In vitro and *in situ* Antibacterial and Insecticidal Activity of *Fragaria ananassa* Essential Oil

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Abstract
In folk medicine, the fruits and leaves of Fragaria ananassa have been used as a source of vitamins, as an agent in the treatment of inflammation, and as a diuretic, diarrhea and choleretic agent. It is used in the treatment of anaemia, in diseases of the gastrointestinal tract. Besides, the leaves have a styptic, astringent, wound-healing effect and are used for preparation of infusions, tinctures and tea. Infusion and decoction of leaves are applied at inflammation of mucous membranes of an oral cavity, at a headache, at jaundice, as an antiseptic and externally - at rashes and dermatitis. Infusion of leaves of *F. ananassa* shows antibacterial activity with respect to various microorganisms. The essential oil of *F. ananassa* was used in this study to determine its antibacterial potential against Gram-negative and Gram-positive bacteria and its insecticidal activity against insect. Bacteria from the Czech Collection of Microorganisms *Bacillus subtilis, Paenibacillus larvae* and *Listeria ivanovii* from the group of Gram-positive bacteria and *Enterobacter aerogenes, Klebsiella pneumoniae* and *Shigella sonnei* from the group of Gram-positive bacteria were used in the study to test the antibacterial activity. *Megabruchidius dorsalis* was used as the model insect for insecticidal activity. The best antimicrobial activity was found against *P. larvae* under *in vitro* conditions, as well as under *in situ* conditions on pear and parsley models. Insecticidal activity against *M. dorsalis* was observed at the highest concentrations. *F. ananassa* essential oil demonstrated very good antibacterial activity against various bacterial species under *in vitro* and *in situ* conditions, as well as good potential against insects.

Keywords: insecticidal activity, antibacterial potential *in vitro and in situ, Fragaria ananassa* essential oil.

1. Introduction

The four primary pillars that promote food security are as follows: food access, food usage, food stability, and food preservation. The latter is chiefly concerned with the deterioration of foodstuffs and the presence of microbiological contaminants. It is imperative to implement measures that will prevent food spoilage. A plethora of chemical preservatives have been developed and their efficacy in mitigating this deterioration has been demonstrated. Nevertheless, due to their non-green origin, they have frequently evoked concerns among consumers. Due to their extensive array of antibacterial, antifungal, antimycotoxigenic, and antioxidant properties, essential oils (EOs) and their active constituents are being investigated for their potential application as preservatives [1]. As EOs can be added directly to edible items or utilised for active packaging and edible coatings, their use in the food sector is expanding [2]. The employment of essential oils in the food industry engenders a dual effect, attributable to their inherent antibacterial and antioxidant properties [3]. Furthermore, the bioactive substances found in EOs may have applications in cosmetic and medicinal fields [4]. In general, EOs are

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substances that are typically extracted from agro-industrial by-products, which include leaves, bark, seeds, fruit peels, roots, flowers, buds, and stems. The complex blend of terpenes, terpenoids, and phenylpropanoids that constitute essential oils has been demonstrated to be associated with their antioxidant properties. It has been established that carvacrol, thymol, and eugenol are amongst the substances capable of preventing oxidation processes [5]. It has been demonstrated that the same is true of the EOs' antibacterial activity. In this instance, however, it is the precise chemical components that determine the method. The most prevalent process appears to be associated with changes in membranes that alter their permeability and dynamicity, thereby releasing the contents of the cytoplasm. However, the effect varies for each microbe based on the cellular metabolic activity, membrane composition, and thickness variations [1].

The utilisation of essential oils (EOs) as preservatives within foodstuffs may be constrained by several factors. Firstly, their pungent scent can be a hindrance. Secondly, their high reactivity can be problematic. Thirdly, their hydrophobicity can compromise their solubility, which can result in unfavourable interactions with other substances. These interactions can lead to changes in intestinal absorption and organoleptic qualities. However, the advent of newly researched and created processes for encapsulating such EOs has enabled their avoidance. By enhancing their stability and solubility, these techniques can shield essential oils (EOs) from environmental interactions [6].

In this work, the antibacterial capabilities of *Fragaria ananassa* essential oil against both Gram-positive and Gram-negative bacteria as well as its insecticidal efficacy against insects were assessed.

2. Materials and methods

2.1. Essential oil

Fragaria ananassa essential oil was purchased from Inevita SK (Bratislava, Slovakia). The essential oil was stored at 4 °C before use. The essential oil was produced by CO_2 extraction of the fruit. The country of origin was India.

