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Original Article

Ocotea nutans (Nees) Mez (Lauraceae): chemical composition, antioxidant capacity and biological properties of essential oil

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The present study was undertaken to assess the potential uses of the essential oil obtained from *Ocotea nutans* (Nees) Mez. The hydrodistilled essential oil from O. nutans leaves was analyzed by gas chromatography-mass spectrometry. Fifty-eight compounds representing 87,29% of the total leaf essential oil components were identified, of which biciclogermacrene(11.41%), germacrene-D (4.89%), bisabolol-11-ol(3.73%) and spathulenol (3.71%) were the major compounds. The essential oil from O. nutans were tested for antibacterial activity using the minimum inhibitory concentracion (MIC) method, *Artemia salina* method, larvicidal activity in *Aedes aegypti,* and antioxidant capacity.The antioxidant activity measured by the phosphomolybdenum complex and Prussian blue method had positive results. The minimum inhibitory concentration for the microorganisms tested allowed moderate inhibitionfor Enterococcus faecalis (MIC=500 µg/mL). *Artemia salina* were toxic to the organisms in the study (LC_{50} = 71,70 µg /mL). The essential oil showed remarkable larvicidal activity potencial (LC_{50}= 250 µg/mL). The present results showed that *O. nutans* essential oil has potential biological uses.

Keywords: *Ocoteanutans*/essential oil/evaluation. Antioxidant capacity.*Aedesaegypti.*Toxic potential.

INTRODUCTION

Lauraceae is a family of flowering plants that contain approximately 2,500 species distributed in the Neotropics of America and several species found in Africa and Madagascar (Van der Werff, 2002). This family has great economic importance,with several species (particularly from the genera *Ocotea, Nectandra* and *Aniba*) having high commercial value because they are aromatic plants that produce essential oils commonly used in different industries (Marques, 2001). *Ocotea* is one of the largest genus of the family Lauraceae that is located in America. This genus includes approximately 350 species distributed in tropical and subtropical America (from Argentina to México). In Brazil, there are approximately 150 species of *Ocotea*. According to a study by Brotto, Cervi and Santos(2013), 31 species of *Ocotea* occur in Paraná State (Brazil), with the Atlantic Forest having the highest number of species (26), followed by the Araucaria Forest (14 species) with an endemic seasonal (11 species) and theCerrado (7species) with two endemic and fields (1 specie).

Ocotea nutans (Ness) Mez (Lauraceae) is a 10-30 meter high tree, that is found in the Mixed Ombrophilus Forest, often distributed in the first and second plateau (Brotto, Cervi, Santos, 2013). This species is popularly named 'canelinha' and 'canela'.

Essential oils from the genus *Ocotea* have evidenced pharmacological effects:e.g., cardiovascular (Barbosa-Filho *et al.*, 2008), antifungal and antibacterial (Garrett *et al.*, 2007), anti-inflammatory (Ballabeni *et al.*, 2010) and antiplatelet (Ballabeni *et al.*, 2007) activities.

In the present study, the chemical composition of essential oils from leaves of *O. nutans* were examined by gas chromatography-mass spectrometry (GC–MS), the evaluation of antioxidant activity was undertaken, and testing of potencial biological activities (antibacterial, toxicological and larvicidal properties) occured.

MATERIAL AND METHODS

Plant material

The leaves of *O. nutans* were collected from the

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city of Colombo, Paraná State, Brazil (25°17'03.5"S, 49°08'44"W). Plant identification was performed by forest engineer Marcelo Leandro Brotto, from the Botanical Garden of Curitiba Herbarium by comparison with the voucher specimen deposited under number 56552 at the Herbarium of the Federal University of Paraná.

The present study was authorized by the Genetic Heritage Management Council (CGEN), a legislative and deliberative body under the Ministry of the Environment of Brazil, under number 02001.001165 / 2013-47.

Extraction of essential oil

The essential oil was extracted from 100 g of dried leaves that had been ground in a knife mill and hydrodistilled for 6h using a modified Clevenger type apparatus. The essential oil was stored in a sealed amber jar glass at -18 °C until subsequent analysis. The essential oil yield was expressed in v/w% of essential oil per 100 g of the drug (Brasil, 2010).

