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Use of GC/MS to identify chemical constituents and cytotoxic activity of the leaves of *Phoradendron mucronatum* and *Phoradendron microphyllum* (Viscaceae)

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ABSTRACT

Phoradendron mucronatum and *P. microphyllum* are plants that found in tropical and subtropical areas, used in traditional medicine and popularly known as mistle-thrush. The aim of this study was to identify the chemical constituents of different leaf extracts from *P. mucronatum* and *P. microphyllum* and assess cytotoxic activity against strains from a human tumour cells. Extracts obtained with hexane, dichloromethane, chloroform and ethyl acetate from the leaves were analysed by gas chromatography coupled with mass spectrometry (GC-MS) and the cytotoxicity was assessed by the MTT method (bromide (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)). The tested human tumour cells were NCI-H292 (human pulmonar mucoepidermoid carcinoma), MCF-7 (human breast adenocarcinoma) and HEp-2 (epidermoid carcinoma of the larynx). Analysis by GC/MS of the extracts from leaves of *P. microphyllum* and *P. mucronatum* detected 51 different compounds, such as alkaloids, diterpenes, triterpenes, sterols, alcohols, aldehydes, fatty acids and hydrocarbons. In the cytotoxic evaluation, hexane and ethyl acetate extracts from the leaves *P. microphyllum* inhibited cell growth of NCI-H292 strains (72.97%) and HEp-2 (87.53%), respectively. The extracts of *P. mucronatum* species showed an inhibitory effect towards NCI-H292 (83.19%/hexane), MCF-7 (88.69%/dichloromethane) and HEp-2 (93.40%/hexane). The extracts showed cytotoxic activity against the tested strains, especially the *P. mucronatum*, which presented the highest percentages of inhibition of cell growth.

Key words: Cytotoxicity, Gas Chromatography, *Phoradendron microphyllum, Phoradendron mucronatum*.

INTRODUCTION

Most current drugs are derived, directly or indirectly from the chemical constituents of higher plants. About 60% of the drugs used for the treatment of cancer have been isolated from vegetable products (Gordaliza 2007).

The main plant-derived compounds with anticancer properties are alkaloids and terpenoids (Gupta et al. 2005). Examples are derivatives of

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etoposide and teniposide lignans (Allen et al. 2003, Choi et al. 2008), vincristine and vinblastine from *Catharanthus roseus* (L.) G. Don (Johnson et al. 1963, Carvalhaes et al. 2002), semi-synthetic derivatives of podophyllotoxin isolated from species of the genus *Podophyllum* (Berberidaceae) (Jacobo-Herrera et al. 2016), taxol, and docetaxel from *Taxus brevifolia* Nutt. (Wani et al. 1971) and semi-synthetic derivatives from camptothecin, irinotecan and topotecan isolated from *Camptotheca acuminata* (Nyssaceae) (Cragg et al. 1993, 1994, Wang 1998).

The analysis of anticancer activity is based on assays that use established cell lines where the toxic effect of plant extracts can be measured. Cytotoxic screening models provide important preliminary data for selecting plant extracts or compounds with antineoplasic properties (Alonso-Castro et al. 2011).

Phoradendron is a genus of the Viscaceae family with approximately 234 species. Mistletoes are plants that occur in tropical and subtropical areas of both hemispheres (Cronquist 1981). Several species have wide use in traditional medicine. For example, *P. carneum* Urb., *P. galeottii* Trel., *P. reichenbachianum* (Seem.) Oliver and *P. serotinum* (Raf.) M.C. Johnst are used to treat dermatological disorders and skin tumors (Alonso-Castro et al. 2011).

Phoratoxinas isolated from *P. tomentosum* manifests cytotoxic activity in different cell lines (Johansson et al. 2003). The methanol extract of the leaves of *P. vernicosum* Greenm. presented activity against nasopharyngeal carcinoma (Caamal-Fuentes et al. 2011). The aqueous extract the leaves of *P. serotinum* (Raf.) MC Johnst. caused toxic effects against breast carcinoma (Jacobo-Salcedo et al. 2011) and ethanol extract shows cytotoxic activity against lung epithelial cells (Alonso-Castro et al. 2012).

