Research, Society and Development, v. 9, n. 10, e8129108447, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8447

Antimicrobial activity of the essential oil from *Bixa orellana* L.

Atividade antimicrobiana do óleo essencial de *Bixa orellana* L.

Actividad antimicrobiana del aceite esencial de *Bixa orellana* L.

Received: 09/14/2020 | Reviewed: 09/21/2020 | Accept: 10/18/2020 | Published: 10/20/2020

Yuri Nascimento Fróes
ORCID: https://orcid.org/0000-0002-0928-0980
Universidade CEUMA, Brasil
E-mail: yurifroes@outlook.com
Ana Patrícia Matos Pereira
ORCID: https://orcid.org/0000-0003-4478-4209
Universidade Federal do Maranhão, Brasil
E-mail: ap.matos11@hotmail.com
Paulo Victor Serra Rosa
ORCID: https://orcid.org/0000-0003-1782-5896
Universidade Federal do Maranhão, Brasil
E-mail: paullovictorserra@gmail.com
Laiane Araújo da Silva Souto
ORCID: https://orcid.org/0000-0002-8101-6973
Universidade Federal do Maranhão, Brasil
E-mail: laianesouto@gmail.com
Mariana Oliveira Arruda
ORCID: https://orcid.org/0000-0001-7097-7843
Faculdade Uninassau, Brasil
E-mail: mariana_o.arruda@yahoo.com.br
Luís Felipe Castro Araújo
ORCID: https://orcid.org/0000-0002-8597-1329
Universidade Estácio de Sá, Brasil
E-mail: luisfelipecastroaraujo@gmail.com
Andressa Teixeira Costa
ORCID: https://orcid.org/0000-0002-3236-2722
Universidade Estácio de Sá, Brasil
E-mail: nutriandressateixeira@gmail.com

Danielly Fonseca
ORCID: https://orcid.org/0000-0002-8500-4777
Faculdade Uninassau, Brasil
E-mail: danyfonseca2002@gmail.com
Dorileira Pereira do Nascimento
ORCID: https://orcid.org/0000-0001-9929-2661
Faculdade Uninassau, Brasil
E-mail: daranscimento@gmail.com
Gustavo Oliveira Everton
ORCID: https://orcid.org/0000-0002-0457-914X
Universidade Federal do Maranhão, Brasil
E-mail: gustavooliveiraeverton@gmail.com
Victor Elias Mouchrek Filho
ORCID: https://orcid.org/0000-0003-2855-7292
Universidade Federal do Maranhão, Brasil
E-mail: victor.mouchrek@ufma.br
Joicy Cortez de Sá Sousa
ORCID: https://orcid.org/0000-0001-8678-0244
Universidade CEUMA, Brasil
E-mail: joicyvet@hotmail.com

Abstract

The application of natural substances with bactericidal action is the target of research aimed at a more sustainable production, implying in various sources of substances for new therapeutic formulations. This study presents the chemical characterization, toxicity and antimicrobial activity of essential oil (EO) of *Bixa orellana Labill* leaves. EO was extracted by hydrodistillation at 100°C for 3h. Physicochemical parameters were determined and chemical characterization used Gas chromatography coupled with Mass Spectrometry (GC/MS). The toxicity assay was performed through the bioassay with *Artemia salina* Leach. The methods of Disc Diffusion and Dilution in Broth in front of the bacteria *Escherichia coli* and *Staphylococcus aureus* were used to evaluate the antimicrobial activity. LC_{50} in the toxicity assay was verified in 355 mg L^{-1} and was classified as nontoxic. The OE presented antimicrobial activity against the microorganisms tested and totaled 320 mg EAT g^{-1} of total

phenolics. The results obtained highlight the importance of pointing out this product as a therapeutic alternative, encouraging its application potential.

Keywords: *Bixa*; Essential oil; Toxicity.

