ORIGINAL

The essential oil of *Curcuma longa* rhizomes as an antimicrobial and its composition by Gas Chromatography/Mass Spectrometry

Óleo essencial de Curcuma longa L. como um antimicrobiano e composição por Cromatografia Gasosa/Espectrometria de massas

Gisele Mara Silva GONÇALVES[1] 0000-0002-3480-5777

Pedro Paulo BARROS[1] 0000-0001-7435-1645

Gustavo Henrique da SILVA[1] 0000-0002-4277-1167

Gabriela Riqueza FEDES[1] 0000-0003-2048-0702

ABSTRACT

Objective

This study aimed to extract the essential oil of *Curcuma longa* rhizomes collected in Brazil, determine its composition by gas chromatography and mass spectrometry, and evaluate its hemolytic action and antimicrobial activity.

Methods

The oil extraction was performed by hydrodistillation; its composition was determined by GC-MS; the Minimum Inhibitory Concentration was evaluated through microdilution, and the hemolytic activity was analyzed in sheep red blood cells.

[1] Pontifícia Universidade Católica de Campinas (PUC-Campinas), Centro de Ciências da Vida, Faculdade de Ciências Farmacêuticas. Av. John Boyd Dunlop, s/n., Prédio Administrativo, Jd. Ipaussurama, 13060-904, Campinas, SP, Brasil. *Correspondência para*/Correspondence to: GMS GONÇALVES. E-mail: <gmsg@puc-campinas.edu.br>.

Como citar este artigo/How to cite this article

Gonçalves GMS, Barros PP, Silva GH, Fedes GR. The essential oil of *Curcuma longa* rhizomes as an antimicrobial and its composition by CG-MS. Rev Ciênc Med. 2019;28(1):1-10. http://dx.doi.org/10.24220/2318-0897v28n1a4389

http://dx.doi.org/10.24220/2318-0897v28n1a4389

Results

The essential oil's major components are zingiberene (11%), sesquiphellandrene (10%), β-turmerone (10%), and α-curcumene (5%). It proved efficient at inhibiting *Staphylococcus aureus* with Minimum Inhibitory Concentrations of 38.8µl/mL, *Staphylococcus epidermidis* (Minimum Inhibitory Concentrations of 50.0µl/mL), *Escherichia coli* (Minimum Inhibitory Concentrations of 44.4µl/mL), and *Pseudomonas aeruginosa* (Minimum Inhibitory Concentrations of 27.7µl/mL).

Conclusion

Despite provoking hemolysis in sheep red blood cells, the essential oil suggests promising results for a variety of purposes due to its antibacterial properties. Supplementary research is necessary to determine *in vivo* activity and the potential use of the *C. longa* essential oil as an antimicrobial agent in diverse situations.

Keywords: Antimicrobial activity. *Curcuma longa*. Essential oil. GC/MS. Hemolytic activity.

RESUMO

Objetivo

Este trabalho teve como objetivo determinar a composição e avaliar as atividades antimicrobiana e hemolítica do óleo essencial de rizomas da Curcuma longa L.

Métodos

A extração do óleo essencial foi realizada por hidrodestilação e sua composição determinada por cromatografia gasosa com espectrometria de massas. A Concentração Mínima Inibitória foi avaliada por microdiluição e a atividade hemolítica foi analisada em hemácias de carneiro.

Resultados

Os componentes majoritários encontrados foram zingibereno (11%), sesquipelenadieno (10%), β-turmerona (10%) e α-curcumeno (5%). O óleo essencial provocou hemólise e inibiu Staphylococcus aureus (Concentração Mínima Inibitória=38,8µl/mL), Staphylococcus epidermidis (Concentração Mínima Inibitória=50,0µl/mL), Escherichia coli (Concentração Mínima Inibitória=44,4µl µl/mL) e Pseudomonas aeruginosa (Concentração Mínima Inibitória=27,7µl/mL).

Conclusão

Apesar de provocar hemólise, o óleo essencial analisado tem potencial para diversas finalidades, devido às suas propriedades antibacterianas. Estudos complementares são necessários para determinar a atividade in vivo e o potencial uso de óleo essencial de C. longa como agente antimicrobiano em diversas situações.

