Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 15 (1)
January 2022
DOI: http://dx.doi.org/10.36560/15120221506
Article link: https://sea.ufr.edu.br/SEA/article/view/1506

ARCHIVES
ISSN 2316-9281

Chemical profile and antioxidant, antibacterial, and cytotoxic activities on *Artemia salina* from the essential oil of leaves and xylopodium of *Cochlospermum regium*

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Abstract. *Cochlospermum regium* is a shrub plant species from the Cerrado domain used in traditional medicine. This study aimed to evaluate the chemical profile and antioxidant, antibacterial and cytotoxic activities on *Artemia salina* from the essential oil of fresh leaves and xylopodium. Fresch leaves and xylopodium of *C. regium* were collected in an area of Cerrado domain in Goiás State, Brazil, 2021. The essential oil was obtained by hydrodistillation, the yield was quantified and the chemical profile determined by gas chromatography with mass spectrometry (GC-MS). Physicochemical analyzes were carried out for organoleptic analysis (color and appearance), solubility, relative density (g mL^{-1}), refractive index, optical rotation (α_D), antioxidant activity in DPPH radical reduction (IC_{50} µL mL^{-1}), antibacterial activity on *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella serovar* Enteritidis and *Salmonella serovar* Thyphymurium by the disc method (mm), and cytotoxicity bioassay on *Artemia salina* (LC_{50} µg mL^{-1}). The major compounds for the essential oil of fresh leaves were viridiflorol 10.21%, Copaen-4-α-ol <β>, longiborneol 9.07 and β-bisabolene 11.48%, and for the essential oil of xylopodium β-selinene 26.17%, aromadendrene 8.66 % and thujopsene 8.09%. The yield was 0.58 and 0.33%, color slightly yellow and yellow for fresh leaves and xylopodium, respectively. Positive solubility, refractive index of 1.3468 and 1.3347, optical rotation +48.8 and +21.5, relative density 0.932 and 0.936 g mL^{-1}, antioxidant activity IC_{50} = 47.65 and 111.16 µL mL^{-1} for fresh leaves essential oil and xylopodium, respectively. The essential oil from fresh leaves showed high antibacterial potential for all strains, as well as for cytotoxic activity on *A. salina* with LC_{50} = 90.17 and 625.08 µg mL^{-1}, respectively.
Keywords: Bixaceae family, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*.

Introduction

Cochlospermum regium (Schrank.) Pilg. the Bixaceae family is one of 13 species included in the genus *Cochlospermum* found in the Americas, Africa, Asia and Australia (Hislop; Thiele; Brassington, 2013; Cowie; Kerrigan, 2015;

Mahendra et al., 2017). *C. regium* is native to Brazil, being present in areas with phytophysiognomy of campo Cerrado, typical Cerrado or restricted sense, where it is popularly known as "algodãozinho-do-Cerrado or algodãozinho-do-campo) presenting beautiful yellow flowers and deep xylopodium (source). According to Galvão et al. (2020) the species is used as animal, ornamental and medicinal forage.

In traditional medicine *C regium* xylopodium, is used to cure gastric problems, ulcers, rheumatism, vaginal discharge, prostatic infections, abscesses, in analgesia, anti-hypertensive, anti-inflammatory, in the treatment of arthritis and acne (Carvalho et al., 2018) . Studies evaluating the phytomedicinal properties for xylopodium and leaves showed important biological activities explored mainly from hydroethanolic, hydromethanolic, ethanolic, methanolic extracts and their fractions, as an antimicrobial agent on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida tropicalis* and *Cryptococcus gattii* (Leme et al., 2017; Carvalho et al., 2018; Almeida-Apolonio et al., 2018).

Several studies have demonstrated the presence of important phytomolecules in extracts and their fractions from *C. regium* roots such as pinoresinol, excelsin, flavones, naringenin, aromadendrin, dihydrokaempferol-3-*O*-β-(6″-galloyl), two triacyl benzenes, 1-hydroxytetradecanone-3 and the flavonoid and 3-*O*-glycosyl dihydrokaempferol, glucopyranoside, cochlospermins A and B (Ritto et al., 1996; Castro et al., 2004), dihydrokaempferol-3-*O*-β-glucopyranoside (DHK- glucoside) was the major, together with other minor constituents, such as dihydrokaempferol, gallic acid and ellagic acid (Oliveira et al., 1996; Solon et al., 2009), flavonoids and tannins (Solón et al., 2012).

