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ASSESSMENT OF ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF CINNAMON AND CLOVE ESSENTIAL OILS

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Abstract

The present research paper aims to evaluate the antifungal and antibacterial activity of two essential oils, cinnamon essential oil (Cinnamomum zeylanicum) and clove essential oil (Eugenia caryophyllata) on three fungal strains (Aspergillus brasiliensis, Botrytis cinerea, Penicillium expansum) and one bacteria strain (Bacillus subtilis). The studied essential oils (EOs) are used in the food industry as bioactive compounds and are known to have antimicrobial activity on the growth of some bacteria strains as well as several fungal strains. The trend worldwide is to develop novel and healthy methods used to prologue the shelf-life of food products that are naturally spoiled by different types of microorganismsn thus EOs are a perfect solution in potentially solving this problem. The major problem that occurs with the use of EOs as natural preservatives used to prolong the shelf life of different food products is that in high concentrations they affect the sensory qualities. Also, the EOs have several limitations like variability in efficacy, stability issues, low water solubility and regulatory challenges. The results showed that the minimum inhibitory concentration (MIC) values ranged between 10µl and 50µl. The results suggest that both EOs could be used as potential natural alternatives that may lead to the growth inhibition of some microorganism's strains, thus prolonging the shelf life of food.

Key words*: antimicrobial activity, bioactive compounds, food safety, shelf life.*

INTRODUCTION

Natural deterioration of agri-food products refers to the gradual loss of quality, nutritional value, or overall viability of agricultural and food products over time, as a result of various natural factors, such as exposure to air, humidity, temperature changes, and microbial activity. This process can lead to changes in taste, texture, nutritional composition and in the end to the loss of the products quality. These changes can reduce the shelf life and affect the value of these products in the market (Iulietto et al., 2015).

To understand and explore this phenomenon in more detail, researchers and experts in the food industry focus on aspects related to agricultural economics, food science, and related fields. In recent years, there have been a lot of researches and studies in finding and developing novel and healthy alternatives to extend the shelf-life and nutritional value of agri-food products (Lee et al., 2015). The main purpose of antimicrobial preservation is to extend the shelf life of products and maintain their quality by preventing microbial contamination, growth, and spoilage. This applies to various industries, including food and beverages, pharmaceuticals, cosmetics, and personal care products. Types of antimicrobial agents, these can be chemical, physical, or biological in nature. Chemical antimicrobial agents include preservatives such as organic acids, salts, nitrites, and sulphites. Physical methods include heat treatment, filtration, and irradiation, while biological agents may include bacteriocins and certain beneficial microorganisms (Wang et al., 2023).

1. Microorganisms that spoil fruits and vegetables

Microorganisms that can grow on fresh produce have often developed sophisticated biochemical mechanisms to break down food components, which provide them with the energy sources in order for them to develop. Fruits and vegetables have often been associated with having a high risk of microbial contamination with fungi, yeasts and bacteria, because of their short shelf

