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Antibacterial activity of *Theobroma grandiflorum* Schum leaves' extract against *Escherichia coli* and *Staphylococcus aureus*

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Abstract

The present study aimed to obtain ethanol extract of *Theobroma Grandiflorum* Schum (cupuaçu) leaves and evaluate the antibacterial activity of the obtained extract against *Escherichia coli* and *Staphylococcus aureus*. The leaves were collected in two different periods of the year and divided into two groups (Group 1 and Group 2), in order to investigate the influence of season of leaves collecting on the effectiveness of the extract. The leaves were cleaned, dried (50 °C/12 h) and grinded for posterior addition of ethanol. Grinded leaves and ethanol mixture was heated (50 °C/1 h) and left to rest (24 h). Solid and liquid parts were separated by filtration. Soxhlet technique was used to recover the ethanol from the separated liquid part. Complete ethanol removal was achieved using an oven, to obtain the dry extract (DLE). The yield obtained was 1.46% and 1.54% for the first and second group, respectively. The extract obtained was diluted in DMSO in distinct proportions (1:3, 1:7, 1:9 (extract: DMSO (w/w)) and tested against *Escherichia coli* and *Staphylococcus aureus*, both at a concentration of 10⁹ and 10⁵ CFU mL⁻¹, based on the disk diffusion technique. Using the extract from the first group of leaves against 10⁹ CFU mL⁻¹ density population, both bacteria were classified as intermediate sensitive. Using the extract obtained from the second group of leaves against 10⁵ CFU mL⁻¹, it was observed that *S. aureus* kept classified as intermediate sensitive while *E. coli* was classified as resistant. The results suggest that the DLE can inhibit the growth of tested bacteria. Thus, further studies may be carried out in order to (a) investigate the DLE as a natural product for food preservation and (b) support the search of food industry for alternatives to control such microorganisms.

Keywords: antimicrobial activity; inhibition zone; cupuaçu extract

Atividade antibacteriana do extrato das folhas de *Theobroma grandiflorum* Schum contra *Escherichia coli* e *Staphylococcus aureus*

Resumo

O presente estudo teve como objetivo obter o extrato etanólico das folhas de *Theobroma Grandiflorum* Schum (cupuaçu) e avaliar a atividade antibacteriana do extrato obtido contra *Escherichia coli* e *Staphylococcus aureus*. As folhas foram coletadas em duas épocas diferentes do ano e divididas em dois grupos (Grupo 1 e Grupo 2), a fim de verificar a influência da época da coleta das folhas na eficácia do extrato. As folhas foram limpas, secas (50 °C/12 h) e moídas para posterior adição de etanol. Folhas moídas e mistura de etanol foram aquecidas (50 °C/1 h) e deixadas em repouso (24 h). As partes sólidas e líquidas foram separadas por filtração. A técnica de Soxhlet foi usada para recuperar o etanol da parte líquida separada. A remoção completa do etanol foi realizada em estufa, para obtenção do extrato seco (DLE). O rendimento obtido foi de 1,46% e 1,54% para o primeiro e segundo grupos, respectivamente. O extrato obtido foi diluído em DMSO em proporções distintas (1:3, 1:7, 1:9 (extrato: DMSO (w/w)) e testado contra *Escherichia coli* e *Staphylococcus aureus*, ambos na concentração de 10⁹ e 10⁵ CFU mL⁻¹, baseado na técnica de difusão em disco. Utilizando o extrato do primeiro grupo de folhas contra população de densidade de 10⁹ UFC mL⁻¹, ambas as bactérias foram classificadas como sensíveis intermediárias. Utilizando o extrato obtido do segundo grupo de folhas contra 10⁵ UFC mL⁻¹, observou-se que *S. aureus* se manteve classificado como intermediário sensível enquanto *E. coli* foi classificado como resistente. Os resultados sugerem que o DLE pode inibir o crescimento das bactérias testadas. Assim, novos estudos podem ser realizados a fim de (a) investigar o LED como um produto natural

para a preservação de alimentos e (b) subsidiar a busca da indústria de alimentos por alternativas para o controle de tais microrganismos.