According to Menezes Filho et al. (2020) *C. regium* have important phytochemical groups belonging to special metabolites such as phenols and essential oils present in xylopodium, leaves and flowers. Essential oils are rich in terpenes, especially for the class of sesquiterpenes 96.87%, β-copaen-4-α-ol (18.73%) and viridiflorol (12.67%) (Inácio et al., 2014; Leme et al., 2017), β-selinene (34.1%), β-elemene (5.4%), *Trans*-caryophyllene (4.8%), α-pinene (3.4%), α-humulene (2.8%), α-selinene (1.2%), δ-cadinene (0.8%), and 45.4% of other unidentified elements (Brum et al., 1997).

Essential oils are volatile for the most part due to the low number of structural carbons where at temperatures above 25 °C they already volatilize. This important group of phytomolecules has large and peculiar characteristics and also a complex morphostructure containing monoterpenes, diterpenes, sesquiterpenes and phenylpropanoids, producing an aroma that is most often an artifice of plant pollination (Prins et al., 2006).

Although *C. regium* is widely studied regarding its biological properties, especially extracts, little is known about the antioxidant, antibacterial and cytotoxic activities of essential oils and little is known about the composition of the chemical profile of this species.

Thus, this study aimed to obtain and characterize the essential oils from fresh leaves and xylopodium of *C. regium*, as well as to evaluate the physicochemical parameters, and the antioxidant, antibacterial and cytotoxic activities on *Artemia salina*.

Materials and Methods

Reagent and equipments

2,2-diphenyl-1-picrylhydrazyl (DPPH) Sigma-Aldrich, *n*-Alkanes (C_7-C_{40}) (Sigma-Aldrich),

Refractometer (Hanna Instruments, Mod. HI96800), polarimeter (Novainstruments, Mod. WXG-4), UV-*Vis* spectrophotometer (Belphotonics, Mod. M-51).

Plant material

Leaves and xylopodium of *C. regium* were collected in the Cerrado region at the University of Rio Verde, in Rio Verde, Goiás, Brazil, (S and W) in September 2021. The plant was identified by the Biologist Msº. Antonio Carlos Pereira de Menezes Filho and as sample was deposited at the Herbarium Goiano Federal Institute at exsiccate number HRV 14064.

Essential oil extraction

The essential oil was obtained from fresh leaves and xylopodium (150 g), which were reduced by a home processor and had their essential oil extracted by the distillation method carried out by a Clevenger type apparatus at 100 °C for 5 h (Estevam et al., 2017) adapted. Thereafter, the hydrolate was submitted to liquid-liquid partition in a separatory funnel. Three washes of the hydrolate were performed with three 10 mL portions of dichloromethane. Essential oil samples were stored at -12 °C until further chemical profile and biological assay.

Physicochemical analysis

Total oil yield was expressed as percentage (g 100 g^{-1} of fresh plant material) and color, and appearance were evaluated by the organoleptic analysis method (Gomes et al., 2018). The solubility of essential oil was determined in a solution of 70% (*v/v*) ethanol as described by Alarcón et al. (2019). In an *Eppendorf* tube an aliquot of 100 μL of hydroethanolic solution 70% about 2 μL of essential oil was added. The tube was homogenized for 5 minutes.

The refractive index test was performed in a refractometer with refractive indexes between (1.3330 - 1.5080) and resolution of 0.0001 to 20 °C, according to Alarcón et al. (2019). The optical rotation test was determined in polarimeter provided with a 10 mL cell at a temperature of 25 °C and line α_D of the Sodium lamp at 589 nm with a measurement range of (-180° to +180°) on the Vernier scale. The sample was prepared with 10%

(*w/v*) of essential oil in 98% ethanol, as described by Alarcón et al. (2019).

