ARTIGO ORIGINAL

Chemical composition of essential oils from the leaves of *Curcuma longa* and *Bixa orellana* and antimicrobial activity in *Pseudomonas aeruginosa* and *Listeria monocytogenes*

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ABSTRACT: The objective of this work was to identify the chemical constituents of the essential oils of dry leaves of turmeric and annatto, to verify their bactericidal action, determining the MIC on each bacterium and to evaluate the action mechanism on the planktonic cells through TEM. It can be observed that the yield of the oils was 2.78 and 0.21%, and moisture of the dry turmeric and annatto leaves was 13% and 10.6%, respectively. In the essential oil of the turmeric leaves were the majority components: α-phellandrene, terpinolene and 1,8-cineol, while in the essential oil of the annatto leaf, were α- humulene, E-nerolidol and spathulenol. The MIC of the essential oils was 0.5%, and the essential oils, when in synergism at the concentration of 5%, presented the largest inhibition halo for *Pseudomonas aeruginosa* (14.67 mm).
Key words: Annatto, Inhibition, Microorganisms, Turmeric.

RESUMO: Composição química dos óleos essenciais das folhas de *Curcuma longa* e *Bixa orellana* e atividade antimicrobiana em *Pseudomonas aeruginosa* e *Listeria monocytogenes*. Objetivou-se identificar os constituintes químicos dos óleos essenciais de folhas secas de cúrcuma e urucum, para verificar sua ação bactericida, determinando a CIM em cada bactéria e avaliar o mecanismo de ação sobre as células planctônicas através da TEM. Pode-se observar que o rendimento dos óleos foi de 2,78 e 0,21%, e a umidade das folhas secas de cúrcuma e urucum foi de 13% e 10,6%, respectivamente. No óleo essencial das folhas de açafrão foram os componentes majoritários: α-felandreno, terpinoleno e 1,8-cineol, enquanto no óleo essencial da folha de urucum, foram α-humuleno, E-nerolidol e espatulenol. A CIM dos óleos essenciais foi de 0,5%, e os óleos essenciais, quando em sinergismo na concentração de 5%, apresentaram o maior halo de inibição para *Pseudomonas aeruginosa* (14,67 mm).
Palavras-chave: Urucum, Inibição, Microrganismos, Açafrão.

INTRODUCTION

The investigation of new antimicrobial agents has been gaining prominence in recent decade mainly due to the declining of the number of new approved drugs and the imminent loss of patent protection. Furthermore, an increase is observed in the number of bacteria to resistant sanitizing agents used in the medical area as well as in the food industry (Li and Vederas 2009).

The extracts of higher plants have been and still are widely used to obtain substances with antimicrobial action. However, many times their low concentration in the extract makes the purification processes or the synergistic action of the different compounds unfeasible, causing major problems for industries. Seeking to reduce the parameters involved in the isolation and purification of compounds, the essential oils have been studied. These presents high antimicrobial efficiency and in appropriate concentrations they are considered safe

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(GRAS) (Dimitrijevic et al. 2007).

At the present time, approximately 3000 plant essential oils are known and around 300 are marketed. There are countless international conglomerates that negotiate essential oils, the most important use them as raw material for the production of aromas and fragrances and also in the food industries and in agriculture, with the market presenting growth over 11% a year (Bizzo et al. 2009).

Various plants are used for the extraction of essential oils, and some studies have demonstrated that those originating from oregano, cinnamon, thyme and rosemary are among the most active antimicrobials (Dimitrijevic et al. 2007; Jeong et al. 2014; Choi et al. 2016; Mahboubi et al. 2018), that is due mainly to the presence of majority compounds such as thymol, eugenol and the carvacrol, substances with high microbial activity (Liolios et al. 2009).

It is well defined in the literature that the essential oils containing phenolics as majority compounds possess higher antimicrobial activity (Burt 2004), however, the antimicrobial activity can also be attributed to terpenoid compounds (Liolios et al. 2009).

In search of new compounds with high antimicrobial activity, the essential oils of various plants are being analyzed quantitatively and qualitatively, among them turmeric and the annatto (Hu et al. 2017). However, only the rhizomes of turmeric and annatto seeds have been the focus of studies. There are few references in the literature to the antimicrobial activity of turmeric and annatto leaves, however, some studies show that the majority components of the essential oils of those plants belong to the class of the terpenes, whose bactericidal activity is proven.

