http://dx.doi.org/10.5935/0103-5053.20140253

*J. Braz. Chem. Soc.*, Vol. 26, No. 2, 233-238, 2015.
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0103 - 5053 $6.00+0.00

Article

# Chemical Composition and Cytotoxic Activity of the Root Essential Oil from *Jatropha ribifolia* (Pohl) Baill (Euphorbiaceae)

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The essential oil of roots of *Jatropha ribifolia*, obtained by hydrodistillation, was characterized in terms of its chemical composition by chromatographic method with flame ionization detection (GC-FID) and gas chromatography coupled to electron ionization mass spectrometry (GC-MS). The analyses and identification pointed by mass fragmentation pattern and retention index revealed the presence of 49 compounds, representing 91.4% of the total oil, with 39.5% of monoterpenes, 43.0% of sesquiterpenes and 8.5% of phenylpropanoids. The major compounds of the oil were β-pinene (9.2%), isoeugenol methyl ether (8.5%), vatirenene (8.4%), α-gurjunene (7.0%), *endo*-8-hydroxy-cycloisolongifolene (6.6%), α-pinene (6.4%) and p-menth-1-en-8-ol (5.2%). The fractionation by preparative thin layer chromatography (TLC) allowed obtaining five fractions (F1-F5) with different compound contents from the original oil. Some essential oil components showed a significant increase in their levels after fractionation, as borneol (17.9%, F1), 3-thujopsanone (19.1%, F4), isoeugenol methylether (21.2%, F2), 8-oxo-9H-cycloisolongifolene (21.4%, F4), 8-*cis*-5(1H)-azulenone,2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene) (23.1%, F4) e *endo*-8-hydroxy-cycloisolongifolene (38.6%, F2). These fractions and oil were tested *in vitro* against nine human cancer cell lines by sulforhodamine B assay. The *Jatropha* oil was more effective in inhibiting the growth of cells NCI-H460 (drug resistant ovarian; $GI_{50}$ 6.2 μg $mL^{-1}$) and OVCAR-3 (ovarian; $GI_{50}$ 8.0 μg $mL^{-1}$). The cancer cells line PC-3 (prostate) was more sensitive to the effects of the fractions showing significant values of $GI_{50}$ such as for fraction F1, F2 and F4 (< 0.25 μg $mL^{-1}$). In general the antiproliferative activity of the fractions was more pronounced than that of crude oil.

**Keywords:** *Jatropha ribifolia,* roots essential oil, cancer cells, antiproliferative activity

## Introduction

The genus *Jatropha* (Euphorbiaceae) contains approximately 170 known species. These species are woody trees, shrubs, and sub-shrubs of disjunct distribution in the seasonally dry tropics of the Old and the New World. Species of the genus *Jatropha* have been extensively investigated as sources for natural products with potential antitumoral, antimicrobial, antifungal, anti-inflammatory and other activities.[1] The roots of some species of *Jatropha* (*J. glandulifera*, *J. gossypiifolia*, *J. multifida*) have been applied to treat people suffering from leprosy and gonorrhea.[1,2] Investigations of the chemical constituents of *Jatropha* plants resulted in the isolation of alkaloids, cyclic peptides, terpenes (monoterpenes, sesquiterpenes, diterpenes and triterpenes), flavonoids, lignans, coumarins and fatty acids.[1]

*Jatropha ribifolia* (Pohl) Baill, Euphorbiaceae, is found throughout the Brazilian northeastern region, popularly known as "pinhão-de-purga" (purgin nut). The latex is used in folk medicine for treatment of snake bites and to treat upper tract decongestions. This species is considered

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endemic in the state of Mato Grosso do Sul and known as "minâncora-do-campo".[3] The cytotoxicity of a hexanic fraction and isolated compounds obtained from roots of *J. ribifolia*, was evaluated against ten human cancer cell lines with good results in inhibiting cell growth.[4] A comparison of the profiles of volatile compounds obtained by hydrodistillation and solid phase micro extraction (SPME) was also performed with the roots of *J. ribifolia*.[5]

As part of our work on the characterization of aromatic and medicinal plants from Mato Grosso do Sul state, Brazil,[6,7] we are now reporting the chemical composition and antiproliferative activity of oil and fractions from *J. ribifolia* roots essential oil. To our knowledge, there are no previous reports on the composition and biological activities of this oil.

