Chemical composition and \textit{in vitro} biological activities of the essential oils of the rhizomes of \textit{Zingiber officinale} Roscoe and \textit{Curcuma longa} L. (Zingiberaceae)

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ABSTRACT

Zingiber officinale Roscoe and Curcuma longa L. (Zingiberaceae) essential oils were obtained by the hydrodistillation method. The oils offered yields of 0.120% and 0.044% respectively. GC-MS analyzes allowed the identification of 11 constituents in the Curcuma longa essential oil, with major components: ar-turmerone (36.8%), β-tumerone (32.3%) and curlone (19.2%) For the Zingiber officinale essential oil 19 constituents were identified, being: neral (22.9%), zingiberene (15.5%) and geranial (14.9%) the main components. The result of the toxicity test showed significant activity against Artemia salina for both oils. The dose values required to eliminate 50% (LC50) of the larvae were 100 µg / mL for Z. officinale and 25 µg / mL for C. longa. The antioxidant activity was performed against DPPH free radical. The most significant activity was exhibited by Z. officinale with an IC50 value of 215.8 µg / mL. The antibacterial assay with Z. officinale oil showed efficacy against S. aureus and E. coli, with MIC values of ≥ 213.3 µg / mL and 85.3 µg / mL respectively. For C. longa, MIC values ≥ 1024 µg / mL were observed for both bacteria tested. Modulation with aminoglycoside antibiotics showed synergism of C. longa oil combined with amikacin against S. aureus, with a decrease in MIC from 1024 µg / mL to 14.7 µg / mL. Meanwhile, the Zingiber officinale oil obtained a MIC reduction to 6.7 µg / mL, demonstrating greater efficacy. The results observed here demonstrate the biological potential of the species as cytotoxic and antimicrobial.

Keywords: Antioxidant; antibiotic modulation; essential oil.

RESUMO

Os óleos essenciais de Zingiber officinale Roscoe e Curcuma longa L. (Zingiberaceae) foram obtidos pelo método de hidrodestilação. Os óleos ofereceram rendimentos de 0,120% e 0,044%, respectivamente. As análises por GC-MS permitiram a identificação de 11 constituintes no óleo essencial de Curcuma longa, com componentes principais: ar-turmerona (36,8%), β-tumerona (32,3%) e curlone (19,2%). Para o óleo essencial de Zingiber officinale 19 constituintes foram identificados, sendo: principal (22,9%), zingibereno (15,5%) e geranial (14,9%) os principais componentes. O resultado do teste de toxicidade mostrou atividade significativa contra Artemiasalina nos dois óleos. Os valores de dose necessários para eliminar 50% (CL50) das larvas foram 100 µg / mL para Z. officinale e 25 µg / mL para C. longa. A atividade antioxidante foi realizada contra o radical livre DPPH. A atividade mais significativa foi exibida por Z. officinale com um valor de IC50 de 215,8 µg / mL. O ensaio antibacteriano com óleo de Z. officinale mostrou eficácia contra S. aureus e E. coli, com valores de CIM ≥ 213,3 µg / mL e 85,3 µg / mL, respectivamente. Para C. longa, foram observados valores de MIC ≥ 1024 µg / mL para ambas as
bactérias testadas. A modulação com antibióticos aminoglicosídeos mostrou sinergismo do óleo de C. longa combinado com amicacina contra S. aureus, com uma diminuição na CIM de 1024 μg / mL para 14,7 μg / mL. Enquanto isso, o óleo de Zingiber officinale obteve uma redução de CIM para 6,7 μg / mL, demonstrando maior eficácia. Os resultados aqui observados demonstram o potencial biológico da espécie como citotóxico e antimicrobiano.

