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ANALYSIS OF PLANT EXTRACTS WITH ANTIBACTERIAL EFFECTS AND THEIR APPLICATION IN EDIBLE ALGINATE-PECTIN FILMS

Ayten Solak*, Svetla Dyankova, Iliana Nacheva, Maria Doneva, Iliana Lazova-Borisova

Institute of Cryobiology and Food Technologies - Bulgaria, Sofia, Bulgaria
*e-mail: asolak@abv.bg

ABSTRACT

The existing potential risks for consumers from the synthetic antimicrobial additives and the aspiration towards reduction of the possibilities for emergence of microbial resistance reinforce the trends of searching for alternative solutions to protect foods from microbial contamination. One of these solutions is the use of natural plant antibacterial compounds. The aim of this study was to examine the antibacterial activity of extracts derived from sour cherry (*Prunus cerasus*), St. John's Wort (*Hypericum perforatum*), clove (*Syzygium aromaticum*) and smoke tree (*Cotinus coggygria*). The total content of phenols was highest in the extract from smoke tree (31.80 mg GAE / mL) and clove (23.12 mg GAE / mL). All extracts had an inhibiting effect on the tested microorganisms (*Staphylococcus aureus* ATCC 43300 and *Escherichia coli* ATCC 25922). The largest inhibition zones were observed in the extracts from St. John's Wort and smoke tree. A very good result was obtained through the combination of extracts from St. John's Wort and clove (39 mm for *Staphylococcus aureus* and 20 mm for *Escherichia coli*). The extracts from smoke tree and the combination of St. John's Wort and clove extracts preserved their high degree of antibacterial activity when they were included in edible alginate-pectin films. The films obtained in this way may be used in packaging to help reduce the microbial population on the surface of food products.

Key words: antimicrobial activity, biopolymer films, plant extracts, alginate-pectin films.

INTRODUCTION

Approximately 1.3 billion tonnes of food is wasted globally each year, which is about a third of the total food produced for human consumption (FAO, 2019). This assessment covers all food production sectors and all levels of the food supply chain, including final consumers (households). In many cases, food loss is due to food deterioration caused by contamination with spoilage bacteria, yeasts or molds. The growth of microorganisms and their biochemical activity leads to the accumulation of metabolites that make the products unacceptable for consumption (Lianou et al., 2016; Gonelimali et al., 2018). Diseases caused by food-borne pathogens are another widespread problem that directly affects public health.

Synthetic antimicrobial preservatives prevent food spoilage by inhibiting the growth of unwanted microorganisms, but they can also be dangerous due to potential toxic or allergenic effects (Rangan & Barceloux, 2009).

For this reason, the efforts of research groups are directed towards new, natural and safe methods to inhibit the growth of spoilage and pathogenic bacteria and extend the shelf life of food products, without the use of chemical preservatives. The possibilities of using extracts of spices and medicinal plants as natural antimicrobial food additives are being studied. Plant antimicrobial compounds are grouped into different classes based on their chemical structure and properties: essential oils, phenols, alkaloids, saponins, and peptides (Ferdes, 2018).