## Experimental

### Plant material

The roots of *J. ribifolia* were collected in February and March 2011 at the rural area of Navirai, Mato Grosso do Sul state, Brazil. A voucher specimen (CGMS 31.481) was deposited at the herbarium of the Department of Botany of the University of São Paulo (USP), Brazil.

### Essential oil isolation

The stem roots were subjected to hydrodistillation for 5 h using a modified Clevenger-type apparatus. The extraction yield and the physical properties (density, refractive index and optical rotation) of the oil were determined according to the literature.[8]

### GC/FID analysis

Sample analyses (in triplicate) were performed on a HP5890 SERIE II Gas Chromatograph system series equipped with a flame ionization detector (FID) using a fused silica capillary column (DB-5; 30 m × 0.25 mm, film thickness 0.2 μm). Oven temperature was programmed from 50 to 250 °C at a rate of 4 °C $min^{-1}$, with injector and detector temperatures at 230 and 250 °C, respectively. The split ratio was (1:20). The volume injected was 2.0 μL. A $C_7$-$C_{21}$ n-alkanes mixture diluted in n-hexane was prepared for determination of the temperature programmed retention indices. Samples were analyzed in n-hexane solution. Internal standards (n-alkanes) were then added to each sample to aid in the standardization of retention times, and the samples analyzed again. Then, retention indices (RI) for all compounds were determined. The identification of the chemical constituents was based on comparison of their retention indices (RI) and mass spectra with those obtained from authentic samples and/or the Wiley and NBS/NIST libraries and those published by Adams.[9] The quantitative data regarding the volatile constituents were obtained by peak-area normalization using chromatographic method with flame ionization detection (GC-FID) operated under similar conditions to the gas chromatography coupled to mass spectrometry (GC-MS). Compounds with concentrations equal or greater than 0.1% were considered for quantification. Percentage values were the mean of three sample injections.

### Gas chromatography/mass spectrometry analysis

GC-MS analysis was performed on a gas chromatograph coupled to a mass spectrometry (GCMS Thermo-Finnigan, Focus DSQ II) with a quadropole mass analyzer, electron impact ionization (70 eV), and autosampler model Triplus. The analysis was carried out using a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Analytical 5.0 grade helium was used as carrier gas at a flow rate of 1.0 mL $min^{-1}$. The inlet was operated in split mode (ratio 1:15) with injection volume of 2.0 μL of the oil diluted in ethyl acetate. The GC temperature program used was 40 °C (1 min) and 4 °C $min^{-1}$ up to 280 °C. The injector, ionization source, and transfer line temperatures were set at 230, 250, and 280 °C, respectively. In the TIC mode operation the mass ranged from 50 to 500 amu. Data acquisition was performed by Software Xcalibur 1.4 SR1. Data analysis was performed by NIST MS Search 2.0 library.

### Chromatographic fractionation

Part of the resulting oil from the roots of *J. ribifolia* (100 mg) was further subjected to repeated preparative thin layer chromatography (PTLC) ($SiO_2$; hexane-EtOAc, 85:15) and five fractions were scraped after development: fraction 1 ($F_1$, $R_f$ 0.85, 11 mg), fraction 2 ($F_2$, $R_f$ 0.66, 7 mg), fraction 3 ($F_3$, $R_f$ 0.58, 8 mg), fraction 4 ($F_4$, $R_f$ 0.41, 5 mg) and fraction 5 ($F_5$, $R_f$ 0.25, 3 mg). All the fractions were obtained and gathered, according to their chromatographic profiles by GC-FID. Detection and fractioning in thin layer chromatography (TLC) was achieved by UV light (254 nm) and by spraying with solutions of 2% of vanillin in EtOH/$H_2SO_4$ (90:10), followed by heating.

