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# Chemical composition and thermal properties of commercial essential oils and their antimicrobial and antioxidant activities

## Composição química e propriedades térmicas de óleos essenciais comerciais e suas atividades antimicrobianas e antioxidantes

## Composición química y propiedades térmicas de los aceites esenciales comerciales y sus actividades antimicrobiana y antioxidante

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**Abstract**
This study aimed to chemically characterize commercial Essentials oils and determine their thermal properties and their antimicrobial and antioxidant activities. Essential oils extracted from leaves of Rosemary, lemongrass, cloves, orange, Tahiti lemon and thyme were studied, with chemical characterization by gas chromatography/mass spectrometry, thermoanalytical characterization and antimicrobial (microdilution) and antioxidant (ferric reduction activities power - FRAP). The chemistry of the essential oils composition was consistent with literature data and justified their thermal behavior. Comparing the thermal stability between the studied essential oils results revealed the optimization of the da $T_{onset}$ = 106.6°C to clove`s essential oils. The results of differentiated antimicrobial and antioxidant activity justified by factors that influence the biological functions of Essentials oils, such as the origin of the plant (climate conditions), forms of cultivation and harvesting of the raw material, parameters and extraction method. Thus, it emphasizes the importance of a Brazilian production of essentials to take advantage of the technological and analytical capacity of national research centers, to provide the market with traceable products with certified identity.
**Keywords:** Thermogravimetric analysis; Chemical characterization**;** Essencial oils; Antimicrobial sensitivity; Antioxidant potential.

**Resumo**

O presente estudo teve como objetivo caracterizar quimicamente óleos essenciais comerciais e determinar as suas propriedades térmicas, atividade antimicrobiana e antioxidante. Foram estudados os óleos essenciais de alecrim, capim-limão, cravo, laranja, limão – tahiti e tomilho extraído das folhas. Os óleos foram analisados por meio de Cromatografia Gasosa acoplada ao Espectro de Massas (CG-EM), caracterização termoanálitica e atividades antimicrobiana (por microdiluição) e antioxidante via método de poder de atividade de redução férrica (FRAP). A composição química dos óleos essenciais se mostrou consistente com dados estudados na literatura e justificou seu comportamento térmico. Comparando a estabilidade térmica entre os óleos essenciais estudados resultados revelaram a otimização da $T_{onset}$ = 106,6℃ do óleo essencial de cravo. Resultados das atividades antimicrobiana e antioxidante foram justificados por fatores que influem nas funções biológicas dos óleos essenciais como as condições climáticas da origem da planta, formas de cultivo e colheita da matéria-prima, parâmetros e métodos de extração. Deste modo, este trabalho tem como fundamento prioritário enfatizar a importância de uma produção brasileira dos óleos essenciais e aproveitar a capacidade tecnológica e analítica dos centros de pesquisa nacionais para fornecer ao mercado brasileiro produtos rastreáveis e com identidade certificada.

**Palavras-chave:** Análise termogravimétrica; Caracterização química; Óleos essenciais; Sensibilidade antimicrobiana; Potencial antioxidante.

**Resumen**

El presente estúdio tuvo como objetivo más grande caracterizar quimicamente los aceites esenciales comerciales y determinar sus propiedades térmicas, actividad antimicrobiana y antioxidante. Los aceites esenciales de romero, limoncillo, clavo, naranja, limón-tahití y tomillo extraídos de las hojas. Los aceites se analizaron utilizando Cromatografía de gases acoplada a espectro de masas (GC-MS), caracterización termoanalítica y actividades antimicrobianas (por microdilución) y antioxidante a través del método de potencia de actividad reductora férrica (FRAP). La composición química de los aceites esenciales fue consistente con los datos estudiados en la literatura y justificó su comportamiento térmico. Comparando la estabilidad térmica entre los aceites esenciales estudiados, los resultados revelaron la optimización de la $T_{onset}$ = 106,6℃ de aceite esencial de clavo. Los resultados de las actividades antimicrobiana y antioxidante se justificaron por factores que influyen en las funciones biológicas de los aceites esenciales como las condiciones climáticas del origen de la planta, formas de cultivar y cosechar la materia prima, parámetros y métodos de extracción. De esta manera, este trabajo tiene como fundamento prioritario enfatizar la importancia de una producción brasileña de aceites esenciales y aprovechar la capacidad tecnológica y analítica de los centros de investigación nacionales para abastecer el mercado brasileño productos trazables con identidad certificada.

**Palabras clave:** Análisis termogravimétrico; Caracterización química; Aceites esenciales; Sensibilidad antimicrobiana; Potencial antioxidante.

## 1. Introduction

Brazil has a world production of essential oil, but it also lacks the maintenance of standard representation problems related to the maintenance of the quality paper of essential products and the difficulty of bringing the productive sector closer to the national standard maintenance centers, as a way of adding value to the primary product through the parameters of identity and quality of essential oils (Bizzo *et al.*, 2009).

As a market, the public has expressed growing interest in the trend and production processes of consumption from origins. In this way, environmental preservation policies have been allied to marketing strategies, creating an opportunity to develop processes for exploring biodiversity.

There are several international conglomerates that trade essential oils, mainly as raw material for the production of aromas and oils. However, essential products have antimicrobial and agricultural activity, including antimicrobial and antifungal resistant components, with great potential for chemical application in pharmaceutical, sanitary, antimicrobial and foodstuffs (Majolo *et al.*, 2019).

Considering the importance of knowing the properties related to the chemical composition of batches of essential oils, with consequent implications for their biological activities, this study aimed to characterize chemically essential oils and determine their thermal properties and their antimicrobial and antioxidant activities.

## 2. Methodology

Essential oils of rosemary (Rosmarinus officinalis), lemongrass (Cymbopogon schoenanthus), clove (Eugenia caryophyllus) - extracted from the leaves, orange (Citrus aurantium), Tahiti lemon (Citrus aurantifolia) and thyme (Thymus vulgaris), kindly donated by the company By Samia.

### 2.1 Chemical characterization

Essential oil samples were analyzed by Gas Chromatography coupled to Mass Spectrometry. A Thermo device, model Focus GC, was used as a DB5-MS capillary column (30 m x 0.25 mm and 0.25 µm), with helium gas for the drag. Temperatures used were 230 °C in the inlet and detector. Initial temperature of the column was 60 °C, being programmed to increase by 3 °C every minute, until reaching the maximum temperature of 220 °C. The injection volume was 1 µL. A mixture of linear alkanes (C4 to C20) was injected into the chromatograph under the same conditions used for the analysis of essential oils for the calculation of the retention index with linear temperature programming. Identification of compounds was performed by comparing the linear retention index with the Adams library (2017).

### 2.2 Thermal properties

Thermoanalytical characterization of essential oils was performed in a high resolution simultaneous equipment (STA 6000 Simultaneous Thermal Analyzer PerkinElmer Frontier), in the temperature range of 25 to 250 °C. Tests were submitted to a heating rate of 20 °C min$^{-1}$. Approximately 10 µL of sample were placed in an open platinum-rhodium crucible, under a dynamic $N_2$ atmosphere (30 mL min$^{-1}$). By thermogravimetry (TG) it was possible to observe information related to mass changes, as a function of temperature under dynamic atmosphere. The experiments were performed on a thermobalance of high sensitivity, reproducibility and response to mass variations. The equipment was previously calibrated with indium at a melting temperature of 156.6 °C.

