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STUDY ON THE ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT EXTRACTS AND EMULSION PRODUCTS WITH INTEGRATED HERBAL EXTRACTS

SUMMARY

The antimicrobial activity of water and oil extracts of five identified medicinal plants – thyme (*Thymus callieri* Borbás ex Velen), St. John's wort (*Hypericum perforatum* L.), cirsium (*Cirsium ligulare* Bois), hawthorn – flowers with leaf (*Crataegus monogyna* Jacq.), hawthorn–berries (*Crataegus monogyna* Jacq.) and juniper (*Juniperus communis* L) was investigated. The antimicrobial activity of the water extracts was tested against two groups of microorganisms: pathogenic (*Escherichia coli* ATCC 8739, *Salmonella enteretidis* ATCC 13076, *Klebsiella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Candida albicans* NBIMCC 74 and *Listeria monocytogenes* ATCC 8632) and saprophytic (*Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Saccharomyces cerevisiae* ATCC 9763, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Fusarium moniliforme* ATCC 38932). The obtained water extracts from all plant sources suppressed the growth of *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 8632, as well as the fungi *A. niger* ATCC 1015, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *F. moniliforme* ATCC 38932. The oil extracts showed inhibitory effect only on *S. aureus* ATCC 25923. In order to investigate their food biopreservation effect, the obtained plant extracts were

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incorporated in food matrix – mayonnaise, and stored for 20 days. During the storage, samples for microbiological analyses were taken. It was found that until the 12th day of the storage, in the controls as well as in the three samples of mayonnaise and the three samples of mayonnaise sauce, the yeasts and fungal counts were below the permissible values, while on the 20th day of the storage, visible fungal growth in all samples was observed.

Keywords: antimicrobial activity, pathogenic and saprophytic microorganisms, medicinal plants, mayonnaise, mayonnaise sauce

INTRODUCTION

Since ancient times, medicinal and flavoring plants and their essential oils have been known to have varying degrees of antimicrobial activity (Zaika, 1988). Historically, the products from natural sources, used in medicine, predate today's widespread antibiotic therapies and various medicinal drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief (Kafaru, 1994).

They are used as spices in the preparation of foods, improving their sensory profile and increasing their storage life. Spices or edible herbs have been shown to possess aromatic properties and exert antimicrobial effects on different pathogenic microorganisms (Brandi *et al*., 2006).

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Lewis *et al*., 2006; Mc Lauchlin, 2004).

The antimicrobial and antioxidant properties of edible plants and the aromatic products obtained from them are due to substances with different chemical composition – essential and glyceride oils, alkaloids, flavonoids, tannins, glycosides and other compounds (Dobreva, 2010; Souza *et al.,* 2005; Suhaj, 2006; Obad *et al*., 2016; Teneva *et al*., 2016).

Even without a detailed understanding of the natural antimicrobial substances action, efforts to develop new effective methods aimed to improved the food safety are growing (Ayala Zavala *et al.*, 2008; Brandi *et al*., 2006; Lopez *et al*., 2007; Murdak *et al*., 2007; Nazef *et al*., 2008).

Thyme (*Thymus callieri* Borbás ex Velen) is a representative of the herbs with antimicrobial and antioxidant activity, which can be incorporated into food matrices preventing the growth of pathogenic and spoilage microorganisms and increasing their safety. However, the mechanisms of action, as well as the toxicological and sensory effects of the natural antimicrobial resources, are not fully understood (Burt, 2004; Davidson and Naidu, 2000; Gaysinsky and Weiss, 2007; Gutierrez *et al*., 2008b; Gutierrez *et al*., 2009; Lopez *et al*., 2008; Moriera *et al*., 2007; Patrignani *et al*., 2008; Periago *et al*., 2006; Ponce *et al.*, 2008). A number of studies consider the application of thyme extracts with proven antimicrobial activity and recommended its use as natural food preservatives (Bajpai *et al*., 2008; Burt, 2004; Sofia *et al.*, 2007). It has been reported that the thyme extracts added to meat products reduce the presence of pathogenic

microorganisms (Grosso *et al*., 2008, Emiroğlu *et al*., 2010; Krishnan *et al.*, 2014).

Nowadays it is well known that the plants of the *Asteraceae* family have strong antimicrobial activity. The antimicrobial activity of *Achillea* spp. (Niño *et al.*, 2006; Paulo, 2006), *Arctotis auriculata* Jacq., and *Eriocephalus africanus* L. (Salie *et al*., 1996; Stojanovic *et al*., 2005) have been reported. Further, there are also many reports on the bioactivities, including antimicrobial activity, of extracts and essential oils from species of the genus Artemisia (Ramezani *et al*., 2004).

According to Tadic *et al.* (2008) extracts of dried hawthorn (*Crataegus monogyna* Jacq. and *Crataegus oxyacantha* L) can be used as an anti-inflammatory, gastroprotective and antimicrobial agent. Studies conducted by Kostić (2012), prove that hawthorn-berries extracts (*Crataegus oxyacantha* L.) are rich in polyphenolic compounds and exhibit good antioxidant and antimicrobial properties.

Rezvani and Mahmoodi (2009) demonstrated that the essential oils of *Juniperus communis* possessed antibacterial activity against *Staphylococcus aureus* NCIB 6751 and *Escherichia coli* NCIB 8879.