life, high water content and natural microbiota that is present in the environment. Contamination of fruits and vegetables can occur during the pre-and post-harvest stages of food production, such as growing, processing and transport. Contaminated water used for rinsing vegetables and subsequent spraying to keep them fresh longer is a common source of contamination. Other possible sources of food contamination with microorganisms include: soil, faecal matter, ice, product handling and contaminated processing equipment. Microorganisms that can be found on the surface of vegetables can be adventitious environmental contaminants, present on soil particles, dust and/or as airborne spores (Kaczmarek et al., 2019). Salmonella is the leading cause of foodborne illness worldwide. In the last few years, Salmonella outbreaks have been increasingly linked to the consumption of fresh vegetables. This pathogen is the main challenge for the microbiological safety of vegetables. Jeddi et al. investigated the occurrence of *Salmonella*, *Escherichia coli*, coliforms, total aerobic and spoilage bacteria, fungi and yeasts on several fruits species. Most of the reported counts for total aerobic bacteria ranged between 4 and 8 log CFU/g and between 0.7 and 6 log CFU/g for coliforms. *E. coli* strains were often observed at low prevalence and low numbers. Pathogens such as *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* were rarely present (Jeddi et al., 2014). Another study was conducted in order to isolate and characterize different bacterial populations associated with fruit spoilage in Gwagwalada market Abuja, Nigeria. Seven different commonly consumed fruits namely: orange (*Citrus sinensis*), tomato (*Lycopersicon lycopersicum*), banana (*Musa* spp.), mango (*Mangifera indica*), red pepper (*Capsicum spp*.), guava (*Psidium guajava*) and watermelon (*Citrullus lanatus*) were used for the study. Fruits showing spoilage symptoms were collected and transported to the laboratory where the rotten parts were isolated and cultured on four bacteriological culture media. The result obtained revealed eight different species of bacteria associated with spoilage of fruits at Gwagwalada market. These bacteria are *Staphylococcus aureus* (25%), *Escherichia coli* (17%), *Bacillus* (19%), *Klebsiella* (10%), *Pseudomonas* (10%), *Lactobacillus* (10%), *Micrococcus* (0.7%) and *Salmonella* (0.7%) (Mairami et al., 2018).

2. Microorganisms that spoil meat and fish products

Meat spoilage typically occurs under conditions of optimal water availability, low oxygen, and low temperature, where growth conditions are ideal for microbial growth (Fletcher et al., 2018). Meat spoilage can be caused by natural processes in meat, such as lipid oxidation or autolytic enzymatic reactions in the muscle cells of the animal after slaughter. However, the major cause of spoilage is the inevitable contamination with microorganisms (mainly bacteria) during the processing. Each processing step can influence microbial contamination, and storage conditions can shape the structure of bacterial communities, consequently influencing the occurrence of microbial spoilage over time (Luong et al., 2020).

Fresh fish is a highly perishable product and spoils due to microbiological activity, chemical oxidation of lipids and autolysis. However, microbiological spoilage is the main mechanism affecting the quality of fresh fish. As bacteria grow, they utilize nutrients and produce by-products. It is well established that the accumulation of metabolic products is the main cause of organoleptic rejection of fresh fish. It is also known that spoilage is caused by only a fraction of the initial microbial population, known as specific spoilage microorganisms, which produce metabolites (chemical index of spoilage) responsible for abnormal odours and which cause organoleptic rejection of the product. The initial fraction of microbiota that dominates and the metabolic products that are produced are largely determined by the temperature and atmospheric conditions during storage (Boziaris, 2015).

Microbial growth and metabolism is a major cause of fish spoilage, as they produce biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable aromas. Trimethylamine (TMA) levels are universally used to correlate with the degree of microbial spoilage responsible for fish spoilage. Trimethylamine oxide (TMAO) is used as an osmoregulator to avoid dehydration in marine environments. Bacteria such as

Shewanella putrifaciens, *Aeromonas* spp., *Enterobacteriaceae*, *Pseudomonas phosphoreum* and *Vibrio* spp. can obtain energy by reducing TMAO to TMA, creating off-flavors resembling ammonia. *Pseudomonas putrefaciens*, *Pseudomonas fluorescentis* and other spoilage bacteria grow rapidly in the initial stages of spoilage, producing many proteolytic and hydrolytic enzymes (Rathod et al., 2022). Similar to other types of meat, not all microorganisms in fish meat have the potential for corruption, except for specific spoilage organisms. In Table 1 is a list of some bacterial groups associated with fish meat spoilage.

Table 1. List of bacterial groups associated with fish meat spoilage (Source: Zhu et al., 2022)

Bacteria type	Fish meat
Shewanella	Raw lobster tails, whole lobster, salmon
Pseudomonas	Raw lobster tails, whole lobster, salmon, carp
Photobacterium	Raw salmon
Psychrobacter	Whole lobster, cod
Brochothrix thermosphacta	Raw salmon, catfish, sea bass, sea bream
Aeromonas salmonicida	Shrimp, salmon, pheasant

Olafsdottir et al. reported the proliferation of specific spoilage organisms in mackerel fillets stored at 0, 7 and 15°C and found that *Photobacterium phosphoreum* was predominant among the bacteria. *Pseudomonas* spp. appears to be responsible for the sweet, fruity odors, while *Shewanella putrefaciens* is responsible for the production of H_2S (Ghaly et al., 2010).