Palavras-chave: atividade antimicrobiana; zona de inibição; extrato de cupuaçu.

Introdução

The Amazon region has a wide diversity of plants and a large part of this biome (more than 80%) has yet to be studied related to its medicinal properties (Silva & Franco, 2010). Among the most studied plants in the Amazon region is the species *Theobroma grandiflorum* Schum, especially its fruit (cupuaçu). Carvalho et al. (2009), for an example, developed a protein isolate from the seeds of cupuaçu fruit. However, little is investigated about the other components of the cupuaçu tree, such as leaves and their compounds. In this sense, it can be mentioned the study of Sprenger et al. (2016) about the antibacterial effect of an hydroalcoholic extract obtained from cupuaçu leaves in which it was detected the presence of phenolic compounds and tannins that are considered to be natural antimicrobials.

Studies on plant leaves are important since its compounds can have therapeutic potentials besides of being able to inhibit the growth of pathogens, presenting antimicrobial activity in vitro and in vivo. According to Askari et al. (2012), substances isolated from vegetables are a promising way to ensure food safety and increase the shelf life, thus the use of plants extracts in the food industry, in order to achieve greater conservation and desirable qualities to consumers (such as clean label and natural ingredients), is a trend.

Food preservation has been considered a challenge for the food processing industry, mainly for a wide variety of foods that have not been subjected to heat treatment, or have been moderate intensity (known as ready-to-eat foods), which can be contaminated by pathogenic bacteria that may be present in the raw material or contaminated the post-processing products through handling operations (i.e. cutting, peeling and packaging). Therefore, food industry is encouraged to find effective processing treatments or combination of treatments (e.g. natural antimicrobial compounds and refrigeration temperature) in order to avoid the consuming of foods contaminated with harmful bacteria (that can cause foodborne illnesses) and also to extend the shelf-life of food products (Rawat, 2015).

Among the most common ways to preserve food, the use of chemicals is an alternative to control microbial growth. However, the interest of people for chemical-free foods has increased, pressuring the industry to create methods to replace them effectively, leading to the use of natural compounds for food preservation. Many authors, like Logue et al. (2003), Sartoratto et al. (2004), Moreira et al. (2007), Lucera et al. (2012), Alahakoon et al. (2015), argument in favor of the use of natural compounds, claiming that some microorganisms are becoming resistant to existing antimicrobials. In this perspective, studies have been carried out aiming the use of extracts from plants in the food industry, introducing new active antibacterial agents, with less toxicity, and higher efficiency (Simonetti et al., 2016; Bona et al., 2014).

Foodborne illness may occur when the consumed food is contaminated with pathogens (e.g. viruses, bacteria, parasites) or its toxins and establishes itself in the human host. As bacteria are the most common cause of foodborne diseases, the hygienic standard of food can be measured by the analysis of the indicator microorganisms (Bintsis, 2017), as *Staphylococcus aureus* and *Escherichia coli*.

Staphylococcal food poisoning, caused by toxins of *Staphylococcus aureus*, is considered one of the most frequently occurring foodborne diseases worldwide. This bacterium is naturally present in the nose, throat, skin, and hair of humans and animals and its growth in the food can be associated with foods that were handled extensively and/or at improper temperatures (Bibek, 2005).

Escherichia coli is a bacterium present in the lower intestinal tract of humans and warm-blooded animals. Its presence in raw foods is considered an indication of fecal contamination which means that the contamination occurred during the processing of raw foods of animal origin and through poor personal hygiene of food handlers (Bibek, 2005).

