS	4.5
2	12.45	α -Phellandrene	1000	1002	RI, MS	0.1
3	12.90	α -Terpinene	1013	1014	RI, MS	tr
4	13.17	p-Cimene	1021	1020	RI, MS	0.2
5	13.33	Limonene	1025	1024	RI, MS, Co-I	0.4
6	13.42	1,8-Cineole	1028	1026	RI, MS, Co-I	1.8
7	14.02	(<i>E</i>)- β -Ocimene	1044	1044	RI, MS	0.4
8	14.42	γ -Terpinene	1056	1054	RI, MS, Co-I	0.1
9	18.69	Terpinen-4-ol	1176	1174	RI, MS	0.5
10	19.15	α -Terpineol	1189	1186	RI, MS, Co-I	1.4
11	19.40	Methyl chavicol	1196	1195	RI, MS, Co-I	0.1
12	24.95	Eugenol	1363	1356	RI, MS, Co-I	48.5
13	25.44	α -Copaene	1378	1374	RI, MS	0.1
14	25.92	β -Elemene	1393	1389	RI, MS	0.3
15	26.37	Methyl eugenol	1407	1403	RI, MS	35.0
16	26.86	(<i>E</i>)-Caryophyllene	1423	1417	RI, MS	2.5
17	27.90	α -Humulene	1457	1452	RI, MS	0.4
18	29.14	α -Selinene	1498	1498	RI, MS	0.2
19	29.81	Myristicine	1521	1517	RI, MS	0.3
20	29.91	δ -Cadinene	1525	1522	RI, MS	0.2
Total identified (%)						97.0
Grouped components (%)						
Monoterpene hydrocarbons (1-5, 7, 8)			5.7			
Oxygen-containing monoterpene (6, 9-12, 15, 19)			87.6			
Sesquiterpene hydrocarbons (13,14, 16-18, 20)			3.7			

$t_{ret.}$: Retention time; RI^{lit} : Retention indices from the literature [23]; RI^{exp} : Experimentally determined retention indices using a homologous series of n-alkanes (C_7 - C_{22}) on the HP-5MS column. MS: constituent identified by mass-spectra comparison; RI: constituent identified by retention index matching; Co-I: constituent identity confirmed by GC co-injection of an authentic sample; tr = trace amount (<0.05%).

The yield of total extractive matter

The yield of total extractive matter (TEM) isolated from the allspice fructus during the reflux extraction process (solvomodule 1:10 m/v, extraction time 120 min) was 6.99 ± 0.09 g/100 g p.m.

The yield of the extractive matter depends on the solvomodule, the extraction time, the solvent (solvency), the quality of the plant material itself and the chemical composition of the plant material used [41-43]. The yield of TEM from allspice, obtained by the extraction with ethanol as a solvent using a solvomodule of 1:10 m/v is up to 7% according to current literature data [44], which agrees with the results obtained in this paper.

Total phenolics and total flavonoids content (TPC and TFC) in the ethanolic allspice extract

The content of total phenolics and total flavonoids in the allspice ethanolic extract was determined based on the calibration curve equations (Equations 1 and 2, Experimental Section). The results were expressed per

gram of the dry extract (d.e.). The amount of total phenolic and total flavonoid compounds were 273.29 ± 0.68 mg GAE/g d.e. and 22.21 ± 0.24 mg RE/g d.e., respectively. According to recent research, methanolic and aqueous extracts of allspice fruit are also a significant source of phenols and flavonoids. The total phenolic content in both methanol and water extracts of allspice fruit were 728.4 ± 137.5 GAE/g and 678.3 ± 141.1 GAE/g. The total flavonoid content in methanol and water extracts were 595.31 ± 51.77 mgCE/100g and 255.99 ± 9.89 mgCE/100g (mg catechin equivalents/100g) [45].

The phenol composition in plants depends on many factors such as variety, genotype, climatic conditions, raw material sweetening, processing and various treatments of the plant material [46]. Even earlier, phenolic

compounds have been associated with the antioxidant activity and played an important role in stabilizing lipid peroxidation [47].

Polar solvents frequently used for extracting polyphenols include ethanol, ethyl acetate, methanol and acetone [48]. Acetone is better suited for extracting flavonoids, and methanol for extracting polyphenols [49]. These solvents can also be combined to provide better extraction of phenolic compounds [40]. Methanol can extract more polyphenols from the sample compared to the water extraction method [50,51]. Ethanol and methanol could be used for the extraction of these compounds, with ethanol being selected for extraction in this work because of its less toxicity [52,53].

Figure 2. GC/FID chromatogram of the allspice (*Pimenta dioica* (L.) Merr.) essential oil

Eugenol

Methyl eugenol

Figure 3. Structures of the major components identified in the allspice essential

Antioxidant activity of essential oil and extract

The antioxidant activity of the obtained essential oil and extract was studied using DPPH assay. DPPH radical absorbs at 517 nm (violet in color). When exposing the radical to free radical scavengers, its absorbance significantly decreases because of the hydrogen atom transfer from antioxidant to radical. Thereby, the absorb-

ance decrease indicates the antioxidant potential of the studied sample [54].