2.2. Bacterial strains

The antibacterial activity of *Fragaria ananassa* essential oil (FAEO) was evaluated against a range of Gram-negative (G^-) bacterial strains including *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* CCM 2318, and *Shigella sonnei* CCM 1373, and Gram-positive (G^+) bacteria *Bacillus subtilis* subsp. *Spizizenii* CCM 1999, *Listeria ivanovii* CCM 5884, *Paenibacillus larvae* CCM 4483**.** All bacterial strains were obtained from the Czech Collection of Microorganisms in Brno, Czech Republic. Bacterial inocula were cultured in Mueller-Hinton broth (MHB, Oxoid, Basingstoke, UK) for 24 h at 37 °C before analysis. The optical density of the inocula was adjusted to 0.5 McFarland standard on the day of the experiment.

2.3. Disc diffusion method

In an effort to assess the antimicrobial activity, we opted for the disc diffusion method, a technique that we have previously outlined. We proceeded with the preparation of small discs (6 mm in diameter) that were saturated with FAEO and placed them on Mueller-Hinton agar (MHA) for the bacterial strains. The bacterial strains were then incubated at 37 °C for a duration of 24 hours. To conclude the process, we measured the inhibition zones in mm. The blank discs were used as negative controls, while the antibiotic discs (cefoxitin for Gram-positive bacteria, gentamicin for Gram-negative bacteria, from Oxoid, Basingstoke, UK) served as positive controls [7].

2.4. In situ antimicrobial activity

The present study set out to assess the in situ antimicrobial activity of FAEO. A range of substrates were tested for this purpose, and these included commercial pear and parsley, as well as specific Gram-positive and Gram-negative bacteria. The substrates were sliced into pieces measuring 0.5 mm, thoroughly cleaned, and placed in 60 mm Petri dishes. These dishes had previously been inoculated with bacterial samples. FAEO was dispersed in ethyl acetate at concentrations of 500, 250, 125, and 62.5 µg/ml. Ethyl acetate filter sheets served as controls. The plates were hermetically sealed and then placed within an incubator set at 37 °C for a period of

seven days. Assessment of microbial colony growth was facilitated using the ImageJ software to calculate bacterial volume densities. This was undertaken alongside standard methods for measuring in situ colony development [7].

2.4. Insecticidal activity

Megabruchidius dorsalis Fahreus, 1839, was used as the model organism to evaluate the insecticidal activity of FAEO. Petri dishes lined with sterile filter paper were used to hold groups of fifty *M. dorsalis* insects. In order to create different concentrations of FAEO (100, 50, 25, 12.5, 6.25 and 3.12 %), the FAEO was diluted with a 0.1 % polysorbate solution. After saturating sterile filter paper discs with 100 µL of each FAEO concentration, the plates were sealed with parafilm and left at room temperature for 24 hours. One hundred microliters of the 0.1 % polysorbate solution were given to the control group. The quantity of both living and dead insects was counted after a whole day. Three different research have successfully duplicated this experimental process.

3. Results and discussion

The best antibacterial activity of FAEO was found against *Paenibacillus larvae* (14.33 mm). The lowest antibacterial was found against *Shigella sonnei* (7.33 mm). The results of our experiments compared antibiotic resistance as a positive control in relation to antimicrobial activity. We found that the antibiotic resistance values were higher when compared with the antimicrobial activity of FAEO (Figure 1).
In situ analyses show the best antibacterial activity in lower concentration 62.5 µg/mL on both models against *Paenibacillus larvae* (Figure 2 and 3).