The biocidal potential of an oil or of its components varies according to the physiological characteristics of the microorganism, a fact sustained by several works that show that the Gram-positive bacteria are more sensitive to the essential oils than the Gram-negative (Khorshidian et al. 2018). The bacteria *Listeria monocytogenes* and *Pseudomonas aeruginosa* present resistance to a variety of sanitizers considered of great importance in the food and/or medical areas, causing economic losses and even problems related to public health.

The objective of this study was to identify the chemical constituents of the essential oils of dry leaves of *Curcuma longa* L. (turmeric) and *Bixa orellana* L. (annatto), to determine the moisture level and yield of the essential oils of the dry leaves, to verify the bactericidal action of the essential oils on *Listeria monocytogenes* ATCC 19117 and *Pseudomonas aeruginosa* ATCC 25853 determining the minimum inhibitory concentration of the oils on each bacterium; and, to evaluate the effect of the oils on the planktonic cells through Transmission Electronic Microscopy.

MATERIALS AND METHODS

Raw material and extraction of essential oils

Fresh leaves of turmeric *(Curcuma longa* L., Zingiberaceae) and annatto (*Bixa orellana* L., Bixaceae) were obtained from cultures from the Mara Rosa (GO) and Presidente Olegário (MG) regions respectively, in December 2008. After collected, the samples were shade-dried at room temperature and transported to the Chemistry Laboratory in paper bags.

The essential oils were obtained by the hydrodistillation technique, using a modified Clevenger distiller, for two hours. After that period, the essential oils were separated from the water (hydrolate) using rotary evaporation (annatto) and centrifugation techniques (turmeric). The obtained essential oil was stored in amber glass wrapped with aluminum foil and stored under refrigeration.

The yield of the essential oil was expressed in % volume/mass, in other words, volume (ml) of essential oil per mass (g) of dry plant material.

Parallel testing was performed for moisture, according to Pimentel et al. (2008).

All the procedures were conducted in triplicate.

Identification and quantification of the chemical constituents of essential oils

For the qualitative evaluation, the essential oils were submitted to gas chromatography coupled with mass spectrometry (GC-MS), using a Shimadzu model CG-17A apparatus, with mass selective detector model QP 5000. The compounds were identified for comparisons with spectra existent in the library (Wiley, 229) and by the Kovat's index (Adams 1995).

For the quantitative evaluation, a Shimadzu gas chromatograph, model 17A, coupled with a hydrogen flame ionization detector (FID), capillary column DB5 was used. Three injections were conducted for each tested oil, obtaining the average concentration and the standard deviation for each constituent, obtaining the quantification through normalization of the area.

Standard microorganisms

The bacteria used in the development of this work were *Pseudomonas aeruginosa* ATCC 27853 and *Listeria monocytogenes* ATCC 19117. The stock culture was stored in freezing medium (glycerol - 15 ml; bacteriological peptone – 0.5 g; yeast extract –

0.3 g; NaCl – 0.5 g; distilled water 100 ml).

The number of cells per ml of each culture was quantified using the standard curve. The bacterial cultures were standardized to about 10^7 CFU/ml.

Determination of minimum inhibitory concentration (MIC)

The methodology used was the agar well diffusion (Bauer et al. 1966, with modifications), using TSA (Tryptic soy agar) with added 0.5% Tween 80. Initially, a fine agar layer was added on 140 mm diameter Petri dishes and solidified in a laminar flow chamber at room temperature. On the solid medium 9 sterile glass spheres with a diameter of 3 mm and volume of 7 mm^3 were placed.

The TSA culture medium, with 0.5 added Tween 80, was inoculated with the standardized culture (10^7 CFU/ml) and deposited on the solid agar layer. After the solidification, the spheres were removed and, later, the formed slots were filled with 8 µl of essential oil, at the different concentrations. The dilutions of the turmeric, annatto and synergism oils were prepared in dimethylsulfoxide (DMSO), obtaining the concentrations of 10%, 5%, 2.5%, 1.5% and 0.5%. To verify the effect of DMSO on the bacteria, this was used as control at 100%. The procedure was carried out in triplicate and the dishes were incubated at 37.0 °C for 24 h. After that interval, the measurement of the formed inhibition halos was conducted with a digital caliper rule (Nedorostova et al. 2009) with modifications.

Evaluation of the essential oil performance on microorganisms by transmission electron microscopy

After the standardization of the cultures, 1 ml aliquots of each were transferred to eppendorf tubes and centrifuged at 5000 rpm for 5 min. The pelet was treated with the essential oils and their mixture at the concentration of 0.5% for 15 min. After that period, the cultures were centrifuged, and the supernatant discarded; the pelets were embedded by the addition of 0.5 ml of agarose 1.5%.