### 2.3 Antimicrobian activity

By the microdilution method in microplates (CLSI, 2002; CLSI, 2009) the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the oils were determined. Essential for concentrations of 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 µL mL$^{-1}$ against the Staphylococcus aureus bacteria INCQS 00381 (ATCC 29213) and Escherichia coli INCQS 00033 (ATCC 25922) in quadruplicate. For MIC and Minimum Fungicide Concentration (MFC) of essential oils, concentrations of 16.384, 8.192, 4.096, 2.048, 1.024, 0.512, 0.256, 0.128, 0.064 and 0.032 µL mL$^{-1}$ were tested against three strains of Candida albicans yeast, ATCC 26790 (in triplicate), ATCC 24433 (in triplicate), and ATCC 90029 (in duplicate). All microorganisms came from the Collection of Reference Microorganisms in Sanitary Surveillance – CMRVS, FIOCRUZ-INCQS, Rio de Janeiro, RJ, Brazil.

The highest concentrations were prepared in Mueller Hinton (MH) broth with 0.5% Tween 80, in double concentration. From this concentration, serial dilution was performed in the microplate, obtaining 50 µL of each concentration (columns) in the microplate wells.

Inoculums were activated in 3 mL of Tryptic Soy Broth (TSB) broth and incubated at 35 °C for 24 hours. Standardization of cultures was performed on the 0.5 MacFarland scale (1.0 108 CFU mL$^{-1}$) using a spectrophotometer (Kasuaki, IL-227) with a wavelength of 625 nm for bacteria and 530 nm for strains of yeast. Then, the inoculum was prepared with two serial dilutions, the first dilution at a ratio of 1:10 in saline solution (1.0 107 CFU mL$^{-1}$) and the second dilution 1:10 in MH broth (1.0 106 CFU mL$^{-1}$) for bacteria and the second dilution 1:20 in MH broth enriched with 2% glucose (5.0 x 104 CFU mL$^{-1}$).

In the microplate, 50 µL of the inoculum were added to each well, obtaining a cell concentration of 5.0 105 CFU mL$^{-1}$ of bacteria and 2.5 104 CFU mL$^{-1}$ of yeast. The microplates were incubated in a bacteriological oven at 37 ºC for 24 hours. The MIC was determined by adding 50 µL of the 0.01% resazurin indicator to each well. After two hours of incubation at 37 °C, the visual color reading was performed, considering bacterial inhibition as blue, and bacterial growth as pink. From verify the CBM and CFM, each well of the microplates was subcultured in Petri dishes containing MH agar, after incubation and before the addition of the resazurin indicator. MH agar plates were incubated under aerobic conditions for 24 hours at 37°C. The lack of colony development in each well indicated bactericidal or fungicidal activity.

### 2.4 Antioxidant activity

Antioxidant activity of essential oils was verified by determining the ferric reducing power (FRAP) following the method described by Benzie and Strain (2020). The calibration curve was prepared with a solution of Trolox and the results were expressed in μmol of Trolox mL$^{-1}$ of sample.

## 3. Results and Discussion

Gas chromatography is an ideal technique for the separation of volatile and semi-volatile constituent substances. The mass spectrum attached to the chromatogram has an additional advantage whereby each separate constituent can be fragmented and the fragmentation pattern can be compared with the spectra available in the databases, and finally, all available constituents can be separated, fragmented and characterized simultaneously (Al-Asmari *et al.*, 2017). Compositions of Rosemary, lemongrass, clove, orange, Tahiti lemon and thyme essential oils are shown in Tables 1 to 6, respectively.

**Table 1** - Chemical composition of rosemary essential oil.

| **Elution`s ordem** | **Retention time (min)** | **Linear Retention Index** | **Retention Index** | **Identified Compound** | **Area (%)** |
|---|---|---|---|---|---|
| 1 | 8.18 | 930 | 935 | α-Pinene | 16.28 |
| 2 | 8.78 | 946 | 946 | Camphene | 5.43 |
| 3 | 9.95 | 976 | 976 | β-Pinene | 4.70 |
| 4 | 10.33 | 986 | 988 | β-Myrcene | 2.52 |
| 5 | 10.92 | 1002 | - | 1,8 Cineol | 27.13 |
| 6 | 11.11 | 1006 | 1008 | 3-Carene | 0.70 |
| 7 | 11.47 | 1014 | 1014 | α-Terpinene | 0.99 |
| 8 | 11.80 | 1021 | - | β-Cymene | 2.16 |
| 9 | 12.00 | 1026 | 1028 | Limonene | 4.26 |
| 10 | 12.12 | 1029 | 1031 | Eucalyptol | 2.93 |
| 11 | 13.26 | 1055 | 1055 | γ-Terpinene | 1.32 |
| 12 | 14.48 | 1082 | 1080 | Terpinolene | 0.70 |
| 13 | 15.17 | 1098 | 1098 | β-Linalool | 2.10 |
| 14 | 15.37 | 1102 | 1102 | Linalool | 1.76 |
| 15 | 17.28 | 1144 | - | L-Camphor | 20.72 |

| | | | | | |
|---|---|---|---|---|---|
| 16 | 18.42 | 1160 | 1164 | Borneol | 0.35 |
| 17 | 19.50 | 1192 | 1192 | α-Terpineol | 3.34 |
| 18 | 23.55 | 1281 | 12.84 | Bornylacetate | 0.71 |
| 19 | 29.38 | 1415 | 1415 | Caryophyllene | 1.84 |
| **Total identified identified** | | | | | **99.94** |

(*): tabled Kováts index [Adams, 2017]. (-): no reference *webbook.nist.gov*. Source: Authors.

**Table 2** - Chemical composition of lemongrass essential oil.

| **Elution`s ordem** | **Retention time (min)** | **Linear Retention Index** | **Retention Index** | **Identified Compound** | **Area (%)** |
|---|---|---|---|---|---|
| 1 | 8.80 | 947 | 946 | Camphene | 1.07 |
| 2 | 10.14 | 982 | 985 | 6-Methyl-5-heptene-2-one | 1.21 |
| 3 | 15.2 | 1099 | 1096 | α-Linalool | 1.23 |
| 4 | 18.89 | 1179 | - | Limonene oxide,cis- | 1.01 |
| 5 | 21.54 | 1237 | 1240 | β-Citral | 38.42 |
| 6 | 22.10 | 1249 | 1249 | 3,7-Dimethyl-2,6-octadien1-ol | 3.42 |
| 7 | 22.89 | 1267 | 1240 | α-Citral | 45.39 |
| 8 | 27.80 | 1378 | 1379 | Geraniol ester | 3.94 |
| 9 | 29.43 | 1416 | 1417 | Caryophyllene | 3.06 |
| 10 | 33.30 | 1510 | - | δ-Muurolene | 1.24 |
| **Total identified identified** | | | | | **99.99** |

(*): tabled Kováts index [Adams, 2017]. (-): no reference *webbook.nist.gov*.

**Table 3** - Chemical composition of clove essential oil.

| **Elution`s ordem** | **Retention time (min)** | **Linear Retention Index** | **Retention Index** | **Identified Compound** | **Area (%)** |
|---|---|---|---|---|---|
| 1 | 26.63 | 1351 | 1356 | Eugenol | 87.33 |
| 2 | 29.42 | 1416 | 1418 | Caryophyllene | 11.84 |
| 3 | 30.90 | 1452 | 1459 | α-Caryophyllene | 0.83 |
| **Total identified identified** | | | | | **100.00** |

(*): tabled Kováts index [Adams, 2017]. Source: Authors.