**Palavras-chave:** Antioxidante; modulação antibiótica; óleo essencial.

1 INTRODUCTION

The Zingiberaceae family is known to contain plants with pleasant aromas and fleshy rhizomes. This family has approximately 50 genera and 1300 species distributed in the tropics, especially in Southeast Asia (ALBUQUERQUE, NEVES, 2004; HABSAH et al., 2000; BARBOSA et al., 2017). Its species are commonly used in cooking, perfumery and folk medicine. Among these the species *Zingiber officinale* Roscoe (ginger) and *Curcuma longa* L. (turmeric) are widely used for food purposes as they have nutritional and therapeutic properties. In ethnopharmacology, ginger and turmeric are cited for the treatment of liver disease, gastritis, inflammation, and have antimicrobial, antioxidant and anticarcinogenic properties (ALONSO, 2016; LORENZI, MATOS, 2002).

Several species of the Zingiberaceae family are recognized for their therapeutic properties. In the work of Jantan et al. (2008), the action of compounds isolated from species of this family with inhibitory effect on platelet aggregation was evidenced. For the curcuma its intestinal protective action against mucositis, antiinflammatory and antioxidant actions were reported, such activities being related to its chemical composition, coming from both fixed compounds and flavonoids, as well as volatile compounds such as β-sesquiphellandrene and terpinolene (PRIYA et al., 2012; BASTOS et al., 2016; KIM et al., 2016).

The essential oil produced by curcuma rhizomes is rich in compounds such as α-turmerone, 1,8-cineol, curlone (GOUNDER, LINGAMALLU, 2012; OYEMITAN et al., 2017). The chemical composition of this species depends on factors such as harvest season, genetic group, processing and storage conditions, which means that their therapeutic properties may be subject to modifications in the chemical composition. The work of Mishra et al, (2018) showed the genetic diversity and chemical and biological variation of essential oils obtained from 65 sixty-five curcuma genotypes, showing variation in the presence of compounds and their antioxidant and antimicrobial activities.

Despite the diversity of studies related to the species mentioned above it is important to point out that depending on the condition of the natural product its chemical and biological properties change totally, so it is important to chemically and biologically study the *Z. officinale* and
C. longa species as they are directly linked to the local culture and customs, serving as food and therapeutic products.

In this perspective, the objective of the present study is to analyze the chemical composition of the essential oils of the rhizomes of Z. officinale (ZOEO) and C. longa (CLEO), and to evaluate their toxicological and antioxidant potential.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The rhizomes of Z. officinale (192g) and C. longa (260g) were purchased in the central market in Juazeiro do Norte, Ceará, Brazil.

2.2 OBTENTION OF THE ESSENTIAL OILS

The essential oils were obtained by hydrodistillation (Clevenger-type apparatus). Samples of dried rhizomes were triturated and submitted to distillation for 4 h. The essential oils obtained were dried using anhydrous sodium sulfate (\( \text{Na}_2\text{SO}_4 \)) and yields were of 0.12 % for the Z. officinale essential oil (ZOEO), and 0.05 % for the C. longa essential oil (CLEO).

2.3 CHEMICAL COMPOSITION

GC/MS analysis of the essential oils was carried out on a Shimadzu GC-17 A/MS QP5050A (GC/MS system) using a DB-5HT fused silica capillary column (30 m x 0.25 mm i.d., 0.25 m film thickness); carrier gas helium, flow rate 1.7 mL/min and with split mode. Injector and detector temperatures were 270 °C and 290 °C, respectively. The column temperature was programmed from 35 °C to 180 °C at 4 °C /min and then 180 °C to 250 °C at 10 °C /min. Mass spectra were recorded from 30 – 450 m/z. injected volume: 1 mL of 5 mg/mL solution ethyl acetate. Solvent cut time was 3 min. Mass spectrometer operating conditions 70 eV ionization energy. Identification of Individual components was based on their mass spectral fragmentation using two computer library MS searches (wiley229), retention indices and comparison with literature data (ADAMS, 2001).

