



Chemical Composition and Cytotoxic Activity of *Lepechinia speciosa* (St. Hill) Epling

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SUMMARY. The cell viability of *Lepechinia speciosa* (St. Hill) Epling fractions was measured by cell membrane integrity (lactate dehydrogenase assay) on rat basophilic leukemia cells (RBL-2H3). All fractions and extract tested (100 µg/ml) increased the release of lactate dehydrogenase (LDH), being the ethyl acetate and dichloromethane fractions with LDH release of 94.5% and 91.2%, respectively. As these fractions showed decrease of cell viability, the antiproliferative activity on human breast adenocarcinoma cells (MCF-7) through sulphorhodamine B (SRB) assay was performed with them. The dichloromethane fraction (50 µg/ml) displayed the maximum activity (95% of inhibition) (IC₅₀ = 1.99 ± 0.06 µg/ml). From this fraction was obtained a mixture containing two triterpenes (ursolic and oleanolic acids) and one fatty acid (palmitic acid), which were identified by gas chromatography coupled to mass spectrometry (GC-MS) and had their structures confirmed by ¹³C NMR. Rosmarinic acid and verbascoside were isolated from the ethyl acetate fraction and had their structures confirmed by ¹H NMR.

INTRODUCTION

The Lamiaceae family is comprised approximately of 224 genera and 5600 species distributed across the world. One of these genera is *Lepechinia*, which comprises 40 species that are mainly distributed in South America and Mexico¹.

Plants of this genus are used in folk medicine for the treatment of uterine tumors, stomach ailments, diabetes mellitus control and diarrhea¹⁻⁵; in addition, previous investigations showed that *Lepechinia* species display hypoglycemic, vasorelaxant, antimicrobial, cytotoxic and antioxidant activities^{2,4,6-8}. Phytochemical studies have revealed the presence of tricyclic

diterpenes, flavonoids and pentacyclic triterpenes¹ and some species of this genus have been studied for their volatile compounds⁹⁻¹².

Some species belonging to the Lamiaceae family also exhibit cytotoxic activities, such as *Lamium album*¹³, *Salvia miltiorrhiza*¹⁴, *Marrubium cylleneum*¹⁵, *Marrubium velutinum*¹⁵, *Glossocarya calcicola*¹⁶ and *Lepechinia bullata*⁶, and diterpenes are the main responsible of this activity.

In this work we studied the cell viability on rat basophilic leukemia cells (RBL-2H3), the antiproliferative activity on human breast adenocarcinoma cells (MCF-7) and analyzed the

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chemical composition of the bioactive fractions of *Lepechinia speciosa*, contributing to its limited scientific knowledge.

MATERIALS AND METHODS

Plant material

Lepechinia speciosa (St. Hill) Epling (Lamiaceae) was collected at Parque Nacional de Itatiaia, Rio de Janeiro, Brazil, in February 2004. Its authenticity was confirmed by Dr Regina Braga de Moura, (Universidade Estácio de Sá) using morphological and anatomical techniques. Voucher specimens were deposited and registered in the Herbarium of the Departamento de Botânica da Universidade Federal do Rio de Janeiro (Brazil) with RFA-28365.

Chemicals

All solvents used were of analytical grade and purchased from Tedia and the sodium carbonate (Na_2CO_3) was purchased from Sigma. The chromatography analyses were realised in silica gel 60 (70-230 mesh) columns from Merck and in thin-layer chromatography with silica gel plates 60 F254 also from Merck.

Extract preparation

The dried and powdered aerial parts (574 g) were extracted with 96% ethanol at room temperature for at least 24 h (20 x 400 ml). Thereafter, the ethanol crude extract (ET) was filtered, dried under reduced pressure (68.21 g). This crude extract was suspended in water (300 ml), with posterior ethanol (200 ml) addition and this solution was submitted to a liquid-liquid extraction procedure with solvents (10 x 100 ml) of different polarity: *n*-hexane; dichloromethane, ethyl acetate, *n*-butanol. Thus, the following fractions were obtained: *n*-hexane fraction (FH) (12.34 g), dichloromethane fraction (FD) (6.49 g), ethyl acetate fraction (FAE) (5.6 g), butanolic fraction (FB) (5.48 g) and an aqueous fraction (FAQ) (3.25 g).