A significant amount of research has focused on phenolic compounds, which are the most numerous group of secondary metabolites in plants. Secondary metabolites are biosynthesized by plants as a result of biotic (e.g. contamination by phytopathogenic microorganisms) and abiotic factors (UV light). From a safety perspective, only edible plants or herbs with no known toxic effects can be used as potential sources of alternative food preservatives (Oulahal & Degraeve, 2022). Another characteristic of many plant phenols and polyphenols is their variable content in plants as a function of climatic conditions, which must be considered when the goal is to produce plant extracts with standardized antimicrobial activity.
The fruits of the sour cherry *(Prunus cerasus* L.*)* are used in the food industry in the production of jam, juices, liqueurs, etc. There are a limited number of articles on the antibacterial effect of extracts from different parts of the plant. Fruit stems and leaves have been found to be rich in polyphenols (Demiray et al., 2011). Piccirillo and coworkers, investigated ethyl acetate, ethanol and acetone extracts of fruit stems and leaves of Ginja (sour cherry variety, native of Portugal). They found some antibacterial activity in all the extracts, which was most pronounced in the samples extracted with ethyl acetate for all the tested strains of microorganisms (Piccirillo et al.,2013).
A number of studies have demonstrated the antimicrobial properties of *Cotinus coggygria*, *Syzygium aromaticum* and *Hypericum perforatum* extracts against a wide range of microorganisms (Saddiqe et al., 2010; Cortés-Rojas et al., 2014; Matić et al., 2016). The strength and range of the antibacterial effect depend to a large extent on the type of extract, the solvent used and the technological conditions of preparation.
Control of microbial growth in food products by plant extracts can be achieved by applying them in the product itself or by including them in the packaging. In order to achieve effective antimicrobial activity when using the extracts in food, a high concentration is required, which in some cases can negatively affect the aroma and taste of the products. An alternative solution is to incorporate natural antimicrobials into biopolymer films and coatings used as packaging material for food products (Matić et al., 2016).
The aim of the study was to investigate the antibacterial activity of ethanolic extracts obtained from sour cherry, St. John's wort, clove and smoke tree and to develop antibacterial packaging for food products by incorporating extracts of smoke tree and a combination of St. John's wort and clove in a biopolymer film based on sodium alginate-pectin.

MATERIAL AND METHODS

Materials

Sodium alginate was supplied by Biosynth AG, high methoxyl apple pectin (DE - 61.80%) - Herbstreinth & Fox GmbH. Methanol, calcium chloride and Folin - Ciocalteu phenol reagent were supplied by Merck, ethanol and glycerol by Valerus. All reagents and chemicals used are analytical grade.

Plant materials

Sour cherry, St. John’s wort, clove and smoke tree were obtained from a herbal pharmacy in the city of Sofia. The common name, scientific name and plant part used are presented in Table 1.

Table 1. Scientific name of plants and used parts in extraction.

Name	Scientific name	Part used	Pre-treatment of material
Sour cherry	*Prunus cerasus* L.	Stems	Dried and chopped
St. John's wort	*Hypericum perforatum* L.	Aerial part	Dried and chopped
Clove	*Syzygium aromaticum* L.	Flower buds	Dried and chopped
Smoke tree	*Cotinus coggygria Scop.*	Leaves	Dried and chopped

Methods

Plant extracts preparation: The extraction was carried out with 70% V/V ethanol at room temperature, as a stationary 7-day process with raw material 1:10. The samples were filtered through a sintered glass filter (pore size 40μm), the clarified extract was collected and then evaporated to dryness using a rotary evaporator at 50 °C.

Determination of dry matter content in the plant extracts: Dry matter in plant extracts was quantified according to the BDS EN 12145:2000. Quantity of extractive substances is expressed in g/100 mL.

Determination of total phenolic compounds: The content of total phenolic content (TPC) in the extracts was determined by spectrophotometric method with Folin- Ciocâlteu reagent and expressed as gallic acid equivalents - mg GAE /mL (Singleton et al., 1999).

Preparation of a composite alginate-pectin film with plant extracts: Sodium alginate (2.5% w/V) and high methoxyl pectin (4.0% w/V) were dissolved in distilled water. Film forming solution (FFS) was prepared by mixing sodium alginate and high methoxyl pectin aqueous solutions 3:1. The mixture was homogenized by magnetic stirring at room temperature and the relevant ethanolic extract was added at concentration 5mL /100 g FFS. In the control series, the extract was replaced with the corresponding amount of 70% ethanol. Glycerol was used as a plasticizer (0.6 g / g polymer) and obtained mixtures were poured onto Petri dishes (0.325 g FFS / cm^2) and dried under vacuum (20 kPa, SPT-200 Vacuum Drier) at 45°C. Dried samples were immersed for 30 min in 0.3 M $CaCl_2$ solution to allow cross-linking, washed with distilled water to remove excess Ca^{2+} and dried at 35°C.

Moisture content: The moisture content of the obtained films (g/100 g) was measured with Sartorius Thermo Control YTC 01L balances.

Film thickness: The thickness of the films was determined with a digital micrometer with an accuracy of 0.01 mm ± 5% in five randomly selected sections of the sample.