**Table 4** - Chemical composition of orange essential oil.

| **Elution`s ordem** | **Retention time (min)** | **Linear Retention Index** | **Retention Index** | **Identified Compound** | **Area (%)** |
|---|---|---|---|---|---|
| 1 | 10.42 | 989 | 988 | α-Myrcene | 1.34 |
| 2 | 12.10 | 1029 | 1027 | m-Mentha-6,8-diene | 98.66 |
| | | | **Total identified identified** | | **100.00** |

(*): tabled Kováts index [Adams, 2017]. Source: Authors.

**Table 5** - Chemical composition of Tahiti lemon essential oil.

| **Elution`s ordem** | **Retention time (min)** | **Linear Retention Index** | **Retention Index** | **Identified Compound** | **Area (%)** |
|---|---|---|---|---|---|
| 1 | 7.91 | 923 | 924 | 3-Thujene | 0.72 |
| 2 | 8.17 | 930 | 932 | α-Pinene | 2.43 |
| 3 | 9.67 | 969 | - | β-Phellandrene | 1.88 |
| 4 | 9.86 | 974 | 974 | β-Pinene | 11.79 |
| 5 | 1.,32 | 987 | 988 | a-Myrcene | 1.18 |
| 6 | 1.79 | 1022 | 1022 | m-Cymene | 0.80 |
| 7 | 12.04 | 1027 | 1025 | D-Limonene | 55.32 |
| 8 | 13.28 | 1055 | 1054 | ƴ-Terpinene | 18.10 |
| 9 | 14.47 | 1082 | 1088 | Terpinolene | 0.63 |
| 10 | 19.49 | 1192 | 1186 | α-Terpineol | 0.24 |
| 11 | 21.46 | 1235 | 1240 | β-Citral | 0.80 |
| 12 | 22.8 | 1265 | 1270 | α-Citral | 1.19 |
| 13 | 26.84 | 1356 | 1359 | Neryl Acetato | 1.02 |
| 14 | 27.7 | 1376 | 1379 | Geraniol ester | 0.12 |
| 15 | 29.36 | 1415 | 1417 | Caryophyllene | 0.56 |
| 16 | 29.98 | 1430 | 1430 | 2-Norpinene, 2,6,-dimethyl-6-(4-methyl-3-pentenyl) | 1.40 |
| 17 | 33.06 | 1504 | 1501 | 1,5-Heptadiene, 6-methyl-2-(4-methyl-3-cyclohexen-1-yl) | 1.80 |
| | | | **Total identified identified** | | **99.98** |

(*): tabled Kováts index [Adams, 2017]. (-): no reference *webbook.nist.gov.* Source: Authors.

**Table 6** - Chemical composition of thyme essential oil.

| Elution`s ordem | Retention time (min) | Linear Retention Index | Retention Index | Identified Compound | Area (%) |
|---|---|---|---|---|---|
| 1 | 7.98 | 925 | 924 | 3-Thujene | 0.22 |
| 2 | 8.23 | 932 | 931 | α-Pinene | 0.50 |
| 3 | 9.90 | 976 | 974 | β-Pinene | 2.23 |
| 4 | 10.38 | 988 | 988 | α-Myrcene | 0.34 |
| 5 | 11.86 | 1023 | 1021 | m-Cymene | 48.04 |
| 6 | 13.34 | 1057 | 1062 | δ Terpinen | 34.60 |
| 7 | 23.89 | 1289 | 1290 | Thymol | 14.06 |
|  |  |  | **Total identified identified** |  | **99.99** |

(*): tabled Kováts index [Adams, 2017]. Source: Authors.

Were identified as majores compounds for rosemary essential oil.1,8-cineole (27.13%), L-camphor (20.72%) and α-pinene (6.28%). For lemongrass essential oil were β-citral (38.42%), α-citral (45.39%) and 3,7-dimethyl-2,6-octadien1-ol (3.42%). For clove essential oil (leaf) were eugenol (87.33%), caryophyllene (11.84%) and α-caryophyllene (0.83%). For orange essential oil, almost all was m-Mentha-6,8-diene (98.66%). For Tahiti lemon essential oil were D-limonene (55.32%), ɣ́-terpinene (18.10%) and β-pinene (11.79%). And for thyme essential oil were m-cymene (48.04%), δ-terpinen (34.60%) and thymol (14.06%).

` Major volatile components of the essential oils in this study were found to be consistent based on previously published studies in which researchers identified the same volatile compounds, only with variations in concentrations. Clove essential oil was reported in the literature with eugenol (69.68%) and caryophyllene (15.38%) as main compounds (Kaur *et al.*, 2019).

The citrus essential oils (lemon grass, orange and Thaiti lemon) showed a chemical composition formed by terpenes, oxygenated compounds and terpenoids, generally the main components found are α-pinene, sabinene, β-pinene, β-myrcene, D-limonene, linalool, m-cymene and 4-terpineol. However, D-limonene deserves to be highlighted as it is the main component representing 66 to 93% (Bozkurt *et al.*, 2017).

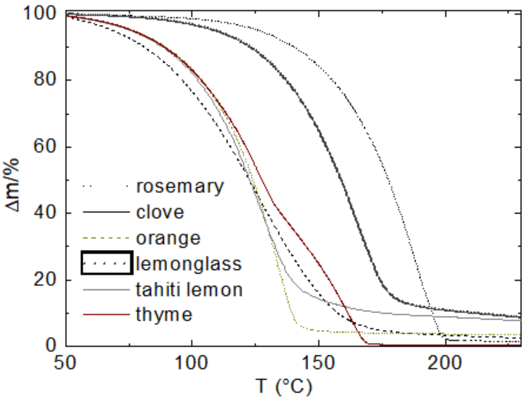
Main compounds reported in the literature for thyme essential oil, from different regions, were thymol (46.2 to 67.5%), carvacrol (5.7 to 7.3%) and caryophyllene oxide (1.7% at 7.3%) (Mancini et al.; 2015).

Composition essential`s oils can be influenced by many factors, such as cultivar, climatic and geographical factors, handling during and after harvest. For these reasons, plants of the same species, but from different contexts, can express different characteristics and chemical compositions (Al-Asmari *et al.*, 2017).

### 3.2 Thermal properties

Thermogravimetric Analysis (TG) was applied to evaluate the thermogravimetric decomposition of essential oils, according to the profiles shown in (Figure 1).