MATERIALS AND METHODS

In the *in vitro* analyses performed in order to evaluate the antimicrobial effect of the two essential oils, three strains of fungi (*Aspergillus brasiliensis*, *Botrytis cinerea*, *Penicillium expansum*) and one bacterium strain (*Bacillus subtilis*) were selected. The disk diffusion method was used in order to determine the inhibition halo and the inhibition rate and Potato Dextrose Agar and Nutrient Agar were used as culture media. For each Petri Dish, a quantity of 100 µl of fungal suspension (spores/ml) and 50 µl of bacterial suspension (spores/ml) was spread with a Drigalsky wand, and left to rest for 30 minutes to facilitate the incorporation of the microorganism into the culture medium, as presented in Figure 1.

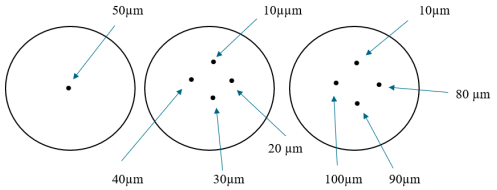


Figure 1. Distribution of Whatman diffusion discs (Ϥ=6 mm) in Petri dishes

The distribution of the essential oil was done by pipetting the analysed essential oils on Whatman discs in concentrations between 10 and 50 µl/disc. Two repetitions were performed for each determination.

The procedure for evaluating the samples involved evaluating the proportion of the surface area on which the mycelium developed compared to the total surface area of the plate.

At the same time, a control sample was also performed using the same procedure, except that no essential oils were added. The Petri dishes were incubated at 25°C for 7 days for fungi and at 37°C for 24-48 hours for bacteria. The microorganism growth was visually monitored by two evaluators and degree of GI inhibition (%) was evaluated. The minimum inhibitory concentration (MIC) was calculated for each essential oil and was defined as the minimum amount of essential oil for which the inhibition halo diameter was at a minimum of 1 cm.

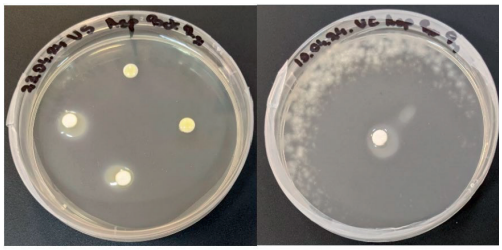
RESULTS AND DISCUSSIONS

The objective of the research was to determine the antimicrobial activity effect of two essential oils on four types of microorganisms, three fungal strains and one bacterial strain that have a high incidence in food spoilage. The EOs used in the experiments were bought from a supermarket and were commercial grade.

The antifungal activity of the essential oils used on the three strains of fungi tested (*Aspergillus brasiliensis*, *Botrytis cinerea* and *Penicillium expansum*) and the antibacterial activity of the essential oils used on the *Bacillus subtilis* strain were evaluated by determining the degree of inhibition by measuring the inhibition halo and the minimum inhibitory concentration (MIC).

Antifungal activity of cinnamon essential oil and clove essential oil on *Aspergillus brasiliensis*

The antifungal activities of essential oils against *Aspergillus brasiliensis* are summarized in Table 2 and Figure 3. The visual representation of the inhibition diameter zones obtained by paper disk diffusion method is presented in Figure 2.