Food emulsions are the basis of many products, and a significant part of them are from the mayonnaise type, sweet and salty emulsion sauces and dressings. Nowadays, their composition includes not only glyceride oils, which are rich in polyunsaturated fatty acids (Nikovska 2008a, b; Nikovska and Stamov, 2009), but also oil extracts containing various biologically active substances (Boeva *et al*., 1990; Georgiev and Stoyanova, 2006).

The aim of the present study was to determine the antimicrobial activity of water and oil extracts from five types of medicinal plants against pathogenic and saprophytic microorganisms, as well as to investigate the possibility of their application in model formulations of various food products – mayonnaise, mayonnaise sauces and salad dressing.

MATERIAL AND METHODS

Materials

Culture media

Plate count agar (PCA). This medium was used for determination of the total plate count of mesophilic aerobic and facultative anaerobic microorganisms. A quantity of 23.5 g of the PCA agar medium base (containing 5 g casein peptone, 2.5 g yeast extract, 1 g dextrose and 15 g agar) was dissolved in 1 L of deionized water, pH 7.0±0.2. The medium was sterilized by autoclaving (regime:121 °C/15 min).

Chloramphenicol glucose agar (CGA). CGA is a selective medium for the enumeration of yeasts and fungi. A quantity of 40 g of the CGA agar medium base (containing 20 g dextrose, 5 g yeast extract, 0.1 g chloramphenicol and 15 g agar) was dissolved in 1 L of deionized water, pH 6.6±0.2. The medium was sterilized by autoclaving (regime: 121 °C/15 min).

Tryptone bile glucuronic agar (TBX). TBX is a selective medium for *Escherichia coli*. A quantity of 36.5 g of the TBX agar medium base (containing 20 g tryptone, 1.5 g bile salts, 0.075 g X-β-D-glucoronide and 15 g agar) was dissolved in 1 L of deionized water, pH 7.2±0.2. The medium was sterilized by autoclaving (regime: 121 °C/15 min).

Xylose Lysine Deoxycholate (XLD) Agar. XLD is a selective medium for *Salmonella*. A quantity of 55.2 g of the XLD agar medium base (containing lactose 7.5 g, sucrose 7.5 g, sodium thiosulfate 6.8 g, l-lysine 5.0 g, sodium chloride 5.0 g, xylose 3.75 g, yeast extract 3.0 g, sodium deoxycholate 2.5 g, ferric ammonium citrate 0.8 g, phenol red 0.08 g, agar 15.0) was dissolved in 1 L of deionized water, pH 7.4±0.2. The medium do not autoclave or overheat. After preparation the medium was directly transferred to water bath (50 °C).

Chapman agar (Mannitol salt agar). Chapman agar is a selective medium for *Staphylococcus aureus*. A quantity of 111 g of the Chapman agar medium base (containing 1 g beef extract, 5 g pancreatic digest of casein, 5 g peptic digest of meat, 75 g NaCl, 10 g D-mannitol, 0.025 g phenol red and 15 g agar) was dissolved in 1 L of deionized water, pH 7.4±0.2. The medium was sterilized by autoclaving (regime: 121 °C / 15 min).

Luria-Bertani agar medium supplemented with glucose (LBG agar). LBG agar was used for cultivation of test bacteria and implementation of antimicrobial activity assay. The LGB mixture contained following substances: tryptone (10 g), yeast extract (5 g), NaCl (10 g), glucose (10 g) and agar (15 g). The total quantity of 50 g was used. This mixture was dissolved in 1 L of deionized water, pH 7.5±0.2. The prepared medium was sterilized by autoclaving (regime: 121 °C/15 min).

Malt extract agar (MEA). Yeasts and fungi were cultivated in this medium. The MEA mixture contained following substances: malt extract (30 g), mycological peptone (5 g), agar (15 g). The total quantity of 50 g was used. This mixture was dissolved in 1 L of deionized water, pH 5.4±0.2. The medium was autoclaved at 115 °C for 10 min.

All culture media were prepared in accordance with the manufacturer's (Scharlab SL, Spain) instructions.

Plant sources

Five different Bulgarian medicinal plants that grow in the Western Rhodopes, Dospat municipality, Bulgaria were selected. The plants were collected during the period of their flowering, in sunny and dry weather – May-September 2019. To obtain the two types of extracts (water and oil) different morphological parts of the plants were used, namely: flowers of the cirsium (*Cirsium ligulare* Boiss) and hawthorn (*Crataegus monogyna*), flower-bearing stems – stalks with flowers of St. John's wort (*Hypericum perforatum* L.) and thyme (*Thymus callieri* Borbás ex Velen.). Hawthorn-berries (*Crataegus monogyna*) and juniper cones (*Juniperus communis* L) were also used, but they were harvested after the ripening of the berry and the cones – September-October

2019. The geographical coordinates from where herbs were harvested (presented in table 1) give the exact location of every single population on the map.