Resumo

A aplicação de substâncias naturais com ação bactericida é alvo de pesquisas voltadas para uma produção mais sustentável, implicando em diversas fontes de substâncias para novas formulações terapêuticas. Este estudo teve por objetivo a caracterização química, toxicidade e atividade antimicrobiana do óleo essencial (OE) das folhas de *Bixa orellana* Labill. O OE foi extraído por hidrodestilação a 100°C por 3h. Foram determinados os parâmetros físico-químicos e a caracterização química por Cromatografia Gasosa acoplada a Espectrometria de Massas (GC/MS). O ensaio de toxicidade foi realizado através do bioensaio com *Artemia salina* Leach. Os métodos de Difusão de Disco e Diluição em Caldo frente às bactérias *Escherichia coli* e *Staphylococcus aureus* foram utilizados para avaliar a atividade antimicrobiana. O CL_{50} no ensaio de toxicidade foi verificado em 355 mg L^{-1} e o OE foi classificado como não tóxico. O OE apresentou atividade antimicrobiana contra os microrganismos testados e totalizou 320 mg EAT g^{-1} de fenólicos totais. Os resultados obtidos destacam a importância de apontar este produto como uma alternativa terapêutica, incentivando seu potencial de aplicação.

Palavras-chave: *Bixa*; Óleo essencial; Toxicidade.

Resumen

La aplicación de sustancias naturales con acción bactericida es el objetivo de la investigación dirigida a una producción más sostenible, lo que implica varias fuentes de sustancias para nuevas formulaciones terapéuticas. Este estudio fue dirigido a la caracterización química, toxicidad y actividad antimicrobiana del aceite esencial (AE) de las hojas de *Bixa orellana* Labill. AE se extrajo por hidrodestálo a 100 °C durante 3h. Se determinaron los parámetros fisicoquímicos y la caracterización química por cromatografía de gases acopladas a la espectrometría de masas (GC/MS). El ensayo de toxicidad se realizó a través del bioensayo con *Artemia salina* Leach. Los métodos de difusión de disco y dilución en el caldo contra las bacterias *Escherichia coli* y *Staphylococcus aureus* se utilizaron para evaluar la actividad antimicrobiana. El CL_{50} en el ensayo de toxicidad se verificó en 355 mg de L^{-1} y se clasificó como no tóxico. La EO mostró actividad antimicrobiana contra los microorganismos analizados y totalizó 320 mg de fenólicos GAT g^{-1} totales. Los resultados obtenidos ponen de

relieve la importancia de señalar este producto como una alternativa terapéutica, fomentando su potencial de aplicación.

Palabras clave: *Bixa*; Aceite esencial; Toxicidad.

1. Introduction

Brazil tops the list of the richest countries in biodiversity in the world, which implies several sources of substances for therapeutic formulations, including about 55,000 species and only 25% of registered herbal medicines come from plant species present in South, factors that attract the attention of health care programs and the attention of researchers around the world, for their medicinal and organoleptic properties (Santos *et al.*, 2011).

Much of what is known today about plant treatments comes from popular knowledge. Despite the evolution of scientific knowledge, the use of alternative methods of cure for plant use is still very frequent, a fact occurred mainly due to the high cost of synthetic drugs and the ease of obtaining of these plants (Silva & Oliveira, 2018).

The properties of these medicinal plants are directly related to their essential oils (EOs), which according to Simões (2010) are components that integrate the secondary metabolites of plants, that is, they are part of the non-system of these organisms, possessing protective functions against elements external to plants.

EO is a natural product derived from medicinal plants, which have potential in the control of diseases in plants, as they have antifungal, antibacterial and insecticide characteristics, in addition are not toxic to the environment and the human being (Tomazoni *et al*., 2013). In addition, they are used in the pharmaceutical and perfumery industry, in the manufacture of hygiene and cleaning products, besides being able to be incorporated into the formulation of synthetic products, in order to reduce or even replace the use of toxic elements (Danielli *et al*., 2017).

Among plants with medicinal properties, the annatto stands out, scientifically known as *Bixa orellana* L., a plant native to the tropical region of America (Aparecido *et al*., 2017). Its application occurs both in industry and popular use as food and textile dye and for pharmacological purposes, since it has antimicrobial activities, antioxidant, diuretic, antifungal and antileishmanial (Monzote *et al.*, 2014; Giovannini *et al*.; Shahid-Ul-Islam *et al*., 2016).