The *Phordendron microphyllum* (Pohl ex DC.) Trel and *Phordendron mucronatum* (DC.) Krug & Urb species are popularly known as "herbal" or "bird dung" and can be found as parasite species in the northeastern semiarid region of Brazil (Ferreira et al. 2007).

Scientific reports concerning this species are scarce. The present study then was mounted to identify the chemical constituents of hexane, dichlomethane, chloroform and ethyl acetate extracts from the leaves of *P. mucronatum* and *P. microphyllum* and to evaluate their cytotoxic activity against human tumour cell lines.

MATERIALS AND METHODS

PLANT MATERIAL

The species *P. mucronatum* was collected in the municipality of Buique – Pernambuco, (08°37'23" S 37°09'21'' W). *P. microphyllum* (Pohl ex DC.) Trel (Viscaceae) was collected at Fazenda Canto dos Passaros in Sao Jose do Espinharas, Paraiba, (06°52'56" S 37°17'12" W). Samples of *P. mucronatum* and *P. microphyllum* were identified by curators Rita Pereira and Olivia Cano, respectively, and deposited in the Dárdano of the Andrade Lima Herbarium of the Agricultural Institute of Pernambuco (IPA) under registration numbers 63330 and 87746, respectively.

PREPARATION OF THE EXTRACT

A dry power of the *P. microphyllum* and *P. mucronatum* (30 g) leaves was extracted at room temperature for 30 minutes three times consecutively by maceration in an ultrasonic bath (Uniqu USC - 1400) using solvents with different polarities: hexane (Hx), dichloromethane (DCM), chloroform (CF) and ethyl acetate (EtOAc). Then it was filtered and the crude extracts were obtained after evaporation of the solvents under reduced pressure at 40 °C. Ten mg of the extracts was dissolved in 2 ml of ethyl acetate for analysis by Gas Chromatography coupled to Mass Spectrometry (GC/MS).

GC-MS ANALYSIS

Analysis of compounds from extracts of *P. microphyllum* and *P. mucronatum* was performed at the Research Center of Natural and Synthetic Products, University of São Paulo (USP-Ribeirão Preto), using GC/MS, Shimadzu, model QP 2010. Separation of the chemical constituents was carried out using a DB-5MS column [(5%-Phenyl)-methylpolysiloxane] brand Agilent J&W GC Columns, 30 m long, 0.25 mm internal diameter, thickness of the film 0.25 μm. The carrier gas was helium. The operating conditions of the gas chromatograph were: column internal pressure of 97.4 kPa, column flow of gas at 1.3 ml min^{-1} column, injector temperature 260 °C, detector temperature at the interface (GC/MS) of 290 °C. The initial column temperature was 100 °C for 4 min, followed by an increase of 3 °C min^{-1} up to 300 °C and kept constant for 90 min. The split ratio was 5:1. The mass spectrometer was programmed to perform readings in a range of 50 to 500 Da at intervals of 0.30 s, with ionization energy of 70 eV. One μl of the different extracts was injected (10 mg dissolved in 2 ml ethyl acetate). A mixture of linear hydrocarbons (C9-C20, C21-C40) was injected under the same conditions in order to identify the chemical constituents. The identification of the constituents was performed by analyzing and comparing the mass spectra based on data libraries (FFNSC1.3.lib, WILEY7.LIB, NIST08s.LIB, MY LIBRARY.lib) using a GC/MS instrument, whose índices showed a similarity of greater than or equal to 90%. Relative quantification of the components of each sample was obtained from the relative area of the peaks in the chromatograms.

