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FULL PAPER

Chemical Characterization and Antimicrobial Activity of Essential Oils of Mint (*Mentha spicata* L.) and Surinam Cherry (*Eugenia uniflora* L.)

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Abstract:

Essential Oils (EOs) of plants are commonly commercially produced for their scents. However, they have also aroused great interest due to their functional properties as antimicrobial substances. The aim of this work was to characterise the chemical composition and evaluate the antimicrobial activity of mint (*Mentha spicata* L.) and surinam cherry (*Eugenia uniflora* L.) EOs. The EOs were obtained by water vapour entrapment in a Clevenger-type distiller and the chemical characterization was performed using gas chromatography and mass spectrometry (GC-MS). The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) for bacteria causing foodborne diseases. Chromatographic analysis of mint EO revealed the presence of 28 distinct components, of which 18 were identified, composing about 90% of the total mass. The major component linalool (58.51%), carvone and compound 19 (total of 15.1%, compounds with overlapping curves on the chromatogram), and terpinen-4-ol (5.73%) were the most abundant compounds. In the chemical characterization of the surinam cherry EO, 16 compounds were found, of which 10 were identified, with more than 75% of the mixture comprising selina-1,3,7(11)-trien-8-one and selina-1,3,7(11)-trien-8-one epoxide. Mint EO had a MIC between 1.60 and 3.20 $\mu L.mL^{-1}$. The surinam cherry EO did not inhibit bacterial growth in this study (MIC> 25.60 $\mu L.mL^{-1}$).

Keywords: essential oils; antimicrobial; aromatic plants

1. Introduction

Essential Oils (EOs) are obtained from aromatic plants and are frequently commercialised for their scents. They also have antimicrobial properties, so are considered to have a great potential as natural additives for food preservation [1].

Depending on the mode of extraction, which is usually by steam distillation, essential oils contain a variety of volatile molecules such as terpenes, aromatic compounds derived from phenol and aliphatic components. The presence of these compounds in EOs explains their broad spectrum of action against bacteria, viruses, fungi, parasites and insects and their potential for use in the pharmaceutical, health, cosmetics, agriculture and food industries [2].

Especially in the food industry, interest in the possibility of using natural compounds in the prevention of microbial growth in food has shown remarkable growth as a response to the consumer pressure for the reduction or even elimination of synthetic additives in industrially produced foods

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[3]. However, the direct application of essential oils as antimicrobials in foods still presents obstacles in their actual industrial application, because of a lack of data in the literature regarding their effects on food, their intense aromas, and high costs [4]. Although several studies show essential oils to be efficient in *in vitro* tests against foodborne pathogens, the same effects were found only when EOs were used at higher concentrations in food. The use of these higher concentrations would have unacceptable impacts on food products [5].

In recent years, researchers reported alternative methods for the use of EOs in food to minimise negative sensory effects. In the present study, the use of EO in primary and secondary packaging [6] or as an edible coating for minimally processed products is proposed [7].

The objective of this work was to perform chemical characterization and evaluate the antimicrobial activity of essential oils of peppermint and surinam cherry by determining their Minimum Inhibitory Concentrations (MICs) against foodborne disease-causing agents.

2. Results and Discussion

Table 1 presents the chemical characterization of peppermint and surinam cherry EOs. Analysis of the peppermint EO by GC-MS revealed the presence of 28 distinct components, of which the 18 identified compounds constituted approximately 90% of the total mass of the oil. For the surinam cherry EO, 16 compounds were distinguished, of which 10 could be identified.

The MIC results are shown in Table 2. Mint EO inhibited the growth of *Staphylococcus epidermidis*, *Bacillus cereus*¸ *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Salmonella enterica* subsp. *enterica* serovar Typhi with a MIC of 1.6 $\mu L.ml^{-1}$. For the other tested bacteria the MIC was 3.2 $\mu L.mL^{-1}$. Inhibitory activity of surinam cherry EO was not detected for the tested bacteria, with the MIC being >25.6 $\mu L.mL^{-1}$.

The major components in the mint EO were linalool (58.51%), carvone and compound 19 (total of 15.1%, compounds with overlapping curves on the chromatogram), and terpinen-4-ol (5.73%), constituted approximately 80% of the total mass of the oil.