This is popularly known as "annatto", a word that derives from the "Guaraní (ru-ku)" the "annatto" meaning red was the first vegetable dye to be marketed in large quantities to

Europe (Hagiwara *et al*., 2003). Its seeds consist of substances such as cellulose (40 to 45%), sugars (3.5 to 5.2%), essential oils (0.3 to 0.9%), proteins (13 to 16%), alpha pigments and beta carotenes (4.5 to 5.5%), and compounds such as tannins and saponins (Barrozo *et al*., 2013).

The dye extracted from *Bixa orellana* is considered harmless and its toxicity is virtually nil, not only after ingestion but after contact with the skin (Pérez, 2003). In addition, the plant is also used in wounds, bruises, burns, sore throats and diseases such as bronchitis and asthma, because it has anti-inflammatory and healing activity (Braga *et al.*, 2007).

This study reports for the first time the chemical characterization, toxicity and antimicrobial activity of EO of *Bixa orellana* Labill leaves. in order to deepen knowledge with regard to their characteristics and medicinal properties making possible the population's knowledge about possible contraindications and emphasis on some warnings and precautions to be taken.

2. Methodology

2.1. Botanical material

The collection of the plant material used in this research was carried out in October 2019. The leaves of *Bixa orellana* (annatto) were collected in the Attic Herbarium Seabra of the Federal University of Maranhão (UFMA) and deposited under registration of n° 00815. After collection, the plant material was transported to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA), where it was submitted to the kiln of convective air drying FANEM 520 to 45ºC for 24 hours, and later crushed in knife mill.

2.2. Essential oil

For extraction of the EO, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottomed balloon packed in an electric blanket as a heat generating source. 90g of the dried leaves of *Bixa orellana* were used, adding distilled water (1:10). Hydrodistillation was conducted at 100°C for 3h collecting the extracted EO. Each EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifugate. These operations were carried out in triplicates and samples stored in amber glass ampoules under 4°C cooling. Subsequently submitted the analyses.

2.3. Chemical analysis

The physicochemical parameters of the EOs were determined: density, solubility, color and appearance according to the Farmacopeia Brasileira (2019) and the EO constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS) at the Catalysis, Fuels and Environmental Center of the Federal University of Maranhão (NCCA-UFMA). The AMDIS (Automated Mass spectral Deconvolution Mass & Identification System) program was used to identify the compounds in the sample.

The determination of total phenolic compounds of the EO was performed with adaptation of the Folin-Ciocalteu (Waterhouse, 2002). The readings were performed in a spectrophotometer at 760 nm, and the standard curve expressed in mg of tannic acid.

2.4. Toxicity

This test was performed according to the methodology described by Meyer *et al*. (1982). For the evaluation of the lethality of *Artemia salina* Linnaeus (1758), a saline solution was prepared stock of each EO in the concentration of 10.000 mg L^{-1} and 0.02 mg of Tween 80 (active tense). Rates of 5, 50 and 500 µL of this were transferred to test tubes and supplemented with saline solution previously prepared up to 5 mL, obtaining at the end concentrations of 10, 100 and 1000 mg L^{-1}, respectively. All tests were carried out in triplicates, where ten larvae in the nauplium phase were transferred to each of the test tubes.

For white control, 5 mL of the saline solution was used for positive control $K_2Cr_2O_7$ and for negative control 5 mL of a 4 mg L^{-1} solution of Tween 80. After 24 hours of exposure, the count of the live larvae was performed, considering dead those that did not move during observation or with the slight agitation of the vial. The criterion established by Dolabela (1997) was adopted for classification of EOs toxicity, being considered highly toxic when $LC_{50} \leq 80$ mg L^{-1}, moderately toxic to 80 mg $L^{-1} \leq LC_{50} \geq 250$ mg L^{-1} and slightly toxic or nontoxic when $LC_{50} \geq 250$ mg L^{-1}. Statistical analysis of the data for the toxicity test was performed according to the Reed & Muench (1938).

2.5. Antimicrobial activity

Two strains of bacteria were used: *Escherichia coli* T. Escherich (1885) (ATCC 25922) and *Staphylococcus aureus* Rosenbach (1884) (ATCC 25923). These were previously identified and confirmed by biochemical tests.

