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ESSENTIAL OILS AND THEIR APPLICATION IN A FOOD MODEL

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ABSTRACT

The aim of the study was to investigate the chemical composition, antioxidant, and antimicrobial activity of essential oils (*Canarium luzonicum* CLEO, *Melaleuca leucadenron* MLEO, *Amyris balsamifera* ABEO). There was Gas chromatographic-mass spectrometric analysis used for the characteristic of the semiquantitative composition of the essential oils. The DPPH method was used to determine the antioxidant activity. Minimum inhibitory concentrations (MIC) of essential oils against *Stenotrophomonas maltophilia* were analyzed in a 96-well plate. The broth microdilution method was used for the minimal inhibitory concentration. A gas-phase antimicrobial assay was used to determine inhibitory concentrations in a food model. CLEO proved to be the best with the lowest MIC 50 and 90 of 6.67 µL.mL^{-1} respectively 6.81 µL.mL^{-1} and antioxidant activity of 33.43% among the tested essential oils. The main volatile compounds CLEO were limonene 36.38%, elemol 16.65%, α-fellandren 12.18% and elemicin 9.59%. It showed inhibition of *S. maltophilia* growth in the food model at the lowest concentrations among the essential oils.

Keywords: *Stenotrophomonas maltophilia*; *Canarium luzonicum*; *Melaleuca leucadenron*; *Amyris balsamifera*; essential oil; food model

INTRODUCTION

In recent years, natural substances have come to the fore due to their low toxicity, pharmacological effects, and economic advantage **(Dias, Urban, and Roessner, 2012)**.

Elemi (*Canarium luzonicum*) essential oil comes from an evergreen tree that reaches a height of more than 30 meters and a trunk diameter of more than 1 meter. *C. luzonicum* naturally occurs in the Philippines **(Barwick, Schans and Claudy, 2004)**.

Oleoresin is one of the aromatic components. Therefore, it has a wide range of uses in the pharmaceutical and food industries. It is also used for its rubefic, expectorant, antifungal, antibacterial and antirheumatic effects **(Nikolic et al., 2016)**.

Kajeput essential oil (*Melaleuca leucadendron*) is used for its antifungal, antiviral, antibacterial, antiseptic, and anti-inflammatory effects. The plant occurs predominantly in Indonesia **(Pujiarti, Ohtani and Ichiura, 2011)**. Many of the compounds present in this plant are considered to be bioactive substances **(Cleber et al., 2007)**.

Amyris, essential oil (*Amyris balsamifera*) comes from always green small trees and it has high flammability. It occurs in the Caribbean and near the Gulf of Mexico **(Rohmer, Schwartz and Anton, 2012)**. Amyris is rich in sesquiterpene alcohol. It has antiseptic effects **(Khan and Abourashed, 2009)**.

Stenotrophomonas maltophilia is a non-fermentative, gram-negative, aerobic bacillus. These bacteria can form biofilm structures. It is most often found in raw milk, vegetables, fruits and fish products regarding the food industry **(An and Berg, 2018)**.

We aimed to determine chemical composition, antioxidant activity, and minimal inhibitory concentrations of these essential oils against the bacterium *Stenotrophomonas maltophilia.*

Another aim was also to evaluate the inhibitory effect of essential oils against *S. maltophilia* from the surface of carrots, potatoes and apples by using a vapor phase antimicrobial test.

Scientific hypothesis

We assume the presence of biologically active substances and the antioxidant potential of essential oils. Given the available literature, we assume the inhibitory effect of essential oils on the bacteria *Stenotrophomonas maltophilia*. We believe that essential oils could also have an inhibitory effect in the vapor phase.

MATERIAL AND METHODOLOGY

Essential oil

The tested essential oils (*Canarium luzonicum* CLEO, *Melaleuca leucadenron* MLEO, *Amyris balsamifera* ABEO) were bought from the Hanus s.r.o (Slovakia).

Microorganism

Bacteria *Stenotrophomonas maltophilia* was got from the dairy industry. It was identified by 16S rRNA sequencing and MALDI-TOF MS Biotyper.

