Illicium verum Essential Oil Antimicrobial Activity *in vitro* and *in situ*

Natália Čmiková[1], Simona Kunová[2], Miroslava Kačániová[1]

[1]*Slovak University of Agriculture, Faculty of Horticulture and Landscape Engineering, Institution of Horticulture, Tr. A. Hlinku 2, 94976 Nitra, Slovakia*
[2]*Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Institution of Food Sciences, Tr. A. Hlinku 2, 94976 Nitra, Slovakia*

Abstract
Star anise, or *Illicium verum*, is a widely used term. Star anise, a culinary flavor that is primarily used in Asian nations, is derived from the evergreen Chinese plant *Illicium verum*. In addition to being flavorful, it has significant medicinal value. *Illicium verum* has strong antimicrobial properties, and was once used to treat a variety of illnesses with microbial origins. In our study the antimicrobial activity of *Illicum verum* essential oil (IVEO) against ten microorganisms *Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis* CCM 8223, *Staphylococcus aureus* subsp. *aureus* CCM 4223, *Micrococcus luteus* CCM 732, *Listeria monocytogenes* CCM 4699, *Escherichia coli* CCM 3954, *Pseudomonas putida* CCM 1977, and *Enterobacter aerogenes* CCM 2531 were tested *in vitro* and *in situ*. The antimicrobial activity of CCEO was evaluated using the disc diffusion method *in vitro* and in vapor phase on carrot *in situ*. The Chinese star anise essential oil showed the best antibacterial and antifungal activity against *Listeria monocytogenes* and *Candida glabrata in vitro*. The best results of antimicrobial activity in vapor phase on carrot were found against *Micrococcus luteus* and *Candida tropicalis in situ*. The results of this study suggest that IVEO is more active against Gram-positive bacteria and star anise essential oil could be used as a natural antimicrobial agent.

Keywords: *Illicium verum* essential oil, antimicrobial activity, *in vitro*, *in situ*, bacteria, candida

1. Introduction

An aromatic evergreen tree in the Illiciaceae family called *Illicium verum* has purple-red flowers, and star-shaped fruit that has an anise fragrance. It primarily grows in Vietnam and southern China, and is important for Asian cuisine as a spice [1, 2]. Before they ripen, the fruits are picked and sun-dried. The fruits ripen from September to October, and the blossoms are visible from March to May [3]. It is a well-regarded medicinal plant with numerous therapeutic benefits [4, 5]. Common applications for star anise essential oil include antibacterial, and topical treatment for rheumatism [6–10].

* Corresponding author: Natália Čmiková, +421376414715, n.cmikova@gmail.com

Consequently, the main focus of chemical research on *Illicium verum* has been on essential oils (IVEO). Depending on the harvest period, the season, the country of origin, and whether the plant is fresh or dry, different factors affect the composition and quantity of essential oils [11]. IVEO has number of possible uses, including estrogenic effects, antimicrobial, antifungal, anthelmintic, insecticidal, secretolytic, antinociceptive, anti-inflammatory, gastroprotective, and sedative properties [12–14]. Fruits contain numerous alkaloids, and tannins, as well as limone, α-pinene, safrol, β-phellandrene, α-terpineol, and farnesol, which are both cis- and trans-anethole and range in concentration from 85 % to 90 % [15–17].
According to a study by Huang et al. [6], which tested both the essential oil and trans-anethole as well as their potent inhibitory effect against the tested fungi, most of the antifungal properties were

likely caused by the presence of trans-anethole in the oil, which could be developed as natural fungicides for plant disease control in fruit and vegetable canning.

The aim of the study was to determine antimicrobial activity of IVEO against ten microorganisms *in vitro* and *in situ* on a carrot model.

2. Materials and methods

Essential oil

Illicium verum essential oil (IVEO) origin of China was purchased from Hanus s.r.o. (Nitra, Slovakia). Trans-anethole, methyl chavicol, limonene, cis-anethole, alpha pinene, cubebene, pheniculin, α-caryophyllene, beta caryophyllene, p-anisaldehyde, eugenol, and linalool were the main components of the IVEO made by distillation of dried fruits.

Microorganisms

Three Gram-positive bacteria *Staphylococcus aureus* subsp. *aureus* CCM 4223, *Micrococcus luteus* CCM 732, *Listeria monocytogenes* CCM 4699, three Gram-negative bacteria *Escherichia coli* CCM 3954, *Pseudomonas putida* CCM 1977, and *Enterobacter aerogenes* CCM 2531, and four yeasts *Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis* CCM 8223, were obtained from Czech Collection of Microorganisms (CCM, Brno, Czech republic).