Antimicrobial activity was performed according to the disc diffusion technique of the Clinical and Laboratory Standards Institute (CLSI, 2015) that standardizes antimicrobial sensitivity tests by disc-diffusion, using standardized suspensions of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) strains distributed in plates containing Agar Mueller Hinton (AMH) culture medium plus discs containing 50 µL of EO. Gentamycin (30 µg) was used positive control. The plates were incubated in a bacteriological greenhouse at 35 °C/24 h. Inhibition halo diameters were measured, including disc diameter. These trials were done in triplicate.

To determine the Minimum Inhibitory Concentration (MIC), the dilution technique was used in broth. With serial EO dilutions in Broth Mueller Hinton (BMH), resulting in concentrations of 1000, 500, 250, 100, 50, 25, 10 and 5 µg mL^{-1}, performing sterility controls and incubation at 35ºC for 24 h. After the incubation period, MIC was verified, being defined as the lowest concentration that visibly inhibited bacterial growth (absence of visible cloudiness). Trials carried out in triplicate. The Minimum Bactericidal Concentration (MBC) was measured from the inoculation of 10 µL of the tubes resulting from dilution in BMH, performed a plate count after 24 h, where plaques that did not grow colonies were classified as bactericidal concentrations for the action of the EO.

3. Resultados e Discussão

3.1. Physicochemical parameters

Physicochemical parameters are presented in Table 1.

Table 1. Physicochemical parameters of EO *Bixa orellana***.**

Physicochemical parameters	
Refractive index	**1,5300** (nD 25°)
Density	**0,9980** (g mL^{-1})
Color	**Amarelo**
Yield	**2,23 %**

Source: Authors (2020).

Using the hydrodistillation process to obtain the EO of *Bixa orellana*, a yield of 2.23% can be observed. By comparing this yield to the results obtained by Caixeta (2010) that extracted the EO from the dried leaves of annatto and obtained yield of 0.21%, one can infer the importance of using the EO in this study, since a value well above the yield was found, since a value well above the yield was found obtained by the author.

The studies with the objective of evaluating the physicochemical parameters of EO *Bixa orellana* were scarce, making it difficult to analyze the other factors. However, they are in accordance with the criteria established by the Farmacopeia Brasileira (2019), and it is important to emphasize the yield of 2.23% of the EO that was observed in results higher than the literature, encouraging its production.

3.2. Total phenolics

The equation of the straight obtained was y = 0.05857x + 0.06000 (R^2= 0.9998), where y represents absorbance and x the equivalent concentration of tannic acid. The total phenolic content of the sample analyzed was 320 mg EAT g^{-1}, which can be inferred a significant amount of the compound. Phenolic compounds are pointed out as considerable bioactive, associated with various health-friendly effects, among other functions, are mainly related to antioxidant activity in plants (Rocha *et al.*,2011).

Using the same methodology Rincón *et al*. (2016), they quantified the content of total phenolics of the extract of the annatto leaves, evaluating the effect of extraction time and the solvent/leaf ratio, obtained a slightly similar result, with a total maximum phenol content of 144.77 ± 9.66 mg EAT g^{-1}. Lemos *et al*. (2011), found total phenolic contents of five genotypes analyzed with mean values from 776.02 to 1,498.48 mg GAE 100 g^{-1} sample (dry weight) and 297.08 to 450.97 mg GAE 100 g^{-1}, for hydroethanolic and ethanolic extracts, respectively, the that makes the present study more reliable.

3.3. Chemical constituents

Table 2 shows the components found in the annatto EO constituting 20 identified components. The majority constituents were 1R-α-pinene (26.50%), β-bisabolene (19.71%), caryophyllene (10.42%) and pinene (9.23%), belonging to the classes of terpenes, monoterpenes and sesquiterpenes.