Chemical Composition of Essential Oils

There was Gas chromatographic-mass spectrometric (GC-MS, Agilent 7890B, Agilent 5977A, Agilent Technologies Inc., Palo Alto, CA, USA) and CombiPal autosampler 120 (CTC Analytics AG, Zwingen, Switzerland) parsing test of the essential oil used as well as in a previous study **(Kačániová et al., 2020)**. The results were set as the average mean and standard deviation of three repeated measurements.

Radical Scavenging Activity—DPPH Method

The activity of capturing free radicals with essential oil was determined in the same way as in the study of **Kačániová et al. (2020)** by using 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich, Germany) method.

Minimum Inhibitory Concentration (MIC)

The bacterial culture was cultivated in the Muller Hinton broth (MHB, Oxoid, Basingstoke, UK)) at 37 °C for 24 h. One hundred μL inoculum with a density of 0.5 McF was inoculated into each well of a 96-well microtitration plate. There was 100 μl of the essential oil with a concentration from 0.3125 μL to 10 μL per well added after inoculation. Negative control contended a mixture of MHB with essential oil, while a mix of MHB with bacterial inoculum was used as a control of maximum growth **(Hassan et al. 2011)**. The absorbance was measured and evaluated in the same way as it was in the study of **Kačániová et al. (2020)**. The experiment was carried out in three repeated measurements.

Statistical analysis

The measurements were repeated three times. Statistical variability of the data was processed with Microsoft™ Excel® software.

Table 1 Main components of essential oil *Canarium luzonicum.*

Name	Synonyms	TIC% Area [a]
β-phellandrene		4.54 ±0.04
α-phellandrene		12.2 ±0.05
β-pinen		0.61 ±0.03
α-terpinene		0.61 ±0.01
d-limonene		36.4 ±0.16
cis-sabinene		3.06 ±0.03
α-ocimene		0.38 ±0.01
γ-terpinen		0.58 ±0.02
cymene		3.35 ±0.01
α-terpinolen		1.59 ±0.02
4,8,8-trimethyl-2-methylene-4-vinylbicyclo[5.2.0]nonane		0.51 ±0.02
terpinen-4-ol		1.15 ±0.01
α-terpineol		3.83 ±0.01
α-phellandrene epoxide		0.39 ±0.03
1,3,4-eugenol methyl ether	4-allylveratrole	0.69 ±0.01
elemol		16.7 ±0.18
guaiol		0.43 ±0.02
10-epi-γ-eudesmol		1.59 ±0.01
γ-eudesmol		0.84 ±0.01
rosifoliol		1.08 ±0.01
elemicin		9.59 ±0.11

Note: [a] mean value ±SE.

Results The MIC value (concentration causing 50% and 90% reduction in bacteria growth) was determined by logit analysis. Statistical evaluation of the antioxidant activity of the obtained data was performed using the GraphPad Prism 8.0.1 (GraphPad Software Incorporated, San Diego, California, USA). One way analysis of variance (ANOVA) followed by the Tukey test was used for statistical analysis.

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils

The main volatile compounds of the analyzed essential oil CLEO based on reduced percentage were limonene 36.38%, elemol 16.65%, α-fellandren 12.18% and elemicin 9.59% **(Table 1)**. **Swift (2002)** has stated 59.4%, α-phelandrene 8.01%, and sabinene 3.35% as the main constituents of *Canarium luzonicum*.

Orchard et al. (2017) found out that the main components of the essential oil *C. luzonicum* were limonene 47.5%, elemol 18.4%, and α-phelandrene 9.2%.

Malik (2019) indicated sabinene 5.7%, α-phelandrene 17.6%, limonene 56%, and elemol 6.3% as the main components of the essential oil *C. luzonicum*. **Silva et al. (2012)** identified limonene, β-Cymene, β-fellandren, α-phellandren and β-pinene as the main components of C. luzonicum essential oil.

The main volatile compounds of the analyzed MLEO based on the reduced percentage were eucalyptol 49.23%, α-terpineol 9.92%, limonene 8.12%, and caryophyllene 5.65% **(Table 2)**. **Pujiarti et al. (2011)** in their study tested 9 varieties of *M. leucadendron* from Java and Indonesia, in which twenty-six compounds were identified.