Palavras-chave: *Atividade Antimicrobiana. Curcuma longa. Óleo Essencial. GCMS. Atividade Hemolítica.*

INTRODUCTION

Curcuma longa L. (*Zingiberaceae*) has been widely studied by the scientific community due to its diverse properties, usually attributed to substances present in its rhizomes. Its essential oil generally contains turmerone, dehydroturmerone, and aromatic ketones, in addition to a variety of other volatile components such as aliphatic or oxygenated mono- and sesquiterpenes [1-5].

Studies carried out by several authors have shown that the essential oil of *Curcuma longa* can possibly be used as an anti-inflammatory, antioxidant, antimicrobial, anti-cancer, and anti-viral. Essential oils have

currently been characterized by gas chromatography and mass spectrometry systems, as well as through research of their antimicrobial and antioxidant activities [6]. Variations in climatic conditions may interfere with the composition of the essential oils [7].

Hassan *et al*. [8] determined that the major components of a sample of *C. longa* essential oil that showed high antimicrobial and antioxidant actions were β-sesquiphellandrene, α-curcumene, and p-mentha-1,4(8)-diene. Another study [9] pointed to the presence of α-turmerone, β-turmerone, and ar-turmerone, mainly, and to antifungal and antimycotoxigenic activities.

Considering the aspects above, the present study analyzed the chemical composition of the essential oil extracted from *Curcuma longa* fresh rhizomes collected in Brazil by gas chromatography and mass spectrometry, as well as its antimicrobial and hemolytic activities.

METHODS

Curcuma longa was purchased from a commercial plantation (-14.012490, -49.174368) during the months of September and October 2015. The plant samples were authenticated by means of gross and microscopic analysis, as defined in the Brazilian Pharmacopoeia [10].

After confirming the plant material's identity, sliced rhizomes of fresh turmeric (200g) were mixed with distilled water. The essential oil was extracted by hydrodistillation using a Clevenger apparatus, in accordance with the Brazilian Pharmacopoeia [10]. The method was based on Gonçalves *et al*. [11]. Thus, the liquid-liquid partitioning of the hydrolate was carried out with chloroform (three 50ml portions) in a separatory funnel. The organic fraction (chloroform and the essential oil) was treated with anhydrous sodium sulfate in order to remove the residual moisture. Next, the fraction was filtered with filter paper and the remaining contents were transferred to a round-bottom flask. The solvent's evaporation was carried out in a rotary evaporator at 50°C and 40rpm for 30 minutes. The essential oil was stored in sealed glass vials at -10°C in the dark until its use.

The essential oil analysis was then performed by Gas Chromatography/Mass Spectrometry (GC/MS). Five hundred microliters of *Curcuma longa* essential oil were diluted in 1mL of hexane, and 1μL of the mixture was injected into a GC-MS apparatus (GC-MS QP 2010 Ultra, Shimadzu, Kyoto, Japan) equipped with an auto sampler (AOC 5000, Shimadzu). The injection was carried out in split mode (1:10) at 220°C. Separation was achieved in an Rtx-5MS column (30mx0.25mm, 0.25μm, Restek, Pennsylvania, United States) according to the chromatography and mass spectrometry conditions previously described by Gonçalves *et al*. [11]. The identification was carried out based on mass spectra and the Kovatz retention index. Mass spectra from the sample peaks were compared to the Wiley mass spectra library. The Kovatz retention index of the compounds found in the sample were calculated in relation to a series of n-alkane (C6-C25) and compared with data published on the National Institute of Standards and Technology (NIST) library [12,13].

Analysis of antimicrobial activity

Suspensions equivalent to the 0.5 standard of the Mac Farland scale (Probac Brazil™) [14] prepared from fresh cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), and *Pseudomonas aeruginosa* (ATCC 13525) were used.