Table 1. Geographical coordinates of the herbs plantations

Herbs	Locality	Geographical coordinates	Altitude
Thyme	Bulgaria, Western Rhodopes, near the town of Dospat	35TKG 63503 16655 Lat. 41.66583 Lon. 24.159444	UTM/MGRS KG61 1214 m
St. John's Wort	Bulgaria, Western Rhodopes, near the town of Dospat, Dulga Barchina locality	35TKG 64164 13506 Lat. 41.6377 Lon. 24.16861	UTM/MGRS KG61 1264 m
Cirsium	Bulgaria, Western Rhodopes, near the town of Dospat	35TKG 63413 16751 Lat. 41.66666 Lon. 24.15833	UTM/MGRS KG61 1207 m
Hawthorn	Bulgaria, Blagoevgrad region, near the village of Satovcha, Aspen locality	35TKG 57029 12827 Lat. 41.62944 Lon. 24.50992	UTM/MGRS KG51 1134 m
Juniper cones	Bulgaria, Western Rhodopes, near the town of Dospat	35TKG 63526 16655 Lat. 41.66583 Lon. 24.159722	UTM/MGRS KG61 1214 m

To obtain the extracts, dried plant materials were used. The fresh plant sources were identified, inspected to remove impurities and dried in a thin layer in the shade at a temperature of 22–25 °C. The dry plant mass was stored in paper, in well-closed bags, in a dry place until analysis.

Ingredients for the extracts and samples

For the preparation of water and oil extracts, experimental samples and control samples of mayonnaise, mayonnaise sauce and salad dressings, the following ingredients were used:

Main raw materials: vegetable oil (refined sunflower oil-oleic type), "Papas Oil" Ltd, Veliki Preslav, Bulgaria and water.

Additional and auxiliary raw materials: sugar (white, crystalline) – "Sweet Life", Serbia; salt (cooking, table, iodized) –"Lubex" Ltd.; vinegar (5%, apple), "Veda" Ltd., Pleven, Bulgaria. The emulsifiers and stabilizers were provided by "Bobal-Boyadzhiev" Ltd., Sofia, Bulgaria. Emulsifiers and egg yolk substitutes – Trecomex Twelve – "Bobal-Boyadzhiev" Ltd., Sofia, Bulgaria and stabilizers –

Mayolys (mixture of vegetable gums) and Swely Gel Soft (modified starch, very strong thickening agent) – "Bobal-Boyadzhiev" Ltd., Sofia, Bulgaria.

Test microorganisms

For the determination of antimicrobial activity, the following test microorganisms from the National Bank for Industrial Microorganisms and Cell Cultures and the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria were used: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella enteretidis* ATCC 13076, *Salmonella enterica* subsp. *enterica* serovar *abony* NCTC 6017, *Klebsiella sp.* (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 8632, *Listeria monocytogenes* NCTC 11994, *Candida albicans* NBIMCC 74, *Saccharomyces cerevisiae* ATCC 9763, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Fusarium moniliforme* ATCC 38932.

The strains were cultivated as follows: *Saccharomyces cerevisiae* ATCC 9763 cultivated at 30°C on Malt extract agar, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Penicillium* sp., *Rhizopus* sp., *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Fusarium moniliforme* ATCC 38932 were cultivated at 30°C on LBG-agar, and *Escherichia coli* ATCC 8739, *Salmonella enteretidis* ATCC 13076, *Klebsiella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Candida albicans* NBIMCC 74, *Listeria monocytogenes* ATCC 8632, *Listeria monocytogenes* NCTC 11994, *Salmonella enterica* subsp. enterica serovar abony NCTC 6017 – at 37 °C on LBG-agar.

Methods

Preparation of oil extracts

The extraction procedure was carried out according to the methods of Stoyanova (1986) and Georgiev and Stoyanova (2006). To obtain oil extracts, pre-ground and moistened to a 70% humidity (Georgiev, 1998) dry plant raw materials from the studied plant species were used and they were subjected to extraction by soaking for 24÷48 hours at 80 °C and with continuous stirring.

Preparation of water extracts

To obtain decoction-type water extracts, a hydromodule of 1:20 was maintained. In our case, 15 g of the raw material was weighed and transferred to a flask containing 300 cm^3 of extractant – water heated to 60–65 °C. The temperature was maintained for 1 h, after which the mixture was filtered through kapron cloth. The residue was returned to the flask and poured with 200 cm^3 of the appropriate extractant (water). The second extraction lasted 1 hour, at the same temperature (60–65 °C), and then filtered. The two filtrates were combined and homogenized well.

Determination of oil extracts antimicrobial activity

The antimicrobial activity of the oil extracts was determined by a modification of the "diffusion in agar" method, by measuring the inhibition zones of the pathogens growth around metal rings in which a certain amount of oil

extract was introduced. The selective media for *Listeria monocytogenes* NCTC 11994, *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *enterica* serovar *abony* NCTC 6017 and *Staphylococcus aureus* ATCC 25923 were inoculated with suspensions of the pathogens prepared from a 24 hour culture on slanted PCA. From a suitable ten fold suspension dilution, the melted and cooled to 45–50 °C selective media were inoculated. The effective concentration of the cells in the agar was equated to the concentration of the 1.0×105 dilution suspension, as 1 cm^3 of suspension was inoculated into 99 cm^3 of medium. After solidification of the media, sterilized metal rings (Ø=6 mm) were placed on their surface, in which 0.05; 0.10 and 0.15 cm^3 extract was added. Petri dishes were incubated at 37 °C. The diameter [mm] of the growth inhibition zones of tested microorganisms at 24 and 48 hours was measured and a comparative assessment of their antibacterial activity was made.

Determination of water extracts antimicrobial activity

The antimicrobial activity of water extracts was determined by the agar-well diffusion method, while the minimum inhibitory concentration (MIC) was determined by the serial dilution method (Jirovetz *et al.*, 2006).