Linalool and 4-terpineol are terpenoid alcohols of the monoterpene class. Yang et al. [8] reported that among the compounds found in the essential oil of *Glossogyne tenuifolia*, linalool and 4-terpineol had the strongest antimicrobial effects, exhibiting activity at a maximum concentration of 3 $mg.Kg^{-1}$ against Gram positive (*Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus mutans* and *S. sanguinis*) and Gram-negative pathogens (*Escherichia coli* O157: H7, *Vibrio parahaemolyticus* and *Salmonella enterica*).

It is believed that the relatively high solubility in water and the presence of an alcohol group contribute to the antimicrobial activity of alcoholic monoterpenes [9], which may cause protein denaturation or dehydration of vegetative cells [10].

Carvone is a monocyclic ketone also belonging to the monoterpene class for which fungistatic activity against *Fusarium solani* and *Fusarium sulphureum* and bacteriostatic activity against *Streptococcus thermophilus*, *Lactococcus lactis* and *Escherichia coli* has been reported [11]. Although the mechanisms of action of carvone are not fully characterised, they may be associated with its lipophilic character and thus with accumulation in membranes resulting in a loss of energy by the cells through the dissipation of the proton-motive force [11-13], as described for other small terpenoids and phenolic compounds [14].

It is well established in the literature that carvone is one of the most abundant components of *M. spicata* EO. However, using specimens originary from Botucatu (São Paulo State, Brazil) we found that the essential oil from this specie presented high levels of linalool (58.5%). This modification in EO composition may be triggered by several factors including ambiental ones (temperature, relative humidity, irradiation and photoperiod), cultural practices (plant age; number and time of plant harvest) and genotype, whose may change gene expression [15, 16], and consequently, the production and quality of the EO [17]. Additionally, such changes occur due to the chemotype (chemical race) of the plant, corresponding to identical botanically specimens but with different chemical constitution.

Three different chemotypes were also identified in *M. spicata* specimens from Turkey,

rich in carvone (I), pulegone (II) and linalool (III) [18]. Linalool and linalyn acetate were the main constituints from EOs of several mint specimens: *M. arvensis, M. longifolia* and *M. spicata* [19]. All these interesting findings corroborate with our data and may explain the linalool-rich mint EO obtained.

Table 1. Chemical composition of mint and surinam cherry Essential Oils (EOs) determined by gas chromatography and mass spectrometry (GC-MS).

Mint EO			Surinam cherry EO		
	Compounds	**%**		**Compounds**	**%**
1	α-Pinene	0.38	1	β-Elemene	2.93
2	Octanone	0.40	2	β-Caryophyllene	2.83
3	Octanal	5.78	3	Germacrene A	2.69
4	p-Cymene	0.31	4	Not identified	4.21
5	Limonene	0.33	5	β-Selinene	1.04
6	Eucalyptol	1.07	6	Not identified	0.54
7	Not identified	0.37	7	Spathulenol	1.01
8	Linalool	58.51	8	Caryophyllene oxide	1.96
9	Not identified	0.22	9	Not identified	0.76
10	Not identified	0.35	10	Germacrone	1.06
11	Camphor	0.59	11	Apiol	2.14
12	Menthol	0.35	12	Selina-1,3,7(11)-trien-8one	39.45
13	Terpinen-4-ol	5.73	13	Not identified	0.41
14	α-Terpineol	1.43	14	Not identified	1.31
15	Dihydrocarveol	0.36	15	Not identified	-
16	Not identified	0.65	16	Selina-1,3,7(11)-trien-8-one epoxide	36.71
17	Not identified	0.76			
18	Carvone*	15.1 total			
19	Not identified*	(10.73 +4.37)			
20	Elemene	0.24			
21	β-caryophyllene	2.02			
22	Not identified	0.28			
23	Germacrene D	0.41			
24	Caryophyllene oxide	0.35			
25	Apiol	0.39			
26	Not identified	1.08			
27	Not identified	1.56			
28	Not identified	0.85			
	Total identified	89.38		**Total identified**	91.82
	Total compounds	99.87		**Total compounds**	99.05

*Compounds with overlapping curves on the chromatogram.

It should be noted that in addition to the major components, the mint EO presented other compounds such as camphor, menthol and limonene, which, although not abundant in the oil, are also recognised as antimicrobial agents [8, 20-22] and thus may contribute to the antimicrobial activity. In fact, most of the compounds identified in the mint EO belong to the monoterpene class, to which its antimicrobial effect can be attributed. However, 10 compounds (approximately 30%) have not yet been identified. These data show the potential for the discovery of new antimicrobial substances, present at low concentrations, that could contribute to a synergistic effect, potentiating the activity of other components. Thus, further studies aimed at the

isolation and characterization of these substances are necessary for a broad understanding of the antimicrobial effect of the mint EO.