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil from *O. nutans* was analyzed by gas chromatography-mass spectrometry using a Shimadzu GC-MS - QP 2010 Plus analyzer equipped with a capillary column Rtx -5MS (30 m × 0.25 mm × 0.25 µm.) with a splitless injector mode at 250 °C, and an ion source and interface at 300 °C. The mass window was analyzed from m/z 40 and m/z 350, using helium as the carrier gas. Ramp injection for analysis had the injector temperature set at 250 °C, pressure of 20 psi column, starting at 50 °C for 5 min and increasing to 200 °C at a rate of 5 °C/min. Identification of the oil components was undertaken by comparing their Kovats indices and mass spectra with the NIST library as well as comparing them with those reported in the literature (Adams, 2007).

Antioxidant activity of essential oil

Formation of the phosphomolybdenum complex method

The essential oil and standard (ascorbic acid and rutin) were diluted in methanol to a concentration of 200 µg/mL.The methodology used has been described previously by Prieto, Pineda and Aguilar (1999). A 0.3 mL essential oil was combined with 1 mL of reagent solution (0.1 mol/L of sodium phosphate, 0.03 mol/L of ammonium molybdate and 3 mol/L of sulfuric acid) and made up to 100 mL with distilled water. The tubes were incubated at 95 °C for 90 min, after which they were cooled to room temperature (25-30 °C). The absorbance of the solution was measured at 695 nm. The AA% compared to ascorbic acid was evaluated with the following formula:

$$\text{AA\% compared to ascorbic acid} = [(A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}}) - (A_{\text{blank}})] \text{ X } 100 \quad (1)$$

where A_{sample} is the absorbance of the test compound, A_{blank} is the absorbance of the white and A_{control} is the absorbance of the ascorbic acid.

Reducing power antioxidant (Prussian blue) method

In the antioxidant activity test (Prussian blue method), the essential oil at a concentration of 200 µg/mL was transferred to 25mL test tubes, and 0.2M potassium phosphate buffer (pH 7.0) and 1.0% potassium ferricyanide were added. The mixture was incubated at 45°C for 20 min, and then 1% trichloroacetic acid was added to the test tubes. Approximately 2.5 mL was transferred to 5.0 mL test tubes, and 1.5 mL of distilled H_2O, 1.0 mL of ethanol and 0.5 mL of $FeCl_3$ was added to make up to 1.0% (w/v). The absorbance reading was performed at 700 nm (Yen, Chen, 1995;Jayanthi,Lalitha, 2011).

Antibacterial activity

The antibacterial activity tests were performed with the following strains: *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and *Pseudomonas aeruginosa* (ATCC 27853).

The essential oil was prepared in 0.5% of polysorbate 80, and filtered through a 0.22 µm Milipore membrane (TPP, Trasadingen, Switzerland) to assure its sterility. The suspensions of bacteria were prepared in saline solution at a concentration of 10 x 10^8 CFU/mL, corresponding to a 0.5 McFarland standard tube.

The minimum inhibitory concentration (MIC) values were determined through broth microdilution method (Clinical and Laboratory Standards Institute, 2008). The essential oil was tested at concentrations from 7.81 to 1000 µg/mL in microplates containing Muller Hinton Broth and 5 µl suspensions of bacteria were subsequently inoculated into the wells to obtain a final concentration of 10^4 CFU/mL. After preparation, the microplates were incubated at 35ºC for 16 to 20h. After this time, 20 µL of aqueous solution of 0.5% triphenyltetrazolium chloride (Merck, Darmstadt, Gemany) was added, and the microplates were incubated again for 3h at 35ºC (Souza *et al.*, 2014)

The red color formation in the microplate wells were evaluated as the absence of bacterial growth inhibition. The MIC values obtained were classified as having good inhibitory potential (up to 100 µg/mL), moderate inhibitory activity (between 100 and 500 µg/mL), weak inhibitory activity (between 500 and 1000 µg/mL), and absence of inhibitory activity (higher than 1000 µg/mL) (Ayres *et al.*, 2008).

Preliminary toxicological activities

Toxicity against Aedes aegypti

The methodology applied was adapted from the World Health Organization (2005) and Fujiwara *et al*. (2017). The eggs of *Aedes aegypti* (eggs from the Rockefeller strain made available by Oswaldo Cruz Foundation - Fiocruz) were placed in dechlorinated water and incubated in a B.O.D incubator at a controlled temperature of 27°C and relative humidity of 80%. The larval diet consisted of fish feed (Aldon basic, MEP 200 complex), from hatching until they reach the third developmental larval stage.