The samples for transmission microscopy were prepared according to the protocol of Alves (2004).

Statistical analysis

The statistical analysis was done according to a randomized block design. The statistical analyses were conducted with the R Development (2010) in which variance analysis through the F test was used to verify the difference among the treatments. When significant, the comparison of averages for the oils and their concentrations was carried out through the Scott-Knott test.

RESULTS

Chemical composition of essential oils

Through the use of the hydrodistillation process for obtaining the essential oils of dry leaves of *C. longa* and *B. orellana*, we observed that a high variation exists in the moisture and yield. The moisture of the *C. longa* and *B. orellana* dry leaves was 13.2% and 10.6%, while the oil yields were 2.78 and 0.21%, respectively.

Tables 1 and 2 show the chemical components of the essential oils of the turmeric and annatto leaves, identified by GC/MS.

In the essential oil of *C. longa* leaves 15 chemical constituents were isolated, 88.23% being identified, the majority being α-phellandrene (41.07%), terpinolene (27.38%), followed by 1,8-cineol (7.70%) α-pinene (4.79%) and limonene (3.04%), belonging to the class of the monoterpenes. In the essential oil of the *B. orellana* leaf 21 chemical constituents were found, 75% being identified, of the which α-humulene (43.01%), E-nerolidol (14.40%) and spathulenol (7.57%) were the majority chemical constituents, belonging to the class of the sesquiterpenes.

Antimicrobial activity

The *in vitro* antibacterial activity of the essential oils of *C. longa*, *B. orellana* and their synergism on *Listeria monocytogenes* and *Pseudomonas aeruginosa* was qualitatively and quantitatively analyzed evaluating the presence and the diameter of the inhibition halos.

In Table 3 it can be observed that the essential oils and their mixtures presented antibacterial activity on *P. aeruginosa and L. monocytogenes.* According to variance analysis through the F test, there was significant difference ($p<0.05$) in relation to the oils and concentrations on *P. aeruginosa*, the oils in combination being better than the annatto oil and turmeric, according to the Scott-Knott test at the level of 5% significance.

For *L. monocytogenes* only the effect of the concentration was significant, and the oils of turmeric, annatto and their combinations presented the same behavior.

Evaluating the antimicrobial action of each essential oil, the largest inhibition halos for turmeric were found at the concentrations of 5% (2.00 mm) and 1.5 and 2.5 (2.5 mm), while for annatto they were at the concentrations of 0.5% (6.50 mm) and 10% (3.0 mm), for *P. aeruginosa* and *L. monocytogenes*, respectively. The essential oils when in combination, at the concentration of 5%, promoted the formation of larger inhibition halos, with a diameter of 14.67 mm for *P. aeruginosa* and 3.67 mm for *L. monocytogenes.*

Seeking a better understanding of the action of the essential oils and their mixtures employed

TABLE 1 - Chemical constituents of essential oil of *Curcuma longa* identified by gas chromatography coupled with mass spectrometry (GC-MS).

Compound	Retention time	KI_T	KI_c	Name
1	4.129	804	824	Ethyl butyrate
2	7.701	939	933	α-Pinene
3	9.217	979	979	β-Pinene
4	9.565	990	990	Myrcene
5	10.261	1002	1010	α-Phellandrene
6	10.317	1011	1011	δ-3-Carene
7	10.594	1017	1019	α-Terpinene
8	10.894	1026	1027	*o*-Cymene
9	11.056	1029	1031	Limonene
10	11.189	1031	1035	1,8-Cineol
11	12.126	1059	1060	γ-Terpinene
12	13.192	1088	1089	Terpinolene
13	13.669	1096	1101	Linalool
14	17.038	1182	1191	*p*-Cymen-8-ol
15	17.342	1188	1199	α-Terpineol

KI_T: Kovat's Index tabulated; KI_c: Kovat's Index calculated

TABLE 2 - Chemical constituents of essential oil of *Bixa orellana* identified by gas chromatography coupled to mass spectrometry (GC-MS).