Based on the absorbance values obtained (control, sample and blank), the degree of neutralization of DPPH radical (%) by the samples analyzed was calculated. It increases with the increase of the essential oil or the extract concentration being the lowest for no incubated

samples (Figures 4 and 5).

Figure 4. Antioxidant activity of the allspice essential oil

Figure 5. Antioxidant activity of the allspice ethanolic extract

EC₅₀ values for allspice essential oil were: 0.194, 0.066, 0.037, 0.023 mg/cm³, without incubation, with 20, 40 and 60 min incubation, respectively. EC₅₀ values for allspice ethanolic extract were: 0.060 and 0.011 mg/cm³, without incubation and with 20 minutes incubation, respectively. The extract and the essential oil showed a good, concentration-dependent antioxidant activity (Figures 4 and 5).

Based on the EC₅₀ values obtained, the extract showed a better antioxidant activity (lower EC₅₀ values) than the essential oil comparing the values without incubation (0.060 vs. 0.194 mg/cm³) and after 20 minutes of incubation (0.011 vs. 0.066 mg/cm³) with radical. This behavior of the extract is probably due to the synergistic action of phenolic compounds, especially flavonoids present therein. There are a large number of scientific papers in which the antioxidant activity of phenols and flavonoids has been confirmed [50,55,56]. Phenols and flavonoids are very important plant ingredients. The highly significant correlations obtained in different studies

[57-59] support the hypothesis that phenolic compounds significantly contribute to the total antioxidant capacity of the plants species, due to their ability to "scavenge" free radicals. This is in accordance with the findings of Cai et al. and Djeridane et al. who found a linear correlation between the content of total phenolic compounds and the antioxidant capacity of several plant species [60,61]. Phenolic compounds are effective donors of hydrogen atoms, which make them very good antioxidants [62]. A good antioxidant activity of the essential oil probably comes from the most abundant components, eugenol and methyl-eugenol [63,64].

The results obtained in our study are in agreement with previous studies [28], which reported that the allspice essential oil contains eugenol, methyl eugenol and caryophyllene as its main components. Eugenol is a natural compound present in many aromatic herbs, spices and foods, and is used in cosmetics and pharmaceuticals. Eugenol has a dual effect on oxidative stress, it can act both as an antioxidant or a prooxidant. Also, it exhibits anticancer, cytotoxic, and antitumor activity [65].

The allspice ethanolic extract has shown a better antioxidant activity than the synthetic antioxidant BHT (EC₅₀ value measured after 20 min of incubation: 0.011 mg/cm³ for the allspice ethanolic extract vs. 0.021 mg/cm³ for BHT). The essential oil exhibited a slightly lower antioxidant activity compared to BHT, but only after 60 min of incubation. Considering that BHT is one of the most commonly used antioxidants, but with harmful effects on the organism [66], it could be concluded that the tested allspice essential oil and the extract, especially the extract, represent an alternative to this antioxidant with potential application in pharmaceutical and food products.

Antimicrobial activity

The antimicrobial activity of the obtained essential oil and the extract was determined by the microdilution method [21]. Minimum inhibitory (MIC) and minimum microbicidal concentration (MMC) were determined for all samples. The results of these tests are shown in Tables 2 and 3, respectively.

Table 2. Determination of MIC values for the allspice essential oil and the extract

Microorganism	Source of strain	MIC (mg/cm ³)		Antibiotic/antimycotic
		I	II	
<i>Bacillus cereus</i>	Isolate from food	3.12	1.56	0.001
<i>Bacillus cereus</i>	ATCC 11778	3.12	1.56	0.0008
<i>Candida albicans</i>	Isolate (feces)	1.56	3.12	0.004
<i>Candida albicans</i>	ATCC 24433	1.56	6.25	0.004
<i>Salmonella spp.</i>	Isolate (feces)	6.25	0.39	0.006
<i>Salmonella enterica</i>				
ssp. <i>enterica</i>	ATCC 13078	3.12	1.56	0.003

I-allspice essential oil; II-allspice ethanolic extract

Table 3. Determination of MMC values for allspice the essential oil and the extract

Microorganism	Source of strain	MMC(mg/cm ³)	
		I	II
<i>Bacillus cereus</i>	Isolate from food	/	12.5
<i>Bacillus cereus</i>	ATCC 11778	/	50
<i>Candida albicans</i>	Isolate (feces)	3.1	6.25
<i>Candida albicans</i>	ATCC 24433	6.25	12.5
<i>Salmonella spp.</i>	Isolate (feces)	25	50
<i>Salmonella enterica</i> ssp. <i>enterica</i>	ATCC 13078	6.25	12.5

I-allspice essential oil; II-allspice ethanolic extract

Antimicrobial testing was performed against two types of bacteria (*Bacillus cereus* and *Salmonella enterica*) and one yeast (*Candida albicans*), which are potential intestinal pathogenic microorganisms, with pairs of reference strains and their isolates. The essential oil and the extract obtained from allspice were tested in the concentration range of 0.02-50.0 mg/cm³.