CYTOTOXIC ACTIVITY

The cytotoxic activity was performed using the MTT method, which consists of a dosage based on the colorimetric conversion of the salt 3-(4,5-dimethyl-2-thiazole)-2,5 diphenyl-2-H bromide tetrazolium (MTT) which moved from a yellow color when insoluble [formazan crystals], moving to purple when precipitated due to the action of succinyl-dehydrogenase enzyme present only in metabolically active cells of mitochondria (Alley et al. 1988, Mosmann 1983). The human tumour cell lines used were NCI-H292 (lung mucoepidermoid carcinoma), MCF-7 (breast adenocarcinoma) and HEp-2 (epidermoid carcinoma of the larynx) obtained from the section of cell cultures of Bank Cell in Rio de Janeiro and maintained in accordance with the protocol established by the Cell Culture Laboratory, Department of Antibiotics of the Federal University of Pernambuco. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% antibiotic solution (penicillin and streptomycin) and *L*-glutamine, grown in culture plates with 96 wells at a concentration of 2 x 10^5 cells/well, incubated at 37 °C in a humid atmosphere (5% CO_2) for 24 hours (Butler and Dawson 1992). Then the extracts Hx, DCM, CF and EtOAc leaves dissolved in dimethylsulfoxide (DMSO) (stock concentration 10 mg/ml) were added to the wells in a single concentration (50 μL/ml) and doxorubicin (5 mg/ml) was used as a positive control. This procedure was performed in triplicate. After 72 hours of plate reincubation, 25 μL of MTT (5 mg/ml) was added to each well. The culture medium with MTT was aspirated, after 3 hours incubation, 100 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured in a spectrophotometer at a wavelength of 540 nm. Cell growth inhibition percentage (CI%) was determined considering the mean of negative control as 100% proliferation. An intensity scale was used to assess the cytotoxic potential of the extracts which were as follows: samples without activity, with little activity (cell growth inhibition ranging from 1 to 50%), with moderate activity (cell growth inhibition ranging

from 50 to 75%) and high activity (growth inhibition ranging 75 to 100%) (Fouche et al. 2008).

RESULTS AND DISCUSSION

Analysis by GC/MS of the Hx, DCM, CF and EtOAc extracts from leaves of *P. microphyllum* and *P. mucronatum* detected 51 constituents (Table I and Table II). These extracts contained a mixture of different compounds such as alkaloids, diterpenes, triterpenes, sterols, alcohols, aldehydes, fatty acids and hydrocarbons.

The constituents that showed relative larger areas (%), from the extracts of *P. microphyllum* leaves, were Lup-20(29)-en-3-one (lupenone, 23.07%) and (3β)-Lup-20(29)-en-3-ol (lupeol, 16.95%) in the Hx. In the DCM and CF extracts were lupeol (18.53% and 16.19%), lupenone (14.47% and 12.99%) and (3β)-Estigmast-5-en-3-ol (β-sitosterol, 9.74% and 9.45%), and *N*,*N*-dimethyl-1H-indole-3-ethanamine (dimetiltriptamine 18.36%) in CF. In the EtOAc extract, the compounds that had higher percentages were lupeol (20.86%) and lupenone (18.69%).

Regarding the extracts of the leaves of *P. mucronatum*, the constituents with the highest percentages were: lupeol (8.33%), triacontane (8.71%), pentacosane (11.37%) in Hx; in DCM, (*Z*)-11-octadecenoic acid (*cis*-vaccenic acid, 7.83%), pentacosane (8.35%); the CF, *cis*-vaccenic acid (8:35%), tetracosane (7.85%) and EtOAc, hexacosane (10.72%), tetratetracontane (8.2%), lupenone (10.21%) and lupeol (22.8%).

Studies with ethanol extract of the aerial parts of *P. greggii* identified and isolated oleanoic acid (Dominguez et al. 1971). Rios et al. (2001) isolated a tetracyclic triterpene (3,4-seco-olean-18-ene-3,28-dioic acid) from the aerial parts of *P. reichenhachianum*. López-Martínez et al. (2013) identified and isolated from the acetone extract of the leaves of *P. brachystachyum* morolic acid as the major component, and β-sitosterol, stigmasterol, triacontanol, squalene, α- and β-amyrin, lupeol, lupenone, betulin, oleanolic aldehyde, betulinic acid, betulonic acid, moronic acid, morolic acid, oleanolic acid.