**Figure 1** - TG`s profiles found for essential oils.

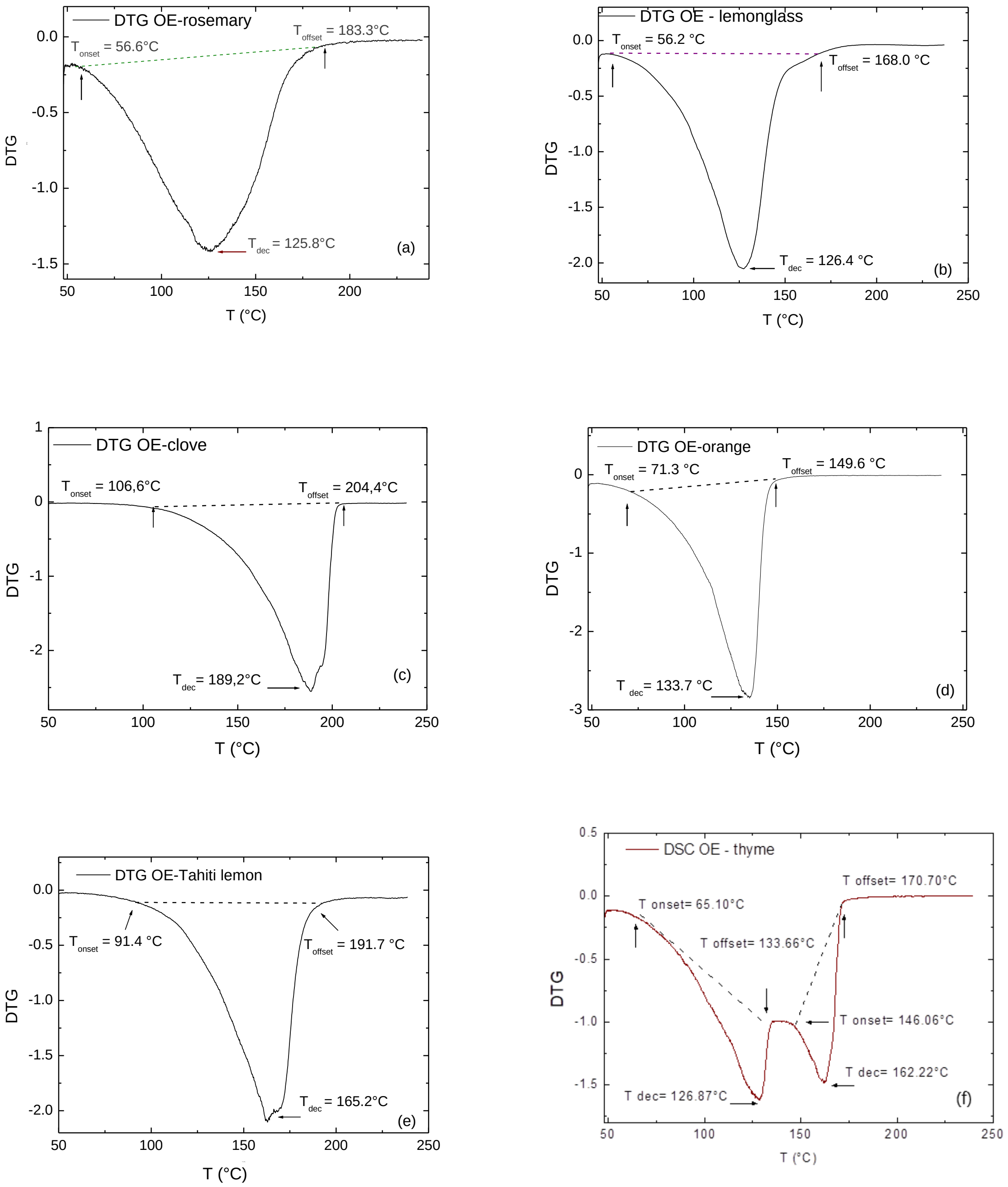


Source: Authors.

Through the TG curves it was possible to contemplate the mass loss of essential oils. Thermogravimetric decomposition is related to the vaporization of volatile compounds. As there was no increase in mass in any of the profiles, it was not possible to say that it existed in essential oils. However, it became unclear to find the thermal behavior of essential oils only by interpolation of the TGs profiles. The problem was then solved using the mathematical technique of Differential Thermal Analysis (DTG), in which, by applying the first-order derivative to the TGs curves, the decomposition start temperatures, $T_{dec}$, the temperature at which the decomposition is maximum or peak temperature, and the temperature at which decomposition ceases, $T_{offset.}$ (Figure 2) shows the DTG curves of essential oils. Thermal behavior at the limit of these characteristic temperatures is determined by interpolation of the concavity in DTG curves.

The study of the thermal degradation of essential oils was performed by thermogravimetry. For this purpose, the essential oils were heated in the range of 25 ºC to 250 ºC in order to study the thermal stability. Thermal decomposition profiles for the commercial oils showed similar characteristics, with the essential oils of rosemary, lemongrass, clove, orange and Tahiti lemon showing a single stage of decomposition, with the exception of the DTG curve obtained for the thyme essential oil which exposed two steps of thermogravimetric decomposition. The temperatures that characterized the thermal behavior of essential oils are shown in Table 7.

**Figure 2** - Derived Thermogravimetric (DTG). Curves of essential oils.



Source: Authors,

**Table 7 -** Temperatures characteristic composition`s stages essential oils.

| **Essential oil** | $T_{on\_set}$ **(ºC)** | $T_{dec\_max}$ **(ºC)** | $T_{off\_set}$ **(ºC)** | $T_{vap\ (DSC)}$ **(ºC)** | $m_{res}$ (%) |
|---|---|---|---|---|---|
| rosemary | 56.6 | 183.3 | 125.8 | 121.2 | 4.3 |
| lemongrass | 56.6 | 168.0 | 126.4 | 126.2 | 11.0 |
| clove | 106.6 | 204.4 | 189.2 | 190.6 | 2.2 |
| orange | 71.3 | 149.6 | 133.7 | 133.7 | 4.6 |
| thaiti lemon | 91.4 | 191.7 | 165.2 | 166.3 | 7.2 |
| thyme (1º stage) | 65.1 | 133.7 | 126.9 | 126.5 | 42.0 |
| thyme (2º stage) | 146.1 | 170.7 | 162.2 | 161.8 | 1.7 |

Source: Authors.

Results revealed that rosemary and lemongrass essential oils showed high sensitivity to heating and thermal stability close to 56.6 ºC, due to the high volatilization of the components. Lemongrass essential oil is rich in citronella aldehyde, esters, citrinellol, while rosemary essential oil is rich in cineole (Guimarães *et al.*, 2012; Paradiso *et al.*, 2020).

Thermal stability temperature of essential oils is an important characteristic to analyze the resistance of each essential oil to the application of heat. The DTG curve of clove essential oil showed Tonset higher than the temperature of the other essential oils studied, suggesting that it can be used in products that undergo treatments up to 106.6 ºC. Decomposition and vaporization of each essential oil depends on the release of the constituent compounds. Clove essential oil is rich in oxygenated monoterpenes and sesquiterpenes (Chaieb *et al.*, 2007).

$T_{offset}$ temperatures pointed to the limit of thermal decomposition. After these temperatures, the essential oils no longer degraded, remaining their residual masses, which are the masses of the compounds remaining in the essential oils (Table 7).

A peculiar result was seen for thyme essential oil, with two stages of decomposition. In the first stage, the main degradation occurred, because most of the essential oil compounds were heat sensitive. Stability of thyme essential oil was maintained until reaching a temperature of 65.1 ºC. This decomposition step was limited to 133.7 ºC, remaining stable with 42% of the mass until it was subjected to a temperature of 146.1 ºC, in which the next stage occurred with decomposition up to 170.7 ºC. Chemically, the majority compounds of thyme essential oil belong to the thymol and carvacrol class, however, the parameters of the essential oil distillation method may have influenced the thermogravimetric degradation behavior.

Decomposition temperature obtained by DTG coincides approximately with the vaporization temperatures ($T_{vap}$) of the DSC curves (Table 7).

DSC`s curves (Figure 3) for essential oils showed similares profiles, analyzing the first-order endothermic transition linked to the vaporization of essential oils. Endothermic transitions refer to the absorption of heat in the system. The peak temperature was discriminated as the main vaporization temperature. Using the DSC technique, the heat flux Q was measured as a function of the controlled temperature.