Acid-base extraction

A sample (50 mg) of the FD was dissolved in chloroform (CHCl_3) (50 ml), treated with 5% sodium carbonate (Na_2CO_3) (5 x 20 ml) and filtered after each treatment. The aqueous fraction was treated with concentrated HCl, and the pH was adjusted to 4.3. Thereafter, the fraction was extracted (x 10) with CHCl_3 (10 ml). The chloroform fraction was treated with ethereal diazomethane.

Fractionation and isolation

FAE (1.4 g) was fractionated by silica gel column chromatography, eluted with a solvent gra-

dient from dichloromethane to methanol. The fractions obtained were analyzed by thin-layer chromatography (TLC) using a mixture of ethyl acetate, acetic acid and water (10:2:3) as developing solvent system and grouped according to their chromatographic profile. Fractions 6-12 (eluted with dichloromethane-methanol 5%) were purified by silica gel column chromatography, eluted with dichloromethane to methanol. The fractions obtained were analyzed by thin-layer chromatography (TLC) using a mixture of ethyl acetate, acetic acid and water (10:2:3) as developing solvent and grouped according to their chromatographic profile. Fractions 30 and 31, eluted with ethyl acetate:methanol (6:1), were combined and yielded rosmarinic acid (69.5 mg). Fraction 28, eluted with ethyl acetate:methanol (6:1) contained verbascoside (4.3 mg).

GC-MS

The FD (derivitised with diazomethane) was dissolved in chloroform and the GC-MS analysis (split inlets) was carried out in a Hewlett-Packard HP 5890 SII gas chromatograph coupled to a mass spectrometer model Hewlett-Packard HP 5973, with a capillary column DB-1 (30 m x 0.20 mm). Helium was used as the carrier gas at 2 ml min^{-1} , and the temperature was programmed from 50 to 270 °C at 4 °C/min. The electron impact ionization was set to 70 eV. The volume injected was 1.0 μl . The injector, detector and column temperatures were 240 °C.

NMR-Analyses

FD (50 mg) was treated with active charcoal, analyzed by ^{13}C NMR nuclear magnetic resonance (also APT technique) and data were compared with literature^{17,18}. The structural elucidation of rosmarinic acid and verbascoside was carried out by comparison of experimental values from ^1H NMR with values previously reported^{19,20}. *Ursolic and oleanolic acids*: ^{13}C NMR (50 MHz, DMSO- D_6 /TMS) data: see Table 1. Rosmarinic acid: ^1H NMR (200 MHz, CDCl_3 /TMS) data: see Table 2. Verbascoside: ^1H NMR (200 MHz, CDCl_3 /TMS) data: see Table 3.

Cytotoxicity

Cell culture

Rat basophilic leukemia cells (RBL-2H3) were cultured in a Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin-streptomycin at 37 °C in 5% CO_2 /humidified air. Human breast adenocarcinoma cells (MCF-7) were

C	1 ^a	2 ^a	1	2
3	78.8	78.7	78.6	78.6
12	125.5	122.1	125.1	122.0
13	138.0	143.4	138.2	143.5
28	177.7	181.0	177.9	178.4

Table 1. ¹³C-NMR spectral data for compound 1, 2 (50 MHz, DMSO-D₆) and data from literature ^{17,18}. ^a Data taken from literature (50 MHz, CDCl₃).

H	4 ^a	4
2	7.03 (<i>d</i>)	7.03 (<i>d</i> , <i>J</i> 1.7Hz)
5	6.76 (<i>d</i>)	6.77 (<i>d</i> , <i>J</i> 8Hz)
6	6.92 (<i>dd</i>)	6.92 (<i>dd</i> , <i>J</i> 1.7 and 8Hz)
7	6.25 (<i>d</i>)	6.26 (<i>d</i> , <i>J</i> 16Hz)
8	7.50 (<i>d</i>)	7.52 (<i>d</i> , <i>J</i> 16Hz)
2'	6.77 (<i>d</i>)	6.67 (<i>s</i>)
5'	6.67 (<i>d</i>)	6.66 (<i>d</i> , <i>J</i> 8.1Hz)
6'	6.62 (<i>d</i>)	6.51 (<i>dd</i> , <i>J</i> 1.7 and 8.1Hz)
7'α	3.10 (<i>dd</i>)	3.02 (<i>m</i>)
7'β	2.93 (<i>dd</i>)	3.02 (<i>m</i>)
8'	5.09 (<i>dd</i>)	4.3 (<i>m</i>)

Table 2. ¹H-NMR spectral data for compound 4 (200 MHz, CDCl₃, *J* values (Hz) are given in parentheses), and data from literature ²⁰. ^a Data taken from literature (50 MHz, CD₃OD).

cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and maintained at 37 °C in a 5% CO₂ /humidified air.