2.4 TOXICITY

Toxicity was evaluated against Artemia salina using the method proposed by Meyer et al. (1982). The test was performed in triplicate with different concentrations (1000, 500, 250, 100, 50, 25 μg / mL), accompanied by a positive control prepared with seawater and potassium chromate (26
mM) and a negative control with seawater. The number of dead larvae was counted after 24 hours. Nonlinear regression analysis was used to determine the LC\textsubscript{50}.

2.5 ANTIOXIDANT ACTIVITY

The antioxidant capacity of the essential oils was measured using the method described by Rufino et al. (2007), with concentrations ranging from 5 to 500 μg / mL. The reaction medium consisted of 20 μL concentrations and 280 μL DPPH solution (60 μM). For positive and negative control, ascorbic acid and methanol were used, respectively. The reaction took place for 30 min in the dark. The reading was performed in Elisa spectrophotometer at a wavelength of 515 nm. The results were calculated according to the equation below, the data evaluated by linear regression, and from that the IC\textsubscript{50} was obtained, which is the concentration capable of scavenging 50% of the free radical.

Equation 1:

\[
\text{AA} \% = 100 - \left\{ \frac{\text{Abs Sample} - \text{Abs blank}}{\text{Abs Negative control}} \right\} \times 100
\]

Where: AA\% = Antioxidant activity percentage; Abs: Absorbances.

Antibacterial Activity

Minimum Inhibitory Concentration

Antibacterial activity was tested by the microdilution method based on document M7-A10 (CLSI, 2015). The assay was performed with bacterial strains: \textit{Escherichia coli} 27 and \textit{Staphylococcus aureus} 358. Essential oils were diluted with sterile distilled water and dimethylsulfoxide (1024 μg / mL), followed by serial dilutions by addition to the wells containing the suspension, reaching concentrations in the range of 512 to 8 μg / mL. The test was performed in triplicate and the plate incubated at 35 ± 2 °C for 24 h. The reading was performed by colorimetry by adding 25 μL of resazurin solution (0.01%) to each well after incubation. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract capable of inhibiting the growth of microorganisms.

Modulation with antibiotics

For the analysis of ZOEO and CLEO as potentiators of aminoglycoside antibiotics (amikacin and gentamicin), the methodology proposed by Coutinho et al. (2008) was used. MIC values of antibiotics were determined by the microdilution method with a concentration range of 1024 - 1 μg / mL. Inoculum containing ZOEO concentrations (213.3 μg / mL for \textit{S. aureus} and 85.3 μg / mL for \textit{E. coli}) and CLEO (1024 μg / mL for both strains tested) in 10% specific culture medium were distributed in microdilution plates followed by the addition of antibiotic solutions with concentrations between 1024 - 1 μg / mL obtained by serial dilution. The plates were
incubated at 35 ± 2 °C for 24 h and read by colorimetry by adding 25 μL of resazurin solution (0.01%).

Statistical Analysis

Values were expressed as means ± S.E.M. (n = 3). For all tests, after normalization of data, a nonlinear regression curve test was performed by the GraphPad Prism version 7.0 statistical program to obtain IC\textsubscript{50} and LC\textsubscript{50} values. With the obtained values, ANOVA and Tukey test were performed for multiple comparison between pairs, and Bonferroni test was performed for microbiological analysis considering significant values of P <0.05.

3 RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION

Thirty constituents were identified in the CLEO and ZOEO, with \( \alpha \)-turmerone (36.8%), \( \beta \)-tumerone (32.3%) and curlone (19.2%) as major components for the first and neral (22.9%), zingiberene (15.5%) and geranial (14.9%) for the second. All constituents are represented in Tables 1 and 2.