The enthalpy variation (ΔH) comprised in the DSC thermal transition process corresponds to the enthalpy of transition from the liquid phase to the gas phase of essential oils. This property measures the amount of thermal energy required for the physical phase transition to occur. There is a direct relationship between heat capacity and enthalpy change (Franciscato *et al.*, 2022). With the aid DSC curve`s, it was possible to calculate the enthalpy variation by equation (1) as a function of the peak area, accentuated between two limit points of the initial and final temperature.

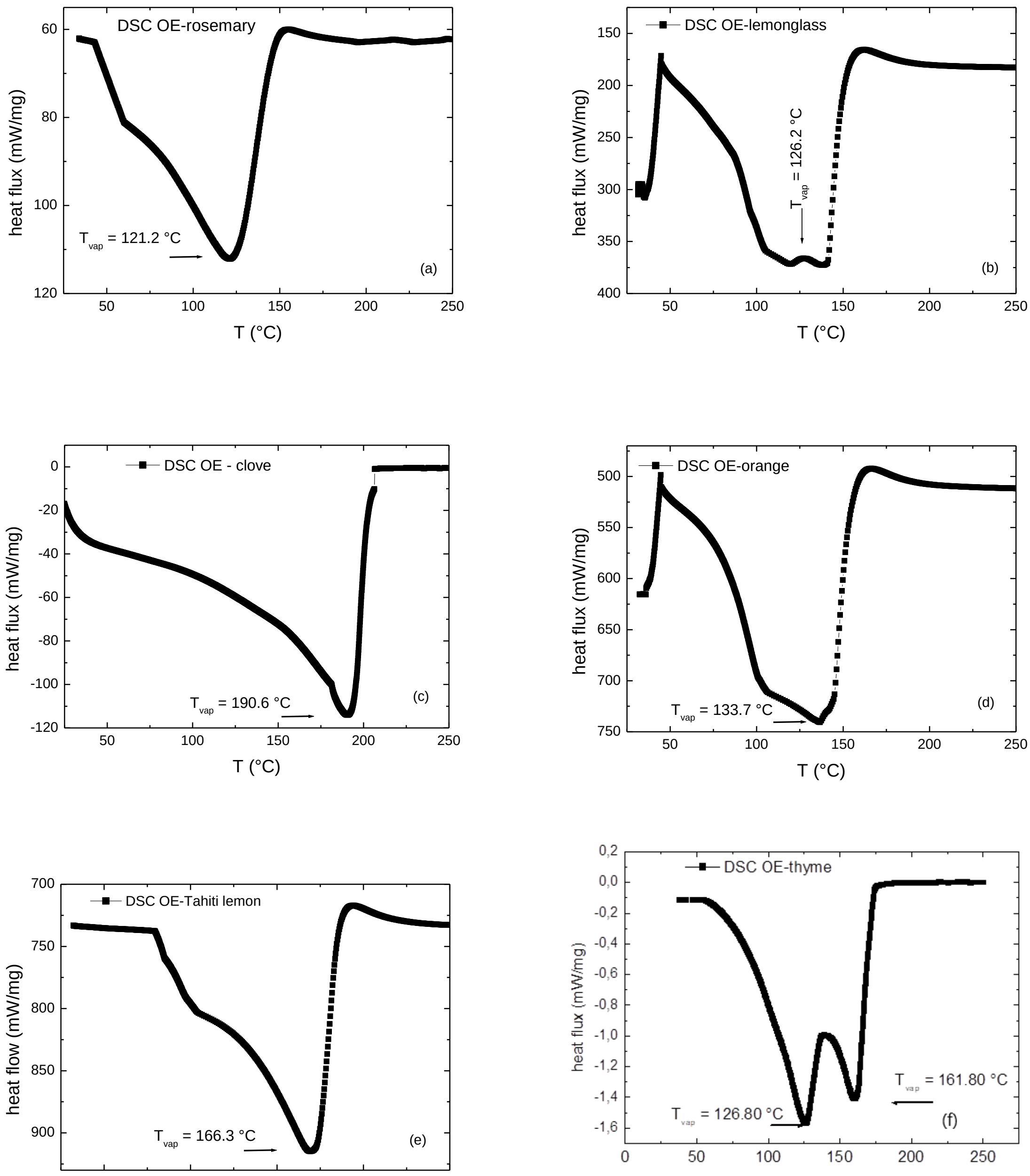
$$\Delta H = \int_{T_i}^{T_f} \left(\frac{dH}{dT}\right)_P dT = \int_{T_i}^{T_f} C_P \, dT \quad (1)$$

Results found for the variation of the enthalpy of vaporization of the essential oils were - 98.52 ± 0.23 KJ $Kg^{-1}$ for the essential oil of rosemary, of - 126.20 ± 0.34 KJ $Kg^{-1}$ for the essential oil of lemongrass, of - 64.08 ± 0.13 KJ $Kg^{-1}$ for the essential oil of clove (leaves), of -133.7 ± 0.47 KJ $Kg^{-1}$ for the essential oil of orange, of - 166.3 ± 0.44 KJ $Kg^{-1}$ for the essential oil of Tahiti lemon, of - 6.08 ± 0.21 KJ $Kg^{-1}$ for the essential oil of thyme in the first stage and of -2.43 ± 0, 14 KJ $Kg^{-1}$ in the second stage.

Negative`s sign in the enthalpy change is due to the endothermic phenomenon. Errors were calculated by standard deviation. The change in enthalpy depends directly on the mass and the change of the heating temperature. During the phase transition, the vaporization temperature remains fixed for a period of time. At this point, the heat absorbed or released Q is proportional to the latent heat, L, typical of each substance. Essential oils are made up of multiple components. Comparing the results between the essential oils studied, it was observed that the essential oils of orange and Tahiti lemon presented high enthalpy variation, suggesting that their components were more thermally resistant to vaporization due to greater thermal stability and decomposition temperature of essential oils. However, the result revealed that clove essential oil identified reduced enthalpy variation when confronted with rosemary, lemongrass, orange and Tahiti lemon essential oils, suggesting that its major compounds, consisting mainly of eugenol, caryophyllene and eugenila, stress lower latent heat to the phase transition.

For thyme essential oil, two enthalpy variations were evaluated for each stage of thermal decomposition. The energy for their compounds to boil was -6.08 KJ $Kg^{-1}$ in the first decomposition stage and -2.43 KJ $Kg^{-1}$ in the second stage. The required enthalpy attenuated with increasing temperature, due to the weak latent heat and powerful volatilization of the compounds.

**Figure 3 -** Differential Scanning Calorimetry (DSC) curves for essential oils.



Source: Authors.

With the application of heat, several molecules of a liquid evaporate to the gaseous state even below the vaporization temperature. In the case of essential oils, when inserted into the crucible of the thermogravimetric analysis equipment, pressures exerted by the molecules in the gas phase changed until the balance between evaporation and condensation was reached. Equilibrium pressure is known as vapor pressure and is an intrinsic property of every substance.