The essential oil was diluted in 0.5% of polysorbate 80, and then dissolved in dechlorinated water to obtain the desired concentration. An aqueous solution of 0.5% of polysorbate 80 was used as a negative control and the insecticide temephos was use as a positive control. The positive control was used at a concentration of 6 µg/mL (twice the lethal concentration that causes 99% mortality of susceptible strains) according to the protocol recommended (WHO, 1981; Lima *et al*., 2003; Grzybowski *et al.*, 2012).

After hatching, 10 larvae in the 3rd stage were placed in contact with the negative and positive control and essential oil at concentrations (1000, 500, 100 and 10 µg/mL) for 24h and then live and dead larvae were counted. Three repetitions were used for each treatment giving a total of 30 larvae to each sample dose.

Brine shrimp (Artemia salina) lethality assay

The essential oil was diluted in 0.5% of polysorbate 80 and then dissolved in artificial seawater to obtain the desired concentration. Artificial seawater and 0.5% of polysorbate 80, was used as the negative control, and sodium dodecyl sulfatewas used as the positive control (Amarante *et al*., 2011). The assay was performed according to the methodology described by Meyer *et al.* (1982) with modifications. The eggs of *Artemia salina* L. were placed in artificial seawater (38 g marine salts dissolved in 1000 mL purified water) and incubated in a B.O.D. incubator at controlled temperature of (30 ± 2) °C for 48h. After hatching, 10 nauplii of *Artemia salina* L. were placed in contact with the negative and posite control and essential oil at concentrations (1000, 500, 250, 100 and 10 µg/mL) for 24 h, and then the live and dead nauplii were counted. Three replicates were used for each treatment, totaling 30 nauplii for each sample dose. The positive control was used in concentrations of 10, 20, 30, 40 and50 µg/mL.

Statistical analysis

The probit method (Finney, 1971) was used to determine the lethal concentration (LC_{50} and LC_{90}) values, as well as the corresponding 95% confidence intervals and chi square values for the assays with *Aedes aegypti* and *Artemia salina* L., using the IBM SPSS Statistics version 20.0 software program.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

GC–MS analysis of the essential oil led to the identification of 58 different compounds, representing 87,29% of the total oil from the leaves. The identified compounds are listed in Table I, according to their elution order on the capillary column.

Essential oil is a mixture of volatile, lipophilic, odoriferous and liquid substances (Simões *et al.*,2004). Chemically the essential oil was composed of terpene hydrocarbons, simple alcohols and terpenes, aldehydes, ketones, phenols, esters, oxides, peroxides, furans, organic acids, lactones, coumarins and sulfur containing compounds, with different concentrations. Among these, the most representative group were the terpenoids, which are formed by the fusion of isoprene units of five carbons,and when subjected to high temperatures, might decompose into isoprenes and occasionally refer to all terpenoids as isoprenoids (Taiz, Zeiger, 2004). Terpenoids are considered a homogeneous group owing to the presence of the isoprene units ($-C_5H_8$), which form hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes (Castro *et al*., 2004; Simões *et al.,* 2004; Brielmann *et al.* 2006).

The essential oils of plants have specific compositions, and in the genus *Ocotea* there is a predominance of monoterpenes and sesquiterpenes, which are often present in several species of the same genus, in varying amounts and sometimes may or may not be present in some species (Bruni *et al.*, 2004; Coutinho *et al.*, 2007; Barbosa-Filho *et al.*, 2008).