Disc diffusion method

The disc diffusion method was used to evaluate the antimicrobial activity of IVEO. Use of IVEO at concentrations of 100 %, 50 %, 25 %, and 12.5 % were made. Bacteria were cultivated for 24 hours on Tryptone soya agar (TSA, Oxoid, Basingstoke, UK), while yeast inoculum was grown for 24 hours on Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK). Inoculums of bacteria and yeast were maintained at 37 °C and 25 °C. The microbial colony was grown to an optical density of 0.5 McFarland standard (1.5 10^8 CFU/mL) using purified water. One hundred microliters of cultures were distributed on either Mueller-Hinton agar (MHA, Oxoid, Basingstoke, UK) for bacteria or Sabouraud dextrose agar (SDA, Oxoid, Basingstoke, UK) for yeasts. The microbial suspension was then covered with sterile 6 mm discs filled with 10 µL of IVEO, and the plates were incubated for 24 hours at 37 °C for bacteria, and 25 °C for yeasts. Inhibition zones were measured on three sides of the filter's edge, and the results were interpreted as follows: inhibition zones greater than 10 mm had extremely potent antimicrobial activity, 10-5 mm had moderate activity, and 5-1 mm had weak activity. The medicines used as yeast and bacteria controls were meropenem (Oxoid, Basingstoke, UK) and fluconazole (Oxoid, Basingstoke, UK). All measurements were performed in triplicate.

Vapor phase

In 60 mm Petri dish (PD) and the lid, warm MHA was added. On the agar were added carrot slices that were 0.5 mm thick. Then, as previously mentioned, an inoculum was made. IVEO was applied to sterilized filter paper after being diluted in ethyl acetate to 500, 250, 125, and 62.5 mL/L. The leftover ethyl acetate was evaporated for 1 minute, then the dishes were sealed and incubated for 7 days at 37 °C.

Stereological techniques were employed to estimate microbial development *in situ*. In this idea, ImageJ software was used to determine the volume density (Vv) of microbial colonies by counting the points of the stereological grid that hit the colonies (P) and those that fell to the reference space (p) (growth substrate used). Therefore, the following formula was used to determine the volume density of bacterial colonies: Vv (%) = P/p. BGI = [(C T)/C] 100, where C and T are the bacterial growth rates (expressed as Vv in the control group and treated group) were used to measure the antibacterial activity of EO. Results that were negative served to stimulate development.

Statistical analyses

Each test and study were run three times. The mean and standard deviation were computed using Microsoft Excel 2020 (SD).

3. Results and discussion

Antimicrobial activity of IVEO against Gram-positive bacteria

The information obtained from the standard IVEO antimicrobial assay was compared to the information obtained from the standard antibiotic e-strips against the same group of clinical MRSA isolates. When compared to standard antibiotic e-strips, the antimicrobial assay test results of IVEO against clinical isolates of the MRSA revealed

encouraging results. The outcomes demonstrated the drug's susceptibility to all MRSA clinical isolates, with inhibition zones measuring between 18 and 28 mm in diameter (mean 24.05 mm). The tested extract was verified to be effective against the extremely virulent Methicillin-Resistant *Staphylococcus aureus* (MRSA) clinical isolates by MBC and MIC results of 0.97 mg/mL and 0.70 mg/mL [7].

The antimicrobial activity of IVEO against *S. aureus* showed Figure 1. The best antimicrobial activity was found with 100 % concentration (18.67±0.58 mm). The antimicrobial activity of IVEO against *M. luteus* showed Figure 2. The best antimicrobial activity was found with 100 % concentration (16.33±0.58 mm). The antimicrobial activity of IVEO against *L. monocytogenes* showed Figure 3. The best antimicrobial activity was found with 100 % concentration (16.67±0.58 mm).