A. B.

Figure 2. Inhibition diameter zones obtained by paper disk diffusion method for A. - cinnamon (CIO) and B. - clove (CLO) on *Aspergillus brasiliensis*

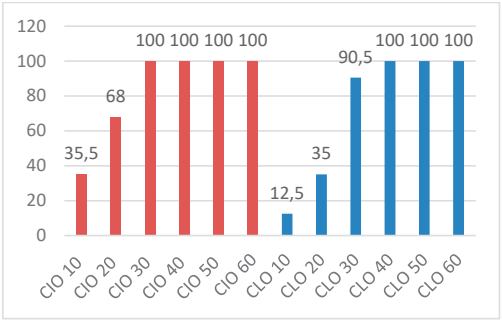
Both essential oils tested showed consistently antimicrobial activity against tested fungal strain at different concentrations. The results showed that total inhibition was registered for CIO at 30 µL and CLO at 40 µL.

Table 2. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Aspergillus brasiliensis*

Essential oil (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
CIO 10	4.8	4	4.4	0.57
CIO 20	7.8	8	7.9	0.14
CIO 30	9	9	9	0.00
CIO 40	9	9	9	0.00
CIO 50	9	9	9	0.00
CIO 60	9	9	9	0.00
CLO 10	2.4	2.8	2.6	0.28
CLO 20	4	5.1	4.55	0.77
CLO 30	6.8	6	6.4	0.57
CLO 40	9	9	9	0.00
CLO 50	9	9	9	0.00
CLO 60	9	9	9	0.00

CIO - cinnamon essential oil, CLO - clove essential oil

The degree of inhibition of the samples was determined by assessing the proportion of the surface area on which the mycelium developed in relation to the surface area of the plate. Visual monitoring was performed by two subjects and the results were noted individually, and at the end of the test period, an average of the two results was calculated.



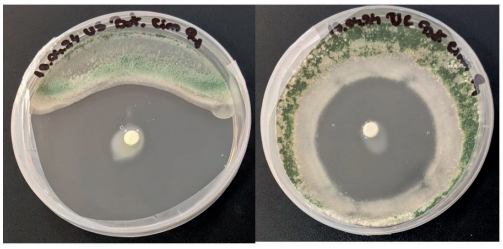
CIO - cinnamon essential oil, CLO - clove essential oil

Figure 3. Graphical representation of the degree of inhibition of essential oils tested on the fungus *Aspergillus brasiliensis*

As it can be seen from Figure 3, starting from concentration of 30 µL, cinnamon essential oil presented a degree of inhibition of 100% over *Aspergillus brasiliensis*. The clove essential oil exhibited a 100% degree of inhibition on the analysed fungal strain at concentration of 40 µL.

Antifungal activity of cinnamon essential oil and clove essential oil on *Botrytis cinerea*

The antifungal activities of essential oils against *Botrytis cinerea* are summarized in Table 3 and Figure 5. The visual representation of the inhibition diameter zones obtained by paper disk diffusion method is presented in Figure 4.



A. B.

Figure 4. Antimicrobial activity of the analysed essential oils: A. - cinnamon (CIO); B. - clove (CLO) on *Botrytis cinerea*

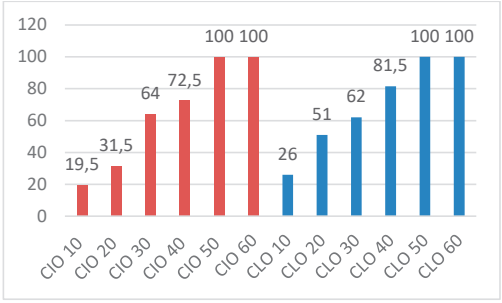
Both essential oils tested showed consistently antimicrobial activity at concentrations of 50 µL against the analysed fungal strain.
The results showed that total inhibition was registered for both EOs at 50 µL.