### Antiproliferative assay

Cancer cells lines U251 (glioma) MCF-7 (breast), NCI-ADR/RES (drug resistant ovarian), 786-0 (kidney),

NCI-460 (lung), OVCAR-3 (ovarian), HT-29 (colon), K562 (leukemia) and PC-3 (prostate) obtained from the Frederick MA, National Cancer Institute/USA, were grown in RPMI 1640 medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 5% fetal bovine serum (Gibco, EUA) and maintained in a humidified atmosphere at 37 °C in 5% $CO_2$. The medium was changed every 2 days until the cells reached confluence, at which point they were subcultured.

The essential oil from the roots of *J. ribifolia* was evaluated for its activity using a previously described sulforhodamine B (SRB) assay.[10,11] The microtiter plates containing cells were pre-incubated for 24 h at 37 °C to allow stabilizations prior to addition (100 µL) of the crude oil and the fractions. The plates were incubated with the test substance for 48 h at 37 °C and 5% $CO_2$ at four concentrations (0.25, 2.5, 25, and 250 µg $mL^{-1}$) each in triplicate wells. Doxorubicin was used as the positive control at concentrations of 0.025, 0.25, 2.5, and 25.0 µg $mL^{-1}$. The substances tested were initially solubilized in dimethylsufoxide (DMSO) (Sigma). The final concentration of DMSO (0.25% at the higher sample concentration) did not affect the cell viability. The stock solution was diluted with complete medium containing 50.0 µg $mL^{-1}$ of gentamicin (Schering-Plough). The plates were air-dried and protein-bound dye was solubilized and the resulting optical density was read in a multiwell plate reader at 540 nm. The antiproliferative activity is expressed as the concentration of drug inhibiting cell growth by 50% ($GI_{50}$). Growth was determined from non-linear regression analysis using the ORIGIN 8.0 (OriginLab Corporation). These results presented here refer to a representative experiment since all assays were run in triplicate and the average standard error was always < 5%.

## Results and Discussion

Hydrodistillation of *J. ribifolia* roots provided a bluish essential oil with yield of 0.2% (v/m), based on their fresh weight. The physical properties for oil were: $d^{25}$: 0.88; $\eta d_{25}$: 1.57; $[\alpha]_D^{25}$ : −5.8 (in $CHCl_3$, $c$ = 0.019).

The analyses and identification pointed by mass fragmentation pattern and retention index revealed the presence of 49 compounds, representing 91.4% of the total oil, with 39.5% of monoterpenes, 43.0% of sesquiterpenes and 8.5% of phenylpropanoids. The qualitative and quantitative composition of roots essential oil, determined after GC and GC-MS analysis is shown in Table 1, listed in order of their elution on a DB-5 column together with their retention indices. The major compounds of oil were β-pinene (9.2%), isoeugenol methyl ether (8.5%), vatirenene (8.4%), α-gurjunene (7.0%), *endo*-8-hydroxy-cycloisolongifolene (6.6%), α-pinene (6.4%), p-menth-1-en-8-ol (5.2%), canfene (4.4%), tricyclene (3.8%), dehydro-aromadendrene (3.5%), 8-*cis*-5(1H)-