Some of these compounds, such as β-sitosterol, squalene, lupeol and lupenone were detected in this study, indicating that various constituents are common among the different species of the genus *Phoradendron*. In addition, common constituents in different extracts of *P. microphyllum* and *P. mucronatum* were observed. These are: 7,11,15-trimethyl-3-methylene-1-hexadecene; (2*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol; (2*R*)-2,5,7,8 tetramethyl-2-[(4*R*,8*R*)-4,8,12 trimethyltridecyl]-6-chromanol; 4,14-dimethyl-9,19-cycloergost-24(28)-en-3-yl acetate and stigmast-4-en-3-one.

Consistent with the diversity of compounds detected, extracts from the leaves of *P. microphyllum* and *P. mucronatum* showed cytotoxic activity against strains analyzed (Table III). The percentages of inhibition of cell growth of extracts from *P. microphyllum* front line HEp-2 were 86.94% for DCM, 82.08% and 87.53% for EtOAc. The latter extract showed moderate activity (72.97%) against NCI-H292.

The extract Hx of *P. mucronatum* inhibited cell growth of NCI-H292 and Hep-2 at 83.19% and 93.40% respectively. The DCM extracts showed inhibitory effect on HEp-2 (85.47%) and MCF-7 (88.69%). The CF showed inhibition of 77.75% for NCI-H292, and 91.46% for HEp-2 and EtOAc to extract inhibited Hep-2 line (72.17%).

The cytotoxic activity of the *Phoradendron* genus has been reported. The ethanol and aqueous extracts of *P. crassifolium* (Polh) Eichier showed low toxicity in cultured HeLa cells (human cervix carcinoma) (Abad et al. 1999); the methanol extract of the leaves of *P. vernicosum* Greenm showed cytotoxic activity against cell line nasopharyngeal carcinoma (KB) (Caamal-Fuentes et al. 2011); the aqueous extract of the leaves of *P. serotinum* (Raf.)

TABLE I
Constituents identified in extracts of leaves of *P. mucronatum* by GC/MS with the highest similarity indices or equal to 90%.

RT (min)	MW	Compound	Molecular formula	Relative Amount %			
				Hx (1)	DCM (2)	CF (3)	ACOEt (4)
30.145 (4) 30.155 (1) 31.030 (2.3)	278	7,11,15-Trimethyl-3-methylene-1-hexadecene	$C_{20}H_{38}$	0.15	0.64	0.78	0.56
31.585	278	7,11,15-Trimethyl-3-methylene-1-hexadecene	$C_{20}H_{38}$	-	-	-	0.56
33.145 (4) 33.150 (1) 34.000 (2)	270	Methyl palmitate	$C_{17}H_{34}O_2$	0.42	0.21	-	0.25
35.200 (2) 35.215 (3)	256	Palmitic acid	$C_{16}H_{32}O_2$	-	6.15	7.25	-
38.365	294	Methyl (9*E*,12*E*)-9,12-octadecadienoate	$C_{19}H_{34}O_2$	0.27	-	-	-
38.590	296	Methyl (9*E*)-9-octadecenoate	$C_{19}H_{36}O_2$	-	-	-	0.36
38.600	296	Methyl (11*E*)-11-octadecenoate	$C_{19}H_{36}O_2$	0.55	-	-	-
38.885 (4) 38.910 (1)	296	(2*E*)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	0.35	-	-	0.36
39.315	302	5,8,11,14,17-Icosapentaenoic acid	$C_{20}H_{30}O_2$	0.16	-	-	-
40.395	280	(9*Z*,12*Z*)-9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	-	2.08	2.12	-
40.650	282	(*Z*)-11-Octadecenoic acid	$C_{18}H_{34}O_2$	-	7.83	8.35	-
41.380	284	Stearic acid	$C_{18}H_{36}O_2$	-	0.89	0.94	-
48.195 (1) 48.210 (4) 48.940 (2,3)	292	12-Hydroxy-14-methoxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1H-2-benzoxacyclododecin-1-one	$C_{17}H_{24}O_4$	1.43	4.51	4.37	5.87
55.320 (1,2,4) 56.175 (3)	354	Aspidospermine	$C_{22}H_{30}N_2O_2$	0.52	1.22	1.20	1.30
57.455 (1,4) 58.370 (2,3)	410	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexene	$C_{30}H_{50}$	4.46	3.25	3.51	3.86
57.820 (4) 58.680 (3)	384	Aspidospermidine	$C_{23}H_{32}N_2O_3$	-	-	2.92	2.59
59.630	366	Hexacosane	$C_{26}H_{54}$	-	-	-	10.72