Table 3. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Botrytis cinerea*

Essential oils (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
CIO 10	1.8	2.1	1.95	0.21
CIO 20	3	3.3	3.15	0.21
CIO 30	6.2	6.6	6.4	0.28
CIO 40	7.2	7.3	7.25	0.07
CIO 50	9	9	9	0.00
CIO 60	9	9	9	0.00
CLO 10	2.4	2.8	2.6	0.28
CLO 20	5	5.2	5.1	0.14
CLO 30	6	6.4	6.2	0.28
CLO 40	7.8	8.5	8.15	0.49
CLO 50	9	9	9	0.00
CLO 60	9	9	9	0.00

CIO - cinnamon essential oil, CLO - clove essential oil

As it can be seen from Figure 5, starting from concentration of 50 µL, cinnamon essential oil presented a degree of inhibition of 100% over *Botrytis cinerea*. The clove essential oil exhibited a 100% degree of inhibition on the analysed fungal strain at concentration of 40 µL.

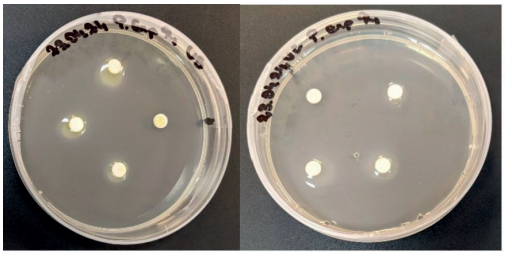


CIO - cinnamon essential oil, CLO - clove essential oil

Figure 5. Graphical representation of the degree of inhibition of essential oils tested on the fungus *Botrytis cinerea*

Antifungal activity of cinnamon essential oil and clove essential oil on *Penicillium expansum*

The antifungal activities of essential oils against *Penicillium expansum* are summarized in Table 4 and Figure 7. The visual representation of the inhibition diameter zones obtained by paper disk diffusion method is presented in Figure 6.



A. B.

Figure 6. Antimicrobial activity of the analysed essential oils: A. - cinnamon (CIO); B. - clove (CLO) on *Penicillium expansum*

It can be seen from Table 4 that the cinnamon essential oil presented complete antifungal activity on *Penicillium expansum* even at concentrations of 60 µL. Contrary, the clove essential oil, at concentrations of 60 µL only presented an inhibition diameter of 6 cm.

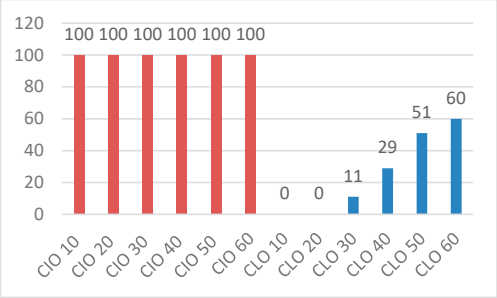
Table 4. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Penicillium expansum*

Essential oil (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
CIO 10	9	9	9	0.00
CIO 20	9	9	9	0.00
CIO 30	9	9	9	0.00
CIO 40	9	9	9	0.00
CIO 50	9	9	9	0.00
CIO 60	9	9	9	0.00
CLO 10	0	0	0	0.00
CLO 20	0	0	0	0.00
CLO 30	1	1.2	1.1	0.14
CLO 40	3	2.8	2.9	0.14
CLO 50	5	5.2	5.1	0.14
CLO 60	6	6	6	0.00

CIO - cinnamon essential oil, CLO - clove essential oil

The degree of inhibition of the samples was determined by assessing the proportion of the surface area on which the mycelium developed in relation to the surface area of the plate.
As it can be seen from Figure 7, starting from concentration of 10 µL, cinnamon essential oil presented a degree of inhibition of 100% over *Penicillium expansum*. The clove essential oil

exhibited a 60% degree of inhibition on the analysed fungal strain at concentration of 60 µL.

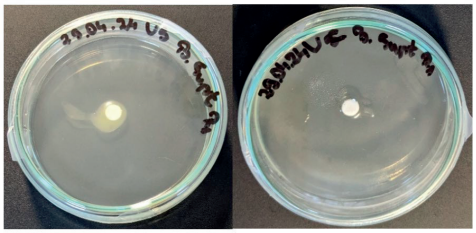


CIO - cinnamon essential oil, CLO - clove essential oil

Figure 7. Graphical representation of the degree of inhibition of essential oils tested on the fungus *Penicillium expansum*

Antimicrobial effect of essential oils on the bacterium *Bacillus subtilis*

At concentration of 50 µL, both EOs, cinnamon essential oil and clove essential oil, inhibited completely the growth of *Bacillus subtilis* (Figure 8).