Table 2. Minimum Inhibitory Concentration (MIC) of mint and surinam cherry Essential Oils (EOs) determined by broth microdilution method.

Bacteria	MIC ($\mu L.mL^{-1}$) of mint EO	MIC ($\mu L.mL^{-1}$) of surinam cherry EO
Staphylococcus aureus (ATCC 14458)	3.2	>25.6
Staphylococcus epidermidis (ATCC 12228)	1.6	>25.6
Bacillus cereus (ATCC 11778)	1.6	>25.6
Listeria monocytogenes (ATCC 7644)	3.2	>25.6
Escherichia coli (ATCC 11229)	3.2	>25.6
Salmonella enterica subsp. *enterica* serovar Typhimurium (ATCC 13311)	1.6	>25.6
Salmonella. enterica subsp. *enterica* serovar Typhi (ATCC 19214)	1.6	>25.6
Shigella flexneri (ATCC 12022)	3.2	>25.6

The MIC values found for the mint EO are consistent with the values presented by Chrysargyris et al. [23], with MICs of 3.125 $mg.mL^{-1}$ for *Listeria monocytogenes* and *Salmonella enteriditis* and 6.25 $mg.mL^{-1}$ for *Escherichia coli* and *Staphylococcus aureus*. On the other hand, the MIC values in this study for the mint EO were higher than those recorded by Sherer et al. [24] who found that 0.67 $mg.mL^{-1}$of EO was able to inhibit the growth of *Staphylococcus aureus* by100% and that of *Escherichia coli* by 51%. Vermaa et al. [25] found MIC values between 250 and 1000 $\mu g.mL^{-1}$ for an essential oil from *Mentha citrata*. However, the authors demonstrated that the methods used for distillation — hydrodistillation or water vapour dragging — influenced the chemical composition and antimicrobial activity of the resulting EOs, as well as the antimicrobial activity and active principles found in the distillate (hydrosol).

Another issue that influences the obtained MIC values is the methodology used for testing and evaluation, which should be carefully defined and standardised. The methodology, lineage of the tested microorganism, culture medium, inoculum density, exposure time of microorganism to oil, use of positive and negative controls and type of emulsifier should be considered as factors of influence [26].

Considering other EOs, Prabuseenivasan, Jayakumar and Ignacimuthu [27] evaluated the antibacterial activity of 21 plant EOs against six bacterial species (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus*), with concentrations varying from 0.2 to 25.6 $mg.mL^{-1}$. *B. subtilis* was the most susceptible and *K. pneumoniae* exhibited lower sensitivity. The study showed that cinnamic EO had better antimicrobial activity: MICs of 3.2 $mg.mL^{-1}$ for *S. aureus* and *K. pneumoniae*, MIC of 1.6 mg $.mL^{-1}$ for *B. subtilis*, *P. vulgaris* and *E. coli*, and MIC of 0.8 $mg.mL^{-1}$ for *P. aeruginosa*. Clove EO was the second with better antimicrobial activity, with MIC of 6.4 $mg.mL^{-1}$ for *S. aureus* and *K. pneumoniae*, MIC of 3.2 $mg.mL^{-1}$ for *B. subtilis* and *P. vulgaris* and MIC of 1.6 $mg.mL^{-1}$ for *P. aeruginosa* and *E. coli*.

For lemon grass EO, Perazzo et al. [28] found MICs of 0.5626 $mg.mL^{-1}$ for *Streptococcus mutans* (ATCC 25175) and MIC of 2.25 $mg.mL^{-1}$ for *S. salivarius* (ATCC 7073) and *S. oralis* (ATCC 1055). Jovanka et al. [29] established for oregano EO, MIC and Minimum Bactericidal Concentration (MBC) of 0.39 and 0.78 $\mu L.mL^{-1}$, respectively, against Gram negative bacteria and MIC and MBC of 0.78 and 1.56 $\mu L.mL^{-1}$, respectively, against Gram positive bacteria. On the other hand, thyme EO had MIC and MBC in the range of 0.39-1.56 and 0.78-3.125 $\mu L.mL^{-1}$, respectively, against Gram negative bacteria and MIC in the range of 3.125-6.25 $\mu L.mL^{-1}$ and MBC of 6.25 $\mu L.mL^{-1}$ against Gram positive bacteria.

