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TESTING THE EFFICIENCY OF 6 ESSENTIAL OILS FOR FOODBORNE PATHOGENS IN ORDER TO SELECT THE MOST SUITABLE FOR APPLICATION IN THE MEAT INDUSTRY

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Abstract

Identifying natural and safe methods for preserving food is an important issue. In this respect, one of the most important foodborne pathogens in ready-to-eat meat products is *Listeria monocytogenes*, which had a high prevalence in global food poisoning outbreaks.

In the study, six essential oils (*Ocimum basilicum, Eucalyptus maculata var. citriodora, Salvia officinalis, Petroselinum crispum, Citrus aurantifolia, Cinnamomum zeylanicum*) were studied for their antimicrobial efficiency against *Listeria monocytogenes* using the Agar Well Diffusion assay, in order to select the three most effective essential oils. Samples were performed in triplicate with positive and negative control.

According to the measured inhibition area, the antimicrobial effect ranking for the studied essential oils was the following: *Cinnamomum zeylanicum* essential oil - 29.00±1.00 mm, *Citrus aurantifolia* - 17.00±0.82 mm and *Ocimum basilicum* - 12.00±0.82 mm.

In conclusion, the efficacy of the selected oils against *L. monocytogenes* is noted, further studies on their activity in food matrices experimentally and naturally contaminated with the studied pathogen being needed.

Key words: essential oils, *Listeria monocytogenes*, ready-to-eat meat products

Listeria monocytogenes is a foodborne pathogen and is the most clinically important species in the genus *Listeria*, along with 28 other distinct species (Rocha et al., 2019). This species is widespread in the environment and can be identified in water, soil, buildings and equipment, with increased incidence also in chicken meat or slaughterhouse waste.

Of the 13 serotypes of *L. monocytogenes*, four are of human health significance. The main source for human listeriosis is through consumption of contaminated food, but vertical or zoonotic transmission is also possible.

Today's lifestyle greatly influences the behaviour and choices of the modern consumer, with the production of Ready-to-Eat (RTE) foods increasing dramatically. *L. monocytogenes* is often associated with such foods, as this bacterium is psychrotrophic. Although some processing steps linked to various operational parameters can inhibit or prevent the growth of *L. monocytogenes*, finished products can be contaminated afterwards (e.g. handling, packaging).

Given the issues related to the ecology of this pathogen and the new outbreaks of listeriosis, research is needed on effective and safe prevention or decontamination methods that align with consumer requirements (Dos Santos et al., 2022).

From this point of view, more and more research is addressing the negative effects of using artificial preservatives, while increasing consumer interest in natural alternatives. A sustainable solution could be the use of plant extracts such as essential oils, many of which have a proven antimicrobial effect on major food pathogens (Georgescu et al.-a, 2018, Georgescu et al.-b, 2018).

In addition to their contribution to food safety, essential oils have been used since ancient times to treat certain diseases or for health maintenance. There are numerous studies on the anti-inflammatory, antioxidant, anti-carcinogenic and other and other beneficial effects which can be the base for functional foods formulations (Bejan et al., 2021).

The aim of this paper was to evaluate the *in vitro* antimicrobial properties against *L. monocytogenes* of 6 commercial essential oils and the selection of the three most efficient for further studies.

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MATERIAL AND METHOD

The essential oils for internal use were purchased in June 2022, from SC BIONOVATIV SRL, Brasov, Romania, being obtained from the following plants: *Ocimum basilicum (OBO), Eucalyptus maculata var. citriodora (EMO), Salvia officinalis (SOO), Petroselinum crispum (PCO), Citrus aurantifolia (CAO), Cinnamomum zeylanicum (CZO).* Based on the producer's declaration and label informations, the purity of the EOs was 100%. The selection protocol was based on a literature survey regarding their antimicrobial and health effects.