Table 2 Main components of essential oil *Melaleuca leucadenron.*

Name	Synonyms	TIC% Area [a]
3-carene		0.25 ±0.02
α-phellandrene		0.26 ±0.03
β-pipene		0.83 ±0.01
α-terpinene		0.48 ±0.01
d-limonene		8.12 ±0.04
eucalyptol	1,8-epoxy-p-menthane; 1,8-cineol	49.2 ±0.18
γ-terpinene		2.91 ±0.01
4-cymene		3.16 ±0.01
α-terpinolen		1.24 ±0.01
α-copaen		0.29 ±0.01
linalyl butanoate	linalyl butyrate	1.13 ±0.01
caryophyllene		5.65 ±0.03
p-menth-1-en-4-ol	1-terpinen-4-ol	0.83 ±0.03
2,4-dihydroxy-2-methylpentane	hexylene glycol	4.11 ±0.02
1,5,9,9-tetramethyl-1,4,7-cycloundecatriene -,		2.91 ±0.01
β-maaliene		0.43 ±0.01
α-muurolene		0.53 ±0.01
β-cadinene		0.65 ±0.01
α-terpineol acetate		1.84 ±0.01
α-terpineol		9.92 ±0.04
α-selinene		2.09 ±0.01
eudesma-3,7(11)-diene	selina-3,7(11)-diene	0.39 ±0.02
bicyclogermacrene	lepidozene; isolepidozene	0.36 ±0.03
caryophylene oxide		0.31 ±0.02
globulol	ledol	1.09 ±0.01
1,2-diacetate-1,2,3-propanetriol	1,2-diacetin	0.26 ±0.02

Note: [a] mean value ±SE.

Table 3 Main components of essential oil *Amyris balsamifera.*

Name	Synonyms	TIC% Area [a]
amorpha-4,11-diene	muurola-4,11-diene	2.58 ±0.02
β-cadinene		0.73 ±0.01
β-chamigrene		0.36 ±0.01
4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene		0.29 ±0.02
δ-bisabolene		0.95 ±0.01
α-zingiberene		2.21 ±0.01
β-bisabolene		0.97 ±0.01
α-maaliene		0.56 ±0.03
β-maaliene		0.32 ±0.02
cedrene	β-funebrene	4.91 ±0.02
α-curcumen		2.44 ±0.01
nerolidol		1.57 ±0.03
α-chamigrene		0.56 ±0.04
elemol		9.62 ±0.01
10-epi-γ-eudesmol		14.7 ±0.01
β-eudesmol		0.78 ±0.02
γ-eudesmol	machilol; selinenol	2.49 ±0.04
β-cadinene		0.60 ±0.01
β-guaiene	azulene	0.46 ±0.01
8-epi-γ-eudesmol		0.40 ±0.03
valerianol		23.2 ±0.16
guaiol		19.4 ±0.16
1,2,3,6-tetramethylbicyclo[2.2.2]octa-2,5-diene		0.53 ±0.02
bisabolone		0.99 ±0.01
selin-6-en-4α-ol	eudesm-6-en-4α-ol	0.27 ±0.03
β-vetispirene	β-vatirenene; β -vetivenene	0.55 ±0.03
isolongifolol, methyl ether		0.94 ±0.01
2-phenylethyl iodide		0.90 ±0.02
7-epi-γ-eudesmol		0.35 ±0.02
drim-7-en-11-ol		1.84 ±0.01

Note: [a] mean value ±SE.

These samples had a very similar composition. The results showed that 1,8-cineole (eucalyptol; 44.76 – 60.19%) was the main compound in these oils, followed by α-terpineol (5.93 – 12.45%), limonene (4.45 – 8.85%) and β-carophyllene (3.78 – 7.64%). **Sharifi-Rad et al. (2017)** reported *M. leucadendron* terpinen-4-ol 30%, 1,8-cyneol 15%, α-terpineol 8%, and limonene 1.5% as the main antimicrobial compounds. **Tia et al. (2013)** reported in their study terpinolene 29.21%, α-terpinene 22.55%, 2-γ-carene 8.53% and α-phelandrene 7.61% as the main components of *M. leucadenron* essential oil. **Fall et al. (2017**) identified the 1.8-Cineol, α-Terpineol and β-Citronellol as main components *M. leucadendron* .