Among the compounds identified in the

surinam cherry EO, the major components were the compounds selina-1,3,7(11)-trien-8-one and selina-1,3,7-(11)-trien-8-one epoxide, which together comprise 76.16% of this essential oil from surinam cherry leaves. These compounds, discovered by Rücker in1977, belong to the sesquiterpene class of compounds, which can have anti-inflammatory, antispasmodic, antiallergic and vermifugic properties and have been used in pharmaceutical products [30]. Other minor compounds identified, such as germacrene A, espatulenol, caryophyllene oxide and germacrone, are also known to be active against bacteria. However, their concentration in the essential oil is low, comprising 6.72% of the total.

There are some previous reports of the identification of chemical components of surinam cherry EO. Morais et al. [31] isolated and identified the components of an EO from surinam cherry leaves harvested in the Northeast region of Brazil, of which the major components were: selina-1,3,5 (11)-trien-8-one and oxidoselina-1,3,7(11)-trien-8-one, comprising 48.52% and 17.33% of the total mass of oil, respectively. Brun and Mossi [32] identified 15 compounds in an oil from the leaves of surinam cherry trees from Erechim - RS - ocimene, β-elemeno, β-caryophyllene, elemeno, trans-caryophyllene, bicyclogermacrene, curzerene, cadinene, germacrenol B, spathulenol, selina-1,3,7(11)-trien-8-one, astragalone, furanedione, germacron and oxidoselina-1,3,7(11)-trien-8-one. Wyerstahl et al. [33] detailing the composition of a *Eugenia uniflora* EO from Nigeria, identified the major compounds ascaryophyllene (5.7%), furanediene (24%), germacrene B (5.8%), (11)-trien-8-one (17%), and oxidoselina-1,3,7(11)-trien-8-one (14%).

Comparing these data with the major chemical constituents of the surinam cherry EO obtained in this study, it is clear that selina-1,3,7(11)-trien-8-one and selina-1,3,7(11)-trien-8-one epoxide are found in other EOs to a greater or lesser extent. In contrast to other published studies, no antimicrobial activity was observed for the surinam cherry EO. Using the disk diffusion method, Souza et al. [34] found activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus* in an extract of surinam cherry leaves. *S. epidermidis*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae* were considered resistant. Brun and Mossi [32] also observed inhibition halos caused by a surinam cherry EO in growth tests of *Micrococcus luteus*, *Staphylococcus epidermidis* and *Xanthomonas campestris*. For a surinam cherry leaf EO, Ogunwande et al. [35] found MICs of 39 $\mu g.mL^{-1}$ for *Bacillus cereus*, 156 $\mu g.mL^{-1}$ for *Staphylococcus aureus* and 625 $\mu g.mL^{-1}$ for *Escherichia coli* and *Pseudomonas aeruginosa*.

The absence of antimicrobial activity of the surinam cherry EO in this study may be explained by the low content of known antimicrobial substances in the obtained oil, as well as by the species of microorganisms and methods used in the tests. Costa et al. [36] highlighted that, depending on the place of origin of an EO, the part of the plant used and/or the climatic conditions in each region, the oil yield and relative concentrations of the substances in the oil can be affected, consequently altering its antimicrobial properties.

3. Material and Methods

3.1. Extraction and chemical characterization of essential oils

The EOs were obtained from the leaves (approximately 2 Kg of fresh leaves) of mint (*Mentha spicata* L.) and surinam cherry (*Eugenia uniflora* L.) by steam trawling in a Clevenger type distiller (model MA480 - Marconi) at the Laboratory of Natural Products of the Department of Microbiology and Immunology, at the Biosciences Institute of Botucatu in the Paulista State University "Júlio de Mesquita Filho".

The mint sample was collected from plants growing naturally in areas near the campus of the Biosciences Institute of Botucatu in the Paulista State University "Júlio de Mesquita Filho" (22º 57' 13.67" S and 48º 30' 23.19" W), and the collection was done in a schedule that did not exceed 9 o'clock in the morning. The surinam cherry sample was obtained in the form of dehydrated leaves of essential oils producer Fazenda Alpina / city of Santa Bárbara, São Paulo, Brazil, owned by Mr. Ivo Gregori (23º 01' 58.5" S and 49º 09' 52.7" O).