TABLE I – Chemical compounds identified in analysis GC-MS of *Ocotea nutans* essencial oil

COMPOUNDS*	CHEMICAL CLASS	RI[a]	% RA[b]	Identification[c]
Pinene<α->	M	939	2.12	RI, MS
Camphene	M	954	0.09	RI, MS
Sabinene	M	975	0.16	RI, MS
Pinene<β->	M	979	2.81	RI, MS
Myrcene	M	990	1.13	RI, MS
Terpinene<α->	M	1017	0.07	RI, MS
Limonene	M	1029	3.24	RI, MS
Cineole<1,8->	OM	1031	0.61	RI, MS
Ocimene<(Z)-β->	M	1037	0.09	RI, MS
Ocimene<(E)-β->	M	1050	0.09	RI, MS
Terpinene<γ->	M	1059	0.16	RI, MS
Terpinolene	M	1088	0.10	RI, MS
Linalool	OM	1096	0.18	RI, MS
Camphor	OM	1146	0.16	RI, MS
Terpinen-4-ol	OM	1177	0.72	RI, MS
Terpineol<α->	OS	1188	0.70	RI, MS
Elemene<δ->	S	1338	1.32	RI, MS
Cubebene<α->	S	1348	1.11	RI, MS
Ylangene<α->	S	1376	0.33	RI, MS
Copaene<α->	S	1376	2.20	RI, MS
Burbonene<β->	S	1388	0.78	RI, MS
Elemene<β->	S	1390	1.94	RI, MS
Gurjunene<α->	S	1409	0.27	RI, MS
Caryophyllene<(E)->	S	1419	2.47	RI, MS
Copaene<β->	S	1432	0.38	RI, MS
Guaiene<α->	S	1439	0.89	RI, MS
Guaiadiene<6,9->	S	1444	0.44	RI, MS
Muurola-3,5-diene<cis->	S	1450	2.09	RI, MS
Humulene<α->	S	1454	1.89	RI, MS
Aromadendrene<allo->	S	1460	3.22	RI, MS
Cadina-1(6),4-diene<trans->	S	1476	1.71	RI, MS
Muurolene<γ->	S	1479	1.13	RI, MS
Germacrene D	S	1485	4.89	RI, MS
Selinene<β->	S	1490	0.72	RI, MS
Muurola-4(14),5-diene<trans->	S	1493	3.09	RI, MS
Bicyclogermacrene	S	1500	11.41	RI, MS
Amorphene<δ->	S	1512	1.01	RI, MS
Cadinene<γ->	S	1513	3.13	RI, MS
Cadina-1,4-diene<trans->	S	1534	1.84	RI, MS
Cadinene<α->	S	1538	0.31	RI, MS
Calacorene<α->	S	1545	0.24	RI, MS

TABLE I – Chemical compounds identified in analysis GC-MS of *Ocotea nutans* essencial oil (cont.)

COMPOUNDS*	CHEMICAL CLASS	RI[a]	% RA[b]	Identification[c]
Elemol	OS	1549	0.62	RI, MS
Germacrene B	S	1561	0.44	RI, MS
Nerolidol<(E)->	OS	1563	0.56	RI, MS
Palustrol	OS	1568	0.28	RI, MS
Germacrene D-4-ol	OS	1575	0.53	RI, MS
Spathulenol	OS	1578	3.71	RI, MS
Caryophyllene oxide	OS	1583	1.74	RI, MS
Globulol	OS	1590	2.45	RI, MS
Ledol	OS	1602	0.80	RI, MS
Cubenol<1,10-di-ep-i>	OS	1619	2.50	RI, MS
Cadin-4-en-7-ol<cis->	OS	1636	0.72	RI, MS
Muurolol<α->	OS	1646	3.18	RI, MS
Valerianol	OS	1658	0.77	RI, MS
Eudesmol<dihydro->	OS	1662	2.29	RI, MS
Bisabol-11-ol <(E)->	OS	1668	3.73	RI, MS
Rosa-5,15-diene<ent->	D	1934	0.38	RI, MS
Kaurene	D	2043	0.55	RI, MS
Total (identified)			87.29	
(M) Monoterpene hydrocarbon (11)			10.06%	
(OM) Oxygenated monoterpene (5)			2.37%	
(S) Sesquiterpene hydrocarbons (26)			50.05%	
(OS) Oxygenated sesquiterpene (14)			23.88%	
(D) Diterpenes (2)			0.93%	

The terpene compounds of several species of *Ocotea*, have as its major components: α-Pinene, β-Pinene, β-Elemene, β-Caryophyllene, α-Humulene, Germacrene D, γ-Cadinene, δ-Cadinene and α-Cadinol (Takaku, Haber, Setzer, 2007). Moreover these nine compounds are common to other genera in the family Lauraceae such as *Cinnamomum, Laurus, Aniba, Nectandra* and *Persea*. These compounds were found in the essential oil from*O.nutans* which reveals the close chemotaxonomic relationship between *O. nutans* and other species of *Ocotea* belonging to the first group of chemical diversity (one whose species contain terpenes compounds). This is typical of the essential oils of several *Ocotea* species from South America (Chaverri *et al.*, 2011).