Agar-well diffusion method. In Petri dishes (d=9 cm), placed on a level surface, 17 ml of pre-melted, cooled to 40–45 °C and inoculated with the test microorganism (1.0×10^6 cfu/cm^3 for fungal spore sand 1.0×10^8 cfu/cm^3 for viable bacterial and yeast cells) LBG-agar medium was poured. After spilling of the inoculated culture media, the Petri dishes were left for 1 hour to solidify the agar. Next, six wells per dish (d=6 mm) were cut, and 60 µl of each water extract were pipetted into the agar wells, in duplicate. The Petri dishes were thermostated at different temperature conditions (depending on the type of test microorganism) for 24÷48 hours. The presence and degree of antimicrobial activity was evaluated by the formed inhibition zones (IZ, mm) around the wells.

Minimum inhibitory concentration (MIC) by serial dilution method: The minimum inhibitory concentration (MIC) of the water extracts was determined in order to calculate their amount for application into food raw materials and products as a biopreservative. For this purpose, the water extracts were diluted twice in 0.9% NaCl. Then, a test for antimicrobial activity was carried out, determining the minimum inhibitory concentration – MIC (the highest dilution of the water extracts which inhibited the growth of the test microorganism around the agar wells).

Preparation of mayonnaise and mayonnaise sauce

The technologies for mayonnaise and dressing-type mayonnaise sauce production was proposed by Perifanova-Nemska and Uzunova (2016). Based on preliminary research on food O/W emulsions with incorporated extracts of the investigated plant materials, three assortments remained as a result of the selection, on which the research was continued. The developed formulation presented in table 2.

Table 2. Model recipe composition of mayonnaise, mayonnaise sauce and salad dressing

Raw materials, %	Assortments					
	Mayonnaise		Mayonnaise sauce		Salad dressing	
	Control Sample	Experimental Samples	Control Sample	Experimental Samples	Control Sample	Experimental Samples
Vegetable Oil	50.00	–	25.00	–	56.00	–
Oil extracts	–	50.00	–	25.00	–	56.00
Mayolys SX	0.30	0.30	0.15	0.15	–	–
Sweelygel	1.50	1.50	0.75	0.75	–	–
Water	45.70	45.70	23.35	23.35	20.00	–
Water extract	–	–	48.00	48.00	–	20.00
Vinegar	–	–	–	–	20.00	20.00
Trecomex	0.50	0.50	0.75	0.75	–	–
Salt	1.00	1.00	1.00	1.00	3.00	3.00
Sugar	1.00	1.00	1.00	1.00	1.00	1.00

All three culinary products were prepared using oil extracts for the oil phase of the assortments. Water extracts were included in the recipe compositions of the mayonnaise sauce and the salad dressings. The six samples were compared with control samples in which refined high oleic sunflower oil and water were included.

Organoleptic analysis of food emulsion products

To conduct the organoleptic analysis, 20 unbiased testers over 18 years old were invited. The sensory evaluation was performed using a five-point hedonic scale, where 5 corresponded to the highest and 1 to the lowest evaluation for the given indicator. The samples were evaluated according to the following quality indicators: appearance, consistency, color, smell, aroma, taste, aftertaste. Based on the conducted tasting, a general perception assessment of the final products was formed.

Microbiological analysis

The total plate count (cfu/g) (mesophilic aerobic and facultative anaerobic microorganisms), yeast and fungal counts (cfu/g), and presence of specific pathogenic microorganisms (cfu/g) were determined according to the Bulgarian State Standards (table 3).

Table 3. Microbiological parameters and standards for foods

Parameter	Culture medium / t°C	Standard
Total plate count*	PCA / 30°C	BSS EN ISO 4833-1, 2013
Yeasts and fungi*	CGA / 30°C	BSS EN ISO 21527-2, 2011
*Escherichia coli**	TBX agar / 44°C	BSS EN ISO 16649-2, 2014
Salmonella sp.**	XLD agar / 37°C	BSS EN ISO 6579-1, 2017
*Staphylococcus aureus***	Chapman agar / 37°C	BSS EN ISO 6888-1, 2005

** –pour-plating method; ** –spread-plating method*

RESULTS AND DISCUSSION

The concentrations of pathogenic and saprophytic microorganisms are presented in table 4.

Table 4. Concentrations of pathogenic and saprophytic test microorganisms

Pathogenic test-microorganism	Concentration, cfu/cm^3
Salmonella enteritidis ATCC 13076	2.0×10^8
Klebsiella sp. (clinical isolate)	2.7×10^8
Escherichia coli ATCC 8739	1.0×10^{12}
Candida albicans NBIMCC 74	2.0×10^{10}
Listeria monocytogenes ATCC 8632	4.6×10^9
Staphylococcus aureus ATCC 25923	4.0×10^8
Salmonella enterica subsp. *enterica* serovar *abony* NCTC 6017	3.5×10^8
Listeria monocytogenes NCTC 11994	7.0×10^8
Saprophytic test-microorganism	Concentration, cfu/cm^3
Bacillus subtilis ATCC 6633	1.0×10^9
Bacillus cereus ATCC 11778	6.5×10^8
Saccharomyces cerevisiae ATCC 9763	2.8×10^7
Aspergillus niger ATCC 1015	4.0×10^8
Aspergillus flavus	4.0×10^8
Penicillium sp.	6.0×10^8
Rhizopus sp.	1.2×10^7
Fusarium moniliforme ATCC 38932	1.2×10^7

Table 5. Antimicrobial activity of oil extracts against pathogenic microorganisms