For relative density, a pycnometer of 1 mL was used. The pycnometer was clean and dry (25 ºC) and weighed empty and the mass determined. Then 1 mL of essential oil wasadded, and subsequently its mass was determined and annotated. The density was expressed in g mL^{-1} at 20 °C according to Alarcón et al. (2019).

Chemical profile by GC-MS

Gas chromatography coupled to mass spectrometry (GC-MS) analysis was done by a PerkinElmer GC Clarus 580 equipment coupled with MS Clarus SQ 8S was used, the auto-injector using a DB-5MS column (30 m x 0.25 mm, 0.25 mm in thickness). The carrier gas was He at pressure of 57.4 kPa and flow rate of 1.0 mL min^{-1}. The split ratio was 1/30, the injector temperature was 240 °C and the injected volume was 0.1 µL. Temperature ranged between 60 °C for 2 min., and 270 °C, having been increased 5 °C min. MS were recorded on electron ionization mode, with ionization energy of 70 eV. The volatile chemical constituents were identified on the basis of their retention indices relative to a homologous series of *n*-alkanes (C_7-C_{40}) and by comparing mass spectra with libraries (Nist 11 spectroteca) and references of previously published data (Adams 2007).

Antioxidant activity

The antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical reduction followed as described by Mezza et al. (2018) adapted. Sample-dichloromethane solutions 2 mL prepared at 0.1-100 mg mL^{-1} were added to 2 mL of DPPH solution in dichloromethane. After 120 min, the absorbance was measured at 517 nm in quartz cuvette. The blank was dichloromethane and the control solution was prepared with 2 mL DPPH solution and 2 mL dichloromethane. The DPPH reduction of percentage was calculated as equation 1.

$$DPPH(\%) = ((AC - AS)/AC)*100 \quad \text{Eq. [1]}$$

Where: AS is the absorbance of the sample solution containing antioxidant and AC is the absorbance of the control solution DPPH.

The inhibition concentration (IC_{50}) was defined as the amount of sample (µL mL^{-1}) that produced a 50% decrease in the initial DPPH concentration. Lower IC_{50} values indicate higher free of radical reduction by essential oil. As antioxidant standards, ascorbic acid and Butylated hydroxytoluene (BHT) were used.

Antibacterial activity

Bacterial strains were obtained from the authors' private microorganism bank. The microbiological assay followed as described by Vieira et al. (2021) adapted, using the paper disc diffusion technique. Were used of strains from *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (LB 29212), *Salmonella serovar* Enteritidis (ATCC 13076) and *Salmonella serovar* Thyphymurium (ATCC 14028).

The activation of microorganisms was carried out in a sterile solution of NaCl conc. 0.85% until reaching the degree of 0.5 on the scale MacFarland conc. ($1x10^8$ CFU mL^{-1}) UV-*Vis* spectrophotometer. *Petri* dishes 10 cm^2 were prepared with Plate Count Agar (PCA) after sterilization. The *Petri* dishes containing specific medium were inoculated using a sterile swab soaked with a microbial suspension, and spread across the plate.

Filter paper discs with a diameter of 7 mm were impregnated with 100 µL of the essential oil in different concentrations (100, 50, 25, 5 and 2.5 mg mL^{-1}), as a negative control, the saline solution used with 10% dimethylsulfoxide (DMSO) (*v/v*), and as positive control discs with antimicrobial agents, Azithromycin (15 µg), Cephalexin (30 µg) and Tigecycline (15 µg). The *Petri* dishes were incubated at 36 °C with an interval between 24-36 h, after that period, the halo of antibiosis when present was measured with a digital caliper. The minimum antibiosis halo was 5 mm. The test was carried out in quadruplicate.

Essential oil bioassay against brine shrimp

Brine shrimp eggs *A. salina* Leach were purchased at home from pet shop products and were hatched in artificial sea water which prepared by dissolving 38 g of sea salt in 1 L of distilled water. After 48 hours incubation at room temperature 25 °C, nauplii (larvae) were collected by *Pasteur* pipette and used in the cytotoxicity experiment described below.