Color: The color parameters of the developed films were evaluated with NR200 Portable Digital Colorimeter (Huanyu), employing CIELab scale. The values of *L* (lightness), *a* (redness) and *b* (yellowness) of the films were measured. The background value was estimated to L^*= 95.98, a^* = 0.24 and b^* = -1.97. Measurements were performed five times for each sample. The parameters of total color difference (ΔE) were calculated by Eq. (1):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (1)$$

Antibacterial activity of extracts

Agar disk diffusion test. The antibacterial properties of the extracts were tested against *Staphylococcus aureus* ATCC 43300 and *Escherichia coli* ATCC 25922. For that purpose, sterile paper discs (6mm) were soaked with 50μL of a 3% solution of the respective dry extract in sterile distilled water and placed on the surface of a Muller-Hinton agar, pre-inoculated with

0.5mL bacterial suspension (10^6 CFU/mL*)*. The plates were incubated at 37°C for 22h. Inhibition zone (IZ) of diameter exceeding 8 mm was considered a positive result. The paper disc impregnated with a solvent was used as a control.

*Determination of minimum inhibitory concentration (*MIC*).* The serial dilution method was used to determine the minimum inhibitory concentration of the extracts. Strains used: methicillin-resistant *Staphylococcus aureus* ATCC 43300 (MRSA), *Escherichia coli* ATTC 25922. Testing was performed in accordance with NCCLS recommendations.

Test for antimicrobial activity of alginate-pectin films with extracts: Discs from the film samples (6 mm) were placed on the surface of Muller-Hinton agar previously inoculated with 0.5mL bacterial suspension (10^6 CFU/mL). After incubation at 37°C for 22 h, the diameter of the zone of inhibition around each disc (IZ) was measured. An IZ greater than 8 mm was considered as a positive result.

Statistical analysis

All tests were done in triplicate. The results were analyzed using the Microsoft Excel 2016 software. Results were presented as mean ± SD and the significance between different data ($p < 0.05$) was evaluated by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The dry matter content in the ethanol extracts of the plant raw materials varied from 1.48 g /100 mL (sour cherry) to 4.38 g / 100mL (smoke tree). The amount of phenolic compounds in the extracts also showed large variations (Table 2 and Figure 1). The highest values for TPC were found in smoke tree (31.80 mg GAE / mL) and clove (23.12 mg GAE / mL) extracts.

Table 2. Dry matter content and total phenols (TPC) in ethanolic extracts of sour cherry, St. John's wort, clove and smoke tree.

Name	Dry matter content (g/100 mL)	TPC (mg GAE / mL)
Sour cherry	1.48 ± 0.05	1.86 ± 0.08
St. John's wort	3.65 ± 0.01	4.42 ± 0.20
Clove	3.41 ± 0.18	23.12 ± 0.35
Smoke tree	4.38 ± 0.26	31.80 ± 0.95

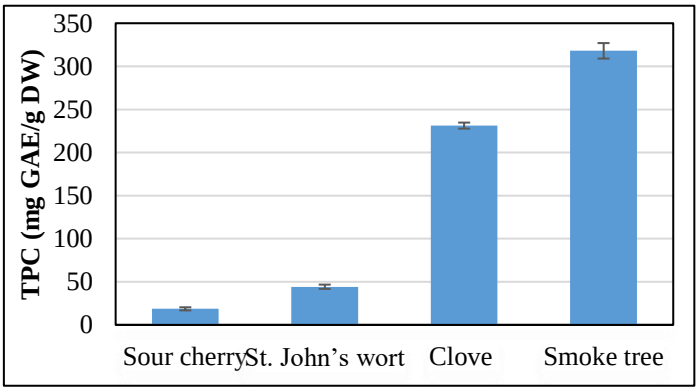

Figure 1. TPC per 1 g plant material (mg / g DW).

All obtained extracts exhibited inhibitory activity against the test microorganisms in the agar disk diffusion test. Regarding *Staphylococcus aureus* (Figure 2), the largest inhibition zones were observed for St. John's wort extract (IZ 40 mm) and the combination of St. John's wort and clove extracts 1:1 (IZ 38 mm), followed by smoke tree (15mm) and clove (14mm).