Methods of investigation

The Agar Well Diffusion assay was used to test the *in vitro* efficacy of essential oils, with samples divided into two test groups (3 oils per plate) (Reeves, 1989). The first testing group was represented by the EOs extracted from *Ocimum basilicum, Eucalyptus maculata var. citriodora, Salvia officinalis*, and the second one, those obtained from *Petroselinum crispum, Citrus aurantifolia, Cinnamomum zeylanicum*.

Samples were performed in triplicate with positive (Ampiciline – AMP) and negative control (sterile peptone saline) (Table 1). The positive and negative control were tested on different Muller Hinton Agar (MHA) plates (Thermo Scientific™ Oxoid™).

The strain used was *L. monocytogenes ATCC 13932*, BioMérieux™, reconstituted according to the manufacturer's recommendations and inoculated onto culture media for fresh use. The colonies formed were suspended in sterile peptone saline.

Bacterial suspensions had an initial turbidity of 0.5 McFarland (1.5×10^8 CFU/ml), with serial dilutions up to 1.5×10^4 CFU/ml. 100 µL of the previously prepared bacterial suspension was inoculated into MHA plates, according to the protocol (9). Subsequently, 8 mm diameter wells were cut using a sterile cork-borer and filled with the tested EOs. After one hour, the samples were incubated at 37^{O}C for 24 hours in TCR100 and Pol-EKO type II W-400 STD thermostats.

After incubation, the results were read by measuring the inhibition zones (clear areas without microbial growth) and expressed in mm.

Statistical analysis was performed using Microsoft Excel 2016 and expressed as mean ± standard deviation. In addition, considering the efficiency of the positive control (AMP) 100%, the percentages were reported according to the observations.

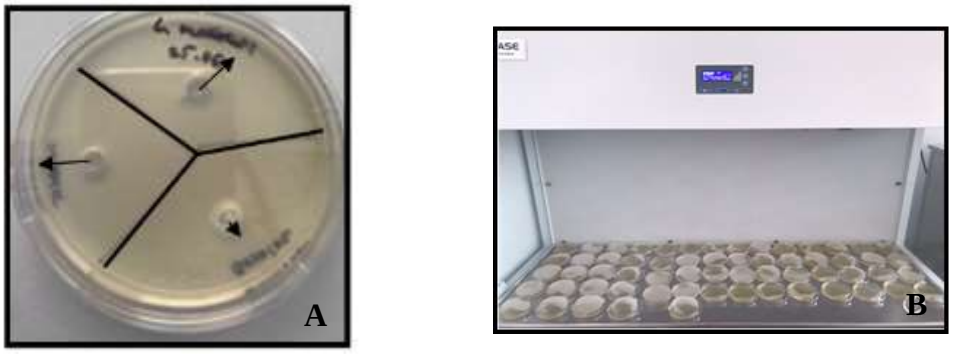


Figure 2 **– A) Inhibiton zone measuring method, B) MHA plates preparation**

RESULTS AND DISCUSSIONS

In our research, CZO had the highest inhibition zone (29.00±1.00). Unlu et al. (2010) obtained similar results in a study conducted to determine the composition, antimicrobial and cytotoxic activity of cinnamon essential oil. Although the working protocol was different, using the Disk Diffusion method and *L. monocytogenes* strains F 1483 and F 1462, the inhibition zones ranged from 32-35 mm (including 6 mm disk diameter). Similarly, Al-Fekaiki et al. (2017), obtained an inhibition zone ranging from 21.30±0.31 to 29.86±0.40 for CZO concentrations ranging from 6µL to 18µL (8).

For CAO, no research on the Agar Well Diffusion or Disk Diffusion assays with the targeted pathogen was identified, a situation also encountered for EMO and PCO.

On the other hand, Costa et. al (2014), observed a minimum inhibitory concentration of 0.25 (% v/v) for *Listeria monocytogenes*, the effect of CAO being demonstrated.