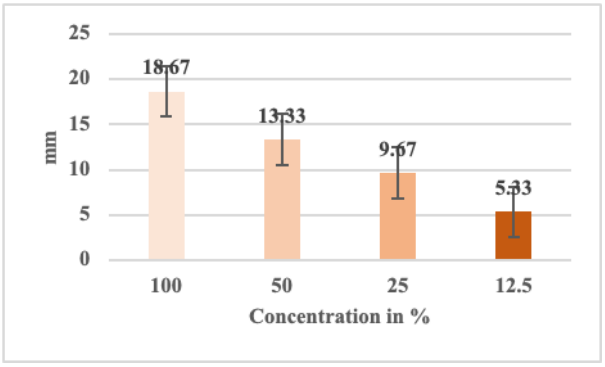

Figure 1. Antimicrobial activity of IVEO against *Staphylococcus aureus*

IVEO potent antimicrobial properties can therefore be ascribed to the substance's phenolic and flavonoid content. Notably, a prior study demonstrated that the antioxidant activity of the star anise ethanol fraction was greater than that of the non-polar petroleum ether fraction because phenolic compounds are more soluble in ethanol [18].

Antimicrobial activity of IVEO against Gram-negative bacteria

Several plants, including *Illicium verum* (badiane or star anise), *Crataegus oxyacantha* ssp. *monogyna* (hawthorn), and *Allium cepa*, have therapeutic promise (onion). A small tree known as *I. verum* (Schisandraceae) is found in the southern part of China and the northern part of Vietnam. It is an aromatic plant that yields oils like anethole and contains some polyphenols, such as flavonols (quercetin and kaempferol), anthocyanins, tannins, and phenolic acids like shikimic and gallic acid. It also includes some flavonols, such as kaempferol [1, 19].

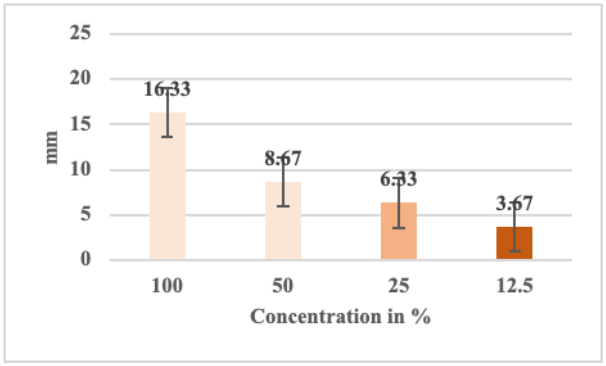

Figure 2. Antimicrobial activity of IVEO against *Micrococcus luteus*

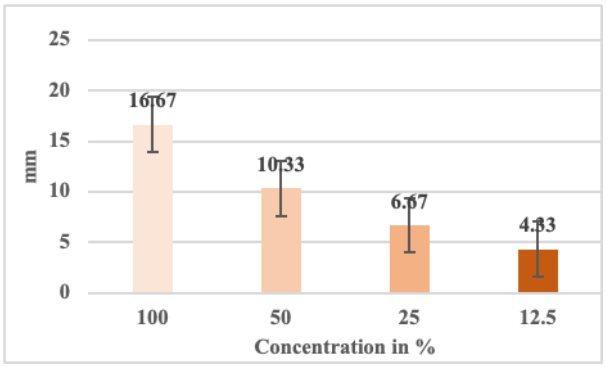

Figure 3. Antimicrobial activity of IVEO against *Listeria monocytogenes*

Antimicrobial, antifungal, and antioxidant activities have been reported for *I. verum* [16, 20, 21].

When antimicrobial activity is evaluated, it is typically anticipated that the majority of the tested materials will be effective against Gram-positive bacteria [22, 23].