The allspice essential oil and the extract have shown an inhibitory effect on all microorganisms. Among the tested samples, the extract exhibited an inhibitory effect at significantly lower concentrations than the essential oil on *Bacillus* and *Salmonella* (isolates and ATCC strains) while the essential oil showed a better inhibitory effect on *C. albicans* (Table 2).

Knowing that *Bacillus* species often cause alimentary toxic infections in humans, they can cause a variety of other diseases: septic meningitis, cellulitis, gangrene, and numerous eye infections [67], these results can be of great importance.

The allspice ethanolic extract has shown a microbicidal effect on all microorganisms, while the obtained essential oil did not affect only *B. cereus* strains (Table 3).

The antimicrobial activity of the extract and the essential oil from allspice has been reported in the literature [68-70] with the emphasis placed on pathogenic bacterial strains. Spices are the most widely used natural antimicrobials in foods being used for thousands of years in food preservation, and as additives to enhance aroma and taste [71]. Phytocompounds which have good solubility in ethanol include tannins, polyphenols, polyacetylene, flavonoids, sterols and alkaloids [72]. The fungicidal action of ethanolic extracts of herbs leads to lysis of the cell wall or cytoplasmic membrane of fungi and the release of antimicrobial substances directly into the cytoplasm [73].

Allspice is rich in glycosides and polyphenols that exhibit antibacterial, fungicidal and antiviral properties [9,74,75]. According to all the literature data available for the antimicrobial activity of allspice essential oil, it was concluded that compounds such as eugenol, methyl-eugenol, (*E*)-caryophyllene, terpinen-4-ol and o-cymene are responsible for this activity [76-79].

Conclusion

The presented results indicate that the allspice essential oil and the ethanolic extract could be used as a potential source of natural antioxidants and antimicrobial agents for the food, pharmaceutical and chemical industry. Therefore, these isolates from allspice fructus represent the alternative to synthetic additives that could be toxic and carcinogenic. So, it is interesting to investigate its application as natural additives in some final food and pharmaceutical products, for preservation and/or extension of shelf life of raw and processed foods, as well as pharmaceuticals. Besides, the presented results suggested that use of allspice is not just reasonable, but it should be even favored in the traditional Serbian cuisine.

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Izvod

HEMIJSKI SASTAV, ANTIOKSIDATIVNA I ANTIMIKROBNA AKTIVNOST ETARSKOG ULJA I EKSTRAKTA NAJKVIRCA (*PIMENTA DIOICA* (L.) MERR.)

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Plod najkvirca (*Pimenta dioica* (L.) Merr.) korišćen je za izolaciju etarskog ulja i etanolnog ekstrakta u ovom istraživanju. Etarsko ulje je dobijeno Clevenger hidrodestilacijom iz ploda najkvirca pri hidromodulu 1:10 m/v u toku 180 minuta, dok je etanolni ekstrakt dobijen refluks ekstrakcijom na temperaturi ključanja pri solvomodulu 1:10 m/v u toku 120 minuta. Kvalitativni i kvantitativni sastav dobijenog etarskog ulja određen je GC/MS i GC/FID metodama, respektivno. Sadržaj ukupnih fenola i flavonoida određen je spektrofotometrijski, Folin-Ciocalteu i AlCl₃ metodama, respektivno. Antioksidativna aktivnost etarskog ulja i ekstrakta ploda najkvirca određena je spektrofotometrijski primenom DPPH testa, dok je antimikrobna aktivnost određena mikrodilucionom metodom na sledeće mikroorganizme: dve vrste bakterija (*Bacillus cereus*, *Salmonella* spp.) i jedan kvasac (*Candida albicans*), pri čemu su testirani parovi referentnih sojeva i njihovih izolata. Na osnovu dobijenih rezultata utvrđeno je prisustvo 20 komponenti u etarskom ulju, uglavnom oksigenovanih monoterpena sa najvišim sadržajem eugenola (48,5%) i metil eugenola (35,0%). Ekstrakt i etarsko ulje su pokazali najbolju antioksidativnu aktivnost posle 20 i 60 minuta inkubacije sa EC₅₀ vrednostima 0,011 mg/cm³ i 0,023 mg/cm³, respektivno. Etarsko ulje i ekstrakt pokazali su inhibitorno dejstvo na sve ispitivane mikroorganizme. Etanolni ekstrakt najkvirca pokazao je bolji inhibitorni efekat na *B. cereus* i *S. enterica* (izolat i ATCC sojeve) od etarskog ulja. Etarsko ulje najkvirca pokazalo je bolji inhibitorni efekat na sojeve *C. albicans*. Dobijeni rezultati ukazuju na to da su i etarsko ulje i ekstrakt izolovani iz aromatičnog ploda najkvirca dobar izvor prirodnih antioksidanasa i antimikrobnih sredstava sa potencijalnom primenom u prehrambenoj i farmaceutskoj industriji kao sigurnija alternativa sintetičkim aditivima.

Ključne reči: Najkvirc, Etarsko ulje, Ekstrakt, Antioksidativna aktivnost, Antimikrobna aktivnost