The cytotoxicity assay on *A. salina* followed as described by Shariffar et al. (2017), proposed by Meyer et al. (1982) adapted. The collected nauplii were treated with various concentrations (1, 2.5, 5, 7, 10, 20, 50, 70, 100 and 1000 µg mL^{-1}) of essential oil of *C. regium*. Various concentrations of each essential oil were dissolved in dimethylsulfoxide (DMSO) 1%, placed in test tubes. Then, 5 mL of artificial sea water was added and 15 active larvae was placed to the tubes and subjected under light. Potassium dichromate was used as positive control. The survivors nauplii were counted after 24 h and the percentage of death were determined by concentrations.

Statistical analysis

The treatment was carried out in quadruplicate and the experimental design was thoroughly randomized. Data were submitted to the analysis of variance (ANOVA), and the means of the treatments were evaluated by the Tukey's ($p < 0.05$) and *Scott-Knott* test at 5% significance level by the Assistat software. For the cytotoxic assay on *A. salina*, the results were evaluated by linear regression.

Results and discussion

The essential oil from fresh leaves and xylopodium obtained by analysis in GC-MS, presented a total of 20 compounds with 90.18% and 17 compounds with 99.95% identified, respectively. The essential oil from the leaves presented the following major compounds viridiflorol, copaen-4-α-ol <β>, longiborneol and β-bisabolene, and for the xylopodium essential oil β-selinene, aromadendrene, and thujopsene (Table 1).

Inácio et al. (2014) evaluated the chemical composition of the essential oil from the leaves of *C. regium* where they verified the presence of 19 compounds, being copaen-4-α-ol <β> with 18.73%, viridiflorol with 12.67%, bicyclogermacrene with 8.26% and longiborneol with 7.13%. Also according Inácio and collaborators, the few chemical profile studies on leaves of plants of the genus *Cochlospermum* show that sesquiterpenes are the major constituents of the essential oil. Essential oil from the leaves of *Cochlospermum angolense* contains 68.8% of sesquiterpenes (Leonardi et al., 2012). The major components of the essential oil from leaves of *Cochlospermum vitifolium*, which also occurs in Brazil, are sesquiterpenes (Almeida et al., 2005). Although the study by Ritto (1996) states that there is no essential oil in the xylopodium, Brum et al. (1997) in a later study, affirmed the positive presence of volatile compounds. Also in the study by Brum and collaborators, the researchers carried out the chemical profile of the essential oil of xylopodium where they found eight compounds, β-selinene with the highest content (34.1%), followed by elemene (5.4%), *Trans*-caryophyllene (4.8%), α-pinene (3.4%), humulene (2.8%), aromadendrin (2.1%), α-selinene (1.2%) and δ-cadiene (0.8%). In the same year, Honda et al. (1997) also evaluated the chemical constitution of xylopodium oil, where they found only one major compound, β-selinene with 34.1%.

Table 1. Essential oil composition of *Cochlospermum regium* in fresh leaves and xylopodium identified by GC-MS.

No	Compound	Percentage[1]	Percentage[2]
1	Viridiflorol	10.21	-
2	β-selinene	-	26.17
3	*Trans*-caryophyllene	-	6.88
4	Orcynil	-	7.41
5	Sydnone, 3(3,3-dymethil buthyl)	-	5.03
6	Bicyclogermacrene	5.90	-
7	Aromadendrene	-	8.66
8	Copaen-4-α-ol <β>	20.05	-
9	Longiborneol	9.07	-
10	α-pinene	-	3.08
11	Humulene	6.00	2.71
12	Germacrene B	4.00	-
13	Guaiol	2.17	-
14	β-ocimene	-	1.19
15	Cedr-8(15)-EM-9-α-ol	1.66	-
16	Muurola-4,10(14)-dien-1-β-ol	7,45	-
17	Myrcene	1.03	-
18	Khusimone	0.97	-
19	Elemene	-	4.03
20	δ-cadiene	-	2.01
21	Aromadendrin	-	6.54
22	Hentriacontane	-	2.27
23	Limonene	1.10	-
24	*Trans*-β-ocimene	-	5.05
25	Germacrene D	1.11	6.77
26	Thujopsene	-	8.09
27	β-bisabolene	11.48	3.11
28	1,13-tetradecadien-3-one	1.01	0.95
29	δ-selinene	0.99	-
30	α-guaiene	1.35	-
31	Panaxene	2.07	-
32	1-decen-3-one	1.00	-
33	3-hexadecanone	1.56	-
	Total	90.18	99.95

Note: [1]Fresch leaves. [2]Xylopodium. (-) Absent.