| Components                  | \( \text{IK}_{\text{Exp}} \) | \( \text{IK}_{\text{Lit}} \) | (%)  |
|-----------------------------|-----------------------------|-----------------------------|------|
| Sesquiterpenes              |                             |                             |      |
| \( \beta \)-sesquiphellandrene | 1144                       | 1149                       | 1.2  |
| Dicumene                    | 1153                       | 1150                       | 1.9  |
| \( \alpha \)-curcumene      | 1469                       | 1471                       | 1.1  |
| \( \alpha \)-zingiberene    | 1491                       | 1495                       | 0.8  |
| \( \text{cis} \)-lanceol     | 1528                       | 1525                       | 1.3  |
| 2-cyclo-hexyl-2-phenyl-propane | 1566                      | 1568                       | 1.6  |
| dyciclo-hexyl-mononitrile   | 1652                       | 1653                       | 0.9  |
| \( \alpha \)-tumerone       | 1666                       | 1664                       | 36.8 |
| cedr-8-en-13-ol             | 1671                       | 1668                       | 0.8  |
| \( \beta \)-tumerone        | 1679                       | 1681                       | 32.3 |
| Curlone                     | 1697                       | 1699                       | 19.2 |
| **Total**                   |                            |                            | 97.9 |

Table 1. Chemical composition (%) of \textit{C. longa} essential oil (CLEO)
Table 2. Chemical Composition (%) of *Z. officinale* essential oil (ZOEO)

| Components             | IK_{Exp} | IK_{Lit} | (%)  |
|------------------------|----------|----------|------|
| **Monoterpenes**       |          |          |      |
| Camphene               | 1095     | 1097     | 3.9  |
| sabinene               | 1152     | 1147     | 6.2  |
| 1,8-cineole            | 1215     | 1219     | 4.7  |
| β-phellandrene         | 1255     | 1257     | 2.9  |
| Linalool               | 1520     | 1522     | 0.6  |
| 2-undecanone           | 1624     | 1628     | 0.8  |
| Borneol                | 1644     | 1642     | 1.4  |
| α-terpineol            | 1673     | 1669     | 1.5  |
| Citronellol            | 1676     | 1675     | 2.8  |
| Geranial               | 1711     | 1714     | 14.9 |
| Neral                  | 1728     | 1724     | 22.9 |
| Nerol                  | 1759     | 1753     | 3.0  |
| **Sesquiterpenes**     |          |          |      |
| Zingiberene            | 1760     | 1758     | 15.5 |
| Farnesene              | 1766     | 1768     | 3.9  |
| β-bisabolene           | 1769     | 1788     | 3.0  |
| Curcumene              | 1782     | 1789     | 1.7  |
| γ-cadinene             | 1815     | 1819     | 1.4  |
| β-sesquiphellandrene   | 1847     | 1849     | 5.0  |
| Elemol                 | 2067     | 2069     | 1.4  |
| **Total**              |          |          | 97.5 |

In study with the chemical composition, Majolo et al. (2014) obtained ar-tumerone as the major component in the CLEO, but did not identify the presence of β-tumerone and curlone that were identified as majority in this research. The results are also in agreement with Gouder and Lingamallu (2011) who reported the presence of Ar-Tumerone and β-Tumerone as majorities in the essential oil composition.
Pinheiro et al. (2017) identified the neral compound as one of the majority in the ZOEO. In the same study the geranial compound was identified as the majority, but it was not found in this work. The compounds 1,8 cineol, sabinene, zingiberene among others are present in both works.

Variations in the presence and quantity of essential oil compounds are determined by genetic factors, but seasonal factors may alter the production of secondary metabolites. Chemical composition and essential oil content may change during the seasons (CHOUDEHURY, BORDOLOI, 1988). Nutrient excess or deficiency may be directly correlated with variation in active substance production. Other determinants that can be cited are the stage of plant development and frequency and intensity of water stress (MARTINS et al., 1995).

3.2 TOXICITY AGAINST ARTEMIA SALINA

Results show that CLEO has more significant activity than ZOEO, as shown in Figure 1. The dose required to eliminate 50% (LC50) of larvae was 100 µg / mL ZOEO and 25 µg / mL for CLEO indicating that both have activities considered significant, for presenting LC50 lower than 1000 µg / mL (MEYER et al., 1982).