Along these lines, the purpose of the present work was to evaluate the antibacterial activity of ethanol extract from *Theobroma grandiflorum* Schum leaves against the bacteria *Escherichia coli* and *Staphylococcus aureus*. The obtained results can aid in the search for new alternatives for food preservation, taking into consideration the progressive demand of offering safe products required of the food industry.

Material and methods

Plant material and extraction

Theobroma grandiflorum Schum (cupuaçu) leaves were collected from trees located in a private rural area (farm) in the countryside of Ariquemes city, Rondônia, Brazil (latitude = -9.891297° S, longitude = -63.050726° W). The leaves were collected manually and the selection was based on the stage of complete maturation, that is, the leaves presented a homogeneous dark green color.

Aiming to investigate the influence of the period of the year, namely the rain incidence, on the effectiveness of the extract activity against the bacteria, the leaves were collected in different periods and separated into two groups. The first group of leaves (Group 1), was collected in the beginning of February of 2019 and the second group (Group 2), was collected in the end of April of 2019. The plant material preparation and extraction experiment were equally carried out for group 1 and 2.

After collected the leaves were kept in polyethylene bags and taken to the Food Engineering laboratory of Rondônia Federal University (UNIR – Ariquemes *campus*) of the where they were cleaned to remove superficial dirt and damaged parts before extraction.

After cleanness, the leaves were dried in incubator (LimaTec, BR) at 50 °C for 12 hours and grinded in a blender (Arno, model Power Mix Plus LQ20, BR) for 5 minutes in high velocity. The powder of dried grinded leaves was stored in glass recipient, hermetically closed and covered with aluminum foil at room temperature.

For extraction, 100 mL of absolute ethanol (Synth, BR) was added to an Erlenmeyer (250 mL) containing 10 grams of the dried leaves powder. The set was taken to a hot plate at 50 °C for 1 hour with occasional manual mixing. After heating the Erlenmeyer was covered with aluminum foil and incubated at room temperature for 24 hours.

After incubating time, the mixture was filtered through qualitative filter paper with grammage of 80 g/m² and diameter of 185 mm (Unifil, BR) and the filtered solution was allowed to evaporate in Soxhlet equipment for about 150 minutes so the ethanol could be mostly recovered. The remaining ethanol leaves extract was divided into three parts (each part in one Petri dish) and taken to ventilated drying oven (Marconi, BR) at 50 °C for 60 minutes so the excess of ethanol was removed and the dried leaves extract (DLE) was obtained. The weight of DLE was measured, in analytical balance (Quimis, BR), for the calculation of extraction yield which was expressed as the percentage on the weight of DLE to the raw material (10 grams) as suggested by Franzen et al. (2018). The DLE was then re-suspended in dimethyl sulfoxide (DMSO) (Neon, BR), at the proportions 1:3, 1:7 e 1:9 w/w, placed in 2.0 mL microtubes covered by aluminum foil and stored in refrigerator at 5 °C. DLE obtained from leaves of Group 1 was named DLE1 and the one obtained from leaves of Group 2 was named DLE2.

It is worth clarifying that the extraction procedure adopted in the present work was defined after previously tests and through grouping distinct previously described methodologies, named: (a) leaf drying and storage in glass bottles, as proposed by Galvão et al. (2011); (b) extract obtaining: adaptation of the methodology applied by Bustamante et al. (2010); (c) removal of the ethyl proportion of the extract, as proposed by Gutierre-Gonçalves & Marcucci (2009) and (d) dilution of the extract in DMSO, in accordance to Delgado et al. (2018). Also, after the definition of the extraction procedure, the product from on single extraction was enough to realize the subsequent analysis (Antibacterial activity test).

Preparation of Inoculum

The *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) strains used were kindly donated by Oswaldo Cruz Foundation (FIOCRUZ/RO). The bacteria were preserved in microtubes stored at -20 °C in Brain Heart Infusion (BHI) broth (LabM, UK) with 20% (v/v) glycerol (stock culture) until testing.