LDH assay

The effect of the *L. speciosa* fractions upon cell viability was determined by lactate dehydro-

genase released method (LDH). The crude extract and fractions of *L. speciosa* (hexane, dichloromethane, ethyl acetate and butanol) were tested on rat basophilic leukemia cells (RBL-2H3) and dissolved in dimethyl sulfoxide (DMSO) at 10mg/ml and diluted in Dulbecco's Modified Eagle's Medium (DMEM) to concentration of 100 µg/ml. Two-fold serial dilutions of the extract or fractions (100 µl) were added to triplicate wells containing 100 µl of cells per well (2 x 10⁶ cell/ml). The cells were incubated at 37 °C with 5% CO₂ and 90 % humidity for 24 h. For the positive control, which permitted the maximum LDH release, 100 µl/well Triton X-100 solution (2% in assay medium) were added to triplicate wells containing 100 µl of cells per well. For the cytotoxic standard, 50 µl/well terfenadine (100 mM) were added to triplicate wells containing 100 µl of cells per well. For the background control, 200 µl assay medium were added to triplicate wells, and for low control 100 µl/well assay medium were added to triplicate wells containing 100 µl of cells per well. The plate was centrifuged at 250 g for 10 min,

	H	5 ^a	5
(3,4-dihydroxyphenyl)ethyl	2	6.72 (<i>sl</i>)	6.70 (<i>sl</i>)
	5	6.55 (<i>d</i>)	6.6 (<i>sl</i>)
	6	6.71 (<i>d</i>)	6.65 (<i>d</i> , <i>J</i> 5Hz)
	β	2.77 (<i>m</i>)	2.97 (<i>d</i> , <i>J</i> 8.8Hz, CH ₂ -β)
Glucose	1'	4.39 (<i>d</i>)	4.4 (<i>d</i> , <i>J</i> 7.8Hz, H anomeric-glucose)
	2'		
	3'	3.85 (<i>t</i>)	3.12-4.26 (CH ₂ -α and H from rhamnose and glucose)
	4'	4.95 (<i>t</i>)	4.76 (<i>m</i>)
	5'		
	6'		3.12-4.26
Caffeoyl	2''	7.09 (<i>s</i>)	7.03 (<i>d</i> , <i>J</i> 1.2Hz)
	5''	6.80 (<i>d</i>)	6.80 (<i>d</i> , <i>J</i> 1.2Hz)
	6''	6.94 (<i>d</i>)	6.92 (<i>dd</i> , <i>J</i> 1.2 and 7.6Hz)
	α''	6.29 (<i>d</i>)	6.26 (<i>d</i> , <i>J</i> 16Hz)
	β''	7.6 (<i>d</i>)	7.51 (<i>d</i> , <i>J</i> 16Hz)
Rhamnose	1'''	5.24 (<i>sl</i>)	5.23 (<i>d</i> , <i>J</i> 2.2 Hz, H anomeric-rhamnose)
	6'''	1.12 (<i>d</i>)	1.2 (<i>d</i> , <i>J</i> 6.8Hz, Me-Rha)

Table 3. ¹H-NMR spectral data for compound 5 (200 MHz, CDCl₃, *J* values (Hz) are given in parentheses), and data from literature ¹⁹. ^a Data taken from literature (50 MHz, CD₃OD).

and 100 μ l/well of supernatant were removed and transferred to a 96-well flat bottom plate. To determine the LDH activity in these supernatants, 100 μ l of assay medium from ELISA kit (reaction mixture) were added to each well and incubated for up to 30 min at 20 °C. Finally, the

absorbance of the samples was measured at 490 nm using an ELISA reader. The test was performed in triplicate on different days.