TABLE I (continuation)

RT (min)	MW	Compound	Molecular formula	Relative Amount %			
				Hx (1)	DCM (2)	CF (3)	ACOEt (4)
59.640	352	Pentacosane	$C_{25}H_{52}$	11.37	-	-	-
60.530	338	Tetracosane	$C_{24}H_{50}$	-	-	7.85	-
60.535	352	Pentacosane	$C_{25}H_{52}$	-	8.35	-	-
61.830	394	Octacosane	$C_{28}H_{58}$	0.92	-	-	-
63.995 (4) 64.900 (3)	619	Tetratetracontane	$C_{44}H_{90}$	-	-	6.36	8.02
64.005	422	Triacontane	$C_{30}H_{62}$	8.71	-	-	-
64.205 (4) 64.210 (1) 65.085 (2)	430	((2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-6-chromanol	$C_{29}H_{50}O_2$	0.32	0.23	-	0.52
64.905	366	Hexacosane	$C_{26}H_{54}$	-	7.20	-	-
67.720 (4) 67.735 (1) 68.625 (2.3)	414	(3β)-Stigmast-5-en-3-ol	$C_{29}H_{50}O$	5.85	5.61	5.08	6.48
68.740 (4) 69.655 (2.3)	424	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	-	7.46	7.19	10.21
69.120 (3) 69.125 (2)	410	1-Octacosanol	$C_{28}H_{58}O$	-	0.84	0.75	-
69.315 (4) 69.320 (1) 70.230 (2.3)	426	(3β)-Lup-20(29)-en-3-ol		8.33	5.63	5.59	8.22
69.975 (1.4) 70.885 (3) 70.895 (2)	468	4,14-Dimethyl-9,19-cycloergost-24(28)-en-3-yl acetate	$C_{32}H_{52}O_2$	3.21	2.19	1.95	2.54
70.225 (1) 70.230 (4) 70.885 (3) 71.120 (2)	412	Stigmast-4-en-3-ona	$C_{29}H_{48}O$	5.62	3.97	3.92	5.64
70.970	268	Stearaldehyde	$C_{18}H_{36}O$	0.53	-	-	-

RT: retention time; MW: molecular weight; (1) Hx: hexane extract; (2) DCM: dichloromethane extract; (3) CF: chloroform extract and (4) EtOAc: ethyl acetate extract.

TABLE II
Constituents identified in extracts of leaves of *P. microphyllum* by GC/MS with the highest similarity indices or equal to 90%.