Before each experiment of antimicrobial activity, *E. coli* and *S. aureus* were resuscitated individually in Tryptic Soy Broth (TSB, Kasvi, BR) at 37 °C. Two consecutive 24 h-subcultures were made for each strain by transferring 0.1 ml of stock culture to tubes containing 9 mL of fresh media and incubating at the same above-mentioned temperature. Then, a third and last subculture was prepared, and tubes were incubated until the initial stationary phase (18 to 20 h), resulting a microbial population density of 10⁹ CFU mL⁻¹, as shown Figure 1.

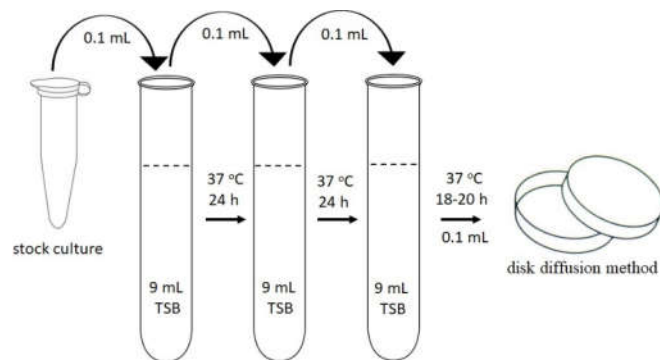


Figure 1. Procedure of reviving stock bacteria's cultures applied in this work.

The procedure showed in Figure 1 was adopted to reach cultures stationary phase (approximately 10^9 CFU mL⁻¹), which, for both tested cultures, can be clearly associated with unsatisfactory conditions of food manufacturing practices, handling and/or safety (Behling et al., 2010). The density population of 10^5 CFU mL⁻¹ was also approached in the present study. Such density was reached by diluting the third sub-culture of each strain (approximately 10^9 CFU mL⁻¹) into a sterilized 0.85% (m/m) NaCl aqueous solution. It is important to emphasize that for both strains, the population densities were ensured by previous assays and the colony-forming units (CFU) were enumerated by pour plate counting method (Equation 1).

$$N = CFU * DF * 10 \quad (1)$$

where N is the number of colony-forming units by milliliter of the inoculant bacteria and DF is the dilution factor.

Antibacterial activity test

The antibacterial activity was tested by disk diffusion method (Bauer et al., 1966). 0.1 mL of the bacterial suspension was uniformly spread on Petri dishes with Tryptic Soy Agar - TSA (Kasvi, BR). Previously sterilized qualitative paper disks (6 mm in diameter) were impregnated with the diluted DLE (1:3, 1:7 e 1:9 w/w in DMSO) and placed on the center surface of each agar plate. The extract obtained from leaves of the Group 1 (DLE1) was tested at 10^9 CFU mL⁻¹ while the extract obtained from leaves of the Group 2 (DLE2) was tested at 10^5 CFU mL⁻¹.

It is important to clarify that the density population of 10^9 CFU mL⁻¹ was applied because such concentration is often reached in the stationary phase of bacterial growth, which can be defined as a state of no net growth (Maier, 2009), functioning as a standard while the density population of 10^5 CFU mL⁻¹ was taken into consideration because such bacterial concentration is presented as the maximum tolerated limit for bacteria presence in food products according to Brazilian current legislation regarding the sanitary microbiological standards for foods (Brasil, 2001).

The inoculated plates were incubated for 24 hours at 37 °C (Fanem, BR), considering the estimated (a) temperature range and (b) time for observing bacteria growth (35-37 °C/18-24 h) in accordance with Silva et al. (2013). After incubation time, antibacterial activity was evaluated by measuring the zone of inhibition formed, with a digital caliper (Jomarca, BR). Each assay in this experiment was performed in triplicate. The measure of the inhibition zone size was used to classify the bacteria sensibility to the extract as suggested by the literature (Moreira et al., 2005), presented in Table 1.