Percent cytotoxicity values were calculated using Eq. [1]. Data are represented as mean values \pm standard deviation.

$$\text{Cytotoxicity (\%)} = \frac{(\text{experimental value} - \text{low control})}{(\text{positive control} - \text{low control})} \times 100 \quad [1]$$

Sulphorhodamine B (SRB) assay

The antiproliferative activity was determined using sulphorhodamine B (SRB) assay. The FD and FAE showed significant effect cytotoxic against rat basophilic leukemia cells (RBL-2H3) that led us to test both fractions against human breast adenocarcinoma cells (MCF-7). The samples were dissolved in DMSO and further diluted with cell culture medium in different concentrations (1, 5, 10, 25 and 50 μ g/ml). For antiproliferative activity, 100 μ l of MCF-7 cells (6.0×10^4) were inoculated onto 96-well plates and incubated at 37 °C in 5% CO₂/humidified air. After 24 h, 100 μ l of the fractions of *L. speciosa* were added and incubated for 48 h. After this treatment, the cells were fixed with 50 μ l of 50% trichloroacetic acid (TCA) for 1h at 4 °C. The plates were washed in distilled water and dried at room temperature. The dyeing was realized by addition of 0.4% sulphorhodamine B (50 μ l) dissolved in 1% acetic acid for 30 min. The plates were incubated at 4 °C, washed four times with 1% acetic acid and dried at room temperature. The bound SRB was solubilized by addition of unbuffered Tris Base (10 mM, pH 10.5) (100 μ l) and shaken for 5 min.

The absorbances were read at 515nm (Lab-systems Multiscan EX plate reader). The extracts which produced an SRB absorbance lower than 25 % that of the time zero control value in the cell line were considered to be cytotoxic and the IC₅₀ values were calculated from the dose response curves.

RESULTS AND DISCUSSION

Extraction and isolation of the compounds.

The GC-MS analysis of the FD revealed a mixture of two triterpene acids (oleanolic and ursolic acids) and one fatty acid (palmitic acid). Ursolic acid (37.08%, RT = 49.3min) (compound 1) was identified as the major component followed by oleanolic acid (16.53%, RT = 46.2 min)

(compound 2) and palmitic acid (1.18%, RT = 19.61 min) (compound 3) as minor component (Fig. 1). The mass spectra obtained for these compounds showed a molecular ion at m/z 270, compatible with the molecular formula C₁₇H₃₄O₂ and in accordance with the structure of palmitic acid methyl ester; the molecular ion at m/z 502 corresponding to the molecular formula C₃₁H₅₀O₅ is in accordance with the structure of oleanolic acid methyl ester; the molecular ion at m/z 470, compatible with the molecular formula C₃₁H₅₀O₃ and in accordance with the structure of ursolic acid methyl ester.

The characterization of the constituents of this fraction was simplified by the assignment of the carbon atoms in the ¹³C NMR. As the chemical shift of a sp² carbon atom is very characteristic for each triterpenoid skeleton, ¹³C NMR spectroscopy is frequently employed for the structural analysis of triterpene mixtures²¹. To distinguish carbon types (multiplicity), the APT (at-

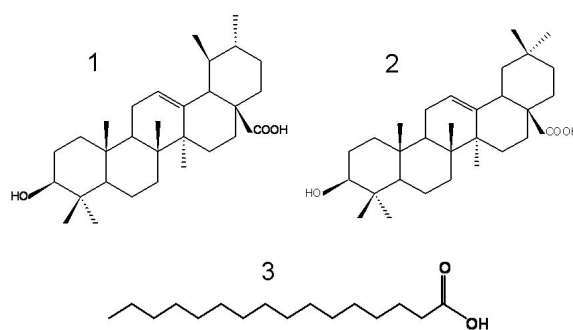


Figure 1. Chemical structure of the main compounds identified within the dichloromethane fraction from *Lepechinia speciosa*: (1) ursolic acid; (2) oleanolic acid; (3) palmitic acid. The dichloromethane fraction (50 mg) was submitted to an acid-base extraction and treated with ethereal diazomethane. After, it was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) and the presence of these compounds was confirmed by ¹³C NMR nuclear magnetic resonance.

tached proton test) was used. The last step of this mixture resolution before the confirmation by GC/MS was the comparison of the attributed signals of ^{13}C NMR spectra obtained for the mixture with previously published data ^{17,18}.