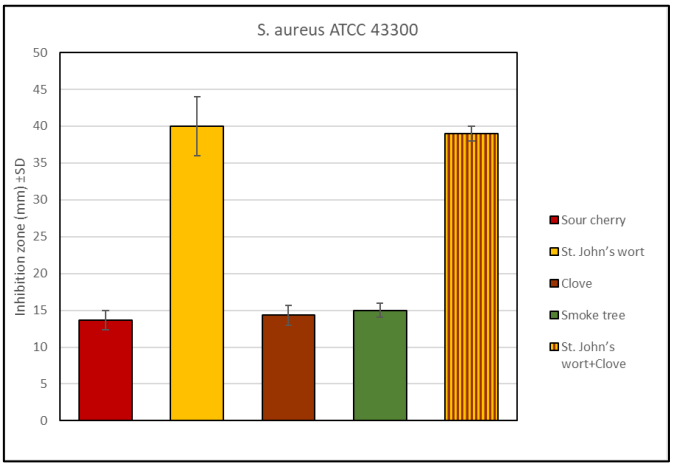

Figure 2. Iinhibition zones of the tested extracts against Staphylococcus aureus ATCC 43300

The antimicrobial activity of the extracts against *Escherichia coli* ATCC 25 922 was weaker, with inhibition zones ranging from 10.7 mm (sour cherry) to 13 mm (smoke tree). It should be noted that the combination of St. John's wort and clove extracts 1:1 showed the highest inhibition zone – 21 mm (Figure 3).

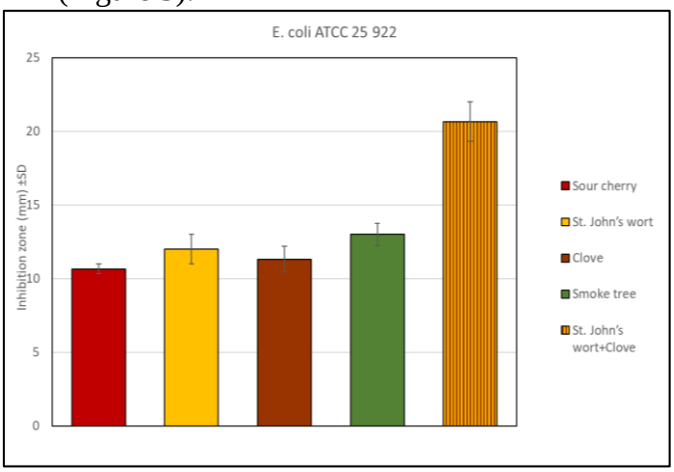

Figure 3. Iinhibition zones of the tested extracts against Escherichia coli ATCC 25 922

Table 3. MIC values (mg / mL) for the extracts.

Extract	Test strains	
	Staphylococcus .aureus ATCC 43300	*Escherichia coli* ATTC 25922
Sour cherry	0.60 mg / mL	ND*
St. John's wort	0.35 mg / mL	3.35 mg / mL
Clove	0.05 mg / mL	1.15 mg / mL
Smoke tree	0.10 mg / mL	0.55 mg / mL

*ND – not detected

The results for a MIC of the individual extracts are presented in Table 3. MIC against *Staphylococcus aureus* varies from 0.05 mg / mL for clove and 0.10 mg / mL for smoke tree, to 0.60 mg / mL for sour cherry. For *Escherichia coli* growth inhibition was observed at significantly higher concentrations - from 0.55 mg / mL (smoke tree) to 3,35 mg / mL (St.