A. B.

Figure 8. Antimicrobial activity of the analysed essential oils: A. - cinnamon (CIO); B. - clove (CLO) on *Bacillus subtilis*

The inhibition diameter zones (diameter of the inhibition halo) values of the analysed essential oils tested on *Bacillus subtilis* are presented in Table 5.

Table 5. Inhibition diameter zones (diameter of the inhibition halo) values of the essential oils tested on *Bacillus subtilis*

Essential oil (µl)	R1 (cm)	R2 (cm)	Mean (cm)	Standard deviation
US 50	9	9	9	0
UC 50	9	9	9	0

CIO - cinnamon essential oil, CLO - clove essential oil

After 48 hours from the inoculation with *Bacillus subtilis* bacterial strain, all tested samples, containing 50 µL of EOs did not exhibit any growth of the microorganism, the inhibition zone (diameter of the inhibition halo) values being of 9 cm (whole surface of the Petri Dish).

The degree of inhibition is presented in Table 6 and it was 100% for both EOs at concentrations of 50 µL.

Table 6. Degree of inhibition of essential oils tested on *Bacillus subtilis*

EOs (µl)	R1 (%)	R2 (%)	Mean (%)	GI (%)
US 50	0	0	0	100
UC 50	0	0	0	100

CIO - cinnamon essential oil, CLO - clove essential oil

Determination of the minimum inhibitory concentration (MIC) of the tested essential oils

The minimum inhibitory concentrations (MIC) of the two essential oils was determined and the results are presented in Table 7.

Table 7. Minimum inhibitory concentration (MIC) of essential oils tested on *Aspergillus brasiliensis*, *Botrytis cinerea*, *Penicillium expansum* and *Bacillus subtilis*

Microorganism	Cinnamon essential oil (CIO)	Clove essential oil (CLO)
	MIC (µl)	
Aspergillus brasiliensis	30	60
Botrytis cinerea	50	50
Penicillium expansum	10	60
Bacillus subtilis	50	50

The minimum inhibitory concentration in the case of cinnamon essential oil was 30 µl for the *Aspergillus brasiliensis*, 50 µl for *Botrytis cinerea* and *Bacillus subtilis* and 10 µl *Penicillium expansum*.

As for the clove essential oil showed that the MIC for *Aspergillus brasiliensis* and *Penicillium expansum* was 60 µl and at 50 µl *Botrytis cinerea and Bacillus subtilis.*

The best results were obtained by the cinnamon essential oil, thus the results obtained are similar to the one in other research articles (El Atki et al., 2019; Gupta et al., 2008).

CONCLUSIONS

There is increasing interest in the development of natural antimicrobial agents and alternative preservation methods in response to consumer demand for natural and clean-label products. Research efforts are focused on exploring new antimicrobial compounds derived from natural sources, as well as innovative preservation technologies that minimize the use of synthetic chemicals. Antimicrobial preservation encompasses a wide range of techniques and strategies aimed at preserving products by inhibiting microorganisms that can cause spoilage or pose a health risk.

Cinnamon essential oil has presented the highest antimicrobial activity for all microroganisms analysed, *Aspergillus brasiliensis*, *Botrytis cinerea*, *Penicillium expansum* and *Bacillus subtilis*.

The degree of inhibition was observed for both cinnamon and clove essential oil, but the best results were obtained for the cinnamon essential oil and concentrations of 10 µl for *Penicillium expansum*. The minimum inhibitory concentration in the case of cinnamon essential oil was between 10 µl and 50 µl for the fungal strains and 50 µl for *Bacillus subtilis*.

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