All the signals obtained in the ^{13}C NMR stated for the presence mainly of two triterpenoid acids: oleanolic and ursolic acids. This has been confirmed by GC/MS through which was also possible to identify a minor component; palmitic acid. Analysis of the ^{13}C NMR (50MHz, DMSO d_6) spectra of oleanolic and ursolic acids and comparison with literature data showed characteristic signals for triterpenoids skeleton at δ (ppm): 177.9 (C-28, C=O, ursolic acid); 178.4 (C-28, C=O, oleanolic acid); 138.2 (C-13, ursolic acid); 143.5 (C-13, oleanolic acid); 125.1 (C-12, ursolic acid); 122.0 (C-12, oleanolic acid) and 78.6 (C3, CHO ursolic and oleanolic acids) (Table 1).

Previous investigations showed significant anti-inflammatory effect of ursolic acid isolated from organic extracts of species belonging to the Lamiaceae family ^{22,23}. The mechanisms of the anti-inflammatory effect of ursolic acid have been attributed to the inhibition of histamine release from mast cells and to the inhibition of complementary activity ^{24,25}.

FAE was fractionated by silica gel column chromatography, affording an ester of caffeic acid, rosmarinic acid (compound 4), and a phenylpropanoid glycoside, verbascoside (compound 5) (Fig. 2). Analysis of the ^1H NMR (200 MHz, CDCl_3) spectrum of rosmarinic acid showed signals at 3.02 ppm (m) relative to $7''\alpha\text{H}$, 4.3 ppm (m) relative to $8''\text{H}$ and two duplet signals: 6.26 ppm (d) and 7.52 ppm (d) respecting to hydrogen of double bond (7H and 8H), with coupling constant of J 16Hz, characteristic of *trans* bond (Table 2).

Analysis of the ^1H NMR (200 MHz, CDCl_3) spectrum of verbascoside evidenced a caffeic acid and a (3,4-dihydroxyphenyl)ethyl units. The presence of glucose was verified through the signal of anomeric hydrogen at 4.44ppm ($1''\text{H}$, d , J 7.8Hz), bonded to phenylethyl unit of molecule under β form. It was determined by its coupling constant that indicate to be in an axial position, because the coupling constant for α form is J 3.5Hz ¹⁹. It was possible to observe the presence of rhamnose due to the signal of anomeric hydrogen at 5.23 ppm ($1'''\text{H}$, d , J 2.2 Hz) and 1.2 ppm ($6'''\text{H}$, d , J 6.8 Hz) relative to methylic hydrogen of C-6. The signal at 4.76 ppm ($4''\text{H}$, m) characterizes the substitution with caffeic acid in the C-4 of glucose. The derivative

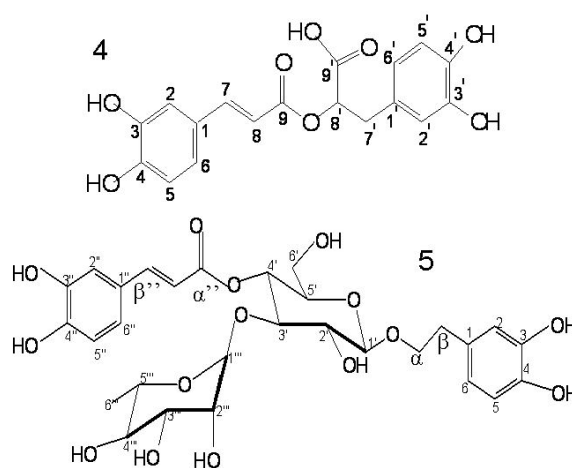


Figure 2. Chemical structure of the compounds isolated of the ethyl acetate fraction from *Lepechinia speciosa*: (4) rosmarinic acid; (5) verbascoside. The ethyl acetate fraction (1.4 g) was fractionated by silica gel column chromatography. The fractions obtained were analyzed by thin-layer chromatography (TLC) and grouped according to their chromatographic profile. Fractions 6-12 were purified by silica gel column chromatography. The fractions obtained were analyzed by TLC and grouped according to their chromatographic profile. The fractions (28; 30-31) obtained had the structure elucidated by ^1H NMR nuclear magnetic resonance, direct comparison with the respective literature data and were identified as rosmarinic acid (fractions 30-31) and verbascoside (fraction 28).