The main volatile compounds of the analyzed ABEO based on reduced percentages were valerianol 23.24%, guaiol 16.56%, elemol 9.62%, and γ-eudesmol 7.95% **(Table 3)**.

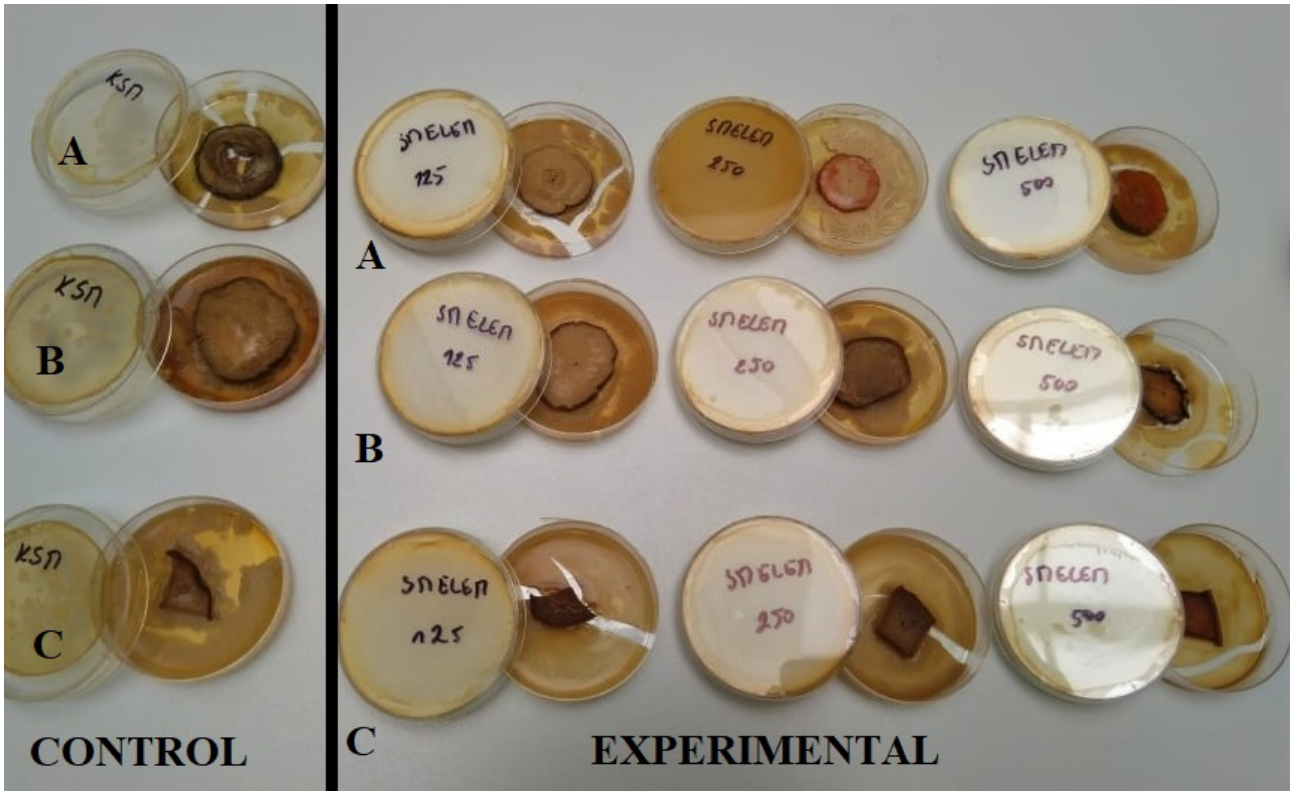

Figure 1 *In situ* antimicrobial analyses of vegetables and fruit with *S. maltophilia* in vapor phase with essential oil *C. luzonicum* (A-carrot, B-potato, C-apple).