Disk diffusion assay

Petri dishes containing Mueller-Hinton (Merck™) agar were seeded with the microbial suspensions with the aid of swabs. Sterile 6-milimiter-diameter paper discs (Whatman™) soaked with 10mL of essential

http://dx.doi.org/10.24220/2318-0897v28n1a4389

oil were distributed on the surface of the agar in duplicate. The incubation occurred overnight at 35°C and the diameters of the zone of inhibition around each disc were measured in millimeters [11,14].

Microdilution test

The Minimum Inhibitory Concentration (MIC) of the essential oil was determined by a broth microdilution method. The essential oil was emulsified in polysorbate 80 and diluted in Mueller-Hinton broth (Oxoid™). The broth cultures were added to a 96-well plate. The essential oil concentrations added to the suspensions ranged from 5.5µL/mL to 111.1µL/mL. After incubating at 35°C for 24 hours, triphenyl tetrazolium was used for reading the microdilution plate. Triphenyl tetrazolium changes the medium's color if there is bacterial growth and facilitates reading. The MIC corresponded to the lowest oil concentration that inhibited visible bacterial growth in the microdilution plate. Each experiment was performed in triplicate [14-16].

Hemolysis assay

Hemolytic activity was measured by determining the lysis of defibrinated sheep blood cells purchased from BioBoavista (*Campinas*, Brazil). This method was based on the Brazilian Pharmacopoeia V [10]. The experiment was performed in triplicate, using phosphate buffer as a negative control (pH7.4), and saponins obtained by decoction of *Aesculus hippocastanum* (commonly known as conker tree or horse-chestnut) as positive controls, in a proportion of 2g of the ground drug to 15mL of distilled water, which has a known hemolytic action. Then, serial dilutions of the essential oil, saponin pattern, and blank trials were performed. The essential oil of *Curcuma longa* was successively diluted in phosphate buffer and 1mL of 2%RBC suspension was added to each tube (Table 1). Tubes were homogenized and rested for 6 hours at room temperature. Subsequently, the presence or absence of hemolysis was assessed.

Table 1. Serial dilution of the essential oil of *Curcuma longa* for determination of hemolytic action, by hemolysis technique in tubes containing sterile defibrinated sheep Red Blood Cells (RBC).

Reagents	Volume per tube (mL)			
	A	B	C	D
Curcuma longa essential oil	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4	0.90	0.80	0.50	-
2%RBC suspension	1.00	1.00	1.00	1.00

RESULTS

The color of the essential oil obtained was yellowish. Its aspect was translucid and slightly viscous, with an intense characteristic odor. Hydrodistillation was carried out for three hours. The yield was 0.70%, based on the weight of fresh rhizomes used.

All concentrations of Essential oil of *Curcuma longa* rhizomes caused hemolysis in the red blood cells of sheep. The GC/MS analysis of the essential oils [12] showed 73 components with a retention time of up to 25 minutes and identified these components (Table 2). The major components were Zingiberene, Sesquiphellandrene, β-Turmerone, and α-Curcumene, in addition to β-Tinene, 1,8-Cineole, a-Terpinolene, β-Cariophellene, β-Bisabolene, Farnesyl Acetate, and α-turmerone, with compositions similar to those shown by Ferreira *et al*. [1] and Qin *et al*. [13].

Table 2. Retention index of each compound identified in the *Curcuma longa* essential oil.