unit of caffeic acid was evidenced by signals at 6.26 ppm ($\alpha''\text{H}$, d , J 16 Hz) and 7.51 ($\beta''\text{H}$, d , J 16 Hz), both with coupling constant of 16 Hz, typical of *trans* hydrogen bonded to carbon of double bond. The presence of caffeoyl at δ (ppm): 6.80 ($5''\text{H}$, d , J 1.2 Hz.), 6.92 ($6''\text{H}$, dd , J 1.2 and 7.6 Hz.) and 7.03 ($2''\text{H}$, d , J 1,2 Hz.) and phenylethyl at δ (ppm): 6.60 (5H, sl), 6.65 (6H, d , J 5Hz) and 6.70 (2H, sl) were confirmed by presence of two signals groups relative to hydrogen of aromatic systems with coupling of *meta*, *orto-meta* and *orto* type (Table 3).

Cytotoxicity

LDH assay

The effect of the *L. speciosa* fractions and extract was studied in the rat basophilic leukemia cell culture (RBL-2H3) through the cell viability by measuring the cell membrane integrity using the lactate dehydrogenase released method (LDH). This is a colorimetric method for the quantification of cell death and lysis, which is based on the measurement of lactate dehydrogenase (LDH) activity released from the damaged cells into the supernatant. All fractions and

extract tested (100 µg/ml) increased the release of LDH from the cells into the surrounding culture medium significantly and were higher than terfenadine (100mM). However, the stronger effect was observed mainly in FAE and FD with 94.5% and 91.2% of LDH release, respectively, while the terfenadine showed 66.43% (Table 4; Fig. 3). That led us to test both FD and FAE against human breast adenocarcinoma cells (MCF-7).

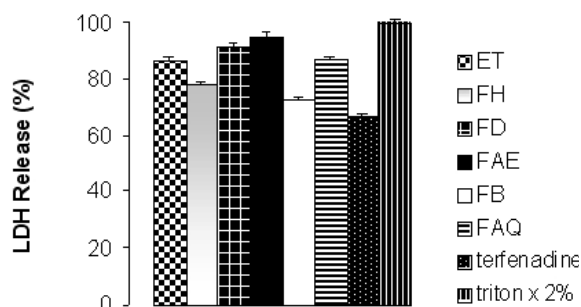


Figure 3. The released of LDH from rat basophilic leukemia cells (RBL-2H3)(2 x 10⁶ cell/ml) after 24 h of treatment with *L. speciosa* fractions and extract (100µg/ml). For the positive control was utilized Triton X-100 solution (2% in assay medium) and as cytotoxic standard, terfenadine (100 mM). The absorbance of the samples was measured at 490 nm using an ELISA reader. ET: ethanol crude extract; FH: *n*-hexane fraction; FD: dichloromethane fraction; FAE: ethyl acetate fraction; FB: butanolic fraction; FAQ: aqueous fraction. Data are presented as mean values ± standard deviation from experiments made in triplicate.

Extract/Fractions	LDH released (%)
ET	86.43 ± 1.2
FH	77.75 ± 0.9
FD	91.02 ± 1.8
FAE	94.5 ± 2.1
FB	72.79 ± 0.8
FAQ	86.68 ± 1.2
Terfenadine	66.43 ± 1.1
Triton X-100	100 ± 1.2

Table 4. The released of LDH (%) from rat basophilic leukemia cells (RBL-2H3)(2 x 10⁶ cell/ml) after treatment with *L. speciosa* fractions and extract (100 µg/ml). Triton X-100 solution was utilized as positive control and terfenadine (100mM) as cytotoxic standard. ET: ethanol crude extract; FH: *n*-hexane fraction; FD: dichloromethane fraction; FAE: ethyl acetate fraction; FB: butanolic fraction; FAQ: aqueous fraction. Data are presented as mean values ± standard deviation from experiments made in triplicate. All the values have *p* < 0.001 when compared with terfenadine, used as a positive control. ANOVA + Bonferroni Test.