Compound	Retention time	KI_T	KI_c	Name
1	4.127	804	823	Ethyl butyrate
2	7.695	939	933	α-Pinene
3	9.207	979	979	β-Pinene
4	23.769	1376	1379	α-Copaene
5	24.218	1390	1391	β-Elemene
6	25.301	1419	1424	E-Caryophyllene
7	26.169	1447	1450	Myltayl-4(12)-ene
8	26.547	1454	1461	α-Humulene
9	27.035	1466	1476	Ishwaranol
10	27.336	1485	1485	Germancrene D
11	27.455	1488	1489	Aristolochene
12	27.711	1497	1496	Valencene
13	27.879	1495	1502	γ-Amorphene
14	28.493	1523	1522	δ-Cadinene
15	28.601	1522	1525	7-Epi-α-selinene
16	29.776	1563	1563	E-Nerolidol
17	30.435	1578	1584	Spatulenol
18	31.969	1636	1635	Cadin-4-en-7-ol
19	32.439	1642	1651	Epi-α-muurolol
20	32.921	1660	1667	Neointermedeol
21	33.076	1671	1672	Bulnesol

KI_T: Kovat's Index tabulated; KI_c: Kovat's Index calculated.

TABLE 3 - Average halo size values at different concentrations of *Curcuma longa*, *Bixa orellana* and in synergism.

	Inhibition zone diameter (mm)					
	P. aeruginosa			*L. monocytogenes*		
Concentration (%)	Turmeric	Annatto	Synergism	Turmeric	Annatto	Synergism
Control (DMSO)	0.00 a	0.00a	0.00a	0.00a	0.00a	0.00a
0.5	0.83a	6.50b	1.50a	2.33b	1.83b	2.33b
1.5	0.83a	4.00b	2.67a	2.50b	2.17b	1.83a
2.5	0.83a	3.00b	7.67b	2.50b	2.17b	2.67b
5.0	2.00a	4.67b	14.67b	2.33b	2.50b	3.67b
10.0	0.83a	0.00a	9.83b	2.17b	3.00b	2.83b

Averages followed by same letter do not differ by Scott-Knott test at 5% significance.

on the structure of *P. aeruginosa* (Figure 1) and *L. monocytogenes* (Figure 2) transmission electron micrographs of the cells were analyzed.

The *P. aeruginosa* culture was treated with DMSO (Figure 1B) seeking to evaluate possible structural damage to the cells caused by the diluent used in the experiment, however compared to the non-treated cells (Figure 1A) no alteration was observed in the cytoplasmic content as well as in the cell wall. On the other hand, after the treatment of the cells with the essential oils of turmeric, annatto and their mixtures, alteration of the cell structure was observed (Figures 1C, 1D and 1E). It was verified that the highest damage was caused by the turmeric essential oil (Figure 1C), because there was cell wall loss and apparent alteration in the density of the cytoplasm, besides the cells being close to each other. The treatment with annatto essential oil (Figure 1D) and in synergism (Figure 1E) only caused cell wall rupture and damage to the