The essential oil from *O.nutans* presented sesquiterpenes as another major constituent which could be related to the antibacterial activity detected. Although the essential oil is a complex mixture of metabolites, this activity might influence the composition of the major components.

Another major compound identified in the essential oil from *O.nutans* was bicyclogermacrene (11.41%). The bicyclogermacrene is effective in microbiological activities (Constantin *et al.*, 2001). Germacrene-D showed anti-cancer activity (Sylvestre *et al.*, 2006), antibacterial activity (Deuschle *et al.*, 2007) and insectifugal (Frascescato, 2007). Bisabol-11-ol showed a known pharmacological activity and is used as an anti-inflammatory agent (Robbers, Speedie, Tyler, 1997), for wound healing, and also as anatimicrobial (Darra *et al.*, 2008). Spathulenol has atimicrobial properties (Veiga-Junior, Pinto, 2002; Limberger *et al.*, 2004). Limonene has proven effective in anti-inflammatory activities (Hirota *et al.*, 2010), for gastroprotection (Rozza*et al.*, 2011), chemopreventive activity for liver, lung, skin and breast cancers (Crowell,1997) and as an atininociceptive (Amaral *et al.*, 2007; Murali, Saravanan, 2012). Allo-

aromadendrene has antibacterial activity (Scalco *et al.*, 2014).

The 0,54% (v/w) yield of the *O.nutans* essential oil was good when compared to other species of the same genus: *O. notata* 0,12% (v/w) (Garrett *et al.*,2007), *O. leucoxylon* 0.13% (v/w), *O. minor* 0.11% (v/w) (Yamaguchi, 2011), and*O. nigrescens* 0.23% (v/w),however, it was lower than the yield from*O. odorifera* 0,86% (v/w) (Castellani *et al.*, 2006).Environmental factors that the plants species are subjected to (e.g., natural factors, light, temperature and humidity), botanical origin, and chemotype, have been demonstrated to significantly influence the emission of volatile compounds, altering their yield and composition (Simões *et al.,* 2004).

Antioxidant activity of essential oil

The ability to reduce a fraction serves as an indicator of its antioxidant potential. Evaluation of the antioxidant activity by the reducing power method (Prussian blue) allowed the antioxidant potential of the *O. nutans* essential oil to be verified. The standards used for comparison were ascorbic acid and rutin.

The analytical method based on Fe^{3+} reduction, that determines the reducing power was applied to evaluate antioxidant activity. This method is based on the capacity of phenolic compounds to reduce Fe^{3+}, with consequent formation of a colored complex with Fe^{2+}. The ferricyanide ion is reduced to ferrocyanide which, in the presence of the ferric ion (from $FeCl_3$), forms the complex of Prussian blue, $Fe_4[Fe(Cn)_6]_3$(Yen, Chen, 1995; Jayanthi; Lalitha, 2011).

The antioxidant activity measured by the formation of the phosphomolybdenum complex is described in Table II. This method measures the inhibition of ammonium molybdate by the action of the essential oil which is based on the reduction of molybdenum IV to molybdenum V and subsequently the formation of a green complex corresponding to phosphate/molybdenum V in acid pH. The assay is undertaken at an elevated temperature for a prolonged period and has the advantage of evaluating antioxidant capacity in both lipophilic substances and hydrophilic substances in an acid medium (Prieto, Pineda, Aguilar, 1999).

The differences between the results obtained by the two techniques might be associated with the action mechanisms involved in the methods and the hydro/lipophilicity of the antioxidant substances present in the essential oil.

Antibacterial activity

This is the first known study of showing the antibacterial activity of essential oil from *O.nutans*. The essential oil has constituents that could be considered as having antibacterial activity. The activity *in vitro* effects of the essential oil were measured in the present study. The values obtained from the microbiological assays were MIC=500 µg/mL in *E. faecalis* and MIC=1000 µg/mL for *S. aureus, S epidermidis* and *P aeruginosa.* No activity was observed for *E. coli* and *K. pneumoniae*.