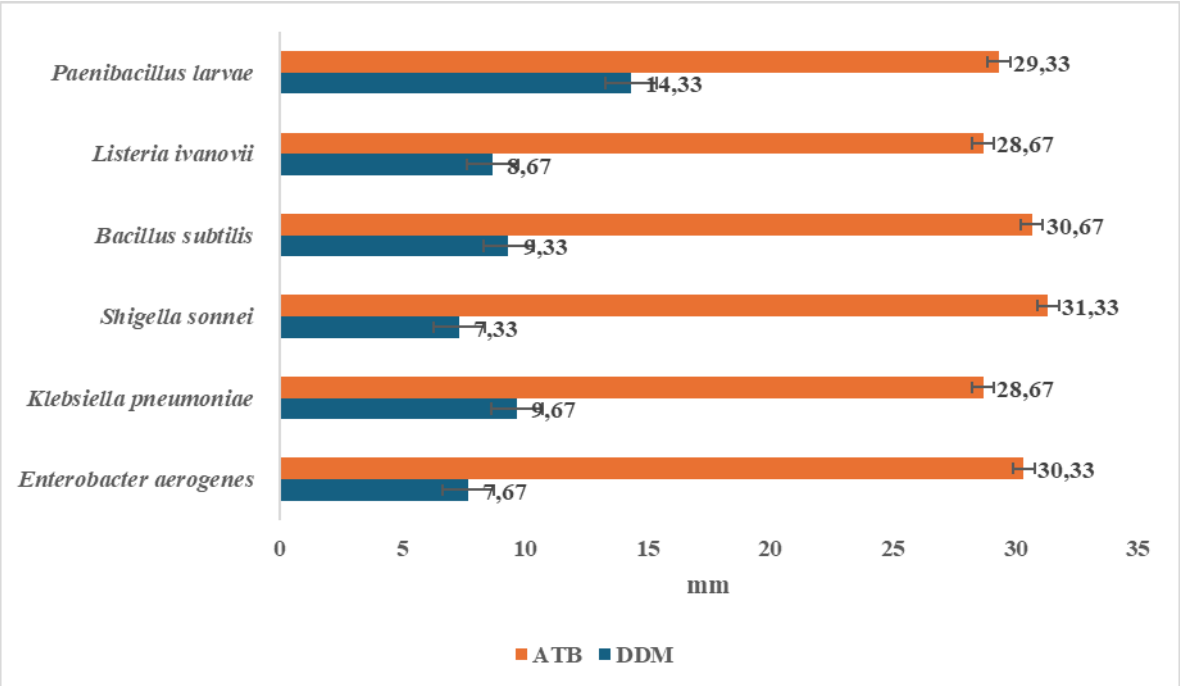

Figure 1. Antimicrobial activity of FAEO with disc diffusion method (DDM) and antibiotic resistance (ATB) in mm