Table 1. Microorganisms sensibility according to Moreira et al. (2005)

Classification	Total inhibition zone size (mm) of the extract
Extremely Sensitive	≥ 20
Sensitive	15 – 19
Intermediate sensitive	9 – 14
Resistant	≤ 8

In order to investigate the influence of the DLE dilution in DMSO, on-way analysis of variance (ANOVA) was applied so it could be verified if a statistical difference is presented in the medium sizes of the inhibition zone (expressed in mm) formed by each diluted DLE against each bacterium at each density population tested. If applicable, a Tukey multiple comparison test was run with statistically significant differences between treatments in growth reported for $p < 0.05$

Results and discussion

Extraction yield

The obtained extraction yields for the Group 1 and Group 2 of leaves were, respectively, 1.46% and 1.54%. Although high yields on extraction processes are often desirable, it is important to notice that there is not a standard value since variables as (a) the proportion plant:solvent and (b) the characteristics of the solvent are strongly related to the extraction yield. Dhawan & Gupta (2017) described that the use of methanol, as solvent to obtain extracts of plant leaves, presented higher yields (when compared to distilled water, acetone, chloroform, ethyl acetate and hexane). The better performance of methanol as solvent can be explained due to its lower polarity and consequent better interaction with the components of the plant material. However, because of its toxicity, methanol is better replaced by ethanol, which is considered to be less toxic to human health and environmentally friendly (Zhou & Yu, 2004; Jesus et al. 2018).

According to Garcia et al., (2016), despite extractions performed by solvents can well dissolve the constituents (sometimes resulting in better extraction efficiency), small proportions of solvent and plant material, as performed in the present study, can cause low yields due to the process of mass transfer that leads to equilibrium reaching and no difference of concentration of the metabolite in the liquid phase and inside the plant matrix. Therefore, the choice of appropriate volumes of solvents is a particularly important task.

It is worth noticing that, in the present study, the experiments were carried out on a laboratory scale, using a small amount of plant material (10 grams), but leaves of cupuaçu tree are abundant in the State of Rondônia since the planting of cupuaçu comprises about 2000 hectares (Ribeiro, 2000). In this sense, in contrast with the obtained low yield of the extraction process, the supply of vegetable material does not present itself as a limiting factor for obtaining the ethanolic extract of *Theobroma grandiflorum* Schum leaves.

Inhibition zone determination

The mean values and standard deviation of diameters zone of inhibition formed by the extract obtained in the present work (DLE) are shown in Table 2.

Table 2. Inhibition zone sizes (mm) of ethanol *Theobroma grandiflorum* Schum leaves extract against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) based on disc-diffusion method

Microorganisms	Dilution on DMSO	Inhibition zone size (mm)	
		10 ⁹ CFU mL ⁻¹ (DLE1)	10 ⁵ CFU mL ⁻¹ (DLE2)
<i>S. aureus</i> (ATCC 25923)	1:3	10.25 ^a ± 2.04	15.01 ^a ± 1.88
	1:7	9.15 ^a ± 1.81	13.80 ^a ± 2.03
	1:9	9.73 ^a ± 1.22	11.96 ^a ± 1.65
<i>E. coli</i> (ATCC 25922)	1:3	14.11 ^a ± 0.63	*NIZ
	1:7	10.26 ^b ± 2.27	NIZ
	1:9	9.47 ^b ± 1.28	NIZ

*NIZ = no inhibition zone presented. For each microorganism at each concentration (CFU mL⁻¹), equal overwritten letters indicate that values do not presented statistically significant differences by Tukey multiple comparison test for $p < 0.05$.

As well as it was found in the studies of Rabanal et al. (2002) and Teanpaisan et al. (2014), in the present study, the DLE diluent (DMSO) did not interfered on bacteria growth since no inhibition zone was observed when exposing the tested bacteria to pure DMSO. Once confirmed that such diluent did not presented antibacterial activity, the observed inhibition zones observed in the present work were thus, attributed solely to the obtained extract (DLE).