Through the thermochemical properties measured by DSC, it was possible to determine the vapor pressure performance of essential oils. For the calculation of vapor pressure, at the vaporization (boiling) temperature the vapor pressure in 1 atm, can be described by equation (2):

$$p_1 = p_o e^{-\left(\frac{\Delta H}{RT_{vap}}\right)} \quad (2)$$

Where $p_1$=1atm, $p_o$= Initial pressure and R = 8.314 J mol$^{-1}$ is the Universal Gas constant. Thus, substituting these values in equation (3), the initial pressure is:

$$p_o = 1atm\, e^{\left(\frac{\Delta H}{RT_{vap}}\right)} \quad (3)$$

Vapor pressure in the container depends only on temperature. When a container with an initial volume of liquid is heated, the pressure increases, since the probability that the substance is in the gaseous state is greater. Vapor pressure ($p_{vapor}$) is an intrinsic property (characteristic) of every substance. Then, we have equation (4):

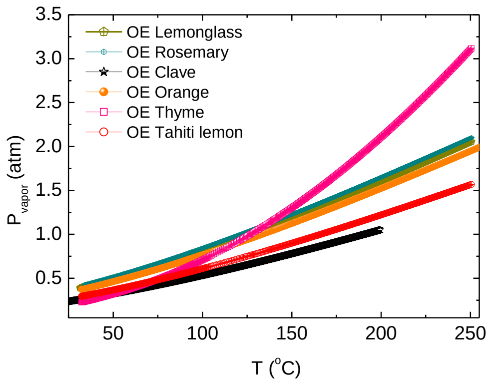
$$p_{vapor} = p_o e^{-\left(\frac{\Delta H}{RT}\right)} \quad (4)$$

Substituting equation (3) in equation (4), we have equation (5). Lower the vaporization temperature, the greater the evolution of vapor pressure. This relationship was verified in the analysis Figure ureure`s 4, in which the order of highest vapor pressure was for the essential oils of: rosemary, lemongrass, orange, Tahiti lemon and clove. Vapor pressure curves demonstrate how the boiling temperature of a substance varies with system pressure. There was the exception of the evolution of the vapor pressure curve for thyme essential oil, which can be seen by the high slope of the vapor pressure curve, since it presented two stages of thermal degradation, consequently, two vaporization temperatures. Vapor pressure curve for thyme saturated at about 3.12 atm near 250°C.

$$p_{vapor} = 1\ atm\, e^{-\frac{\Delta H}{R}\left(\frac{1}{T}-\frac{1}{T_{vap}}\right)} \quad (5)$$

The vapor pressure curves of the essential oils under study are shown in Figure 4.

**Figure 4** - Vapor pressure of essential oils.



Source: Authors.

Operating up to 250 ºC, the vapor pressure of the essential oil of orange reached the mark of 2.74 atm, of the essential oil of rosemary was 2.13 atm, and in the essential oil of clove, the limit of the vapor pressure in the range temperature studied was close to 2.05 atm. Tendency of vapor pressure to increase with temperature tended to be milder in relation to other essential oils because of the variation in enthalpy and temperature of vaporization.

Vapor pressure curves are of great interest in processes such as distillation, as they show a trend in column operating pressure over temperature ranges. In the case of essential oils, the temperature of vaporization is a limiting factor of the process, since at high temperatures the essential oil is easily degraded. In this way, it was possible to estimate a maximum vapor pressure that can be reached in processes involving heating.

### 3.3 Antimicrobian activity

Values found as the MIC and CBM of the essential oils in this study are shown in Table 8.

**Table 8** - Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of essential oils.

| **Essential oil** | ***Staphylococcus aureus subsp. aureus, Rosembach* ATCC 29213** | | ***Escherichia coli* ATCC 25922** | |
|---|---|---|---|---|
| | **CIM (µL mL$^{-1}$)** | **CBM (µL mL$^{-1}$)** | **CIM (µL mL$^{-1}$)** | **CBM (µL mL$^{-1}$)** |
| Rosemary | 12.8 | 12.8 | 3.2 | 3.2 |
| lemongrass | 3.2 | 3.2 | 3.2 | 3.2 |
| Clove | 12.8 | 12.8 | 6.4 | 12.8 |
| Orange | > 25.6 | >25.6 | > 25.6 | >25.6 |
| thaiti lemon | > 25.6 | >25.6 | > 25.6 | >25.6 |
| Thyme | 25.6 | >25.6 | 25.6 | >25.6 |

Source: Authors.

Comparing the MIC results of this study with data from other researches, it was possible to verify the variations in the antimicrobial activity of these essential oils. However, within the scope of this line of research, the various factors that influence the biological activities of essential oils, such as the origin of the plant (climatic conditions), ways of growing and harvesting the raw material, parameters and method must constantly be highlighted. Essential oil extraction, forms of packaging, among other factors that can interfere in the chemical composition of the essential oil, consequently affecting its functional activities. Jordan et al. (2013a) considered the intraspecific chemical influence of the plant and its geographic origin on antimicrobial properties of essential oils.

Even considering the different analytical units used and the forms of dilution of essential oils, the results found were compared with other scientific studies, but considering the same species of plants used for the extraction of essential oils. In this study by (Imane *et al.*, 2020) rosemary essential oil showed MIC of 1.35 µg mL$^{-1}$ against Staphylococcus aureus and 10.8 µg mL$^{-1}$ against Escheriachia coli. Hashim *et al*. (2017) found a MIC of 4.69 µg mL$^{-1}$ against S. aureus and 9.37 µg mL$^{-1}$ against E. coli for lemongrass essential oil. El Amrani and colaboratters (2019) determined a MIC of 2.5 µL mL$^{-1}$ for S. aureus and 5.0 µL.mL$^{-1}$ for E. coli. For Tahiti lemon essential oil, Costa *et al*. (2014) found MIC of 0.25 and 1.0% against S. aureus and E. coli, respectively. Boskovic *et al*. (2015) obtained MIC of 640 µg mL$^{-1}$ against S. aureus and 320 µg mL$^{-1}$ against E. coli for thyme essential oil.

In this study, no inhibitory activity of orange essential oil (extracted from the fruits at the highest concentration tested with 25.6 µL mL$^{-1}$ was observed). Essential oil of orange flowers against Staphylococcus aureus were 391 and 781 mg mL$^{-1}$ and against Escherichia coli were 781 and 1562 mg mL$^{-1}$, respectively, transforming these concentrations into µL mL$^{-1}$, even considering the weight/volume ratio, it is important to note that concentrations were higher than the concentration of 25.6 µL mL$^{-1}$, thus demonstrating the importance of always highlighting the part of the plant used for essential oil extraction.

Although the MIC of thyme essential oil in this study was the same for Staphylococcus aureus and Escherichia coli, Boskovic *et al.* (2015) pointed out that the antimicrobial mechanism of thyme essential oil is related to the actions of carvacrol and thymol, main constituents, which is based on the ability to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing membrane permeability cytoplasm to ATP.

Costa *et al*. (2014), Alibi *et al*. (2020) and Degirmenci and Erkurt (2020) highlighted the better antimicrobial effectiveness of essential oils against Gram-positive bacteria, precisely because lower concentrations are required to provoke such an effect, when compared to Gram-negative bacteria.

Considering the hypothesis often made in scientific studies that there is better activity of essential oils against Gram positive or Gram negative bacteria, it still lacks robust confirmation to make this simple correspondence. Comparing the MICs found for the essential oils of rosemary and cloves, it is evident that much lower concentrations were needed for the inhibition of Escherichia coli compared to Staphylococcus aureus. However, Jordan and collaborators (2013) pointed out that the moderate antimicrobial activity of rosemary essential oil against Gram-negative bacteria could be related to the combination of several minor components present in the essential oil, which act synergistically with the other volatile components.

Considering the discrepancies in MIC values of essential oils in scientific studies, possibly related to the chemical compositions of these oils, the importance of CBM is highlighted in order to have security in relation to the antimicrobial potential of an essential oil. Moreira (Moreira *et al.*, 2020) also considered this, because for a technological application of essential oils in the food industry, as sanitizing agents in post-harvest processing, it requires the establishment of ideal application conditions, considering the sensitivity of a microorganism, the ideal concentrations of the oils essential oils and the contact time between these oils and the microorganism.