A study carried out with the essential oil of *O.odorifera* (Cansian *et al.*, 2010) presented antibacterial activity against *E. faecalis, S. aureus, S. epidermidis, E. coli* and *K. pneumoniae*, which was considered more effective than the bacteria gram-negative comparisons of inhibition concentrations. Another species used in the antibacterial assay was *O.notata* (Garrett *et al.*, 2007), where essential oil activity of the species showed an inhibition power for *E. faecalis, S. aureus* and *S. epidermidis.*

Preliminary toxicological activities

Toxicity against Aedes aegypti and Brine shrimp lethality assay

The essential oil was submitted to tests against 3rd stage *Aedes aegypti* larvae. The results (Table III) suggest that the toxicity of this fraction occurred owing to the presence of apolar compounds, terpenes and derivates, which have demonstrated larvicidal activity, however, the action mechanism was not completely elucidated (Shaalan *et al*., 2005; Gosh, Chowdhury, Chandra, 2012).

Ocotea nutans essential oil had positive toxicity in *Aretmia salina* (Table III). Based on a study by Meyer *et al.* (1982), samples are considered toxic with a LC_{50}<1000

TABLE II - Antioxidant activity by phosphomolybdenum and complex prussian blue

Antioxidant activity (AA)	***Phosphomolybdenum***	***Complex prussian blue***
AA in relation to rutin (%)	41,88	58,42
AA in relation to ascorbic acid (%)	15,14	59,74

The total antioxidant activity is considered to be 100% in relation to the antioxidant potential of ascorbic acid and rutin

TABLE III - Toxicological activities against *Aedes aegypti* and *Artemia salina*

Aedes aegypti				*Artemia salina*			
LC_{50} (µg/mL) (LCL-UCL)	**LC_{90} (µg/mL) (LCL-UCL)**	**x^2**	**(df)**	**LC_{50} (µg/mL) (LCL-UCL)**	**LC_{90} (µg/mL) (LCL-UCL)**	**x^2**	**(df)**
250 µg/mL (179,86 - 32,75)	744,32 µg/mL (534,41 -1254,67)	3,355	(2)	71,70 µg/mL (61,22 -83,91)	117,49 µg/mL (97,30 -170,99)	1,08	(4)

No mortality was observed in the negative controls; positive control killed 100% larvae in *A. aegypti*;(LC_{50}) lethal concentration that kills 50% of the exposed organisms; (LC_{90}) lethal concentration that kills 90% of the exposed organisms; (UCL) 95% upper confidence limit; (LCL) 95% lower confidence limit; (x^2) chi square; (df) degrees of freedom;

µg/mL. However, Amarante *et al.* (2011), described a relationship between the degree of toxicity in *Artemia salina* and the correlation of the more detailed LC_{50} indicating that samples below (LC_{50}<100 µg/mL) are considered highly toxic. Garrett *et al.* (2007) performed a bioassay with *O.notata* essential oil where a LC_{50} of 2.73 µg/mL, was considered extremely toxic.

The toxic potential of essential oils from species of Lauraceae to *Artemia salina* is well documented in the literature. The brine shrimp bioassay correlates with cytotoxicity in solid tumors and activity against *Trypanosoma cruzi*; (Mclaughlin, Rogers, Anderson, 1998; Alves *et al.*, 2000).

The lethal concentration (LC_{50}) of sodium dodecylsulfatein *Artemia salina* should be contained in the range of 13.1 to 30.9 µg/mL in 24h (Nascimento, Souza, Nipper, 2002), which was confirmed in the results obtained in the present study: $LC_{50\text{sodium dodeculsulfate}}$ = 24.49 µg/mL.This finding demonstrates that the sensitivity of individuals is standardized, which reduces the possibility of generating falsely exacerbated or attenuated results. The acute toxicity evaluation in *Artemia salina* establishes a parameter for the species, since no studies of previous ecotoxicological tests were found to establish comparisons.

CONCLUSION

Evaluation of *O. nutans* essential oil in biological activities showed a possible toxicity to *Aedes aegypti* and *Artemia salina*, although low antimicrobial activity was shown. The present study contributes to the enrichment of the data base concerning the specie *O. nutans* (Nees) Mez that previously was limited toonly botanical surveys, as well as revealing activities that require complementary studies.

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