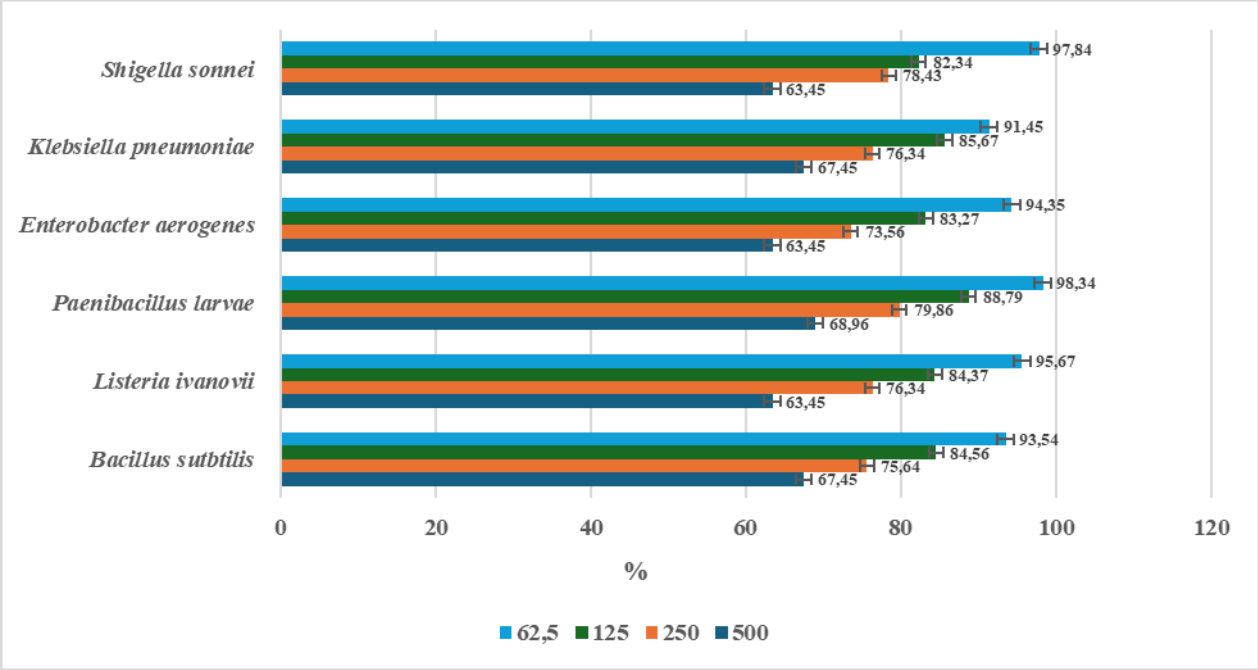

Figure 2. Antimicrobial activity of FAEO *in situ* on pear model in %

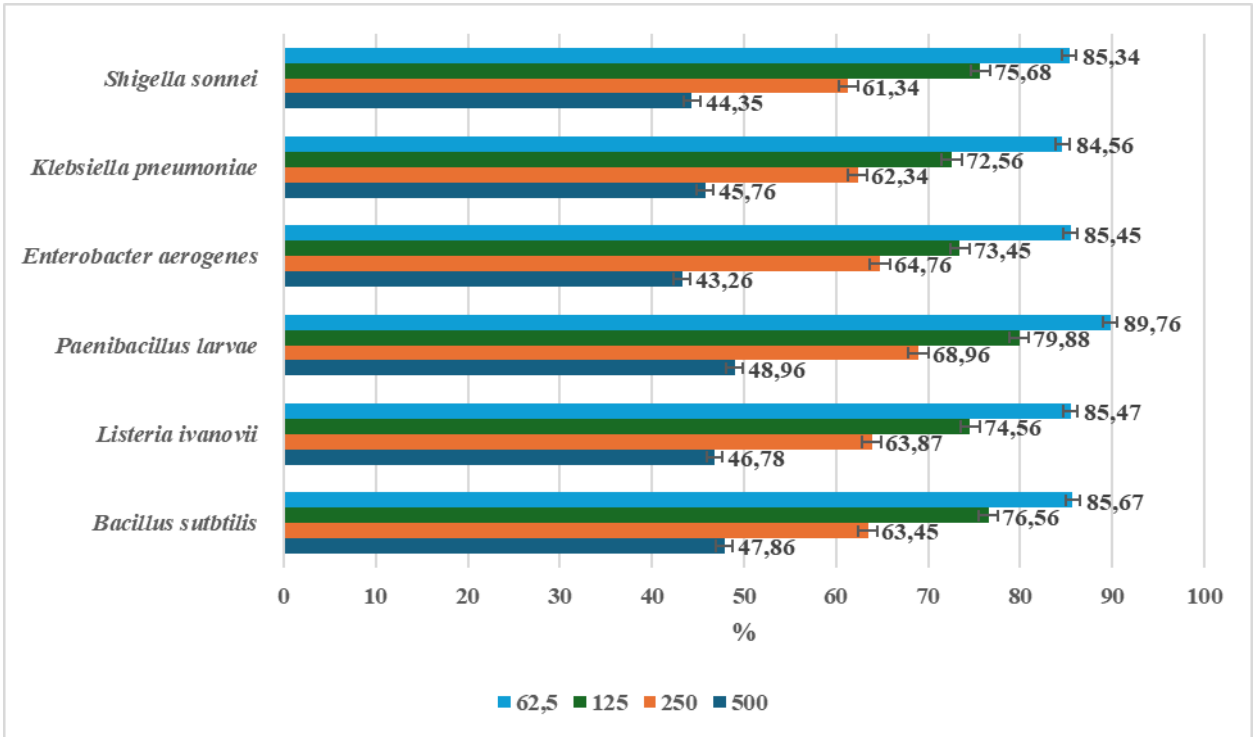

Figure 3. Antimicrobial activity of FAEO *in situ* on parsley model in %

Table 1. Insecticidal activity of FAEO against *Megabruchidius dorsalis* (n=50)

Concentration (%)	Number of Living Individuals	Number of Dead Individuals	Insecticidal Activity (%)
100	10	90	90.00 ± 0.00
50	20	80	80.00 ± 0.00
25	40	60	60.00 ± 0.00
12.5	70	30	30.00 ± 0.00
6.25	100	0	0.00 ± 0.00
3.125	100	0	0.00 ± 0.00
Control group	100	0	0.00 ± 0.00