1 of 2

Peak[a]	Compound	Retention time[b] (min)	Retention index[c]	Compound identification
1	3-Hexen-1-ol	5.204	854.29 ± 0.22	MS[d], RI[e]
2	4-Hepten-2-ol	5.722	882.72 ± 0.29	MS, RI
3	2-Heptanone	5.850	889.74 ± 0.36	MS, RI
4	2-Heptanol	6.006	898.21 ± 0.28	MS, RI
5	α-Thujene	6.612	927.28 ± 0.19	MS, RI
6	α-Pinene	6.771	934.87 ± 0.21	MS, RI
7	Sabinene	7.605	974.61 ± 0.19	MS, RI
8	β-Pinene	7.699	979.09 ± 0.19	MS, RI
9	6-Methyl-5-hepten-2-one	7.844	987.21 ± 2.25	MS, RI
10	β-Myrcene	7.922	990.50 ± 1.35	MS, RI
11	2-Octanol	8.105	998.38 ± 0.17	MS, RI
12	2-Carene	8.183	1002.12 ± 0.23	MS, RI
13	α-Phellandrene	8.261	1005.63 ± 0.16	MS, RI
14	δ-3-Carene	8.395	1011.88 ± 0.23	MS, RI
15	α-Terpinene	8.523	1017.76 ± 0.23	MS, RI
16	p-Cymene	8.700	1025.95 ± 0.23	MS, RI
17	Limonene	8.806	1030.82 ± 0.23	MS, RI
18	1,8-Cineole	8.885	1034.44 ± 0.21	MS, RI
19	β-Ocimene	9.160	1047.17 ± 0.26	MS, RI
20	γ-Terpinene	9.440	1059.96 ± 0.16	MS, RI
21	Sabinene hydrate	9.631	1068.97 ± 0.34	MS, RI
22	α-Terpinolene	10.115	1091.19 ± 0.30	MS, RI
23	2-Nonanol	10.265	1098.10 ± 0.28	MS, RI
24	p-Mentha-1,5,8-triene	10.610	1114.31 ± 0.28	MS, RI
25	p-Mentha-trans-2,8-dien-1-ol	10.789	1122.74 ± 0.22	MS, RI
26	p-Menth-2-en-1-ol	10.816	1124.06 ± 0.27	MS, RI
27	Thujol	11.941	1177.23 ± 0.29	MS, RI
28	Terpinen-4-ol	12.029	1181.33 ± 0.26	MS, RI
29	p-Cymen-8-ol	12.160	1187.55 ± 0.28	MS, RI
30	2-Decanone	12.238	1191.25 ± 0.27	MS, RI
31	α-Terpineol	12.299	1194.10 ± 0.26	MS, RI
32	2-Decanol	12.382	1198.02 ± 0.26	MS, RI
33	2-Pinen-4-one	12.737	1215.48 ± 0.23	MS, RI
34	Cumaldehyde	12.826	1219.85 ± 0.20	MS, RI
35	trans-Chrysanthenyl acetate	12.944	1225.84 ± 0.35	MS, RI
36	cis-Carveol	13.109	1233.86 ± 0.17	MS, RI
37	D-Carvone	13.416	1249.26 ± 0.38	MS, RI
38	cis-p-Mentha-1(7),8-dien-2-ol	13.590	1257.82 ± 0.25	MS, RI
39	Piperitone	13.642	1260.41 ± 0.25	MS, RI
40	2-Undecanone	14.282	1292.16 ± 0.23	MS, RI
41	2-Undecanol	14.407	1298.46 ± 0.33	MS, RI
42	Carvacrol	14.486	1302.60 ± 0.41	MS, RI
43	δ-Elemene	15.265	1343.33 ± 0.29	MS, RI

http://dx.doi.org/10.24220/2318-0897v28n1a4389

Table 2. Retention index of each compound identified in the *Curcuma longa* essential oil.