**Table 1.** Percentage composition of the *J. ribifolia* roots essential oil and corresponding PTLC fractions

| Compound[a,b] | RI[c] | RI (lit)[8] | %[d] | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mass spectral data[e] |
|---|---|---|---|---|---|---|---|---|---|
| Tricyclene | 919 | 927 | 3.8 | | | | | | 136 $[M]^+$, 93 |
| α-Pinene | 931 | 939 | 6.4 | | | | | | 136 $[M]^+$, 93 |
| Camphene | 945 | 954 | 4.4 | | | | | | 136 $[M]^+$, 93 |
| β-Pinene | 974 | 979 | 9.2 | | | | | | 136 $[M]^+$, 93 |
| Myrcene | 990 | 991 | 0.5 | | | | | | 136 $[M]^+$, 41 |
| α-Terpinene | 1014 | 1017 | 0.1 | | | | | | 136 $[M]^+$, 121 |
| *p*-Cymene | 1022 | 1025 | 0.1 | | | | | | 134 $[M]^+$, 119 |
| Limonene | 1026 | 1029 | 1.6 | | | | | | 136 $[M]^+$, 68 |
| γ-Terpinene | 1057 | 1060 | 0.3 | | | | | | 136 $[M]^+$, 93 |
| Terpinolene | 1088 | 1089 | 0.4 | | | | | | 136 $[M]^+$, 93 |
| Linalool | 1100 | 1097 | 0.3 | | | | | | 136 $[M]^+$, 71 |
| *Exo*-fenchol | 1112 | 1117 | 0.4 | | | | | | 154 $[M]^+$, 81 |
| Thujone | 1116 | 1114 | 0.1 | | | | | | 152 $[M]^+$, 81 |
| *Trans*-*p*-menth-2,8-dien-1-ol | 1120 | 1123 | 0.2 | | | | | | 152 $[M]^+$, 94 |
| α-Campholenal | 1125 | 1126 | 0.1 | | | | | | 152 $[M]^+$, 108 |
| *Trans*-pinocarveol | 1137 | 1139 | 0.1 | 1.4 | | | | | 152 $[M]^+$, 92 |
| *Cis*-terpineol | 1139 | 1144 | 0.1 | | 0.8 | | | | 154 $[M]^+$, 43 |
| Camphor | 1142 | 1146 | 0.1 | | | | | | 152 $[M]^+$, 95 |
| Tagetone | 1147 | 1144 | 0.1 | | | | | | 152$[M]^+$, 95 |
| Borneol | 1164 | 1169 | 1.2 | 17.9 | 0.3 | | | | 154 $[M]^+$, 95 |
| *Trans*-β-terpineol | 1165 | 1163 | 2.9 | | | | | | 154 $[M]^+$, 71 |