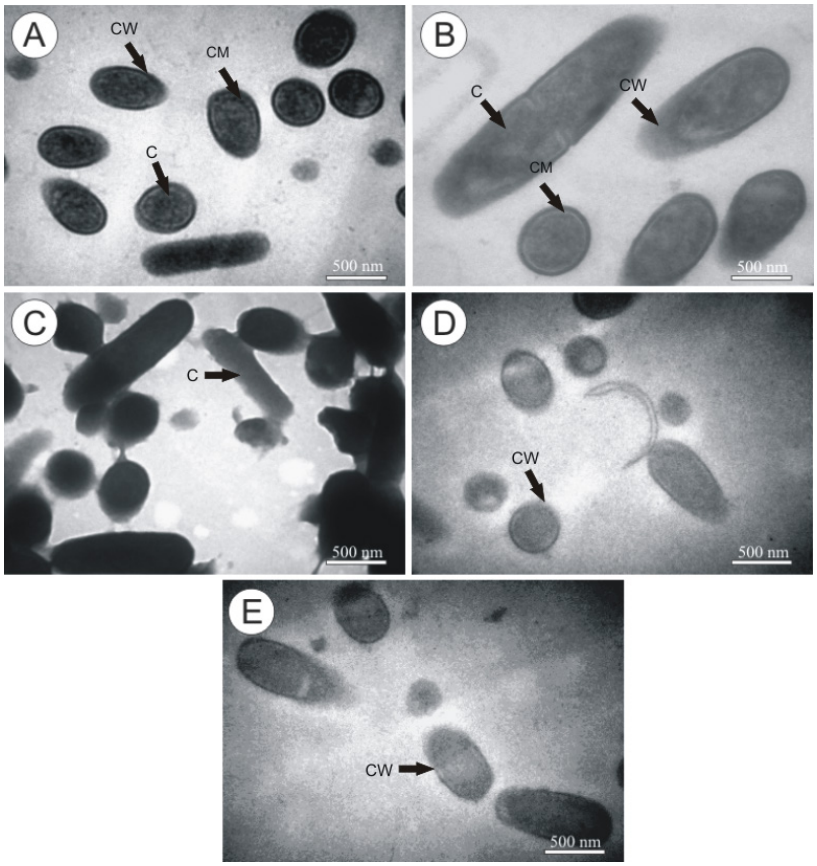

FIGURE 1 - Transmission electron micrograph of *Pseudomonas aeruginosa* exposed to essential oils at 0.5% concentration. Control (A), Bacterial cell exposed to DMSO (B); Turmeric (C); Annatto (D) and Annatto + Turmeric - Synergism (E). CW (Cell Wall); C (Cytoplasm); CM (Cytoplasmic Membrane).

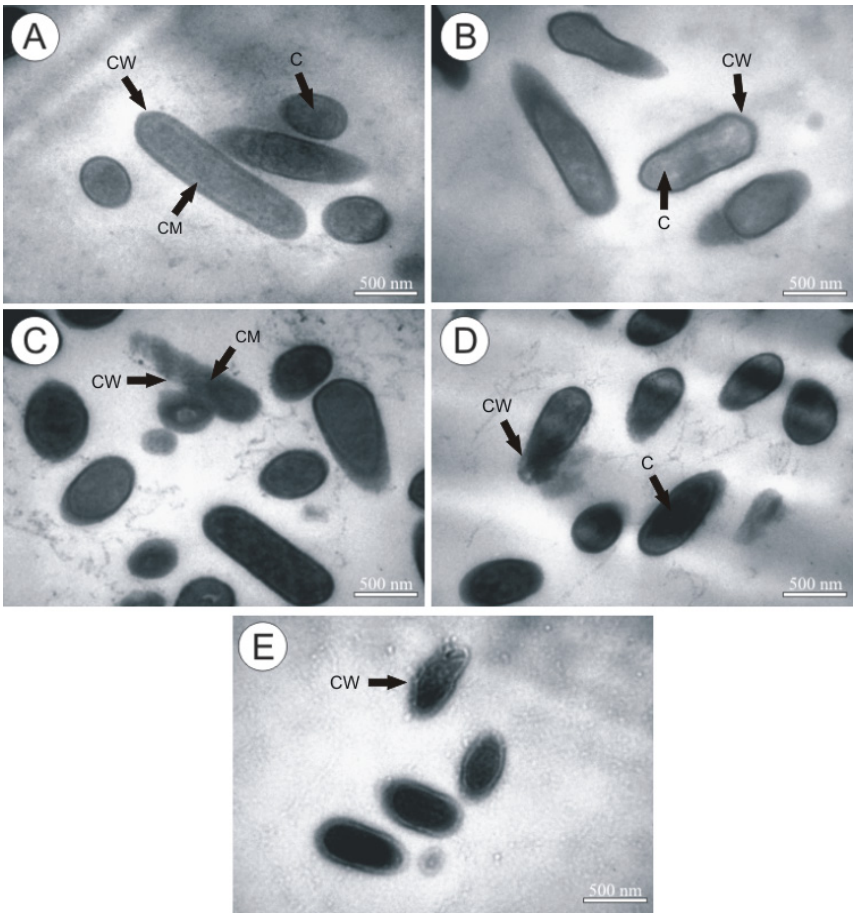

FIGURE 2 - Transmission electron micrograph of *Listeria monocytogenes* exposed to essential oils at 0.5% concentration. Control (A), Bacterial cell exposed to DMSO (B); Turmeric (C); Annatto (D) and Annatto + Turmeric - Synergism (E). CW (Cell Wall); C (Cytoplasm); CM (Cytoplasmic Membrane).

cytoplasmic membrane.

The behavior of the cells of *L. monocytogenes* when treated with DMSO (Figure 2B) was similar to that of *P. aeruginosa*, not observing any alteration in the cell integrity when compared with the non-treated cells (Figure 2A). After the treatments with the essential oils and their mixture, damage was observed in the cell wall (Figures 2C, 2D and 2E) and, as a consequence, extravasation of the cytoplasmic content could be observed, with its partial coagulation. This fact was observed in the cells treated with turmeric (Figure 2C) and annatto (Figure 2D).

DISCUSSION

The essential oil of turmeric leaves had terpenes as preponderant compounds, being α-felandrene being found as the majority compound. That same compound was found in studies carried out by Behura et al. (2002). Plants that contain that compound as majority and other terpenes present high antimicrobial potential. However, in our study the essential oil of turmeric leaves was not very effective against *P. aeruginosa* or *L. monocytogenes*.