Peak[a]	Compound	Retention time[b] (min)	Retention index[c]	Compound identification
44	Piperitenone	15.377	1349.22 ± 0.32	MS, RI
45	α-Cubebene	15.505	1355.90 ± 0.34	MS, RI
46	Carveyl acetate	15.689	1365.64 ± 0.42	MS, RI
47	α-Copaene	15.920	1377.66 ± 0.29	MS, RI
48	α-Ylangene	15.961	1380.16 ± 0.66	MS, RI
49	(-)-β-Elemene	16.324	1398.86 ± 0.30	MS, RI
50	Not identified 1	16.540	1408.95 ± 0.32	-
51	β-caryophellene	16.927	1432.24 ± 0.32	MS, RI
52	α-Bergamotene	17.101	1441.87 ± 0.29	MS, RI
53	β-Farnesene	17.398	1458.25 ± 0.22	MS, RI
54	α-Humulene	17.548	1466.61 ± 0.25	MS, RI
55	Acoradiene	17.621	1470.70 ± 0.31	MS, RI
56	α-Curcumene	17.958	1489.39 ± 0.28	MS, RI
57	Germacrene D	18.035	1493.36 ± 0.03	MS, RI
58	Zingiberene	18.352	1512.06 ± 0.36	MS, RI
59	β-Bisabolene	18.494	1520.48 ± 0.34	MS, RI
60	β-Sesquiphellandrene	18.848	1541.50 ± 0.39	MS, RI
61	trans-α-Bisabolene	18.900	1544.58 ± 0.36	MS, RI
62	Selina-3,7(11)-diene	18.983	1549.49 ± 0.36	MS, RI
63	Sesquisabinene hydrate	19.211	1563.07 ± 0.42	MS, RI
64	Nerolidol	19.291	1567.79 ± 0.37	MS, RI
65	Germacrene B	19.453	1577.44 ± 0.42	MS, RI
66	Ar-Turmerone	19.593	1585.67 ± 0.34	MS
67	Not identified 2	19.833	1599.86 ± 0.31	-
68	Farnesyl acetate	20.228	1624.60 ± 0.46	MS
69	Zingiberenol	20.508	1641.80 ± 0.40	MS, RI
70	β-Turmerone	21.329	1692.95 ± 0.32	MS, RI
71	α-Bisabolol	21.477	1701.22 ± 0.23	MS, RI
72	α-Turmerone	21.780	1711.28 ± 0.19	MS
73	Germacrone	21.818	1712.55 ± 0.18	MS, RI
74	Not identified 3	22.388	1731.61 ± 0.21	-
75	Not identified 4	22.796	1745.17 ± 0.17	-
76	Not identified 5	23.094	1755.14 ± 0.19	-
77	Not identified 6	23.788	1778.26 ± 0.15	-

Note: [a]Numbered according to the elution; [b]Retention time in the Rtx-5MS column (30mx0.25mm, 0.25µm); [c]Values are Mean (M) ± Standard Deviation (SD) (n=3). [d]Mass Spectra (MS) comparison with Wiley library. [e]Kovatz Retention Index (RI) comparison with NIST Chemistry WebBook.

Table 3 shows zones of growth inhibition and the minimum inhibitory concentration of the essential oil of *Curcuma longa* rhizomes against a selection of Gram-positive and Gram-negative bacteria.

Table 3. Zones of growth inhibition (mm) and Minimum Inhibitory Concentration (MIC) (µl/mL) of the essential oil of *Curcuma longa* rhizomes against a selection of Gram-positive and Gram-negative bacteria.

Strains tested	Growth inhibition (mm)	MIC (µl/mL)
Staphylococcus aureus	8.3 ± 0.2	38.8
Staphylococcus epidermidis	9.0 ± 0.1	50.0
Escherichia coli	8.0 ± 0.3	44.4
Pseudomonas aureuginosa	9.3 ± 0.2	27.7

DISCUSSION

The essential oil extracted from *Curcuma longa* rhizomes had 73 compounds identified. The major component was zingiberene (11%), followed by sesquiphellandrene (10%), β-turmerone (10%), and α-curcumene (5%) (Table 2).

Many of the identified substances are found in the essential oils of various potentially antimicrobial species. The four components found by Junqueira *et al*. [17] and Chatterjee *et al*. [18] are similar to the four compounds highlighted in the current study. There was also similarity with the composition demonstrated by Priya *et al*. [19], emphasizing the large amount of sesquiphellandrene. These authors demonstrated antioxidant activity for the essential oil evaluated. In contrast, Singh *et al*. [20] confirmed that ar-turmerone is the major constituent of turmeric rhizome oil of different origins, although this did not occur in the present study. Zhang *et al*. [7] evaluated samples collected in 20 different habitats in China and found that the composition and bioactivity of the essential oils were varied. Among the most abundant components, they found ar-turmerone, β-turmerone, α-zingiberene, ar-curcumene, and β-sesquiphellandrene. These authors found antioxidant and antimicrobial activities, which varied according to the origin of the samples used.