Table 2. Chemical constituents identified in EO *B. Orellana.*

Order	[1]RT (min)	Components	%
1	3.569	1R-α-pinene	26.50
2	5.777	(-)-β-pinene	9.23
3	9.431	2-trans-hexenal	2.55
4	10.141	β-cis-Ocimene	1.55
5	13.670	α-chamigrene	2.83
6	13.944	Copaene	4.28
7	15.197	α-bergamotene	0.60
8	15.260	β-elemene	1.20
9	15.309	caryophyllene	10.42
10	15.443	allo-aromadendrene	0.61
11	15.876	γ-gurjunene	3.28
12	16.127	β-Cedrene	0.59
13	16.207	α-caryophyllene	4.05
14	16.686	Isoledene	2.92
15	16.923	β-bisabolene	19.71
16	16.965	α-chamigrene	3.95
17	17.238	δ-cadinene	0.93
18	17.399	β-cedrene	3.26
19	20.241	nerolidol	0.86
20	21.058	spathulenol	0.68

Note: [1]RT: Retention time of compounds in the column in minutes; Font: Authors (2020).

The results of the research on the chemical constitution of the EO of the leaves of *Bixa orellana* L., were scarce and different from those found in this study, however some similarities regarding the presence of some components. In a similar study conducted by Caixeta (2010) on the EO of the annatto leaves, 28 substances were found, which reached 75% of the total constitution, of which α-humulene (43.01%), E-nerolidol (14.40%) and spathulenol (7.57%) were the majority constituents, belonging to the class of sesquiterpenes. The presence of 73 chemical components in EO *B. orellana* by Monzote *et al*. (2014) was detected, of which it was possible to identify 80%. Among them, α-pinene (3.3%) and β-pinene (2.3%), in much lower concentrations than those found in this study.

3.4. Toxicity

Table 3 shows LC_{50} obtained in the toxicity assay referring to the action of EO against *Artemia salina* and its subsequent classification according to the criterion Dolabela (1997).

Table 3. LC_{50} for EO action against *Artemia salina*.

EO	LC_{50}	Classification
Bixa orellana	**511,6 mg L^{-1}**	**Nontoxic**

Source: Authors (2020).

According to Table 3, it was possible to observe that the annatto EO did not present toxicity by exhibiting LC_{50} of 355 mg L^{-1} ± 3.25 mg L^{-1}, value higher than the framework Dolabela (1997) to classify it as nontoxic, so this EO has its acceptable application potential and encouraged. In a study developed by Kumar *et al*. (2012), using extracts from *Bixa orellana* leaves showed a non-toxic effect on *Artemia salina*, such data corroborate the results of this study. However, Vilar (2015) when testing the toxicity of the oily fraction of the annatto obtained the value of DL_{50} of 285.41 ± 81 µg mL^{-1}, classifying it as highly toxic and unfeasible to be used for biological tests.

3.5. Antimicrobial activity

Table 4 contains the results obtained in antimicrobial assays.

Table 4. Diameter of inhibition halos-DIH (mm) and MIC (µg mL^{-1}) and MBC (µg mL^{-1}) of EO against *S. aureus* and *E. coli.*

Microrganism	GEN (30 µg)	DIH	MIC	MBC
E. coli **(ATCC 25922)**	**25 mm**	**13 mm**	**400 µg mL^{-1}**	**700 µg mL^{-1}**
S. aureus **(ATCC 25923)**	**27 mm**	**20 mm**	**250 µg mL^{-1}**	**500 µg mL^{-1}**

Source: Authors (2020).

According to criteria established by Moreira *et al*. (2005), inhibition halos formed allow classifying all bacterial strains as sensitive to essential oil of *Bixa orellana*. EO was more efficient in inhibiting bacterial growth of *S. aureus* (20mm) when compared to *E. coli* that presented halo of 13 mm. This result is satisfactory since previous studies reported this action in plant extracts with values lower than the of this study.

Silva *et al*. (2018) when evaluating the microbial inhibition activity of methanic extracts obtained from the seeds of different genotypes of *Bixa orellana* L. at different stages of maturation, found that all extracts presented activity of sensitivity about the bacterium *S. aureus*, presenting halo values with diameters ranging from 10-18 mm, while for *E. coli* these did not present inhibition halo. On the other hand, Venugopalan & Giridha (2012), in a similar study, found that inhibition of growth of *E. coli* and *S. aureus* was significantly high for the extract of the annatto leaves, in a concentration above 1500 μg mL^{-1}.