The quality and safety of food is subject to deterioration due to a complex process of food spoilage that incorporates a number of biological and environmental elements [8]. It is imperative to comprehend the underlying causes of spoilage in order to formulate pragmatic strategies aimed at halting or mitigating the deterioration of foodstuffs. The visual appearance, tactile texture, gustatory qualities, and nutritional content of foodstuffs can all be significantly altered by these agents, which can be broadly divided into microbes, enzymes, chemical reactions, physical factors, and pests. The interaction of these elements has been shown to result in a loss of food safety and quality, with the rate of spoilage being accelerated as a consequence [9]. The identification and regulation of these spoilage agents is imperative for the maintenance of food quality, the prolongation of shelf life, and the reduction of waste. The deterioration of foodstuffs can be attributed to a number of factors, including microbes. The primary biological agents responsible for the deterioration of foodstuffs encompass bacteria, and microscopic filamentous fungi. When dietary components are subjected to the action of these microbes, adverse changes in texture, odour, and appearance are the result [10].

In a separate investigation, the efficacy of a number of commercial essential oils in reducing the viability of *Bacillus subtilis* spores was examined. The three EOs that demonstrated the highest level of activity were cardamom, juniper leaf, and tea tree oils. For tea tree and cardamom oils, in particular, the sporicidal effects appeared to be contingent upon both the duration and the ambient temperature. The sporicidal activity was found to be significantly lower than that of the most active oils when the main constituents of these oils were examined separately or in combination [11]. The present study corroborates earlier research demonstrating the efficacy of formulations comprising 5–15 % tea tree oil in reducing the quantity of viable *Bacillus* spores [12].

Three organic acids (OAs) ascorbic, citric, and lactic, and eight essential oils (EOs) lemon, lemongrass, lime, garlic, onion, oregano, thyme, and rosemary—were assessed. Four verified *Listeria* species were used to test these compounds' antibacterial efficacy *in vitro* [13].

There are many medicinal and pharmacological qualities in Brazil, and Amazonia in particular, that should be investigated. There is an untested method for controlling the AFB in the field of honeybee health that involves using several natural ingredients. Treatments that limit residues in honey and wax, have appropriate antibacterial action, have no negative effects on *A. mellifera*, and are a good substitute for reducing antibiotic resistance are all necessary to eradicate *P. larvae* in *A. mellifera* colonies [14].

The IC50 and ILP data were used to calculate the inter- and intraspecies variation when multiple strains of each species were assessed for susceptibility to the antibacterial effects of EOs. Generally speaking, *E. cloacae* was more sensitive than *E. aerogenes*, while *Lactobacillus plantarum* was more sensitive than *L. brevis* [15].

Gram-positive bacteria were generally more sensitive than gram-negative bacteria, and as previously described, strains of *S. aureus* and *Listeria* were the most vulnerable organisms

[16,17]. Because gram-negative bacteria have an outside membrane around their cell wall rather than a single membrane target, they may be less vulnerable to the effects of antibacterials.
The insecticidal efficacy of FAEO is presented in Table 1. The findings indicated that the highest levels of insecticidal activity were achieved when 25 %, 50 % and 100 % of the FAEO solution was applied. Nevertheless, concentrations of 6.25 % and 3.125 % of FAEO did not demonstrate a repellent effect against *M. dorsalis*. No data on the insecticidal activity of FAEO were found in the available literature. In our experiments, this activity was detected.

4. Conclusions

Natural preservatives could be made from the FAEO chosen for this study. In order to lessen and regulate pathogen contamination or the development of native microbiota on sliced fresh fruit, these EOs could be employed as natural sanitizers. Even yet, earlier research has been done to ascertain EO's antibacterial effectiveness both in vitro and in situ. At the product application stages, more research should be done to determine whether plant essential oils can improve product safety and how they will affect the background spoilage microbiota and pathogen presence.