**Table 1.** Percentage composition of the *J. ribifolia* roots essential oil and corresponding PTLC fractions

| Compound[a,b] | RI[c] | RI (lit)[8] | %[d] | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | Mass spectral data[e] |
|---|---|---|---|---|---|---|---|---|---|
| 3-Pinanone | 1173 | 1167 | 0.1 | | | | | | 152 $[M]^+$, 55 |
| α-Terpineol | 1185 | 1189 | 5.2 | 15.3 | | | | | 154 $[M]^+$, 59 |
| 2,6-Octadienoic acid, 3,7-dimetil-ethyl ester [k] | 1195 | – | 0.4 | | | | | | 196 $[M]^+$, 69 |
| Verbenone | 1209 | 1205 | 0.1 | | | | | | 150 $[M]^+$, 107 |
| Bornyl acetate | 1285 | 1289 | 1.2 | | | | | | 196 $[M]^+$, 951 |
| Methyl geranate | 1296 | 1305 | 0.5 | | | | | 2.2 | 182 $[M]^+$, 69 |
| 9,10-Dehydro-isolongifolene | 1361 | 1390 | 0.1 | | | | | | 202$[M]^+$, 131 |
| β-Patchoulene | 1374 | 1381 | 0.3 | | | | | | 204 $[M]^+$, 161 |
| β-Elemene | 1387 | 1391 | 0.1 | | | | | | 204 $[M]^+$, 67 |
| Cyperene | 1383 | 1399 | 0.1 | | | | | 1.8 | 204 $[M]^+$, 204 |
| Isoledene | 1388 | 1376 | 1.4 | | | | | | 204 $[M]^+$, 161 |
| α-Gurjunene | 1400 | 1410 | 7.0 | | | | | | 204 $[M]^+$, 204 |
| Dehydro aromadendrene | 1460 | 1463 | 3.5 | | 2.1 | | | | 202 $[M]^+$, 145 |
| β-Vatirenene | 1486 | 1489 | 8.4 | | | | | | 202$[M]^+$, 202 |
| Isoeugenol methylether | 1494 | 1495 | 8.5 | 0.7 | 21.2 | 0.4 | | | 178 $[M]^+$, 178 |
| Isolongifolene-5-ol [k] | 1534 | – | 0.6 | 1.4 | 1.4 | | | | 220 $[M]^+$, 161 |
| Spathulenol | 1574 | 1578 | 0.7 | 10.5 | | | | 9.3 | 220 $[M]^+$, 43 |
| *Endo*-8-hydroxy-cycloisolongifolene | 1608 | 1690[f] | 6.6 | | 38.6 | 31.6 | | 21.3 | 220 $[M]^+$, 159 |
| Cedrol | 1609 | 1601 | 0.2 | | | | 4.8 | | 222 $[M]^+$, 95 |
| 8-*cis*-5(1H)-azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene) | 1622 | 1692[g] | 3.4 | 1.2 | 11.2 | | 23.1 | 17.1 | 218 $[M]^+$, 218 |
| 3-Iso-thujopsanone | 1645 | 1643 | 1.3 | | | | | | 220 $[M]^+$, 123 |
| 3-Thujopsanone | 1661 | 1655 | 1.4 | | | 9.1 | 19.1 | | 220 $[M]^+$, 123 |
| 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2-ol | 1696 | 1678[f] | 1.2 | 3.7 | | 12.2 | | 13.0 | 220 $[M]^+$, 159 |
| 2,2,7,7-Tetramethyltricyclo [6.2.1.0(1,6)]undec-4-en-3-one | 1713 | 1730[h] | 0.5 | 1.6 | 11.4 | | 7.3 | 4.6 | 218 $[M]^+$, 175 |
| 2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) | 1732 | 1721[i] | 1.2 | 7.8 | | 3.8 | | | 218$[M]^+$, 175 |
| 7-Isopropenyl-1,4a-dimethyl-3-oxo-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl- ethyl ester[k] | 1740 | – | 1.8 | 1.4 | | 9.9 | 4.5 | 1.2 | 276$[M]^+$, 175 |
| 8-Oxo-9H-cycloisolongifolene[k] | 1765 | – | 2.1 | 1.9 | | 3.3 | 21.4 | | 218 $[M]^+$, 175 |
| Aromandendrene oxide-1 | 1782 | 1775 | 0.1 | 2.7 | | | | | 220 $[M]^+$, 41 |
| Methyl hinokiate | 1827 | 1865[j] | 1.2 | 17.8 | | | | 3.6 | 248 $[M]^+$, 123 |
| Monoterpene hydrocarbons | | | 26.8 | – | – | – | – | – | |
| Oxygenated monoterpenes | | | 12.7 | 34.6 | 1.1 | – | – | 2.2 | |
| Sesquiterpene hydrocarbons | | | 20.9 | – | 2.1 | – | – | 1.8 | |
| Ogygenated sesquiterpenes | | | 22.1 | 50.0 | 62.6 | 69.9 | 80.2 | 70.1 | |
| Phenylpropanoids | | | 8.5 | 0.7 | 21.2 | 0.4 | – | – | |
| Others | | | 0.4 | – | – | – | – | – | |
| Total identified | | | 91.4 | 85.3 | 87.0 | 70.3 | 80.2 | 74.1 | |

[a]Compounds listed in order of elution from a DB-5 column; [b]identification: RI, retention indices, GC-MS, gas chromatography-mass spectroscopy; [c]component concentrations were calculated from GC-FID peak areas in the order of DB-5 column elution; [d]programmed temperature retention indices determined on apolar DB-5 column (50-250 °C; 4 °C $min^{-1}$); [e]molecular ion $[M]^+$ and major fragment obtained from GC-MS analyses. Comparison of experimental retention indices and mass spectra data with literature: [f]Chen *et al.*;[18] [g]Griffin *et al.*;[19] [h]Yu *et al.*;[20] [i]Nibret and Wink;[21] [j]Chen-Xing *et al.*;[22] [k]tentative identification.

azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene) (3.4%) and p-menth-1-en-4-ol (2.9%).