In the present study, hemolysis was rapidly observed for all concentrations of the essential oil, about 20 minutes after the test started. However, in a study by Liju *et al*. [21], the oral administration of *Curcuma longa* essential oil failed to produce any damage and was considered safe. Nevertheless, this result is important and should be taken into consideration in the process of research and development of possible medication or food based on the essential oil.

Regarding antimicrobial efficacy, the preliminary study by the disc diffusion has demonstrated that all microbial strains were susceptible to the oil (Table 3). Kamazeri *et al*. [22] evaluated the essential oils of three species of the *Zingerbiraceae* family by Agar diffusion and obtained similar results for the same bacteria.

In the microdilution test, the essential oil was efficient at inhibiting *Staphylococcus aureus*, *Staphylococcus epidermidis, Escherichia coli*, and *Pseudomonas aeruginosa* (Table 3). This is an important result, given that some of these bacteria are multi-resistant and thus difficult to combat with conventional therapy – *P. aeruginosa*, for example. The minimum inhibitory concentration values of the bacteria may be viable therapeutically and for the development of formulations. Although we have not found *in vivo* studies of the effects of this essential oil, we believe it could be administered orally (to study its possible systemic effect) or by pulmonary pathway (to assess its local effect, especially in the case of infections). The safety of use may vary depending on the concentration used and the route of administration of the product containing the essential oil. Supplementary studies are required to ensure safety.

From these results, one may conclude that the essential oil obtained was promising and appeared effective against all tested bacterial strains, despite the need for further proving its use safety.

 http://dx.doi.org/10.24220/2318-0897v28n1a4389

According to Moghadamtousi *et al*. [23], the essential oil of *Curcuma longa* may also be effective against *B. subtilis*, *B. coagulans*, and *B. cereu*s, besides *S. aureus*, *E. coli*, and *P. aeruginosa*. Ferreira *et al*. [1] have evaluated its potential antiaflatoxin activity in comparison to curcumin and found a superior effect in the latter, despite a lack of statistically significant difference in the concentrations used.

In a study of *Thymus vulgaris* [11] essential oil, the authors determined that the MIC and the results obtained for thyme oil were similar in strains of *S. aureus*, *S. epidermidis*, and *E. coli*, with MIC values of 40µl/mL. However, thyme oil had no effect on *P. aeruginosa*. Therefore, the essential oil of *Curcuma longa* may be more efficient at combatting diverse bacteria when compared to a similar concentration of *Thymus vulgaris* essential oil.

In contrast, Singh *et al*. [20] also studied the antimicrobial activity of *Curcuma longa* essential oil and determined a MIC of 1.95µl/mL for *Staphylococcus aureus* (almost 20-fold lower than the concentration found in the current study), in addition to 7.81µl/mL for *P. aeruginosa* (around 3.5-fold lower than the value found). In view of those results, the authors suggested that the difference between the chemical composition and antimicrobial efficacy of the essential oil was a function of possible genetic variability among samples, as well as cultivation conditions. Thus, given this essential oil's potentially therapeutic application, the way rhizomes were obtained and preserved is a relevant and worthy of consideration factor. In a study by Gounder & Lingamallu [24] comparing fresh, dried, and cured rhizomes, those authors noted that the drying method usually has a significant effect on the quality and quantity of volatile oils.

Essential oils constitute a source of antimicrobial substances with many possible practical applications, although the use of these substances in the composition of products implies in modifications in their organoleptic characteristics. Concerning the use of essential oils in food, they might contribute to product acceptability. However, their strong odor may make them undesirable for other uses.

Kunicka-Styczynska *et al*. [25] carried out a study with the essential oils of lavender, tea tree, and lemon in washing liquid and O/W soft body balms. They concluded that essential oils have promising uses as preservative systems, with potential for various purposes, such as in the food, pharmaceutical, and cosmetic industries

Findings in this study indicate that the essential oil of *Curcuma longa* may be considered an eco-friendly alternative for bio-preservation in the food industry. Furthermore, the essential oil may be useful for the treatment of infections and it might be an option for managing resistant microorganisms.