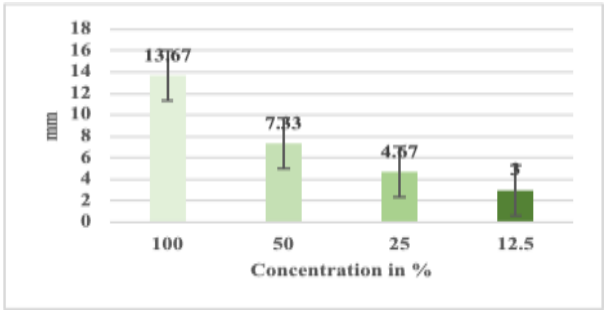

Figure 4. Antimicrobial activity of IVEO against *Escherichia coli*

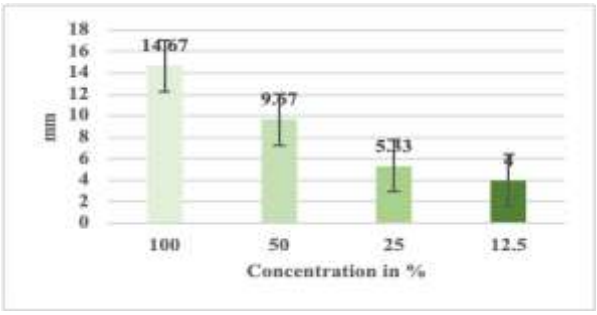

Figure 5. Antimicrobial activity of IVEO against *Pseudomonas putida*

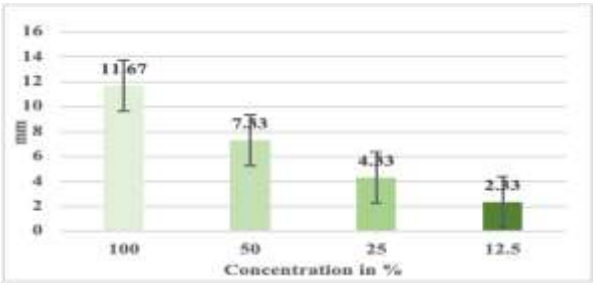

Figure 6. Antimicrobial activity of IVEO against *Enterobacter aerogenes*

The antimicrobial activity of IVEO against *E. coli* showed Figure 4. The best antimicrobial activity was found with 100 % concentration (13.67±0.58 mm). The antimicrobial activity of IVEO against *M. luteus* showed Figure 5. The best antimicrobial activity was found with 100 % concentration (14.67±0.58 mm). The antimicrobial activity of IVEO against *L. monocytogenes* showed Figure 6. The best antimicrobial activity was found with 100 % concentration (11.67±0.58 mm).

Using the disc diffusion method, *Illicium verum* was tested for its antibacterial activity against *E. coli* and *S. aureus*. The width of the inhibition zone provides a clue as to how sensitive the test bacteria are; the larger the inhibition zone, the more potent the antibacterial effect [24].

Anti-candida activity of IVEO

The antimicrobial activity of IVEO against *C. albicans* showed Figure 7. The best antimicrobial activity was found with 100 % concentration (13.33±0.58 mm). The antimicrobial activity of IVEO against *C. glabrata* showed Figure 8. The best antimicrobial activity was found with 100 % concentration (18.33±0.58 mm). The antimicrobial activity of IVEO against *C. krusei* showed Figure 9. The best antimicrobial activity was found with 100 % concentration (14.33±0.58 mm). The antimicrobial activity of IVEO against *C. tropicalis* showed Figure 10. The best antimicrobial activity was found with 100 % concentration (15.67±0.58 mm).

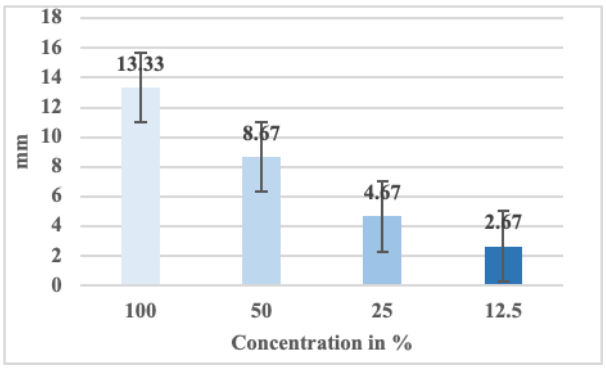

Figure 7. Antimicrobial activity of IVEO against *Candida albicans*

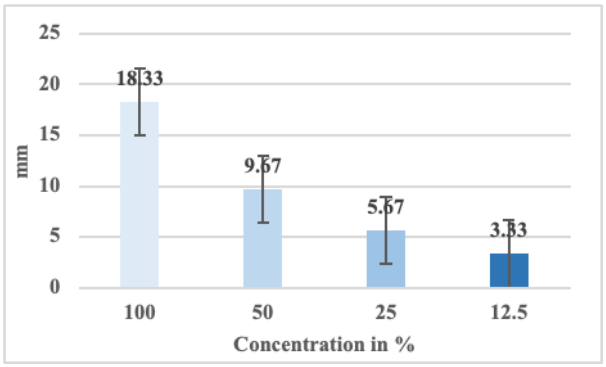

Figure 8. Antimicrobial activity of IVEO against *Candida glabrata*

All of the test fungi were clearly inhibited by the essential oil, and the IC50 values varied from 0.07 mg/mL to 0.25 mg/mL. This suggested that the oil had a wide range of action against all plant pathogenic fungi that had been tested. The comparatively sensitive fungi among them were *A. solani*, *B. maydis*, *F. graminearum*, *P. aphanidermatum*, and *R. solani*, with respective IC50 values of 0.09 mg/mL, 0.07 mg/mL, 0.08 mg/mL, 0.09 mg/mL, and 0.08 mg/mL [18].