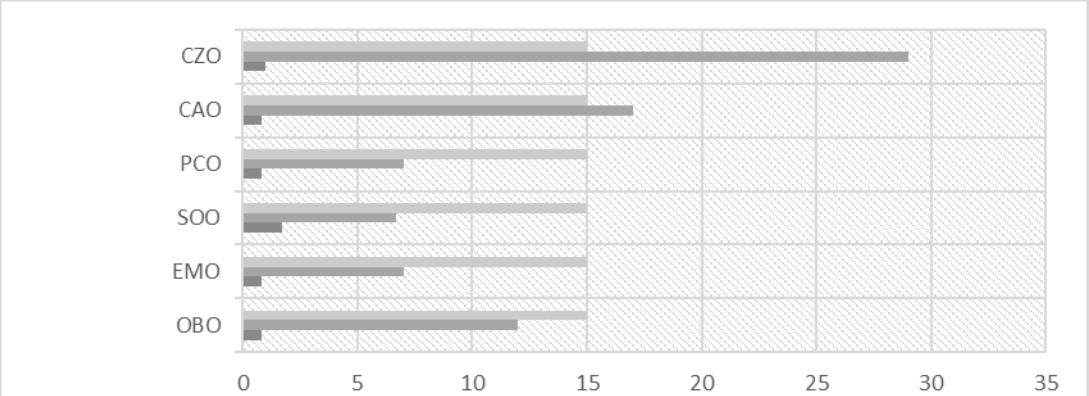
Hossain et al (2010) observed inhibition zones ranging from 15.1±1.5 to 17.1±1.2 for OBO tested by the Disk Diffusion method, these results being higher compared to those obtained in the present research.

SOO had an inhibition zone of 6.67±1.70, which was lower compared to the data obtained by

Ed-Dra et al., (2020) - 10.2±0.1 mm - 17.5±0.3 mm.

Regarding the inhibition zone of the positive control, CAO (113.3%) and CZO (193.33%) showed higher efficiency, other results being presented in Table 1 and in Figure 2.

The antimicrobial potentiation effect was also observed in CAO and EMO, being highlighted by the confluence of the inhibition zones. In this regard, CAO and EMO, are of interest for further studies.



	OBO	EMO	SOO	PCO	CAO	CZO
Positive control	15	15	15	15	15	15
Inhibition zone (mm)	12	7	6,67	7	17	29
Std. dev.	0,82	0,82	1,7	0,82	0,82	1

Figure 2 ***L. monocytogenes* antimicrobial efficiency for the tested EOs**

Table 1

Results of EOs *in vitro* antimicrobial testing

Nr. crt.	*Common name*	*Scientific name*	Inhibition zone (mm) and %	Positive control (AMP)
1	**Basil**	*Ocimum basilicum*	12.00±0.82 80%	15 mm
2	**Brazilian eucalyptus**	*Eucalyptus maculata var. citriodora*	7.00±0.82 46.6%	15 mm
3	**Sage**	*Salvia officinalis*	6.67±1.70 44.46%	15 mm
4	**Parsley seeds**	*Petroselinum crispum*	7.00±0.82 46.6%	15 mm
5	**Lime**	*Citrus aurantifolia*	17.00±0.82 113.3%	15 mm
6	**Cinnamon**	*Cinnamomum zeylanicum*	29.00±1.00 193.33%	15 mm

CONCLUSIONS

The most intense antimicrobial effect on *L. monocytogenes* is noted in *Cinnamomun zeylanicum* and *Citrus aurantifolia* essential oils, with *Ocimum basilicum* having medium efficacy. From this point of view, these essential oils can qualify for further investigations, such as Minimum Inhibitory Concentration and concentrations testing for food products enhancements.

Also, given the potentiation effects between CAO and EMO, further studies to investigate similar effects in relation to other pathogens would be useful.

It is noted that these EOs are natural alternative candidates for traditional chemical food preservatives.

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