Concerning the obtained extract, from Table 2 (columns 3 and 4), it can be observed that DLE dilution on DMSO did not present influence on its antibacterial activity against *S. aureus* at each of tested density population, since no significant difference was noted on the inhibition zone sizes. Related to the results for *E. coli* at 10⁹ CFU mL⁻¹ (column 3), it is seen that the inhibition zone size was significantly affected by the extract dilution. However, the proportions of 1:7 and 1:9 did not present statistically significant differences.

The fact the previously mentioned DLE dilution, did not present influence on the inhibition zone size can indicate that the use of a high dilution might be considered in the sense of increasing the amount (i.e. volume) of antibacterial product.

Related to the bacteria sensibility (as presented in Table 1), values presented in Table 2 indicate that both bacteria tested at 10⁹ CFU mL⁻¹ and *S. aureus* at 10⁵ CFU /mL can be classified as Intermediate sensitive to the obtained extract since the Inhibition zone sizes are mostly in the gap of 9 to 14 mm.

Concerning to *E. coli* at 10^5 CFU mL⁻¹, it is shown in Table 2 that it is classified, as Resistant to the obtained extract in the present study since no inhibition zone was presented. The observed resistance can be explained by the utilization of different group of leaves (Group 2) for ethanol extract obtainment. As Group 2 leaves were collected during a period of year (April), with less rain incidence and consequently higher temperatures, it can be assumed that the chemical composition of the DLE was affected by abiotic factors such as lower humidity and higher temperatures.

Suzuki & Mittler (2006) affirmed heat stress can lead to severe effects on plants since high temperature is responsible for a series of plants biochemical and physiological modifications. In this way, it is noticeable that factors as collect time, season (period of the year) and plant maturation can reflect directly on the vegetal material composition and consequently on the presence and quality of plant active substances against microorganisms (Gobbo-Neto, 2007). In addition, *E. coli* is classified as a gram-negative bacteria and hence presents cell wall composed by greater quantity of amino acids and lipids which, for once, confer greater resistance to chemical compounds penetration (Naik et al., 2010; Pinho et al., 2012; Kalemba & Kunicka, 2003).

Many studies approaching the use of different plant ethanolic extracts for the same bacteria tested in the present study are available in the literature. Although those studies do not use the same vegetable raw material, they can be used as references regarding the sizes of inhibition zones observed. Some of those studies are presented in Table 3.

Table 3. Studies on the use of plant extracts for inhibition of *Staphylococcus aureus* and *Escherichia coli*