Majolo (2013) observed effective antibacterial activity primarily for Gram-positive bacteria such as *S. aureus*, *E. faecalis* and *L. monocytogenes*, while gram-negative bacterium *E. coli* presented the lowest sensitivity. Despite this, Fleischer *et al*. (2003) demonstrated that the ethanolic extract of the annatto leaves obtained a greater inhibition zone against the bacterial strain of *E. coli*, presenting 22.5 mm in diameter.

According to Aligiannis *et al*. (2001), the classification of antimicrobial activity for plant specimens, according to the results of the MIC, is considered strongly inhibition: MIC up to 500 μg mL^{-1}; moderate inhibition: MIC between 600 and 1000 μg mL^{-1}; and weak inhibition: MIC above 1000 μg mL^{-1}.

The MIC value of *Bixa orellana* in front of the *E. coli* and *S. aureus* strains were 400 μg mL^{-1} and 250 μg mL^{-1}, respectively, according to Table 4. According to the results obtained, it is stated that the EO annatto presented potential for strong inhibition in relation to all bacteria tested in this study. However, in the analysis by Irobi *et al*. (1996), the authors reported weak inhibition of the extract of the annatto leaves against *S. aureus*, which exhibited CIM of 4000 μg mL^{-1}.

The MBC assay showed better results for the EO against *S. aureus*, observing bactericidal action from 500 μg mL^{-1}, while action was observed from 700 μg mL^{-1} to *E. coli*. Guedes (2019), observed similar results in relation to bactericidal action against *S. aureus*, with CBM of 625 μg mL^{-1}. In a research conducted by Arruda (2016), he indicated that the bactericidal effect of *Bixa orellana* hydroalcoholic extract was achieved at higher concentrations (64 mg mL^{-1}). A similar result was observed by Irobi et al. (1996), which presented bactericidal action from 16 mg mL^{-1} against *S. aureus* as well.

4. Final Considerations

Regarding this activity on *E. coli*, the research indicated mild bactericidal or non-existent action. Antimicrobial substances present in EO may have influenced bactericidal activity in the susceptible bacteria tested.

Referências

Aligiannis, N., Kalpoutzakis, E., Mitaku, S., & Chinou, I. B. (2001). *Composition and Antimicrobial Activity of the Essential Oils of Two Origanum Species*. 4168–4170.

Aparecido, L. E. de O., Rolim, G. de S., Moraes, J. R. da S. C. de, Rocha, H. G., Lense, G. H. E., & Souza, P. S. (2018). *Agroclimatic zoning for urucum crops in the state of Minas Gerais , Brazil*. *77*(1), 193–200.

Braga, F. G., Maria, L., Bouzada, M., Fabri, R. L., Matos, M. D. O., Moreira, F. O., Scio, E., & Coimbra, E. S. (2007). *Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil*. *111*, 396–402. https://doi.org/10.1016/j.jep.2006.12.006

Caixeta, D. S. (2010). *Ação de óleos essenciais de Curcuma longa L. E Bixa orellana L. sobre Pseudomonas aeruginosa E Listeria monocytogenes planctônicas e sésseis em polipropileno*. 128 p.

CLSI. (2015). *M07-A10: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Tenth Edition*. *January*.

Farmacopeia Brasileira. (2019). Agência Nacional de Vigilância Sanitária. Brasília: Anvisa, (6a ed.).

Fleischer, T. C., Ameade, E. P. K., Mensah, M. L. K., & Sawer, I. K. (2003). *Antimicrobial activity of the leaves and seeds of Bixa orellana*. *74*(02), 136–138. https://doi.org/10.1016/S0367-326X(02)00289-7

García, M., Monzote, L., Montalvo, A. M., & Scull, R. (2011). Effect of Bixa orellana against Leishmania amazonensis. *Forschende Komplementarmedizin*, *18*(6), 351–353. https://doi.org/10.1159/000335280

Giovannini, P., Howes, M. R., & Edwards, E. (2016). Author's Accepted Manuscript America : a review. *Journal of Ethnopharmacology*. https://doi.org/10.1016/j.jep.2016.02.034

Hagiwara A, Imai N, Ichihara T, Sano M, Tamano S, Aoki H, Yasuhara K, Koda T, Nakamura M & Shirai T (2003) A thirteenweek oral toxicity study of annatto extract (norbixin), a natural food color extracted from the seed coat of annatto (*Bixa orellana* L.), in Sprague-Dawley rats. Food and Chemical Toxicology 41: 1157-1164.