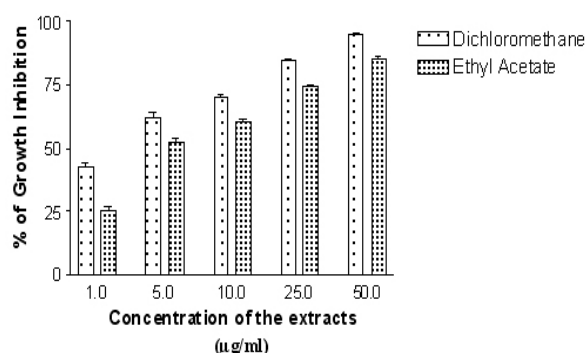


Figure 4. The growth inhibition on human breast adenocarcinoma cells (MCF-7)(6.0 x 10⁴) after 48h of treatment with *L. speciosa* dichloromethane and ethyl acetate fractions. MCF-7 cells (100 µl) were incubated with 100µl of *L. speciosa* dichloromethane and ethyl acetate fractions (1, 5, 10, 25 and 50 µg/ml). The cells were fixed with 50% trichloroacetic acid (TCA) for 1h at 4 °C. The coloration was realized by addition of 0.4% sulphorhodamine B for 30min. The plates were incubated at 4 °C, washed with 1% acetic acid and dried at room temperature. The absorbances were read at a wavelength of 515 nm (Labsystems Multiscan EX plate reader). Data are presented as mean values ± standard deviation from experiments made in triplicate.

Sulphorhodamine B (SRB) assay

When the human breast adenocarcinoma cells (MCF-7) were treated for 48 h with the *Lepechinia speciosa* fractions and extract, the antiproliferative activity was estimated using sulphorhodamine B (SRB) assay, which measures the proliferation of cells. The SRB assay is based on the uptake of the negatively charged pink aminoxanthine dye by basic amino acids in the cell. When the cells are lysed, the released dye and produces a more intense colour and greater absorbance. As shown in Table 5 and Figure 4, both fractions showed growth inhibition in a dose-dependant way, but the FD (50 µg/ml), with higher concentration of triterpene acids, showed 95% of growth inhibition (IC₅₀ = 1.99 ± 0.06 µg/ml), although the FAE (50 µg/ml) has shown a very good result as well, 85% of growth inhibition (IC₅₀ = 5.14 ± 0.23 µg/ml).

Similar to the results obtained for the FD of *L. speciosa*, diterpenes isolated from the dichloromethane fraction of two species of Lamiaceae (*Marrubium cylleneum* and *Marrubium velutinum*) exhibited cytotoxic effect against human tumor cell lines of breast, cervix, melanoma and leukemia¹⁵.

The diterpenes obtained from the methanol fraction of *Lepechinia bullata* and *Glossocarya*

Concentration (µg/ml)	Growth inhibition (%)	
	FD	FAE
1.0	40.79 ± 0.6	23.96 ± 1.2
5.0	63.32 ± 1.1	48.34 ± 1.1
10.0	70.15 ± 0.5	61.53 ± 0.6
25.0	86.88 ± 0.3	75.35 ± 0.8
50.0	94.87 ± 0.2	85.29 ± 0.7

Table 5. The growth inhibition (%) on human breast adenocarcinoma cells (MCF-7)(6.0 x 10⁴) after treatment with *L. speciosa* dichloromethane and ethyl acetate fractions (1, 5, 10, 25 and 50 µg/ml). FD: dichloromethane fraction; FAE: ethyl acetate fraction. Data are presented as mean values ± standard deviation from experiments made in triplicate.

calcicola also showed cell inhibition on nasopharyngeal carcinoma, leukemia cells, ovarian carcinoma, hepatocyte carcinoma, prostate adenocarcinoma and gland adenocarcinoma ^{6,16}.

These studies indicate that fractions or substances isolated from plants belonging to the *Lep-echinia* genus are responsible for the significant cell inhibition in several cancerous cell lines.

CONCLUSIONS

This study showed that the FD and FAE of *L. speciosa* possess strong effect on cell viability of rat basophilic leukemia cells (RBL-2H3); therefore, our study of antiproliferative activity with human breast adenocarcinoma cells (MCF-7) confirms the significant cytotoxic activity of the FD and FAE. The FD predominantly contained a mixture of two triterpene acids (ursolic and oleanolic acids) and one fatty acid (palmitic acid) and the fractionation of the FAE led to the isolation of an ester of caffeic acid, rosmarinic acid, and a phenylpropanoid glycoside, verbascoside. This information can be helpful when estimating the beneficial properties of *Lep-echinia speciosa* fractions or other plant fractions as valuable medicinal raw plant materials to be used for drug development.

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