![Figure 1. Result of Artemia salina toxicity of ZOEO and CLEO essential oils](image)

An analysis by the Departamento de Tecnologia Química e de Alimentos da Universidade Federal da Paraíba, Brazil, shows that the results obtained in the CLEO assays had a LC50 of 319.82 µg / mL (SILVA FILHO et al., 2009). A study by the Centro de Ciências Exatas e Tecnologia da Universidade Federal do Maranhão, Brazil, with ZOEO, showed significant larvicidal activity, eliminating 50% at a concentration of 70 µg / mL and growing exponentially from a concentration of 100 µg / mL causing death of 100% of individuals at a concentration of 160 µg / mL (GOMES et al., 2016). These results show that ZOEO has higher toxicity when compared to CLEO, which differs from the results found in this study.
3.3 INHIBITION OF DPPH RADICAL

The essential oils analyzed were able to neutralize the DPPH free radical. Concentrations indicated values with statistical difference between them, with better results for ZOEO as shown in Figure 2. ZOEO showed significant effect on DPPH inhibition, reaching up to 53.03% at 500 µg / mL concentration, and its IC$_{50}$ was 215.8 µg / ml. CLEO concentrations did not show inhibition percentages up to 50%, and IC$_{50}$ could not be obtained.

In antioxidant studies the DPPH free radical neutralization reaction maintains a degree of proportionality to the amount of proton donating electrons or compounds, the greater the donation capacity, the greater the neutralization (RUFINO et al., 2007). The CLEO obtained in this study had ar-turmerone (36.8%) and b-turmerone (32.3%) as its major components, which are according to Ramos et al. (2003) mainly responsible for the free radical stabilization capacity of this species. Thus, it is possible to relate the antioxidant potential of the essential oil obtained in this research with the high content of the identified major compounds. Sacchetti (2004), when evaluating the antioxidant capacity of ZOEO using DPPH, obtained an efficient concentration in the range of 1000 to 1500 µg / mL. Ramos et al. (2003), using the same methodology, obtained an IC$_{50}$ of 250 µg / mL. The results obtained here demonstrated better efficiency.

![Figure 2](image-url)

**Figure 2.** Antioxidant activity by DPPH free radical scavenging for ZOEO and CLEO and ascorbic acid positive control (p <0.0001; Anova and Tukey test).

4 ANTIBACTERIAL ACTIVITY

4.1 MINIMUM INHIBITORY CONCENTRATION (MIC)

Regarding the antibacterial investigation (Table 3), CLEO presented MIC $\geq$ 1024 µg / mL in both strains, showing an inactive antibacterial activity at these concentrations, while ZOEO
showed higher efficiency on the two bacterial strains tested, especially *E. coli* with MIC ≥ 85.3 μg / mL.

Table 3. Minimum inhibitory concentration of CLEO and ZOEO

| Bacterium | CLEO MIC (μg/mL) | ZOEO MIC (μg/mL) |
|-----------|------------------|------------------|
| *S. aureus* 358 | ≥ 1024          | ≥ 213,3          |
| *E. coli* 27 | ≥ 1024          | ≥ 85,3           |

CLEO- essential oil of *Curcuma longa*; ZOEO- essential oil of *Zingiber officinale*

Among the results obtained for antibacterial activity, ginger showed a higher inhibition potential against the Gram-negative bacterial strain, possibly due to its rich composition of monoterpenes, among them neral (22.9%), geranial (14.9%), zingiberene (15.5%) and 1,8-cineol (4.7%), which are hydrophobic and will probably prefer to move from the aqueous phase towards membrane structures. The accumulation of such constituents in the lipid bilayer of the cytoplasmic membrane will give it a permeability characteristic (BERTINI et al., 2005; ANDRADE et al., 2012). Similar results for ZOEO were found by Silva (2018), in which *S. aureus* showed a MIC of 4.7 μl / mL, while for *E. coli* the MIC was 2.3 μl / mL. By isolating *E. coli* and *S. aureus* from patients’ biological materials, Silva et al. (2009) proved that ginger had a higher efficiency over *E. coli* using the microdilution methodology.