According to Jiang et al. (2006) the diarylheptanoids and the sesquiterpenes, represented by curcumine, demethoxycurcumin and bidemethoxycurcumin, are primarily responsible for the biological and medicinal activities of turmeric rhizomes.

Works conducted with alcoholic extract of the turmeric rhizome show that it has bactericidal effect on *Staphylococcus aureus* and *L. monocytogenes* (Bara & Vanetti 1998) however, the essential oil of the *C. longa* rhizome inhibited the growth of *L. monocytogenes*, S. *aureus* and *B. cereus*, presenting an inhibition halo of 16, 9, and 10 mm, respectively, on the other hand, it was not capable of inhibiting *E. coli* (Natta et al. 2008). Little is found in the literature on the essential oil of annatto leaves, because the plant is perennial, and its cultivation occurs in order to obtain its seeds. However, phytochemical studies show that mono and sesquiterpenes are found in the leaves, ishwaranol being the majority compound (Lorenzi and Matos 2002; Shilpi et al. 2006). In

our study the main component was α-humulene belonging to the class of the sesquiterpenes, that according to Jirovetz et al. (2006) possesses bactericidal action on *P. aeruginosa* and other Gram-negative bacteria, but inefficient against *S. aureus.*

The result of the study conducted by Coelho et al. (2003) revealed that *Pseudomonas aeruginosa* treated with dye prepared from fresh annatto leaves presented larger inhibition halo (11.1±1.7), than those treated with dyes prepared from fresh organs such as stem (7.6±2.7), flower (4.6±1.7), green fruits (8.5±0.9) and root (6.8±0.7). Such results show that *P. aeruginosa* presented high sensitivity to the components existent in the annatto leaves. However, *P. aeruginosa* presented resistance to 10% hydro-alcoholic extract of annatto seeds (Gonçalves et al. 2005).

The antimicrobial activity of the essential oils is attributed mainly to their majority compounds (Bakkali et al. 2008). However, evidence exists that minority components have an important role in the antimicrobial activity of the oil, promoting synergistic action among the others (Burt 2004). Some studies have concluded that the essential oils have higher antibacterial activity when in synergism, because a component can have a minimum effect when isolated and when in combination it can have its effect enhanced. Such a fact was also observed in the present study.

It can be observed that for *P. aeruginosa* there was a synergistic effect among the oils, an effect also noticed for *L. monocytogenes* to a lesser degree. At 5% of each oil there was the highest antimicrobial effect of the mixture of the oils on *P. aeruginosa*, in other words, the highest synergic effect among the oils.

The study of the antimicrobial action of essential oil of *Litsea nakaii* Hayata, shows that it was effective against Gram positive and negative bacteria, being more effective on the Gram positive, mainly S. *aureus.* The majority compounds of that oil were α-humulene (15.5%), δ-cadinene (9.2%), δ-selinene (7.1%), viridiflorene (4.7%) and α-muurolene (4.3%) (Ho et al. 2009). However, the α-humulene does not present biocide activity when appraised on S. *aureus* (Jirovetz et al. 2006). As such, those studies show that although the majority compound is of great importance, the other compounds also have an important role.

Another compound found in the annatto leaf oil with proven antimicrobial activity was *E*-nerolidol. This compound is used in small amounts in various mouthwashes, as a flavoring and an antimicrobial, also being used as a synergistic component in quaternary ammonium-based sanitizers (Shaheen et al. 2002).

The antimicrobial activity of the essential oils is much studied, their action mechanism being under constant questioning. The analysis of the cell morphology of *P. aeruginosa,* treated or not, seen in Figures 1 and 2 leads to interpretation of possible essential oil action mechanisms.

The essential oil of the turmeric leaf seems to interact with the cell walls *P. aeruginosa* and *L.* monocytogenes promoting their partial lysis. A similar result was found for *Pseudomonas fluorescens*, which, after treatment with cinnamaldehyde and limonene, present external modifications, probably due to the penetration of those compounds in the cellular envelope (Di Pasqua et al. 2007). In the same way, the essential oil of annatto leaves also acted on the cell wall of both cells.

It is known that *P. aeruginosa* possesses low susceptibility to antimicrobial agents with diverse structures and features. Various mechanisms can be related to that characteristic of the bacterium including the reduction of the permeability of the compounds in the external membrane and its very active efflux system. In a study conducted by Longbottom et al. (2004) it is clear that the external membrane protects it from the essential oil of *Melaleuca alternifolia* (Maiden & Betche) Cheel and its components, where energy dependent processes are involved. The authors even suggest that efflux processes can be involved. However, *L. monocytogenes* seems to have its glucose use process inhibited in the presence of eugenol (Gill and Holley 2004).