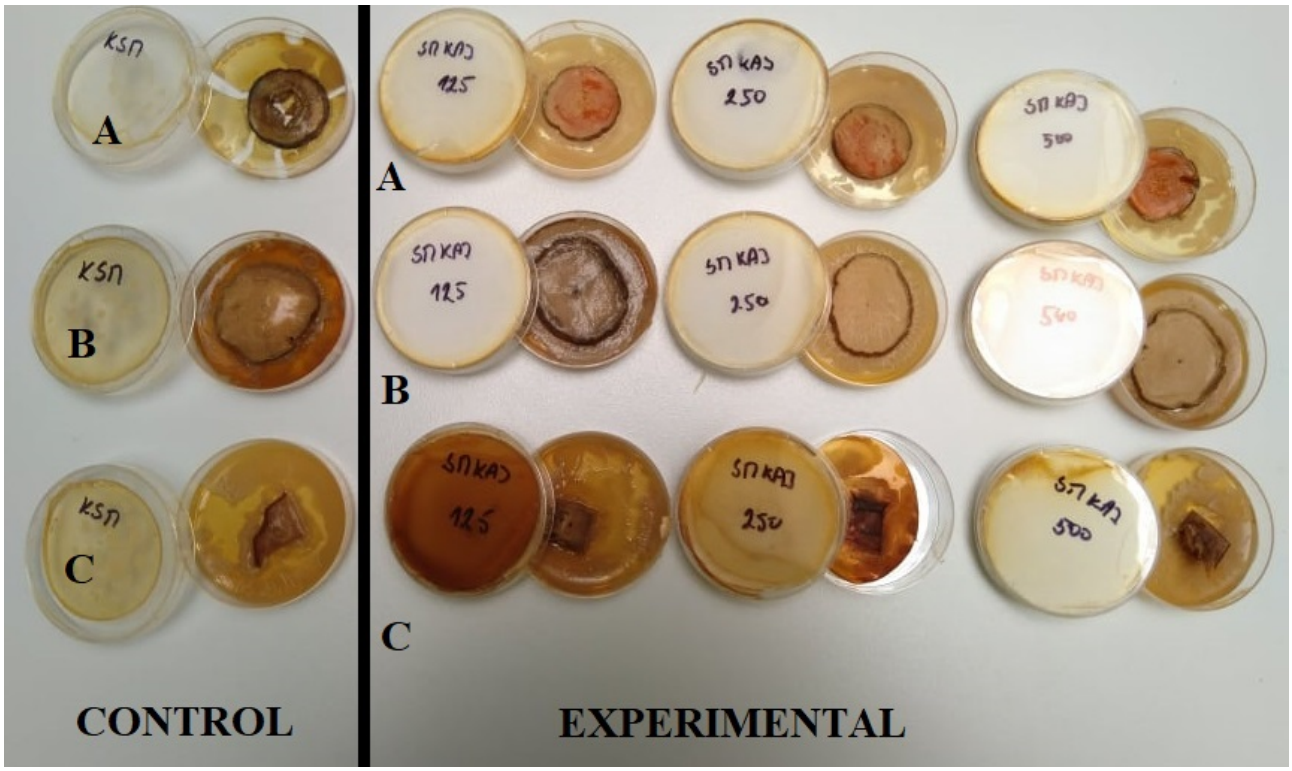

Figure 2 *In situ* antimicrobial analyses of vegetables and fruit with *S. maltophilia* in vapor phase with essential oil *M. leucadendron (*A-carrot, B-potato, C-apple).