C. regium presents non-seed studies on leaves and xylopodium, Menezes Filho et al. (2020a,b) investigated the essential oil in flowers where they identified in the first study five major compounds ocimene, with 15.87%; caryophyllene E, with 11.53%; γ-muurolene, with 20.07%; bicyclogermacrene, with 16.11%; and rosifoliol, with 31.09%. And in the second study, only three caryophyllene E compounds were identified, with 9.76%; γ-muurolene, with 16.68%; and bicyclogermacrene with 39.82%.

The essential oil yield of fresh leaves and xylopodium (Table 2) are similar to the Inácio et al. (2014) with 0.2% for the essential oil of fresh leaves and 0.25% for the essential oil of xylopodium by Brum et al. (1997) and 0.2% by Honda et al. (1997). The essential oil yield of fresh leaves and xylopodium (Table 2) are similar to the Inácio et al. (2014) with 0.2% for the essential oil of fresh leaves and 0.25% for the essential oil of xylopodium by Brum et al. (1997) and 0.2% by Honda et al. (1997). The refractive index showed statistical difference by *Tukey's* test, the same was observed for optical rotation. The relative density did not show statistical difference by the *Tukey's* test, being also in agreement with other essential oils evaluated from different species, genera and families.

As for the DPPH free radical reduction activity, the essential oil from the leaves showed better activity when compared to the essential oil from xylopodium. Although they showed good activity, they presented results inferior to Ascorbic acid and BHT with IC_{50} µL mL^{-1} = 1.96 ± 0.91a and 3.54 ± 0.64b. Statistically there was a difference between both reference antioxidants and *C. regium* essential oils according to *Tukey's* test ($p < 0.05$) (Table 2).

Menezes Filho et al. (2020) found antioxidant activity in the reduction of DPPH between 100-13.18% (50-0.031 mg mL^{-1}) for the essential oil of *C. regium* flower. Pedroso et al. (2019) obtained high inhibition activity from the β-carotene/linoleic acid reduction model (IC_{50} = 85.50 µg mL^{-1}) compared to the natural standard Quercetin (70.65 µg mL^{-1}) from the hydromethanolic extract of the roots of *C. regium*. The researchers also found important reducing activity on DPPH with IC_{50} = 14.68 µg mL^{-1} and for the ascorbic acid standard IC_{50} = 11.50 µg mL^{-1}. As for the FRAP antioxidant model, radical activity = 138.71 µg mL^{-1} was observed for the extract and for the Quercetin 56.76 µg mL^{-1} standard. Abourashed and Fu (2017) also found a potential antioxidant effect from the methanol extract of *Cochlospermum angolensis* barks on the DPPH radical model.

Several volatile molecules such as limonene, thymol, carvacrol, α-terpinene, γ-terpinene, and α-terpinolene, β-caryophyllene among other various terpenes and sesquiterpenes that have considerable antioxidant activity in reducing different reactive molecules such as ROS: singlet oxygen, hydrogen peroxide, organichydrope roxide, hydroxyl radical, superoxide ion and nitrogen (Torres-Martínez et al., 2017; Verma; Verma, 2018; Lin et al., 2019). According with Verma & Verma, (2018) and Menon et al. (2019) the reactive species damages the major living components such as DNA, RNA, protein, biomolecules and lipids and thus various antioxidant protection mechanisms are being evolved by the body (microorganisms, human, animal and plants). Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorder, diabetes, Alzheimer and ischemia.

Table 2. Physicochemical and antioxidant parameters of essential oils from *Cochlospermum regium* from fresh leaves and xylopodium.