In this study, clove essential oil had a higher concentration of bactericidal action than MIC for Escherichia coli and no bactericidal activity was detected for orange essential oils, thaiti lemon and thyme, even at the highest concentration tested (25.6 µL $mL^{-1}$). For the other cases, the CBM was the same as the CIM. For Candida albicans strains, the six essential oils in this study did not show inhibition at the concentrations tested, considering MIC and CFM as > 16.384 µL $mL^{-1}$. According to the results of the study (Castro *et al.*, 2011) rosemary essential oil showed weak antifungal activity for most of the tested Candida albicans strains. And analyzing the results of Cortez and collaborators (Cortez *et al.*, 2015) lemongrass essential oil showed fungicidal activity against ATCC 10231 of Candida albicans. For Silva *et al*. (Silva *et al.*, 2009) all tested strains of the same genus were inhibited from the concentration of 25% of lemongrass essential oil. Nascimento and Fortuna (2020) found that clove essential oil obtained inhibition of all tested strains of Candida albicans up to a concentration of 2%, since this oil has eugenol as its major constituent, where it has proven activity against isolated fungi of this genus.

Few studies investigated the antimicrobial activity of plant species belonging to the genus Citrus and found that orange essential oil showed antifungal activity for all strains of the genus Candida tested, especially against strain ATCC 289065 (Calvacanti *et al*., 2012).

One of the factors that may have significantly interfered with the results of the antimicrobial activity of the present work, different from other studies, are the chemical characteristics of essential oils, such as volatility and insolubility in water, which may reflect variations in antiseptic, antibacterial, antifungal and antiparasitic properties (Škrovánková *et al.*, 2012).

### 3.4 Antioxidant activity

Inhibition of the oxidation process in foods is a challenge of great economic importance in food production, as oxidation decreases the nutritional quality and safety of food (Škrovánková *et al.*, 2012). Thus, antioxidante`s capacity of essential oils is of interest to exert this activity when used in food products.

By the ferric reduction method (FRAP), the antioxidant activity is expressed in µmol of Trolox $mL^{-1}$. A smaller amount of orange essential oil was needed to reduce ferric iron free radicals. Based on this interpretation, orange essential oil (0.083 ± 0.004) showed higher antioxidant activity compared to the other essential oils tested. The antioxidant potential among the other essential oils in descending order was lemon (0.116 ± 0.009), rosemary (0.121 ± 0.012), lemongrass (0.279 ± 0.029), thyme (0.437 ± 0.022) and clove (1.687 ± 0.007).

Methodology applied in this study for the analysis of antioxidant activity showed a low ability to reduce iron, which can be explained by the fact that the FRAP methodology is recommended mainly for aqueous extracts, because the reagents and solvents used have affinity with hydrophilic compounds (Andrade *et al.*, 2012) in the case of essential oils, which have a lipid character, it was necessary to use the methanol solvent to intermediate the solubility and proceed with the analysis.

For proper determination of the performance of the antioxidant capacity of the essential oil, it is necessary to be aware of the extraction technique, properties of the solvent used and applied test methodology (Škrovánková *et al.*, 2012).

## 4. Conclusion

Production of essential oils in Brazil is viable due to the climatic and territorial conditions that the country presents, being of promising profitability. But it is necessary to take advantage of the technological and analytical capacity of national research centers, through partnerships that can result in the real application of modern techniques of cultivation, selection and improvement of plants, in addition to the analytical capacity for the characterization of essential oils, proposing to the market traceable and certified identity products. The determination of antimicrobial and antioxidant activities of OEs in effectively proven studies to evaluate the technological application of Essentials oils in the food industry as sanitizers in the post-harvest process, recognizing the ideal thermal conditions for thermal stability in the face of heat application in industrial processes and

determination of optimized concentrations of oils to combat the proliferation of undesirable microorganisms. Results revealed that Tahiti lime essential oil had the best biological activities and heat resistance. The orange, rosemary and lemongrass OEs showed good antioxidant activities, but low thermal stability, proving to be very volatile and not recommended for processes involving heating at temperatures above 56 ºC.

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## References

Adams, R. P. (2017). Identification of Essential oil components by gas chromatography/mass spectrometry, 4.1rd ed, Carol Stream: Allured publishing.

Al-Asmari, A. K., Athar, M. T., Al-Faraidy, A. A & Almuhaiza, M. S. (2017). Composição química do óleo essencial de Thymus vulgaris coletado do mercado da Árabia Saudita. Asian Pac. *J. Trop. Biomed*, 7(2), 147-150.

Alibi, S., Selma, W. B., Ramos-Vivas J., Smach, M. A., Touati, R., Boukadida, J., Navas, J. & Mansour, H.B. (2020). Atividades de detecção antioxidante, antibacteriana, antibiofilme e antiquórum de quatro óleos essenciais contra isolados clínicos bacterianos multirresistentes. *Curr. Res. Transl. Med.* 68, 59-66. https://doi.org/10.1016/j.retram.2020.01.001.

Andrade, M.A., Cardoso, M.G., Batista, L.R., Mallet, A.C.T & Machado, S.M.F. (2012). Óleos essenciais de *Cymbopogon nardus*, *Cinnamomum zeylanicum* e *Zingiber officinale*: composição, atividades antioxidante e antibacteriana. *Cienc. Agron [online]*. 43, 2, 399-408. https://doi.org/10.1590/S1806-66902012000200025.

Bizzo, H.R., Hovell, A.M & Rezende, C.M. (2009). Óleos essenciais no Brasil: aspectos gerais, desenvolvimento e perspectivas. *Quím. Nova*. 32, 3, 588-594. https://doi.org/10.1590/S0100-40422009000300005.

Boskovic, M., Zdravkovic, N., Ivanovic, J., Janjic, J., Djordjevic, J., Starcevic, M. & Baltic, M.Z. (2015). Antimicrobial Activity of Thyme (Tymus vulgaris) and Oregano (Origanum vulgare) Essential Oils against Some Food-borne Microorganisms. *Procedia Food Sci*. 5, 18-21. https://doi.org/10.1016/j.profoo.2015.09.005.

Bozkurt, T., Gülnaz, O. & Kaçar, Y.A. (2017). Chemical composition of the essential oils from some citrus species and evaluation of the antimicrobial activity. *Journal of Environmental Science, Toxicology and Food Technology*, 11, 10, 29-33.

Cavalcanti, Y.W., Pérez, A.L.A.L., Xavier, G.D.R., Almeida, L.F.D & Padilha, W.W.N. (2012). Atividade antifúngica de extratos vegetais brasileiros sobre cepas de *Candida*. *Rev. Bras. Ciênc. Saúde*. 16, 1, 43-48.

Castro, R.D & Lima, E.O. (2011). Atividade antifúngica dos óleos essenciais de sassafrás (*Ocotea odorifera* Vell.) e alecrim (*Rosmarinus officinalis* L.) sobre o gênero *Candida*. *Rev. bras. plantas med*. 13, 2, 203-208. https://doi.org/10.1590/S1516-05722011000200012.

Chaieb, K., Hajlaoui, H., Zmantar, T., Kahla-Nakbi, A. B., Rouabhia, M., Mahdouani, K & Bakhrouf, A. (2007). Composição química e a atividade biológica do óleo essencial de cravo, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): uma breve revisão. *Phytother Res*. 21, 6, 501-6. 10.1002/ptr.2124.