All the plant samples were identified and the respective exsicates deposited in the Herbarium Profa. Dra. Irina Delanova Gemtchujnicov from the Department of Botany at the Biosciences

Institute of Botucatu in the Paulista State University "Júlio de Mesquita Filho", receiving deposit numbers BOTU 27619 and BOTU 25796, for mint and surinam cherry, respectively.

The chemical characterization was performed in a gas chromatograph coupled to a mass spectrometer (GC-MS) (model QP5050A - Shimadzu) at the Laboratory of Chemistry of the Department of Chemistry and Biochemistry, at the Institute of Biosciences of Botucatu in the Paulista State University "Júlio de Mesquita Filho". A capillary column, CBP-5, 50 m long, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm was used. The injector temperature and the interface temperature were 240°C, with the detector operating in 70eV EI mode and using He as the drag gas. The chromatographic conditions were as follows: an initial temperature of 40°C, followed by heating to 180°C at a rate of 3°C.min^{-1} and then heating to 230°C at a rate of 20°C.min^{-1}. The identification of the EO components was based on the interpretation of the mass spectra, using the NIST (National Institute of Standards and Technology, MD, USA) library. The Retention Index values were used, considering a homologous series of n-alkanes under the same injection conditions as the essential oils and data from the literature [37].

3.3. Antimicrobial activity of the essential oils

The antimicrobial activity was assessed at the Microbiology Laboratory of the Technology Department at the Umuarama Campus of the State University of Maringá. The broth microdilution method [38] was used to determine the MIC of the EOs, the tested concentrations being 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 µL.mL^{-1}. The dilutions were prepared in Mueller-Hinton Broth containing 0.5% Tween 80.

The antimicrobial activity of the EOs was tested against the following cultures: *Staphylococcus aureus* INCQS 00005 (ATCC 14458), *Staphylococcus epidermidis* INCQS 00016 (ATCC 12228), *Bacillus cereus* INCQS 00003 (ATCC 11778), *Listeria monocytogenes* INCQS 00266 (ATCC 7644), *Escherichia coli* INCQS 00032 (ATCC 11229), *Salmonella enterica* subsp. *enterica* serovar Typhimurium INCQS 00084 (ATCC 13311), *Salmonella enterica* subsp. *enterica* serovar Typhi INCQS 00040 (ATCC 19214) and *Shigella flexneri* INCQS 00152 (ATCC 12022). All cultures were obtained from the Reference Microorganism Collection in Sanitary Surveillance - CMRVS, FIOCRUZ-INCQS, Rio de Janeiro - Brazil.

The stock cultures were activated in BHI broth and incubated at 35ºC for 24 hours. The bacterial inoculum were then standardised in 0.85% sterile saline solution at 0.5 on the MacFarland scale, obtaining bacterial suspensions of around 1.0x10^{8} CFU.mL^{-1}. The standardisation was verified by confirming an absorbance reading between 0.08 and 0.10 in a spectrophotometer (Kasuaki, IL-227) at a wavelength of 625nm. After standardisation, each inoculum was diluted 1:10 in saline, followed by further 1:10 dilution in Mueller-Hinton Broth.

In the microdilution plates (96 wells ~ 200µL), 50 µL of EO at double the concentration to be tested was placed into the wells, followed by the addition of 50 µL of inoculum. In this way, the final concentrations of the EOs were obtained with a bacterial concentration of approximately 5.0x10^{5} CFU.mL^{-1}. The same cellular concentration was obtained in one well for each bacteria containing only culture medium for the certification of bacterial growth (positive control).

The color of the wells was read after incubation at 35ºC for 24 hours, after the addition of 15µL of redox indicator (resazurin 0.1%) to each well. Final blue staining indicated a negative result and a pink colour indicated a positive result for bacterial growth. The tests were performed in triplicate for each bacterium and the MIC was considered the lowest concentration in which there was no bacterial growth in at least two replicates after the incubation period.

4. Conclusions

The results in this study show the potential of using mint EO as an antimicrobial agent. The data obtained in the chemical analysis of the oil are in agreement with the antimicrobial tests, as they show the substantial presence (more than 80% in mass) of known antimicrobial compounds. In addition, about 10 compounds still remain to be identified and characterised, which may have potential as new natural antimicrobial

compounds.

In this work, the bacteria studied were not sensitive to surinam cherry EO. There is a need for more detailed information about how variations in plant cultivation may influence the chemical composition of derived EOs. Furthermore, the methodology used to evaluate antimicrobial activity may influence the results obtained for antimicrobial efficacy.)

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