Volatile compounds identified from PTLC essential oil fractions are shown in Table 1. $F_1$ yielded an essential oil free of monoterpene hydrocarbons, with oxygenated monoterpenes borneol (17.9%) and p-menth-1-en-8-ol (15.3%), and sesquiterpenes sphatulenol (10.5%) and methyl hinokiate (17.8%), as major constituents. In $F_2$ it was observed the presence of three major components, phenylpropanoid isoeugenol methyl ether (21.2%), and sesquiterpenes 8-*cis*-5(1H)-azulenone,2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene) (11.2%)

and 8-hydroxy- cycloisolongifolene (38.6%). In $F_3$, four sesquiterpenes were detected, 8-hydroxy-cycloisolongifolene (32.0%), thujopsanone (9.1%), 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol (12.2%), and 7-isoprenyl-1,4a-dimethyl-3-oxo-2,3,4,4a,5,6,7,8-octahydronaphtalen-2-yl ethyl ester (9.9%). An enhanced content of oxygenated sesquiterpenes was possible by fractionation, rising to 80% in $F_4$. In $F_4$, the three major components were 8-*cis*-5(1H)-azulenone,2,4,6,7,8,8a-hexahydro-3,8-methyl-4-(1-methylethylidene) (23.1%), thujopsanone (19.1%), and 8-oxo-9H-cycloisolongifolene (21.4%). In $F_5$, sesquiterpenes *endo*-8-hydroxy-cycloisolongifolene (21.3%), 8-*cis*-5(1H)-azulenone,2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene) (17.1%), and 6-isoprenil-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphtalen-2-ol (13.0%) were the main components.

Therefore, the oil of *J. ribifolia* and its fractions were tested for their cell growth inhibitory effect on nine neoplasic cells. The enrichment of sesquiterpenes through fractionation by TLC fractionation resulted in changes in antitumoral activity, and for some cell lines, the activity of the fractions was much higher than that of crude oil. It is likely that the increase of concentration of the compounds (especially the action of oxygenated monoterpenes and sesquiterpenes) is responsible for this increase in the activity.

The antiproliferative activity was screened using the methodology described by Developmental Therapeutics Program NCI/NIH.[11,23] This methodology aims the evaluation of a sample in many different tumor cell lines in order to evidence an antiproliferative profile of the selected sample. In order to prioritize further chemical evaluations, a threshold for $GI_{50}$ values was assumed following the literature ($GI_{50} \leq 30$ μg mL$^{-1}$).[12,13] The essential oil induced a concentration dependent inhibitory effect on all cell lines tested in the afore mentioned dilution range. The $GI_{50}$ values of the oil and fractions are summarized in Table 2. The essential oil showed more activity against NCI-H640 (6.2 μg mL$^{-1}$) and OVCAR-3 (8.0 μg mL$^{-1}$) cancer cells. However, for some cancer cells, the effect of isolated fractions were more pronounced than the essential oil indicating a possible role of synergism between the different essential oil components.

According to the results, it can be seen that the fractionation of the root essential oil of *J. ribifolia* was effective in increasing antiproliferative activity. $F_1$ showed the best results with lower $GI_{50}$ values than those of crude essential oil, improving activity against eight cell lines. In the case of lines U251, MCF-7, NCI-ADR/RES, 786-0 and HT-29, the oil $GI_{50}$ values were 25 μg mL$^{-1}$, whereas for fractions a reduction was observed. The best overall result found was the action of $F_1$ against the tumor cell lines NCI-ADR-RES ($GI_{50}$ = 1.8 μg mL$^{-1}$), OVCAR-3 (0.51 μg mL$^{-1}$) and PC-3 (< 0.25 μg mL$^{-1}$). In this fraction, the main difference to the original essential oil is the absence of monoterpene hydrocarbons and the content enrichment of oxygenated monoterpenes (borneol and p-menth-1-en-8-ol), and sesquiterpenes sphatulenol and methyl hinokiate. $F_2$ also improved the activity when compared to the oil. The cell lines NCI-ADR/RES ($GI_{50}$ = 0.45 μg mL$^{-1}$), PC-3 (< 0.25 μg mL$^{-1}$) and K562 (1.0 μg mL$^{-1}$) were more sensitive to $F_2$. In the case of fractions $F_3$, $F_4$ and $F_5$, the PC-3 tumor cell was more sensitive, with $GI_{50}$ of 0.88, 0.25 and 0.57 μg mL$^{-1}$, respectively. The U251 cell was the most resistant to the action of the essential oil and fractions. Only $F_2$ was able to induce changes in cell growth of this lineage, reducing the $GI_{50}$ value of 25.0 to 4.2 μg mL$^{-1}$. This fact demonstrates a possible sensitivity of this lineage to isoeugenol methyl ether and oxygenated sesquiterpenes.