Di Pasqua et al. (2007) showed that the thymol, carvacrol, limonene, cinnamaldehyde and eugenol presented high interaction with the cell wall and cytoplasmic membrane of various bacteria, promoting structural alterations. Thus, the antibacterial activity of the essential oils is not attributed to a specific mechanism. Because of their lipophilic character, they pass through the cell wall and cytoplasmic membrane, breaking structures of the different polysaccharide layers, fatty acids and phospholipids, making them more permeable, which causes the ion loss and the reduction of the membrane potential, in the collapse of the proton motivating force, alteration of the electron flow and the transport activity, besides allowing the macromolecule bonding and cellular lysis (Burt 2004). The essential oils can even coagulate the cytoplasm and damage lipids and proteins (Bakkali et al. 2008).

The results revealed by the electron micrographs corroborate data found in the literature, that indicate that one of the main effects caused by essential oils is damage in the cell wall. It is worth emphasizing that the mode of action of each oil is directly related to the constituents present and cell structures.

In the literature there is a lack of studies that apply the transmission electron microscope as an instrument to analyze the effect of essential oils on bacteria. Many of those studies are still conducted using the scanning electron microscope, but only TEM supplies the necessary data on such effects. Therefore, it can be said that the transmission electron microscopy is an additional tool, important for the study of that activity.

CONCLUSION

The results revealed that the essential oils of *C. longa*, *B. orellana* and their synergism showed antibacterial activity against *P. aerguninosa* and *L. monocytogenes.*

Electron micrographs obtained by means of transmission electron microscopy showed the damage caused by essential oils in the cell structure of the *P. aeruginosa* e *L. monocytogenes*.

CONFLICT OF INTEREST

The author declares no competing interests.

REFERENCES

Adams RP (1995) Identification of essential oil components by gas chromatography/mass spectroscopy. Iliois: Allure Publishing Corporation, 469p.

Alves E (2004) Curso: introdução à microscopia eletrônica de varredura e de transmissão. Universidade Federal de Lavras: FAEPE, 43p.

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils: a review. Food Chem Toxicol 46(2):446-475. https://doi.org/10.1016/j.fct.2007.09.106.

Bara MTF, Vanetti MCD (1998) Estudo da atividade antimicrobiana de plantas medicinais, aromáticas e corantes naturais. Rev Bras Farmacogn 7-8(1):22-34. https://doi.org/10.1590/S0102-695X1998000100003.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45(4):493-496. https://doi.org/10.1093/ajcp/45.4_ts.493.

Behura S, Sahoo S, Srivastava V (2002) Major constituents in leaf essential oils of *Curcuma longa* L. and *Curcuma aromatica* Salisb. Curr Sci 83(11):1312-1313.

Bizzo HR, Hovell AMC, Rezende CM (2009) Óleos essenciais no Brasil: aspectos gerais, desenvolvimento e perspectivas. Quim Nova 32:588-594. https://doi.org/10.1590/S0100-40422009000300005.

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94(3):223-253. http://doi.org/10.1016/j.ijfoodmicro.2004.03.022.

Choi O, Cho SK, Kim J, Par CG, Kim J (2016) In vitro antibacterial activity and major bioactive components of *Cinnamomum verum* essential oils against cariogenic bacteria*, Streptococcus mutans* and *Streptococcus sobrinus*. Asian Pac J Trop Biomed 6(4):308-314. http://doi.org/10.1016/j.apjtb.2016.01.007.

Coelho AMSP, Silva GA, Vieira OMC, Chavasco JL (2003) Atividade antimicrobiana de *Bixa orellana* L. (Urucum). Rev Lecta 21(1-2):47-54.

Dimitrijevic SI, Mihajlovski KR, Antonovic DG, Milanovic-Stevanovic MR, Mijin DZ (2007) A study of the synergistic antilisterial effects of a sub-letal dose of lactic acid and essential oils from *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Origanum vulgare* L. Food Chem 104(2):774-782. http://doi.org/10.1016/j.foodchem.2006.12.028.

Di Pasqua R, Betts G, Hoskins N, Edwards M, Ercolini D, Mauriello G (2007) Membrane toxicity of antimicrobial compounds from essential oils. J Agric Food Chem 55(12):4863-4870. http://doi.org/10.1021/jf0636465.