Oil extracts	Concentration, cm^3	*Escherichia coli* ATCC 8739	*Salmonella enterica* subsp. *enterica* serovar *abony* NCTC 6017	*Listeria monocytogenes* NCTC 11994	*Staphylococcus aureus* ATCC 25923
Thyme *Thymus callieri* Borbás ex Velen.	0.15	–*	–	–	–
	0.10	–	–	–	–
	0.05	–	–	–	–
St. John'sWort *Hypericum perforatum* L.	0.15	–	–	–	15
	0.10	–	–	–	–
	0.05	–	–	–	–
Cirsium *Cirsium ligulare* Boiss.	0.15	–	–	–	–
	0.10	–	–	–	–
	70.05	–	–	–	–
Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.	0.15	–	–	–	14
	0.10	–	–	–	12
	0.05	–	–	–	11
Hawthorn /berries/ *Crataegus monogyna* Jacq.	0.15	–	–	–	–
	0.10	–	–	–	–
	0.05	–	–	–	–
Juniper *Juniperus communis* L.	0.15	–	–	–	–
	0.10	–	–	–	–
	0.05	–	–	–	–

– diameter of the zone equal to the diameter of the metal ring is taken as a negative result*

Oil extracts antimicrobial activity

The data from the antimicrobial activity (table 5) of oil extracts indicated that the extracts from thyme, St. John's wort, cirsium, hawthorn (flowers or berries), and juniper had weak antimicrobial activity against pathogenic microorganisms. The tested extracts showed inhibitory activity only against the Gram-positive *Staphylococcus aureus* ATCC 25923. The obtained results showed that *Staphylococcus aureus* ATCC 25923 was more sensitive to the hawthorn (flowers) extract at all three concentrations (0.15; 0.10 and 0.05 cm^3), with inhibition zones: 14, 12 and 11 mm, respectively. For an oil extract obtained from St. John's wort, an inhibition zone of 15 mm was found for the extract concentration of 0.15 cm^3.

Water extracts antimicrobial activity

The inhibition zone (IZ) and the minimum inhibitory concentration (MIC) were determined.

The results from the determination of antimicrobial effect of the water extracts are presented in table 6 and table 7. It was found that all six extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium did not influence the growth of the test microorganisms – the spore-forming *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* ATCC 11778, and the yeasts *Saccharomyces cerevisiae* ATCC 9763.

The experimental data indicated that water extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium, suppressed the growth of the fungi *Aspergillus niger* ATCC 1015 and *Penicillium* sp., in which was established the inhibition zones from 8 to 13 mm and MIC in the range of 60 to 600 ppm. The highest inhibitory activity against *Rhizopus* sp. was found in the thyme extract with inhibition zones of 14 mm and MIC<60 ppm, followed by the extracts of hawthorn (berries), juniper, cirsium and hawthorn (flowers), where the zones of inhibition were between 8 and 12 mm and MIC was 60 ppm. In the water extract of St. John's wort, antimicrobial effect against *Rhizopus* sp. was not observed.

Regarding the fungi *Aspergillus flavus* and *Fusarium moniliforme* ATCC 38932, an inhibitory effect was found only from the extracts of thyme and cirsium.

The data in table 7 indicated that water extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium had weak antimicrobial activity against pathogenic microorganisms. Inhibitory activity only against Gram-positive *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 8632 was found.

A higher antimicrobial effect was found against *Staphylococcus aureus* ATCC 25923 with inhibition zones between 10 to 13 mm and MIC of 600 ppm. The obtained results showed that *Listeria monocytogenes* ATCC 8632 was more sensitive to the extract of hawthorn (berries). The inhibition zones were up to 10.5 mm, while the MIC value was 60 ppm.

The Gram-positive bacteria were more sensitive to the tested extracts (inhibition zones between 10.5 to 13.5 mm), with MIC of 600 ppm. The tested Gram-negative bacteria were less sensitive and inhibitory effect was not found. This is due to the difference in the structure and composition of the cell wall of the two groups bacteria. The presence of an outer membrane in Gram-negative bacteria makes it difficult for the extracts to diffuse through the membrane to the cell cytoplasm, which makes them more resistant to the action of the studied extracts. The obtained results on the different resistance of Gram-positive and Gram-negative bacteria to microbial growth inhibitors were in correlation with the literature data on aromatic products from the studied herbs (Randrianarivelo *et al*., 2009; Souza *et al*., 2005; Teneva *et al*., 2015).

Krtivokapić *et al*. (2021) found, when studying the phenolic composition of *Hypericum perforatum* L and *Melissa officinalis* L from Montenegro, that the type of solvent used and the altitude where the herbs grown had a significant influence on the composition of the extracted substances. This was also consistent with the established different antimicrobial activity found in the different extracts in our study. The composition of the herbs, which determined their antimicrobial activity was extremely influenced by the climate of the area where the plants grown. In this line of thought, Myrtaj *et al*. (2022) demonstrated that climate influenced the essential oil composition of *Salvia officinalis* in 4 populations of the plant grown in a mountainous region of Southern Albania. The importance of the effects of various extracts from plant sources is increasing in view of their broad-spectrum biological activities, part of which is the antimicrobial effect (Petrović *et al.*, 2022).