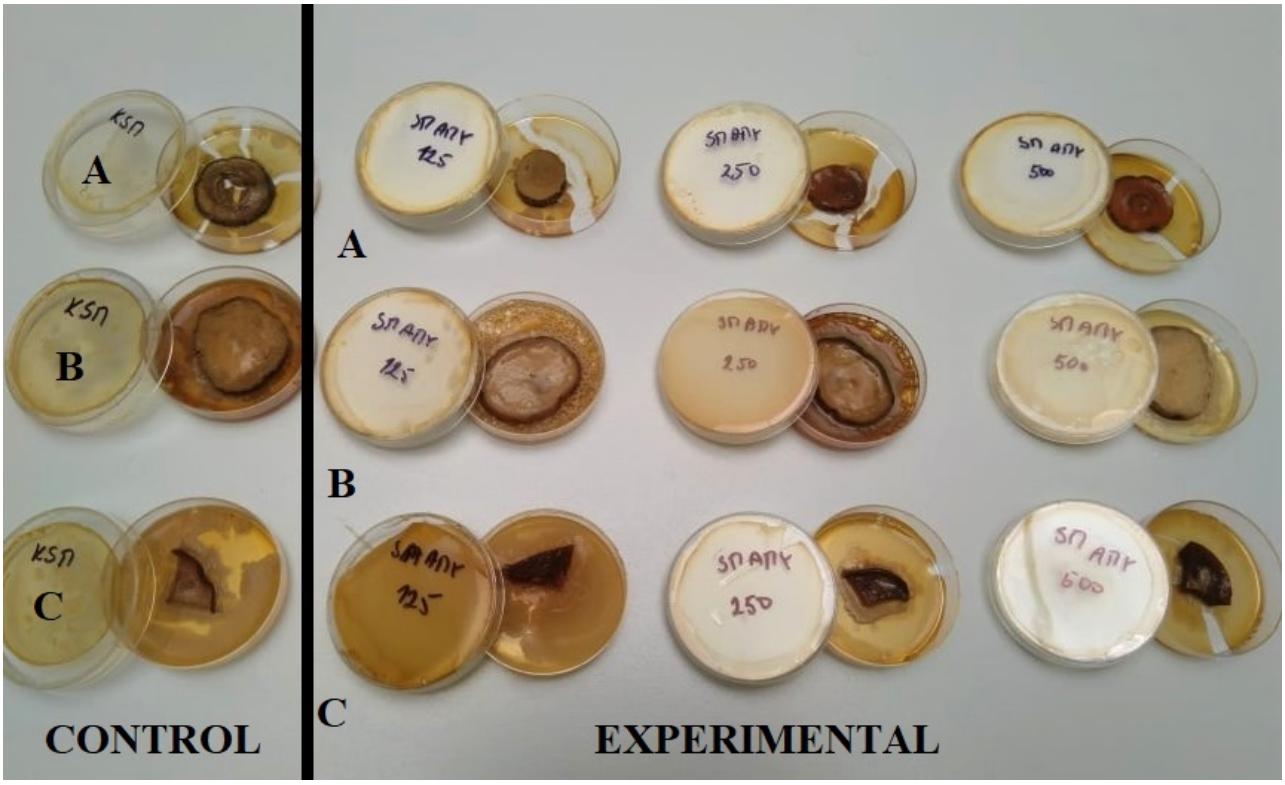

Figure 3 *In situ* antimicrobial analyses of vegetables and fruit with *S. maltophilia* in vapor phase with essential oil *A. balsamifera (*A-carrot, B-potato, C-apple).

Alves et al. (2015) analyzed the chemical composition of amyris essential oil (*Amyris balsamifera* L.) in their study. The main components are eudesmol 23.6%, elemol 14%, and valerianol 12.3%. **Uniyal et al. (2016)** studied the chemical composition of essential oils by gas chromatography and mass spectrometry. Their results show that the main components of amyris oil are β-cadinene 22.66%, (+) - calarene 23.29%, driminol 24%, and linalool oxide 8.48%. **Park and Park (2012)** reported that the major compounds of *A. balsamifera* are β-sesquiphellandrene, elemol, γ-eudesmol and valerianol. **Yun et al. (2012)** they determined chemical compounds essential oil A. balsamifera and the major constituents were elemol, γ-eudesmol and β-sesquiphellandrene.

Antioxidant Activity of essential oils

CLEO essential oil in our study had an antioxidant activity of 33.43%. **Murthy et al. (2016)** determined a DPPH radical inhibition value for the essential oil of 28%. **Lin et al. (2009)** found the antioxidant activity of *C. luzonicum* 11.38%.