Clinical Laboratory Standards Institute (CLSI) (2002). Reference Method for Broth Dilution Tests for Determining Yeast Antifungal Therapy Sensitivity, 2th. Approved standard M27-A2.

Clinical Laboratory Standards Institute (CLSI) (2009). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 7th. Approved standard M7-A8.

Cortez, L.E.R., Yamaguchi, M.U., Cortez, D.A.G & Pesco, D.C.S. (2015). Avaliação da atividade antifúngica dos óleos essencias de Lippia alba (Mill.) N. E. Brown (Verbenaceae) e Cymbopogon citratus (D.C.) Stapf (Poaceae): *O mundo da saúde*. 39, 4, 443 – 440. 10.15343/0104-7809.20153904433440.

Costa, R., Bisignano, C., Filocamo, A., Grasso, E., Occhiuto, F & Spadaro, F. (2014). Atividade antimicrobiana e composição química de *Citrus aurantifolia* (Christm.) Swingle óleo essencial de culturas orgânicas italianas, *J. Essent. Oil Res*., 26, 6, 400-408. 10.1080/10412905.2014.964428.

Degirmenci, H & Erkurt, H. (2020). Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of Citrus aurantium L. flowers. *J. Infect. Public Health*. 13, 1, 58–67. https://doi.org/10.1016/j.jiph.2019.06.017.

El Amrani, S., Lalami, A.E.O., Ez Zoubi, Y., Moukhafi, K., Bouslamti, R &Lairini, S. (2019). Evaluation of antibacterial and antioxidant effects of cinnamon and clove essential oils from Madagascar. *Mater. Today: Proc*. 13, 762–770. https://doi.org/10.1016/j.matpr.2019.04.038.

Everton, G.O., Rosa, P.V.S., Neves, S.C., Pereira, A.P.M., Lima, E.C.S., Mendonça, I.P., Souza, L.S., Fonseca, D., Cunha, J.C.R., Souza, L.S, Oliveira, I.M., Arruda, M.O & Mouchrek Filho, V.E. (2020). Caracterização química, atividade antimicrobiana e toxicidade dos óleos essenciais da Pimenta dioica L. (pimenta da Jamaica) e Citrus sinensis L. Osbeck (laranja doce). *Rev. Colomb. Cienc. Quím. Farm*. 49, 3, 641-655.

Franciscato, L. M. S. dos S., Ariati, A. M., RIATI, A. M., Picolloto, A. M ., Raia, R. Z., Barbosa, V. A., Bittencourt, P. R. S., Souza, M. R. dos R., Sakai, O. A., ÂNGELO, E. A & Moritz (2022). Thermal Properties of Cinnamon (Cinnamomum verum) Essential Oil and Its Antibacterial Activity. *Res. Soc. Dev*. 11, 13, 567111335942. 10.33448/rsd-v11i13.35942.

Guimarães, L.G. L., Cardoso, M.G., Sousa, P. E. de., Andrade, J & Vieira, S. S., Atividades antioxidantes e fungitóxica do óleo essencial de capim-limão e citral. (2011). *Rev. Ciên. Agron*. .42, 2, 464-472.

Hashim, G.M., Almasaudi, S.B., Esam, A & Jaouni, S.K.A., Harakeh, S. (2017). Biological activity of *Cymbopogon schoenanthus* essential oil. *Saudi J. Biol. Sci*. 24, 7, 1458–1464. https://doi.org/10.1016/j.sjbs.2016.06.001.

Imane, N.I., Fouzia, H., Azzahra, L.F., Ahmed, E., Ismail, G., Idrissa, D., Mohamed, K. H., Sirine, F., L'Houcine, O & Bourhim, N. (2020). Chemical composition, antibacterial and antioxidant activities of some essential oils against multidrug resistant bacteria. *Eur. J. Integr. Med*. 35, 101074. https://doi.org/10.1016/j.eujim.2020.101074.

Jordán, M.J., Lax, V., Rota, M.C., Lorán, S & Sotomayor, J.A. (2013). Effect of the phenological stage on the chemical composition, and antimicrobial and antioxidant properties of *Rosmarinus officinalis* L. essential oil and its polyphenolic extract. *Ind. Crops Prod*. 48, 144–152. https://doi.org/10.1016/j.indcrop.2013.04.031.

Kaur, K., Kaushal, S & Rani, R. (2019). Chemical Composition, Antioxidant and Antifungal Potential of Clove (*Syzygium aromaticum*) Essential Oil, its Major Compound and its Derivatives. *J essent oil bear PL*.22,5, 1195 – 1217. https://doi.org/10.1080/0972060X.2019.1688689.

Lima, I.O., Oliveira, R.A.G., Lima, E.O., Farias, N.M.P & Souza, E.L. (2006). Atividade antifúngica de óleos essenciais sobre espécies de *Candida*. *Rev. bras. farmacogn*. 16, 2, 197 - 201. https://doi.org/10.1590/S0102-695X2006000200011.

Majolo, C., Monteiro, P.C, Nascimento, A.V.P, Chaves, F.C.M, Gama, E.P, Bizzo H & Chagas E. C. (2019). Essential oils from five brazilian Piper species as antimicrobials against strains of Aeromonas hydrophila. *J essent oil bear PL*. 22, 3, 746-761.

Mancini, E., Senatore, F., Del Monte, D., De Martino, L., Grulova, D., Scognamiglio, M., Snoussi, M & De Feo V. (2015). Studies on Chemical Composition, Antimicrobial and Antioxidant Activities of Five *Thymus vulgaris* L. Essential Oils. *Molecules*. 20, 7, 12016-12028. https://doi.org/10.3390/molecules200712016.

Nascimento, R. S., SÁ, R. L & Fortuna, J. L. (2020). Antimicrobial activity of the essential oil of leaf and fruit of Schinusterebinthifolius (raddi) against *Staphylococcus aureus* and *Escherichia coli*. *Braz. J. Med. Biol. Res*. 3, 509-521.https://doi.org/10.34188/bjaerv3n2-010.

Paradiso, V.M., Flamminii, F., Pittia, P., Caponio, F. & Mattia, C.D. (2020). Radical Scavenging Activity of Olive Oil Phenolic Antioxidants in Oil or Water Phase during the Oxidation of O/W Emulsions: An Oxidomics Approach. *Antioxidants*. 9, 10, 996. https://doi.org/10.3390/antiox9100996.

Silva, F.S., Ferreira, T.M., Teodoro, G.R., Costa, A.C.B.P., Maria, A., Beltrame Junior, M., Salvador M.J., Paula, C.R. & Khouri, S. (2009). Atividade antifúngica do óleo essencial de *Cymbopogon citratus* frente a cepas de *Candida albicans* e *Candida tropicalis* isoladas de infecções nosocomiais. *Rev. Inst. Adolfo Lutz*. 68, 434-441.

Škrovánková S., Mišurcová L & Machů L. (2012). Atividade antioxidante e efeitos de proteção na saúde de plantas medicinais comuns. *Adv Food Nutr Res*. 67, 75-139. 10.1016/B978-0-12-394598-3.00003-4.

Spiegel, M., Kapusta, K.; Kołodziejczyk, W., Saloni, J., Żbikowska, B., Hill, G.A & Sroka, Z. (2020). Atividade antioxidante de ácidos fenólicos selecionados-Ferric Ensaio de poder antioxidante redutor e análise QSAR das características estruturais. *Moléculas*. 7; 25(13), 3088. 10.3390/moléculas25133088.