Gill AO, Holley RA (2004) Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei.* Appl Environ Microbiol 70(10):5750–5755. http://doi.org/10.1128/AEM.70.10.5750-5755.2004.

Gonçalves AL, Alves Filho A, Menezes H (2005) Estudo comparativo da atividade antimicrobiana de extratos de algumas árvores nativas. Arq Inst Biol *72(3):*353-358.

Ho CL, Wang EIC, Li PY, Su YC (2009) Composition and antimicrobial activity of the leaf essential oil of *Litsea nakaii* from Taiwan. Nat Prod Commun 4(6):865-868.

Hu Y, Zhang J, Kong W, Zhao G, Yang M (2017) Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. Food Chem 220:1-8. http://doi.org/10.1016/j.foodchem.2016.09.179.

Jeong EJ, Lee NK, Oh J, Jang SE, Lee JS, Bae IH, Oh HH, Jung HK, Jeong YS (2014) Inhibitory effect of cinnamon essential oils on selected cheese-contaminating fungi (*Penicillium* spp.) during the cheese-ripening process. *Food Sci Biotechnol* 23(4):1193-1198. http://doi.org/https://doi.org/10.1007/s10068-014-0163-8.

Jiang H, Timmermann BN, Gang DR (2006) Use of liquid chromatography–electrospray ionization tandem mass spectrometry to identify diarylheptanoids in turmeric (*Curcuma longa* L.) rhizome. J Chromatogr 1111(1):21-31. http://doi.org/ 10.1016/j.chroma.2006.01.103.

Jirovetz L, Baill S, Buchbauer G, Denkova Z, Slavchev A, Stoyanova A, Schmidt E, Geissler M (2006) Antimicrobial testings, gas chromatographic analysis and factory evaluation of an essential oil of hop cones (*Humulus lupulus* L.) from Bavaria and some of its main compounds. Sci Pharm 74:189-201. http://doi.org/10.3797/scipharm.2006.74.189.

Khorshidian N, Yousefi M, Khanniri E, Mortazavianc A M (2018) Potential application of essential oils as antimicrobial preservatives in cheese. Innov Food Sci Emerg Technol 45:62-72. https://doi.org/10.1016/j.ifset.2017.09.020.

Li JWH, *Vederas* JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 10(325):161-165. http://doi.org/10.1126/science.1168243.

Liolios CC, Gortzi O, Lalas S, Tsaknis J, Chinou I (2009) Liposomal incorporation of carvacrol and thymol isolated from the essential oil of *Origanum dictamnus* L. and in vitro antimicrobial activity. Food Chem 112(1):77-83.

https://doi.org/10.1016/j.foodchem.2008.05.060.
Longbottom CJ, Carson CF, Hammer KA, Mee BJ, Riley TV (2004) Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. J Antimicrob Chemother 54(2):386-392. http://doi.org/ 10.1093/jac/dkh359.
Lorenzi H, Matos FJA (2002) Plantas medicinais no Brasil: nativas e exóticas. 2.ed. Nova Odessa: Instituto Plantarum, p. 95-96.
Mahboubi M, Heidarytabar R, Mahdizadeh E, Hosseini H (2018) Antimicrobial activity and chemical composition of *Thymus* species and *Zataria multiflora* essential oils. Agric Nat Resour 51(5):1-18. https://doi.org/10.1016/j.anres.2018.02.001.
Natta L, Orapin K, Krittika N, Pantip B (2008) Essential oil from five *Zingiberaceae* for anti-food-borne bacteria. Food Res Int 15:337-346.
Nedorostova L, Kloucek P, Kokoska L, Stolcova M, Pulkrabek J (2009) Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. Food Control 20(2):157-160. https://doi.org/10.1016/j.foodcont.2008.03.007.
Pimentel FA, Cardoso MG, Salgado APSP, Aguiar PM, Silva VF, Morais, AR, Nelson DL (2008) Método para determinação de umidade de plantas aromáticas. Comunicado Técnico. Fortaleza: EMBRAPA, 4p.
R Development Core Team 2010: A language and for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL. Disponível em: < http://www.r-project.org>. Acesso em 14 agosto 2010.
Shaheen EA, Ikawa JY, Blum RL (2002). Enhanced antimicrobial composition. United States n. B1 6361787.
Shilpi JA, Taufiq-Ur-Rahman MD, Uddin SJ, Alam MDS, Sadhu SK, Seidel V (2006) Preliminary pharmacological screening of *Bixa orellana* L. leaves. J Ethnopharmacol 108(2):264-271. http://doi.org/10.1016/j.jep.2006.05.008.