MLEO inhibited the DPPH radical at 18.43%. **Pino et al. (2010)** reported that *M. leucadenron* essential oil achieved a free radical inhibition value of 19.9%. **Zhang et al. (2017)** reported antioxidant activity of *M. leucadenron* of 15.7%.

ABEO essential oil achieved an inhibition value of 9.29%. **Nikšić et al. (2018)** studied amyris essential oil in their study and recorded an antioxidant activity of 10.8%. **Dahiya and Manglik (2013)** determined the antioxidant activity of *A. balsamifera* at 19.89%.

The essential oils tested were statistically significantly different (p <0.0001).

Minimum Inhibitory Concentration (MIC)

We determined the MIC 50 and 90 CLEO for *S. maltophilia* to be 6.67 μL.mL^{-1} respectively 6.81 μL.mL^{-1} by using an agar microdilution method. **Nikolic et al. (2016)** focused on the inhibition of clinical isolates of the genus *Candida* by the influence of essential oils and found out that the MIC for *C. luzonicum* oil was 2.5 mg/ml. **Zhang et al. (2017)** reported MIC *C. luzonicum* for *E. coli* 10 μL.mL^{-1} and *P. fluorescens* 12.3 μL.mL^{-1}. **Angelini et al. (2019)** determined MIC of *C. luzonicum* for *A. tubingensis* 12.7 μL.mL^{-1} and *F. oxysporum* 3.17 μL.mL^{-1}.

There was minimum inhibitory concentration 50 and 90 MLEO for *S. maltophilia* 8.25 μL.mL^{-1} and 8.96 μL.mL^{-1}. **Siddique et al. (2020)** reported in his study for *M. leucadendron* MIC values of 4 μL.mL^{-1} for *B. spizizenii*, 8 μL.mL^{-1} *S. aureus* and resistance to *P. aeruginosa* 250 μL.mL^{-1} and *S. enterica* 250 μL.mL^{-1}. **Lieu et al. (2018)** examined the antifungal activity of *M. leucadendron* in food storage and found out MIC of 20 μL.mL^{-1} for *A. niger*. **Pintas and Quave (2019)** focused on the antifungal activity of essential oils against *Malassezia* spp. They determined a MIC of 64 μL.mL^{-1} for *M. leucadendron*. **Bautista-Silva et al. (2020)** found MIC of *M. leucadendra* for *Salmonella thiphymurium* 7.8 μL.mL^{-1} and *Pseudomonas aeruginosa* 31.2 μL.mL^{-1}.

We determined a MIC 50 and 90 of 10.31 μL.mL^{-1} respectively 10.73 μL.mL^{-1} for ABEO. **Xiao et al. (2020)** studied the essential oils and their activity against the stationary phase of *S. aureus*. They determined the MIC for the essential oil of *Amyris balsamifera* 1.5 μL.mL^{-1}. **Santiago et al. (2018)** examined the antibiofilm activity on *Xylella fastidiosa* and found out that the MIC for *Amyris balsamifera* was 125 μL.mL^{-1}.

In Situ Antimicrobial Effect on Vegetables and Fruit

The antimicrobial study of essential oils was determined by an *in situ* method. CLEO inhibited the growth of *S. maltophilia* on the surface of carrots and potatoes at a concentration of 250 mg/ml. Inhibition for apple was recorded at a concentration of 125 mg.mL^{-1} **(Figure 1)**.

MLEO showed inhibition of *S. maltophilia* growth on carrots at a concentration of 500 mg.mL^{-1}, on potato and apple surface at 250 mg.mL^{-1} **(Figure 2)**.

ABEO inhibited bacterial growth at a concentration of 125 mg.mL^{-1} per carrot, 250 mg.mL^{-1} per apple and up to 500 mg.mL^{-1} per potato **(Figure 3)**.

CONCLUSION

The results of our work demonstrated the inhibitory effect of essential oils on *S. maltophila* in a food model. CLEO proved to be the best with the lowest MIC of 6.67 µL.mL^{-1} and antioxidant activity of 33.43% among the tested essential oils. It showed inhibition of *S. maltophilia* growth in the food model at the lowest concentrations among the essential oils.

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