Previously it has been shown that some chemical constituents act in an additive way to account for the observed pharmacological effects of essential oils, demonstrating the synergistic effect. Synergism has emerged as a research activity and the comparatively

**Table 2.** Antiproliferative activity [$GI_{50}$ (μg mL$^{-1}$)] of *J. ribifolia* essential oil and corresponding PTLC fractions on culture cell lines

| Sample | U251 | MCF-7 | NCI-ADR/RES | 786-0 | NCI-H460 | OVCAR-3 | HT-29 | K562 | PC-3 |
|---|---|---|---|---|---|---|---|---|---|
| O | 25.0 ± 2.8 | 25.0 ± 2.3 | 25.0 ± 2.5 | 25.0 ± 2.7 | 6.2 ± 0.6 | 8.0 ± 1.0 | 25.0 ± 3.1 | 18.3 ± 1.1 | 12.8 ± 1.5 |
| $F_1$ | 25.0 ± 3.4 | 2.5 ± 0.02 | 1.8 ± 0.2 | 3.1 ± 0.7 | 4.5 ± 0.4 | 0.51 ± 0.05 | 3.3 ± 0.4 | 2.7 ± 0.3 | < 0.25 |
| $F_2$ | 4.2 ± 0.04 | 1.0 ± 0.02 | 0.45 ± 0.08 | 3.6 ± 0.3 | 6.5 ± 0.6 | 10.3 ± 1.1 | 6.1 ± 0.2 | 1.0 ± 0.3 | < 0.25 |
| $F_3$ | 25.0 ± 1.9 | 3.9 ± 0.3 | 25.0 ± 2.8 | 25.0 ± 3.0 | 25.0 ± 3.6 | 2.1 ± 0.06 | 25.0 ± 2.0 | 11.3 ± 1.2 | 0.88 ± 0.06 |
| $F_4$ | 25.0 ± 2.4 | 7.8 ± 1.4 | 3.8 ± 0.8 | 25.0 ± 2.5 | 25.0 ± 3.4 | 4.7 ± 0.4 | 25.0 ± 1.9 | 7.9 ± 0.8 | 0.25 ± 0.03 |
| $F_5$ | 25.0 ± 3.1 | 25.0 ± 3.7 | 3.0 ± 0.4 | 25.0 ± 3.7 | 25.0 ± 2.8 | 5.6 ± 0.7 | 25.0 ± 2.7 | 5.8 ± 0.7 | 0.57 ± 0.08 |
| Doxo[a] | 0.025 | < 0.25 | 0.19 | 0.025 | < 0.25 | 0.031 | 0.12 | 0.37 | < 0.25 |

[a]Positive control (doxorubicin); O: essential oil of *J. ribifolia.*

stronger pharmacological effects of different constituents in mixed state than in individual state are well explained by synergism.[14] For instance, the cytotoxicity of the essential oil of *Rosmarinus officinalis* L. against the human tumour cell lines including human ovarian cancer cell lines (SK-OV3, HO-8910) and human hepatocellular liver carcinoma cell line (Bel-7402) shows a probable synergistic effect.[15] The potent cytotoxic effect of essential oil of *Guatteria pogonopus* and *Senecio graciliflorus* is also attributed to the additive/synergistic effects of its main constituents.[16,17] Therefore, the essential oil of *J. ribifolia* roots could be considered as a new potential natural source that exhibits potent cytotoxic effect.

## Conclusions

The present results showed that the essential oil of roots of *J. ribifolia*, here identified for the first time, may have a preventive effect against cancer through the action of its components. This effect could be enhanced by chromatographic fractionation of the oil, leading to fractions displaying antiproliferative activity close to that of the standard doxorubicin.

## Supplementary Information

Supplementary Data (chromatograms of oil and fractions) are available free of charge at http://jbcs.sbq.org.br as a PDF file.

## Acknowledgements

The authors are grateful to FUNDECT, CNPq and CAPES financial support.

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*Submitted: September 16, 2014*
*Published online: November 4, 2014*

**FAPESP has sponsored the publication of this article.**