John's wort). Under the test conditions, the sour cherry extract did not show inhibitory activity against *Escherichia coli.*

A number *Escherichia coli* of studies report the in vitro antibacterial activity of crude extracts of the aerial parts of *Hypericum perforatum*. The extracts exhibit more pronounced activity against Gram-positive bacteria than Gram-negative bacteria (Saddiqe et al., 2010). Mazandarani and coworkers, reported the higher antibacterial activity of ethanolic extracts of *Hypericum perforatum* against *Staphylococcus aureus* and weaker against *Escherichia coli* (Mazandarani et al., 2007). These results are in correlation with the values obtained in our study. The strong antimicrobial activity of the extracts of *Cotinus coggygria* was demonstrated on different bacterial species (Matić et al.,2016). Matić and coworkers, studied the in vitro antimicrobial activity of the methanol extract of smoke tree against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Micrococcus lysodeikticus, Candida albicans* and established inhibitory effect against all test microorganisms (Matić et al., 2011). Our results also confirm the strong antibacterial activity of smoke tree extract against *Staphylococcus aureus* and *Escherichia coli*. Unlike the other tested plants, smoke tree is not suitable for direct application in food products as a food preservative, but it can be incorporated into biopolymer packaging films and coatings to achieve an antibacterial effect.

The antimicrobial activities of clove have been proved against several bacterial and fungal strains, including foodborne pathogens. Many studies also confirm the significant inhibitory effect against *Escherichia coli* and *Staphylococcus aureus* (Cortés-Rojas et al., 2014).

Extracts of smoke tree and the combination of St. John's wort and clove extracts showed the highest and most stable antibacterial activity against the two tested strains. They were used to obtain two types of composite films based on alginate-pectin: AP (1) - with smoke tree extract included and AP (2) - with a mixture of St. John's wort and clove extracts included.

The obtained films were easily peeled from the casting support. They were homogeneous, flexible and semitransparent. The properties of films are presented in Table 4.

Table 4. Characteristics of composite films loaded with smoke tree extract and combination of St. John's wort and clove extracts.

Film Properties	Film Type		
	AP (control)	AP (1)	AP(2)
Thickness (mm)	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Moisture content (g / 100 g)	9.29 ± 0.15	9.58 ± 0.22	9.89 ± 0.45
Color properties			
L value	85.20 ± 0.21	57.36 ± 1.08	48.87 ± 0.84
a value	2.24 ± 0.05	20.49 ± 1.08	22.03 ± 0.36
b value	7.18 ± 1.22	41.78 ± 0.57	37.61 ± 0.47
Δ*E* value	14.31 ± 0.62	61.79 ± 0.90	65.27 ± 0.44
Antimicrobial activity IZ (mm)			
Staphylococcus aureus ATCC 43300	-	18 ± 2.0	17 ± 1.5
Escherichia coli ATTC 25922	-	15 ± 2.0	12 ± 2.0

The incorporation of plant extracts into the biopolymer film resulted in a slight increase in the residual moisture content and film thickness values, but the differences were not statistically significant. The effect of the addition of the extracts on the color parameters of the films is significant. In films with added extracts, the values for *L* decreased significantly ($p < 0.05$). A significant increase in *a* and *b* values was observed for AP (1) and AP (2) compared to the control film ($p < 0.05$). Total color difference values increased for films with extracts as Δ*E*

was greater for AP (2). The analysis of color parameters showed a significant darkening of the films as a result of the inclusion of the plant extracts.
Microbiological studies established a high degree of preservation of the antibacterial activity of the mentioned extracts after their inclusion in composite films based on alginate-pectin. One of the possible applications of the resulting films is as packaging, providing control of microbial growth on the surface of the food product.

CONCLUSION

The content of phenolic substances in the ethanolic extracts of sour cherry, St. John's Wort, clove and smoke tree varied in a wide range. The total phenol content was highest in the extract from smoke tree and clove.
All extracts exhibited antibacterial effect against *Staphylococcus aureus* ATCC 43300 and *Escherichia coli* ATCC 25922. The largest zones of inhibition were observed with St. John's wort and smoke tree extracts, followed by clove and sour cherry. The combination of two extracts - St. John's wort and clove (1:1) showed a strong antibacterial effect.
Extracts of smoke tree and the combination of St. John's wort and clove retained a high degree of antibacterial activity after incorporation into edible composite films based on sodium alginate and pectin. The resulting films can be used for packaging, ensuring a reduction of the microbial population on the surface of the food produc
The variants of composite films with extract of smoke tree and a mixture of St. John's wort and clove extracts had a significant change in color parameters compared to the control series.

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