Organoleptic analysis

Figures 1 and 2 present the results from the organoleptic analysis of mayonnaise with added oil extracts and mayonnaise sauce with added oil and water extracts. From the data, it could be seen that in terms of appearance and color, mayonnaise with added St. John's wort oil extract was the most prefered, followed by that with added thyme oil extract. While in the case of mayonnaise sauces, sample 3 (cirsium) and 4 (hawthorn-flowers) were the best perceived, and with the most liked color was the sauces with integrated hawthorn extracts (flowers and berries). According to evaluators, the dressings had a light, almost white color, which was due to the low volume of the oil phase. Other authors also confirm the light color of low-fat emulsion products, which, according to them, was also due to the presence of water-phase thickening substances – starch and gums (Karas *et al.*, 2002).

The assortments were with the same consistency and stability. There was no delamination and separation of oil on their surface. In terms of viscosity, the evaluators defined the dressings as products with a rather liquid consistency, which in the case of mayonnaise was spreadable.

With the most intense smell, the evaluators defined mayonnaise containing oil extract of thyme, followed by the same one with St. John's wort and hawthorn-berries. This was also the reason why this assortment was accepted even with the most intense taste. The salty taste as an indicator was not commented by the

evaluators, since the amount of salt was the same for all assortments. The evaluators did not report a rancid taste and smell in the products, which was an indicator of the quality of the vegetable oil used.

After consumption, the aftertaste of thyme and hawthorn-berries was more pronounced, which was rated with the highest intensity of perception. The aftertaste of juniper was bitter and unpleasant, followed by St. John's Wort.

From the results of the tasting analysis of the salad dressing, which are presented graphically in figure 3 it was evident that the same trend was maintained. For all indicators, the dressing with thyme received the highest score, followed by the dressing with cirsium and hawthorn-berries. The lowest scores were obtained for the indicators of St. John's wort and juniper.

The results of the general sensory evaluations of the mayonnaise, mayonnaise sauce and salad dressing assortments are placed in table 8.

Table 6. Antimicrobial activity and minimum inhibitory concentration (MIC) of water extracts against saprophytic microorganisms

Saprophytic microorganisms	Thyme *Thymus callieri* Borbás ex Velen.		St. John'sWort *Hypericum perforatum* L.		Cirsium *Cirsium ligulare* Boiss.		Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.		Hawthorn /berries/ *Crataegus monogyna* Jacq.		Juniper *Juniperus communis* L.	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
Bacillus subtilis ATCC 6633, $1.0.10^9$ cfu/cm³	–*	–	–	–	–	–	–	–	–	–	–	–
Bacillus cereus ATCC 11778, $6.5.10^8$ cfu/cm³	–	–	–	–	–	–	–	–	–	–	–	–
Saccharomyces cerevisiae ATCC 9763, $2.8.10^7$ cfu/cm³	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus niger ATCC 1015, $4.0.10^8$ cfu/cm³	13.00 ±1.00	<60	12.00 ±0.00	<600	12.50 ±0.60	<60	10.00 ±0.00	60	10.00 ±0.00	60	8.00 ±0.00	60
Aspergillus flavus, $4.0.10^8$ cfu/cm³	10.00 ±0.00	60	–	–	11.00 ±0.00	<600	–	–	–	–	–	–
Penicillium sp., $6.0.10^8$ cfu/cm³	10.00 ±0.00	60	10.00 ±0.00	60	10.00 ±0.00	60	12.00 ±0.00	<600	12.00 ±0.00	<600	10.00 ±0.00	60
*Rhizopus*sp., $1.2.10^7$ cfu/cm³	14.00 ±0.40	<60	–	–	10.00 ±2.00	60	8.00 ±0.00	60	12.00 ±0.00	<60	11.00 ±0.00	<60
Fusarium moniliforme ATCC 38932, $1.2.10^7$ cfu/cm³	8.00 ±0.00	60	–	–	8.00 ±0.00	600	–	–	–	–	–	–

–* *not detected*

Table 7. Antimicrobial activitv and minimum inhibitory concentration (MIC) of water extracts against pathogenic microorganisms

Pathogenic microorganisms	Thyme *Thymus callieri* Borbás ex Velen.		St. John'sWort *Hypericum perforatum* L.		Cirsium *Cirsium ligulare* Boiss.		Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.		Hawthorn /berries/ *Crataegus monogyna* Jacq.		Juniper *Juniperus communis* L.	
	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm	IZ, mm	MIC, ppm
Salmonella enteritidis ATCC 13076, 2.0. 10^8 cfu/cm³	–*	–	–	–	–	–	–	–	–	–	–	–
Klebsiella sp. (clinical isolate), 2.7. 10^8 cfu/cm³	–	–	–	–	–	–	–	–	–	–	–	–
Escherichia coli ATCC 8739, 1.0. 10^{12} cfu/cm³	–	–	–	–	–	–	–	–	–	–	–	–
Candida albicans NBIMCC 74, 2.0. 10^{10} cfu/cm³	–	–	–	–	–	–	–	–	–	–	–	–
Listeria monocytogenes ATCC 8632,4.6. 10^9 cfu/cm³	–	–	–	–	–	–	–	–	10.50 ±0.60	60	–	–
Staphylococcus aureus ATCC 25923, 4.0. 10^8 cfu/cm³	13.00 ±1.00	600	10.00 ±1.00	600	10.00 ±1.00	600	13.00 ±1.00	<600	13.50 ±2.00	<600	11.50 ±2.00	<600

–* *not detected*

The results of the individual indicators and those of the general perception of mayonnaise showed that the evaluators perceived the best mayonnaise with integrated oil extracts obtained from thyme, St. John's wort and hawthorn-berries. They corresponded to samples 1, 2 and 5.