CONCLUSION

In conclusion, the essential oil of *Curcuma longa* rhizomes obtained exhibited as its major components *zingiberene* (11%), *sesquiphellandrene* (10%), *β-turmerone* (10%), and *α-curcumene* (5%). The essential oil was efficient at inhibiting the growth of *Staphylococcus aureus* (MIC of 38.8µl/mL), *Staphylococcus epidermidis* (MIC of 50.0µl/mL), *Escherichia coli* (MIC of 44.4µl/mL), and *Pseudomonas aeruginosa* (MIC of 27.7µl/mL). Therefore, despite provoking hemolysis in sheep red blood cells, the essential oil suggests promising results for a variety of purposes due to its antibacterial properties. Supplementary research studies are necessary to determine *in vivo* activity, safety, and the potential use of *C. longa* essential oil as an antimicrobial agent in diverse situations.

CONTRIBUTORS

GMS GONÇALVES, responsible for the experimental design, analysis and interpretation of data, revision and approval of the final version of the article. PP BARROS and GH SILVA, responsible for the data analysis and interpretation. GR FEDES, responsible for the experiment and the organization of results.

ACKNOWLEDGEMENTS

The authors wish to thank the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (National Council for Scientific and Technological Development) for the financial support.

REFERENCES

1. Ferreira FD, Kemmelmeier C, Arrotéia CC, Costa CL, Mallmann CA, Janeiro V, *et al*. Inhibitory effect of the essential oil of *Curcuma longa* L. and curcumin on aflatoxin production by *Aspergillus flavus* Link. Food Chem. 2013;136(2):789-93.
2. Liu CH, Chang FY. Development and characterization of eucalyptol microemulsions for topic delivery of curcumin. Chem Pharm Bull. 2011;59(2):172-8.
3. Peret-Almeida L, Naghetini CC, Nunan EA, Junqueira RG, Glória MBA. Atividade antimicrobiana *in vitro* do rizoma em pó, dos pigmentos curcuminóides e dos óleos e dos essenciais da *Curcuma longa* L. Ciênc Agrotec. 2008;32(3):875-81.
4. Prakash B, Singh P, Kedia A, Singh A, Dubey NK. Efficacy of essential oil combination of *Curcuma longa l*. and *Zingiber officinale ros*c. as a postharvest fungitoxicant, aflatoxin inhibitor and antioxidant agent. J Food Safety. 2012;32(3):279-88.
5. Silva Filho CRM, Souza AG, Conceição MM, Silva TG, Silva TMS, Ribeiro APL. Avaliação da bioatividade dos extratos de cúrcuma (*Curcuma longa* L., *Zingiberaceae*) em *Artemia salina e Biomphalaria glabrata*. Braz J Pharm Sci. 2009;19(4):919-23.
6. Lang G, Buchbauer G. A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals: A review. Flavour Fragr J. 2012;27(1):13-39.
7. Zhang L, Yang Z, Chen F, Su P, Chen D, Pan W, *et al*. Composition and bioactivity assessment of essential oils of *Curcuma longa* L. collected in China. Ind Crops Prod. 2017;109(15):60-73.
8. Hassan W, Gul S, Rehman S, Kanwal F, Afridi MS, Fazal H, *et al*. Gas chromatography coupled with mass spectrometric characterization of Curcuma longa: Protection against pathogenic microbes and lipid peroxidation in rat's tissue homogenate. Pak J Pharm Sci. 2016;29(2):615-21.
9. Avanço GB, Ferreira FD, Bomfim NS, Santos PASR, Peralta RM, Brugnari T, *et al*. *Curcuma longa* L. essential oil composition, antioxidant effect, and effect on *Fusarium verticillioides* and fumonisin production. Food Control. 2017;73(Part B):806-13.
10. Agência Nacional de Vigilância Sanitária. Farmacopeia brasileira V. Brasília: Ministério da Saúde; 2010.
11. Gonçalves GMS, Srebernich SM, Bragagnolo N, Adalozzo ES, Merhi VL, Pires DC. Study of the composition of *Thymus vulgaris* essential oil, developing of topi formulations and evaluation of antimicrobial efficacy. J Med Plants Res. 2013;7(23):1736-45.
12. Linstrom PJ. Nist standard reference database number 69. NIST Chemistry WebBook; 2003 [cited 2019 May 25]. Available from: https://webbook.nist.gov/U.S. Secretary of Commerce on behalf of the United States of America.
13. Qin NY, Yang FQ, Wang YT, Li SP. Quantitative determination of eight components in rhizome (Jianghuang) and tuberous root (Yujin) of *Curcuma longa* using pressurized liquid extraction and gas chromatography–mass spectrometry. J Pharm Biomed Anal. 2007;43(2):486-92.
14. Sawaya AC, Palma AM, Caetano FM, Marcucci MC, Silva Cunha IB, Araujo CE, *et al*. Comparative study of *in vitro* methods used to analyze the activity of propolis extracts with different compositions against species of *Candida*. Lett Appl Microbiol. 2002;35(3):107-203.
15. Agência Nacional de Vigilância Sanitária. Metodologia dos testes de sensibilidade a agentes antimicrobianos por diluição para bactéria de crescimento aeróbico: norma aprovada. 6a ed. M7-A6. 2003 [citado 2016 maio 25]; 23(2). Disponível em: http://www.anvisa.gov.br/servicosaude/manuais/clsi/clsi_opasm7_a6.pdf
16. Agência Nacional de Vigilância Sanitária. Método de referência para testes de diluição em caldo para determinação da sensibilidade de leveduras à terapia antifúngica: norma aprovada. 2a ed. M27-A2. 2003 [citado 2016 maio 25]; 22(15). Disponível em: http://www.anvisa.gov.br/servicosaude/manuais/clsi/clsi_OPAS1M27-A2.pdf
17. Junqueira RG, Mata AR, Nelson DL, Afonso RJC, Glória MBA. Identificação de compostos voláteis da cúrcuma empregando microextração por fase sólida e cromatografia gasosa acoplada à espectrometria de massas. Ciênc Tecnol Aliment. 2004;24(1):151-7.
18. Chatterjee S, Variyar PS, Gholap AS, Padwal-Desai SR, Bongirwar DR. Effect of γ-irradiation on the volatile oil constituents of turmeric (*Curcuma longa*). Food Res Int. 2000;33(2):103-6.