Parameters	Essential oil fresch leaves	Essential oil xylopodium
Yield (%)	0.58 ± 0.40a	0.33 ± 0.52b
Color	slightly yellow	yellow
Appearance	homogeneous, clear and crystalline	homogeneous and slightly cloudy
Solubility (*v/v*)	positive	positive
Refractive index (20 °C)	1,3468 ± 0.04b	1,3347 ± 0.05a
Optical rotation (α_D)	+48.8a	+21.5b
Relative density (g mL^{-1} at 20 °C)	0.932a	0.936a
DPPH IC_{50} (µL mL^{-1})	47.65 ± 0.41c	111.16 ± 0.93d

Note: Equal letters in the same column do not differ significantly by *Tukey's* test ($p < 0.05$).

Results obtained in the study of antibacterial activity were satisfactory for the strains of Gram-negative and Gram-positive bacteria, *E. coli*, *S. aureus* and *S.* Enteritidis from the essential oil of fresh leaves, and for the essential oil of xylopodium only for *E. coli*, *E. faecalis* and *S.* Enteritidis. The *S. serovar* Thyphymurium was not sensitive to any of the xylopodium essential oil concentrations (Table 3). Although the essential oil of *C. regium* xylopodium showed antibacterial activity, this growth inhibition sensitivity was only observed at the highest concentrations of 100 and 50 mg mL^{-1}.

According to Riad et al. (2020) in general, Gram-negative bacteria are more resistant to essential oil. This is due to the presence of a restrictive outer membrane surrounding the Gram-negative bacteria cells. In this study, we observed that this resistance to pure essential oil does not apply to the essential oil from fresh leaves but to xylopodium essential oil.

The references antibiotics (Azithromycin, Cephalexin e Tigecycline) have shown a very strong inhibitory effect with respect to the tested microorganisms, as compared to both essential oils of *C. regium* in the study. Statistically all results in both essential oils showed significant difference between the references antibiotics tested.

Carvalho et al. (2020) evaluated the ethanol extract of *C. regium* root, where they observed antibacterial activity for *E. coli* and *S. aureus* strains with inhibition halos ranging from 1.22 to 2.22 mm (0.312-20 mg mL^{-1}). Also in the study by Carvalho and collaborators, the researchers did not observe antibiotic action against the strains of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at the concentrations evaluated. The methanol root extract of *Cochlospermum tinctorium* was effective in inhibiting the isolates at high concentration of 10 mg mL^{-1} on *S. aureus* and *Listeria monocytogene* in the study of Abdulaziz et al. (2019).

Fankibe et al. (2020) found satisfactory antibacterial activity on *S. aureus* by evaluating the extract of *C. planchonii* leaves and roots. Menezes Filho et al. (2020) verified important antifungal activity of the essential oil of the *C. regium* flower on the phytopathogen *Sclerotinia sclerotiorum* with an inhibition rate of 79.98% (100 µL mL-1).

Ouattara et al. (2019) evaluating the essential oil from the root of *Cochlospermum planchonii* obtained highest inhibition of mycelial growth was obtained on *Colletotrichum graminicola* at 0.50% of essential oil. At this concentration, there was high a significant reduction in the mycelial growth of *Colletotrichum graminicola* with 81.70% followed by *Curvularia lunata* with 54.58% and *Macrophomina phasoelina* with 53.89%.

As noted, the genus *Cochlospermum* plays an important role in the study of antibacterial and antifungal activity from special metabolites both in shoots and roots in different species. These biological activities of inhibition on microorganisms are provided from a rich amount of phytochemicals where many of these are volatile (Ouattara et al., 2018; Abdulaziz et al., 2019; Menezes Filho et al., 2020; Galvão et al., 2020).

Several essential oil compounds have high potential on antibacterial activity and these compounds are monoterpenic, sesquiterpenic and phenylpropanoid with proven action as limonene (Goulart et al., 2018), thymol (Majolo et al., 2020), germacrene D (Freitas et al., 2020), caryophyllene and α-pinene (Nelson, 2019), β-bisabolene (Mazaheritehrani et al., 2021) and myrcene (Cabral et al., 2020).

Table 3. Antibacterial activity of essential oils of *Cochlospermum regium* from fresh leaves and xylopodium.