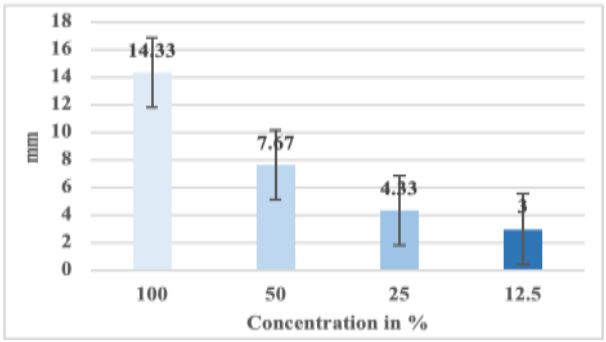

Figure 9. Antimicrobial activity of IVEO against *Candida krusei*

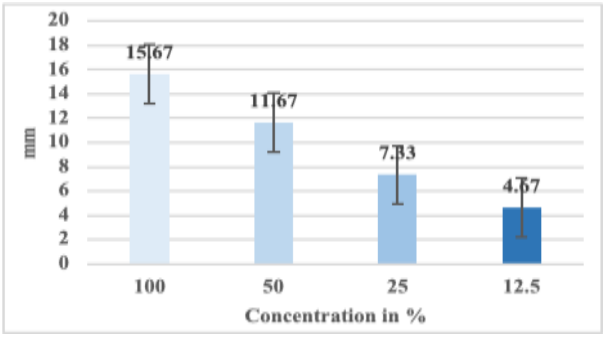

Figure 10. Antimicrobial activity of IVEO against *Candida tropicalis*

Antimicrobial activity *in situ*

In our study, was assessed IVEO vapor phase's antimicrobial potential (Table 1). Examining the impacts of various IVEO vapor phase concentrations on Gram-positive, Gram-negative, and yeasts growing on carrots. Results generally show that IVEO has potent antibacterial properties against all Gram-positive and Gram-negative bacteria. The best antimicrobial action against *M. luteus* and *S. aureus* among the tested Gram-positive bacteria strains was demonstrated by IVEO at the applied concentration of 500 µL/L (95.87±4.58 % and 95.64±3.26 %,).

Two post-harvest pathogens, *P. aphanidermatum* and *B. theobromae*, were selected to evaluate the antifungal activity of the vapor components in *I. verum* essential oil as well as trans-anethole [18]. This suggests that the volatile components of *I. verum* essential oil may be used as fumigants for the control of post-harvest plant diseases in the protection of fruits and vegetables.

Table 1. *In situ* analysis of the antimicrobial activity of the vapor phase of IVEO on carrot

Microbial growth inhibition [%]				
Concentration of EO / **Microorganisms**	**62.5 µL/L**	**125 µL/L**	**250 µL/L**	**500 µL/L**
S. aureus	17.77±1.05	33.56±3.76	67.56±4.67	93.67±5.78
M. luteus	16.84±2.59	35.78±5.16	68.65±4.89	95.87±4.58
L. monocytogenes	13.88±4.88	34.67±3.18	64.67±3.56	95.64±3.26
E. coli	17.55±3.23	31.89±4.55	61.78±6.87	91.56±4.33
P. putida	15.88±2.55	29.67±4.76	62.67±3.89	92.86±3.66
E. aerogenes	14.66±3.76	31.78±2.78	62.56±3.67	94.55±4.67
C. albicans	11.12±0.80	28.67±3.45	58.98±2.45	89.67±4.67
C. glabrata	10.11±1.00	27.67±4.56	52.78±3.78	85.67±4.33
C. krusei	9.18±1.62	28.67±3.67	51.89±5.78	87.89±3.64
C. tropicalis	11.85±3.44	29.78±4.34	58.67±4.67	86.67±5.26

4. Conclusions

This research demonstrated the well spectrum of inhibitory action against Gram-positive bacteria, Gram-negative bacteria, and yeasts that *I. verum* fruit essential oil had. The best antimicrobial effect has *Illicium verum* essential oil against Gram-positive bacteria *in vitro* and *in situ*.

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