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References

1. Maurya, A., Prasad, J., Das, S., Dwivedy, A.K., Essential Oils and Their Application in Food Safety. Front. Sustain, Food Systems, 2021, 5, 133.
2. Amorati, R., Foti, M.C., Valgimigli, L., Antioxidant activity of essential oils, Journal of Agriculture and Food Chemistry, 2013, pp. 10835–10847.
3. Sharma, S., Barkauskaite, S., Jaiswal, A.K., Jaiswal, S., Essential oils as additives in active food packaging, Food Chemistry, 2021, pp. 128403. DOI:10.1016/j.foodchem.2020.128403.
4. Do Nascimento, L.D., de Moraes, A.A.B., da Costa, K.S., Galúcio, J.M.P., Taube, P.S., Costa, C.M.L., Cruz, J.N., de Aguiar Andrade, E.H., de Faria, L.J.G., Bioactive natural compounds and antioxidant activity of essential oils from spice plants: New findings and potential applications, Biomolecules 2020, pp. 988, DOI: doi.org/10.3390/biom10070988.
5. Vianna, T.C., Marinho, C.O., Marangoni Júnior, L., Ibrahim, S.A., Vieira, R.P., Essential oils as additives in active starch-based food packaging films: A review, International Journal of Biological Macromolecules, 2021, pp. 1803–1819. DOI: 10.1016/j.ijbiomac.2021.05.170
6. del Carmen Razola-Díaz, M., Guerra-Hernández, E.J., García-Villanova, B., Verardo, V., Recent developments in extraction and encapsulation techniques of orange essential oil, Food Chemistry, 2021, pp. 129575. DOI: 10.1016/j.foodchem.2021.129575
7. Kačániová, M., Čmiková, N., Vukovic, N. L., Verešová, A., Bianchi, A., Garzoli, S., Ben Saad, R., Ben Hsouna, A., Ban, Z., Vukic, M. D., *Citrus limon* Essential Oil: Chemical Composition and Selected Biological Properties Focusing on the Antimicrobial (*In Vitro, In Situ*), Antibiofilm, Insecticidal Activity and Preservative Effect against *Salmonella enterica* Inoculated in Carrot, Plants, 2004, 524. DOI: 10.3390/plants13040524
8. Karanth, S., Feng, S., Patra, D., Pradhan, A.K., Linking microbial contamination to food spoilage and food waste: The role of smart packaging, spoilage risk assessments, and date labeling, Frontiers in Microbiology, 2023, DOI:10.3389/fmicb.2023.1198124.
9. Damotharan, G. Sudhakaran, M. Ramu, M. Krishnan, K.R.N., Arockiaraj S.J., Biochemical processes mediating neurotoxicity induced by synthetic food dyes: A review of current evidence, Chemosphere, 2024, 143295, DOI:10.1016/j.chemosphere.2024.143295.
10. Subbarayudu, S., Namasivayam, S.K.R., Arockiaraj, J., Immunomodulation in Non-traditional Therapies for Methicillin-resistant *Staphylococcus aureus* (MRSA) Management, Current Microbiology, 2024, pp. 346, DOI:10.1007/s00284-024-03875-7
11. Lawrence, H. A., Activity of Essential Oils Against *Bacillus subtilis* Spores, Journal of Microbiology and Biotechnology, 2009, pp. 1590–1595, DOI:10.4014/jmb.0904.04016
12. Messager, S., Hammer, K.A., Carson, C.F., Riley, T.V., Sporicidal activity of tea tree oil, Australian Infection Control, 2006, pp. 112-121.
13. Pedrós-Garrido, S., Clemente, I., Calanche, J. B., Condón-Abanto, S., Beltrán, J. A., Lyng, J. G., Brunton, N., Bolton, D., Whyte, P., Antimicrobial activity of natural compounds against *Listeria* spp. and their effects on sensory attributes in salmon (*Salmo salar*) and cod (*Gadus morhua*), Food Control, 2020, 106768. DOI:10.1016/j.foodcont.2019.106768

14. Santos, R. C. V., Alves, C. F. dos S., Schneider, T., Lopes, L. Q. S., Aurich, C., Giongo, J. L., Brandelli, A., de Almeida Vaucher, R., Antimicrobial activity of Amazonian oils against P*aenibacillus* species, Journal of Invertebrate Pathology, 2012, 265–268, DOI:10.1016/j.jip.2011.12.002
15. Gutierrez, J., Rodriguez, G., Barry-Ryan, C., & Bourke, P., Efficacy of Plant Essential Oils against Foodborne Pathogens and Spoilage Bacteria Associated with Ready-to-Eat Vegetables: Antimicrobial and Sensory Screening, Journal of Food Protection, 2008, 1846–1854, DOI:10.4315/0362-028x-71.9.1846
16. Lambert, R. J. W., Skandamis, P.N., Coote, P., Nychas. G.J.E., A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol, Journal of Applied Microbiology, 2001, p. 453–462, DOI: 10.1046/j.1365-2672.2001.01428.x.
17. Oussalah, M., Caillet, S., Saucier, L., Lacroix, M., Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella Typhimurium, Staphylococcus aureus* and *Listeria monocytogenes,* Food Control, 2006, pp. 414–420, DOI: 10.1016/j.foodcont.2005.11.009.