	Inhibition zone (mm) fresch leaves essencial oil				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	13.05±0,06b	11.81±0.33b	8.41±0.17c	6.04±0.56c	0.00±0.00d
S. aureus	18.14±0.09b	17.41±0.58b	14.11±1.03bc	9.05±0.12d	0.00±0.00e
E. faecalis	17.01±0.60b	16.45±1.03b	14.68±0.91b	12.44±0.68cb	12.26±1.04cb
S. Enteritidis	9.81±0.23b	8.75±0.39b	5.90±0.00cb	0.00±0.00d	0.00±0.00d
S. Thyphymurium	7.14±0.26b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
	Inhibition zone (mm) xylopodium essential oil				
	100 mg mL^{-1}	**50 mg mL^{-1}**	**25 mg mL^{-1}**	**5 mg mL^{-1}**	**2.5 mg mL^{-1}**
E. coli	8.66±0.39b	7.21±0,99b	5.04±0.20b	0.00±0.00c	0.00±0.00c
S. aureus	0.00±0.30b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
E. faecalis	8,93±1.09b	6.22±0.50b	0.00±0.00c	0.00±0.00c	0.00±0.00c
S. Enteritidis	9.03±0.65b	5.42±1.06cb	0.00±0.00d	0.00±0.00d	0.00±0.00d
S. Thyphymurium	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
Antibiotics	[a]23.52±0.40Aa	[b]28.07±0.19Ba	[a]28.01±0.08Ca	[a]27.53±0.84Da	[c]22.64±0.21Ea

Note: Different lowercase letters on the same line differ statistically by the *Scott-Knott* test with 5% probability. [a]Azithromycin, [b]Cephalexin and [c]Tigecycline. A = *S. aureus*, B = *E. coli*, C = *S. serovar* Thyphymurium, D = *S. serovar* Enteritidis, and E = *E. faecalis*.

The cytotoxic assay on *A. salina* showed LC_{50} = 90.17 ± 1.90b and 625.08 ± 2.88c µg mL^{-1} for the essential oil of fresh leaves and xylopodium of *C. regium*. The control with potassium dichromate presented LC_{50} = 0.00 ± 0.00a with 100% mortality of nauplii. LC_{50} values equal to and less than 1000 µg mL^{-1} indicate considerable biological activity, and above this value are considered non-toxic (Meyer et al., 1982). Muhammad et al. (2020) evaluated in a study from the crude methanol extract of *C. tinctorium* roots potential cytotoxic activity on *A. salina* with 100-83.3% mortality (1000-10 µg mL^{-1}) and *n*-hexane and ethyl acetate fractions with 100-96.7% (1000-10 µg mL^{-1}), and methanol 100-46.7% (1000-10 µg mL^{-1}). The lethal concentration in the study by Muhammad et al. (2020), presented LC_{50} = 3.165 µg mL^{-1}. The result of cytotoxicity revealed that, *n*-hexane and ethyl acetate fractions are more potents with LC_{50} = 1.175 µg mL^{-1} followed by the crude fraction with LC_{50} = 3.165 µg mL^{-1}, and methanol aqueous fraction was least potent with LC_{50} = 15.019 µg mL^{-1}.

The bioassay with *A. salina* presents a quick result which is related to potential biological effects, being, therefore, an important test to know the toxicity levels of a compound or fibroblasts and keratinocytes ((Almeida-Cincotto et al., 2016; Calazans et al., 2019).

Conclusion

The essential oil from fresh leaves and *Cochlospermum regium* xylopodium showed important results on the chemical profile, high antioxidant activity in the reduction of the DPPH free radical, especially for the essential oil of the leaf, as well as for the antibacterial and cytotoxic activity on *Artemia salina*. Further studies should be carried out evaluating essential oils against other models of free radicals, as well as other diverse biological activities.

Acknowledgment

Goiano Federal Institute, Rio Verde Campus, Goiás State, Brazil; Technological Chemistry, Water and Effluent, Microbiology Agricultural, and Phytotechnic and Organomineral laboratories; Multiuser Analytical Center – CEMA.

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