Author (Year)	Row Material	Bacteria tested (CFU mL ⁻¹)	Inhibition zone size (mm)
Sprenger et al. (2016)	<i>Theobroma grandiflorum</i>	<i>S. aureus</i> (10^6 CFU mL ⁻¹)	*NIZ
		<i>E. coli</i> (10^6 CFU mL ⁻¹)	*NIZ
Eller et al. (2015)	<i>Anacardium occidentale</i> L.	<i>S. aureus</i> (10^6 CFU mL ⁻¹)	11 ± 0,96
		<i>E. coli</i> (10^6 CFU mL ⁻¹)	*NIZ
	<i>Stryphnodendron adstringens</i> (Mart.) Coville	<i>S. aureus</i> (10^6 CFU mL ⁻¹)	17 ± 0.82
		<i>E. coli</i> (10^6 CFU mL ⁻¹)	*NIZ
<i>Myracrodruon urundeuva</i>	<i>S. aureus</i> (10^6 CFU mL ⁻¹)	13 ± 0.50	
	<i>E. coli</i> (10^6 CFU mL ⁻¹)	*NIZ	
Bona et al. (2014)	<i>Syzygium cumini</i>	<i>S. aureus</i> (10^8 CFU mL ⁻¹)	15.0 ± 2.6
	<i>Myrciaria cauliflora</i>		11.6 ± 2.8
	<i>Psidium guajava</i>		*NIZ
Ramos et al. (2012)	<i>Zeyheria tuberculosa</i>	<i>S. aureus</i> (10^8 CFU mL ⁻¹)	29
Mendonça et al. (2016)	<i>Eugenia uniflora</i> L.	<i>S. aureus</i> (10^6 CFU mL ⁻¹)	9
		<i>E. coli</i> (10^6 CFU mL ⁻¹)	8
Silva et al. (2014)	<i>Encholirium spectabile</i> Mart.	<i>E. coli</i> (10^8 CFU mL ⁻¹)	*NIZ
	<i>Bromelia laciniosa</i> Mart.		
	<i>Amburana cearensis</i> (Fr. Allem.) AC Smith		
	<i>Hymenaea martiana</i> Hayne		
Lima (2008)	<i>Auxemma onocalyx</i>	<i>E. coli</i> (10^8 CFU mL ⁻¹)	7.5 ± 0.71
	<i>Momordica charantia</i>		9.5 ± 0.71
	<i>Ziziphus joazeiro</i>		9 ± 1.41
Alfadel et al. (2014)	<i>Punica granatum</i> Linn	<i>E. coli</i> (10^8 CFU mL ⁻¹)	10.02 ± 0.77

*NIZ = no inhibition zone presented

As it can be seen from Table 3, regarding *S. aureus*, the inhibition zones presented in the studies of Eller et al. (2015) and Bona et al. (2014) are in close agreement, i.e. the same gap, to those found in the present study which are shown in Table 2. In Table 2, it can be observed that inhibition zones obtained in the present study, for *S. aureus*, varied from 9.73 to 10.25 mm for the population density of 10^9 CFU mL⁻¹ (DLE1) and from 11.96 to 15.01 on the population density of 10^5 CFU mL⁻¹ (DLE2).

On the other hand, the diameters of the inhibition zones obtained in the present study, for both population density tested of *S. aureus*, were inferior when compared to the one found by Ramos et al. (2012) and superior when compared to the one found by Mendonça et al. (2016) and Sprenger et al. (2016).

Related to *E. coli*, it can be seen from Table 3, that the inhibitory zone diameters observed in the present study against such bacteria, at 10^9 CFU mL⁻¹, are in concordance (i.e. close value) with the result found by Lima (2008) and Alfadel et al. (2014). As well as it was observed in the present study, when it was investigated *E. coli* at population density of 10^5 CFU mL⁻¹, some studies also noticed that no inhibition zone was formed (Sprenger et al., 2016; Eller et al., 2015; Silva et al., 2014).

It should be noted that the study of Sprenger et al. (2016), which has used the same raw material of the present study, performed an hydroalcoholic extraction, applying aqueous solution of ethanol (70%) as solvent, indicating that the use of hydroalcoholic solvent can negatively affect the extraction of desirable antibacterial compounds.

Conclusions

From the results obtained by the present study and taking into consideration the growing search for efficient and natural ways for food preservation, some outcome could be reached:

- The ethanol extract obtained from dried leaves (DLE) of *Theobroma grandiflorum* Schum can inhibit the growth of *Staphylococcus aureus* at the tested conditions;
- The DLE can inhibit the growth of *Escherichia coli* at 10^9 CFU mL⁻¹, however the inhibition can be related to the period of leaves collecting (higher rain incidence and mild temperatures);
- The DLE dilution on DMSO (at the applied proportions) do not interfere on the inhibition zone size presented by the tested bacteria;
- The ethanol extract of *Theobroma grandiflorum* Schum leaves may be considered for following studies in order to identify the extract component(s) bonded to the bacteria inhibition, as well as to understand the effect of different variables at the named component(s) (such as plant geographic location and its climate conditions).

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