A higher general evaluation compared to the other assortments was given to mayonnaise sauce dressing type with added oil and water extracts obtained from thyme, cirsium and hawthorn-berries, samples 1, 3 and 5, respectively. Consumers did not perceive dressings with added extracts of St. John's wort and juniper berries.

Based on the obtained results, three samples of mayonnaise were selected, namely variants 1, 2 and 5, which corresponded to mayonnaise with added extracts of medicinal plants – thyme, St. John's wort and hawthorn-berries.

From the six examined samples of mayonnaise sauce, variants 1, 3 and 5 were selected, respectively with added extracts of thyme, cirsium and hawthorn-berries.

Microbiological analysis – microbial contamination of mayonnaise and mayonnaise sauce

Microbiological analysis on day 1, day 4, day 8, day 12 and day 20 of three mayonnaise variants (variants 1, 2 and 5) and three mayonnaise sauce variants (variants 1, 3 and 5), compared with corresponding control samples was performed. The results demonstrated that during the storage, the presence of *Escherichia coli* and coagulase-positive staphylococci was below the acceptable limits. *Salmonella* sp. in the two control samples and in the three variants of mayonnaise and mayonnaise sauce during the entire storage period was not detected.

The experimental results presented in table 9 showed that mayonnaise (variant 2), prepared with St. John's wort, had a longer shelf life compared to variant 1 (thyme) and variant 5 (hawthorn-berries) mayonnaises, while in the other two variants, after the 8^{th} day, an increase in microbial insemination above the permissible values was observed.

From the results showed in table 10 for mayonnaise sauce, it was found that variant 1 (thyme) had a lower microbial population, which on the 12^{th} day reached 1×10^4 CFU/g, which was in accordance with the permissible values compared to the standard requirements. For mayonnaise sauce variant 3 (cirsium) and variant 5 (hawthorn-berries) on the 12^{th} day, the total plate count (mesophilic aerobic and facultatively anaerobic microorganisms) reached value of 1×10^5 CFU/g, therefore, they had a certain shelf life until the 8^{th} day.

It was found that until the 12^{th} day of the storage, in the controls as well as in the three samples of mayonnaise and the three samples of mayonnaise sauce, the yeasts and fungal counts were below the permissible values, while on the 20^{th} day of the storage, visible fungal growth in all samples was observed.

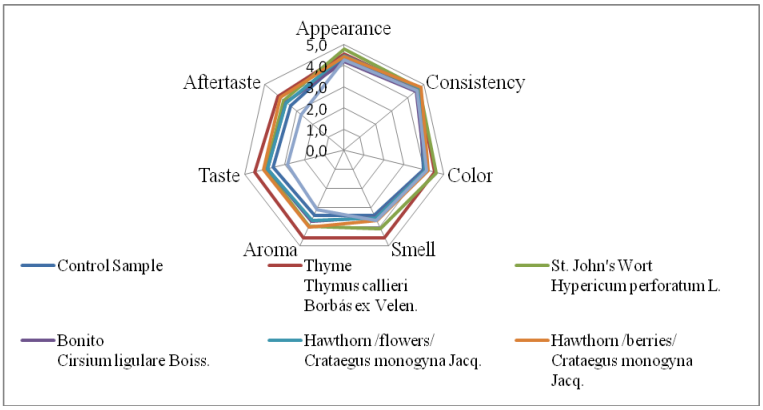

Figure 1. Tasting analysis results of mayonnaise with integrated oil extracts

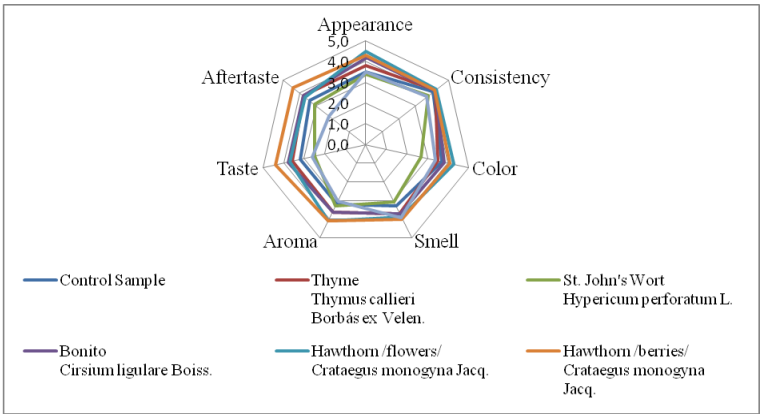

Figure 2. Tasting analysis results of mayonnaise sauce dressing type with integrated oil and water extracts

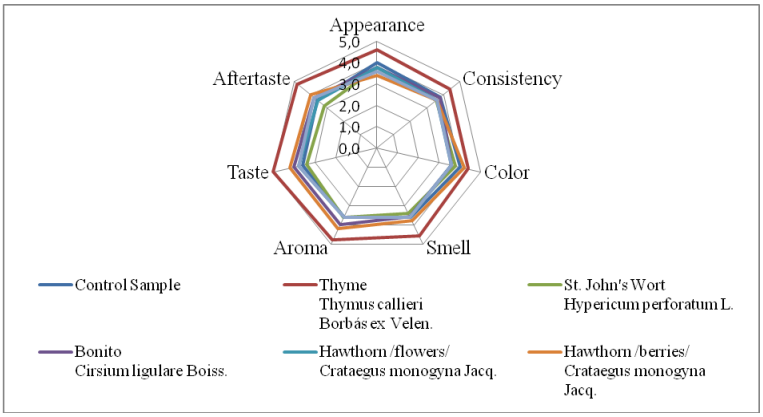

Figure 3. Tasting analysis results of the of salad dressing with oil and water extracts