Regarding the CLEO, studies prove its low antibacterial activity, especially in Gram negative strains. Teramoto et al. (2018), testing the CLEO on *P. aeruginosa, E. coli, E. hirae* and *S. aureus* strains obtained MICs of 2 mg / mL in all strains tested, which gives it poor antimicrobial activity. The author relates these results to some chemical component that undergoes genetic variations within its own cell, and possibly modifies the content of the active ingredient present and the action of compounds not identified by the instrumentation used (FRANCO et al., 2007; LOUREIRO et al., 2016).

4.2 MODULATION WITH ANTIBIOTICS

For modulation with antibiotic action against *S. aureus* and *E. coli*, both samples showed synergistic effect, as shown in Figure 3. The CLEO exerted synergism on amicacin against *S. aureus*, with a decrease in MIC from 1024 μg / mL to 14, 7 μg / mL, while ZOEO reduced the MIC to 6.7 μg / mL and was more effective. These results are possibly due to the fact that *S. aureus* bacteria do not have an outer membrane in their structure, which normally allows the action of antibacterial agents. Regarding gentamicin on *E. coli*, CLEO decreased MIC from 64 μg / mL to 4
μg / mL, while ZOEO decreased from 64 μg / mL to 8 μg / mL, indicating good activity for both essential oils.

There are different mechanisms of bacterial resistance to antibiotics, which may relate *E. coli* resistance to amikacin and gentamicin in both samples; the enzymatic modification of the antibiotic; existence of bacterial cell antibiotic efflux pumps; alterations in the antibiotic target molecules and the production of alternative target molecules, which are not inhibited by the antibiotic, besides the presence of lipopolysaccharides in their outer membrane, which reinforce their resistance (SHANOON et al., 2012; SOLÍS-QUISPE et al., 2019).

![Figure 3. Modulating effect of ZOEO and CLEO on the action of the antibiotics amikacin and gentamicin against *E. coli* and *S. aureus* strains (ANOVA and Bonferroni P test <0.0001).](image)

Studies evaluating the action of ginger and turmeric essential oils have shown greater antibacterial action against Gram-positive bacteria. Similar results were obtained by Nanasombat and Lohasupthawee (2005) where ZOEO demonstrated moderate bacterial activity on *E. coli* with MIC of 4.2 μL / mL. Cutrim et al. (2019), using the disk diffusion method, observed inhibition potential of ZOEO-tested strains on gentamicin (10 μg), reducing the MIC from 13.0 μg / mL to 9.7 μg / mL over *S. aureus*, while for *E. coli* the reduction in its MIC was from 22.5 μg / mL to 10.7 μg / mL.

In a study by Singh et al. (2012), the results obtained from the CLEO when compared to the antibiotic gentamicin (30 μg / mL) showed inhibition potential of 36% against *S. aureus* and 18% for *E. coli*. Soares (2009) associates the CLEO biological activity possibly to its composition containing ketone substances like ar-turmerone (36.8%). Such studies consolidate the results obtained in this work, and affirm the synergism of both essential oils on *S. aureus*. 
5 CONCLUSION

The chemical characterization of both essential oils was consistent with other studies, with variations in compound concentrations. *Artemia salina* toxicity revealed significant LC$_{50}$ of both CLEO and ZOEO. Regarding the ability to neutralize the DPPH radical, there was activity at the tested concentrations, where the best results were from ZOEO. The minimum inhibitory concentration (MIC) showed significant results with ZOEO, however no significant inhibitory activity of bacterial growth of CLEO was observed. Essential oils had significant synergistic activity in modulation with aminoglycoside antibiotics especially against gram-positive bacteria. The present study contributes to the discovery and development of new functional foods as alternative food additives in the prevention and treatment of infections and degenerative diseases caused by oxidative stress.

*Conflicts of Interest:* The authors declare no conflicts of interest.

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