Irobi, O. N., & Anderson, W. A. (1996). *Antimicrobial activity of a nnatto (b ixa orellana)*. *34*(2), 87–90.

Lemos, A. R., Rêgo Junior, N. O., São José, A. R., Pereira, M. L. A., & Silva, M. V. da. (2011). Atividade antioxidante e correlação com fenólicos totais em genótipos de Urucum (Bixa orellana L). *Revista Do Instituto Adolfo Lutz*, *70*(1), 62–68.

Majolo, C. (2009). Atividade antibacteriana in vitro de diferentes acessos de Bixa orellana L. (urucum) e sua relação com o teor de bixina presente nas sementes. *Dissertação*, 57.

Meyer, B. N., Ferrigni, N. A., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & Mclaughlin, J. L. (1982). *Brine Shrimp : A Convenient General Bioassay for Active Plant Constituents*. *45*, 31–34.

Monzote, L., García, M., Scull, R., Cuellar, A., & Setzer, W. N. (2013). *Antileishmanial Activity of the Essential Oil from Bixa orellana*. *July*.

Moreira, M. R., Ponce, A. G., Valle, C. E., & Roura, S. I. (2005). *Inhibitory parameters of essential oils to reduce a foodborne pathogen*. *38*, 565–570. https://doi.org/10.1016/j.lwt.2004.07.012

Reed, L. J., & Muench, H. (1938). *THE AMERICAN*. *27*(3), 493–497.

Rincón, C. T. S., Gómez, G. L. C., & Montoya, J. E. Z. (2016). Extraction of phenolic compounds and antioxidant activity from leaves of Bixa orellana L. (achiote). *Revista Cubana de Plantas Medicinales*, *21*(2), 133–144.

Santos, R. L., Guimaraes, G. P., Nobre, M. S. C., & Portela, A. S. (2011). *Análise sobre a fitoterapia como prática integrativa no Sistema Único de Saúde*. *13*(4), 486–491.

Shahid-ul-Islam, Rather, L. J., & Mohammad, F. (2016). Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications – A review. *Journal of Advanced Research*, *7*(3), 499–514. https://doi.org/10.1016/j.jare.2015.11.002

Silva, M. I. da, & Oliveira, H. B. de. (2018). *Brazilian Applied Science Review*. 1104–1110.

Silva, S. de O., Carvalho, J. M. de, Pereira, E. C., Melo, L. M. da C. L., & Araújo, R. dos S. (2018). *Atividade antimicrobiana e caracterização físico-química de urucum Bixa orellana em diferentes estadios de maturação*. 49–57.

Tomazoni, E. Z., GianI, S. G., Ribeiro, R. T. S., Pauletti, G., & Schwambach, J. (2013). *13846 - Atividade antifúngica do óleo essencial de*. *8*(2), 1–5.

Venugopalan, A., & Giridhar, P. (2012). *Bacterial growth inhibition potential of annatto plant parts*.

Vilar, D. de A. (2015). *Estudo Fitoquímico da Bixina e Fração Oleosa Extraídos da "Bixa orellana" biomonitorado pela atividade leishmanicida.* 71. https://repositorio.ufpb.br/jspui/bitstream/tede/8267/2/arquivototal.pdf

Waterhouse, A. L. (2002). *Wine Phenolics*. *36*, 21–36.

Percentage of contribution of each author in the manuscript

Yuri Nascimento Fróes – 25%

Ana Patrícia Matos Pereira – 5%

Paulo Victor Serra Rosa – 5%

Laiane Araújo da Silva Souto – 5%

Mariana Oliveira Arruda – 5%

Luís Felipe Castro Araújo – 5%

Andressa Teixeira Costa – 5%

Danielly Fonseca – 5%

Dorileira Pereira do Nascimento – 5%

Gustavo Oliveira Everton – 5%

Victor Elias Mouchrek Filho – 5%

Joicy Cortez de Sá Sousa – 20%