http://dx.doi.org/10.24220/2318-0897v28n1a4389

19. Priya R, Prathapan A, Raghu KG, Nirmala Menon A. Chemical composition and in vitro antioxidative potential of essential oil isolated from *Curcuma longa* L. leaves. Asian Pac J Trop Biomed. 2012;S695-9.

20. Singh S, Sankar B, Rajesh S, Sahoo K, Subudhi E, Nayak S. Chemical composition of turmeric oil (*Curcuma longa* L. cv. Roma) and its antimicrobial activity against eye infecting pathogens. JEOR. 2011;23(6):11-8.

21. Liju VB, Jeena K, Kuttan R. Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L). Food Chem Toxicol. 2013;53(3):52-61.

22. Kamazeri TSAT, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of *Curcuma aeruginosa, Curcuma mangga*, and Zingiber cassumunar from Malaysia. Asian Pac J Trop Med. 2012;5(3):202-9.

23. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed Research International. 2014 [cited 2019 Jan 10];1-12. Available from: https://www.hindawi.com/journals/bmri/2014/186864/. doi: http://dx.doi.org/10.1155/2014/186864

24. Gounder DK, Lingamallu J. Comparison of chemical composition and antioxidant potential of volatile oil from fresh, dried and cured turmeric (*Curcuma longa*) rhizomes. Ind Crops Prod. 2012;38:124-31.

25. Kunicka-Styczyńska A, Sikora M, Kalemba D. Lavender, tea tree and lemon oils as antimicrobials in washing liquids and soft body balms. Int J Cosmet Sci. 2011;33(1):53-61.

Received: October 17, 2018
Final version: April 4, 2019
Approved: April 30, 2019