Table 8. Data on the overall sensory evaluation of the three assortments

General sensory evaluation	Control Sample	Thyme *Thymus callieri Borbás exVelen*	St. John's Wort *Hypericum perforatum* L.	Cirsium *Cirsium ligulare* Boiss.	Hawthorn /flowers with leaves/ *Crataegus monogyna* Jacq.	Hawthorn /berries/ *Crataegus monogyna* Jacq.	Juniper *Juniperus communis* L.
		Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6
Mayonnaise	26.9	31.6	29.9	27.5	28.0	29.1	25.3
Mayonnaise sauce	24.4	26.2	22.3	28.5	27.1	29.5	21.9
Salad dressing	26.2	32.6	25.6	26.6	25.6	27.4	24.8

Table 9. Microbiological indicators during storage of mayonnaise (for 20 days) at a temperature of 4 – 6 °C

Sample	Control					Variant 1 Thyme					Variant 2 St. John'sWort					Variant 5 Hawthorn /berries/				
Day	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20
Total count of mesophilic aerobic and facultative anaerobic bacteria, CFU/g	1×10^3	1×10^4	2.5×10^4	2.5×10^4	Visible mold	1.1×10^2	8×10^2	1×10^3	1×10^5	Visible mold	1.0×10^2	1.3×10^2	1.6×10^3	1.6×10^4	Visible mold	1.1×10^2	1.2×10^2	7.5×10^2	1.3×10^3	Visible mold
Yests, CFU/g	<10	<10	<10	<10		<10	<10	<10	<10		<10	<10	<10	1×10^2		<10	<10	<10	1×10^2	
Fungi, CFU/g	10	20	50	50		<10	<10	10	50		<10	10	10	10		<10	10	20	1×10^2	
Escherichia coli, CFU/g	<10	<10	<10	<10		<10	<10	<10	<10		<10	<10	<10	<10		<10	<10	<10	<10	
Staphylococcus aureus, CFU/g	<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100	
Salmonella, CFU/g	–*	–	–	–		–	–	–	–		–	–	–	–		–	–	–	–	

–* *not detected*

Table 10. Microbiological indicators during storage of mayonnaise sauce (for 20 days) at a temperature of 4 – 6 °C

Sample	Control					Variant 1 Thyme					Variant 3 Cirsium					Variant 5 Hawthorn /berries/				
Day	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20	1	4	8	12	20
Total count of mesophilic aerobic and facultative anaerobic bacteria, CFU/g	7×10^3	1×10^4	4×10^4	7.5×10^4	Visible mold	6.8×10^2	1.3×10^3	9.5×10^3	1×10^4	Visible mold	1×10^3	1×10^4	3×10^4	1×10^5	Visible mold	8×10^3	1.5×10^4	4×10^4	1×10^5	Visible mold
Yests, CFU/g	<10	<10	<10	<10		1.1×10^2	1.2×10^2	1.2×10^2	1.5×10^2		1×10^2	1.1×10^2	1.2×10^2	1.4×10^2		1.0×10^2	1.2×10^2	1.5×10^2	1.8×10^2	
Fungi, CFU/g	10	50	50	20		<10	20	10	20		<10	10	1×10^2	1.5×10^2		<10	<10	10	1×10^2	
Escherichia coli, CFU/g	<10	<10	<10	<10		<10	<10	<10	<10		<10	4×10^4	<10	<10		<10	<10	<10	<10	
Staphylococcus aureus, CFU/g	<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100		<100	<100	<100	<100	
Salmonella, CFU/g	–*	–	–	–		–	–	–	–		–	–	–	–		–	–	–	–	

–* *not detected*

CONCLUSIONS

The following conclusions could be made from the studies carried out:

• The oil extracts from the investigated plant sources exhibited weak antimicrobial activity against the used pathogens. This type of extracts showed

inhibitory activity only against the pathogen *Staphylococcus aureus* ATCC 25923.

• The water extracts of hawthorn (flowers), hawthorn (berries), juniper, thyme, St. John's wort and cirsium suppressed the growth of pathogens *S. aureus* ATCC 25923 and *L. monocytogenes* ATCC 8632, and fungi *A. niger* ATCC 1015, *A. flavus*, *Penicillium* sp., *Rhizopus* sp., *F. moniliforme* ATCC 38932 (agents of microbial spoilage).

• The added oil extracts from the medicinal plants – thyme, St. John's wort and hawthorn improved the organoleptic characteristics of the mayonnaise. The same trend was observed in mayonnaise sauce with added extracts of thyme, cirsium and hawthorn-berries.

• The microbiological research and analysis, necessary for the technological production, safety and control, of the three mayonnaises and three mayonnaise sauces rated the highest by the tasters, and compared with the control samples, were made.

• The obtained results of the present study can be used as a basis for developing of technologies related with obtaining of different groups emulsion products with the addition of extracts from plant sources acting as biopreservatives.

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