RT (min)	MW	Compound	Molecular formula	Relative Amount %			
				Hx (1)	DCM (2)	CF (3)	AcOEt (4)
28.535 (2) 28.505 (3) 28.660 (4)	188	2-(1H-indol-3-yl)-N,Ndimethylethanamine	$C_{12}H_{16}N_2$	-	8.21	18.36	3.60
30.160 (2,3) 30.165 (4)	278	7,11,15-Trimethyl-3-methylene-1-hexadecene	$C_{20}H_{38}$	-	0.29	0.62	0.39
37.025	328	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	-	-	-	0.33
38.895 (1,2,4) 38.890 (3)	296	(2*E*)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	7.20	6.61	6.33	7.26
49.945	254	Octadecane	$C_{18}H_{38}$	0.56	-	-	-
52.495	268	Nonadecane	$C_{19}H_{40}$	0.60	-	-	-
54.955	394	Octacosane	$C_{28}H_{58}$	1.49	-	-	-
57.325	338	Tetracosane	$C_{24}H_{50}$	1.59	-	-	-
57.460 (1) 57.465 (2,3,4)	410	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene	$C_{30}H_{50}$	2.46	2.73	2.07	2.62
59.625 (1) 59.630 (2,3,4)	408	Nonacosane	$C_{29}H_{60}$	5.32	3.02	3.00	2.85
61.840	310	Docosane	$C_{22}H_{46}$	1.06	-	-	-
63.995	352	Pentacosane	$C_{25}H_{52}$	0.91	-	-	-
64.220 (1,2,3) 64.225 (4)	430	(2*R*)-2,5,7,8-Tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-6-chromanol	$C_{29}H_{50}O_2$	1.16	1.35	1.42	1.20
67.735 (2,3) 67.730 (4)	414	(3β)-Stigmast-5-en-3-ol	$C_{29}H_{50}O$	-	9.74	9.45	8.37
68.730 (1) 68.735 (2) 68.740 (3,4)	424	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	23.07	14.47	12.99	18.69
69.310 (1) 69.320 (2) 69.315 (3) 69.325 (4)	426	(3β)-Lup-20(29)-en-3-ol	$C_{30}H_{50}O$	16.95	18.53	16.19	20.86

TABLE II (continuation)

RT (min)	MW	Compound	Molecular formula	Relative Amount %			
				Hx (1)	DCM (2)	CF (3)	AcOEt (4)
69.985 (2,3) 69.975 (4)	468	4,14-dimethyl-9,19-cycloergost-24(28)-en-3-yl acetate	$C_{32}H_{52}O_2$	-	3.51	2.77	4.06
70.230 (1) 70.235 (2,3,4)	412	Stigmast-4-en-3-one	$C_{29}H_{48}O$	8.72	6.75	5.68	8.53
73.100 (2,3) 73.105 (4)	278	7,11,15-Trimethyl-3-methylene-1-hexadecene	$C_{20}H_{38}$	-	5.17	4.82	4.25

RT: retention time; MW: molecular weight; (1) Hx: hexane extract; (2) DCM: dichloromethane extract; (3) CF: chloroform extract and (4) EtOAc: ethyl acetate extract.

TABLE III

Cell growth inhibition percentage (%) of the extracts from leaves of *Phoradendron microphyllum* and *Phoradendron mucronatum* in three tumor cell lines at a concentration of 50 µg/ml by MTT method after 72 hours incubation.

	% inhibition		
P. mucronatum **Extracts**	**NCI-H292[a]**	**HEp-2**	**MCF-7**
Hx	83.19 ± 3.86	93.40 ± 0.52	58.89 ± 4.53
DCM	62.25 ± 3.72	85.47 ± 2.40	88.69 ± 5.66
CF	77.75 ± 4.50	91.46 ± 0.97	40.93 ± 4.28
EtOAC	36.29 ± 2.99	72.17 ± 5.87	48.38 ± 2.19
Doxorrubicina[b] (5 µg/ml)	89.80 ± 0.32	86.64 ± 3.48	56.19 ± 5.32
DMSO (10 mg/ml)	0.0	0.0	0.0
	% inhibition		
P. microphyllum **Extracts**	**NCI-H292[a]**	**HEp-2**	**MCF-7**
Hx	0	37.79 ± 0.50	44.86 ± 5.26
DCM	38.48 ± 3.77	86.94 ± 2.23	52.54 ± 0.77
CF	62.98 ± 6.14	82.08 ± 8.43	58.11 ± 5.22
EtOAC	72.97 ± 7.41	87.53 ± 2.38	57.52 ± 5.87
Doxorrubicina[b] (5 µg/ml)	89.80 ± 0.32	86.64 ± 3.48	56.19 ± 5.32
DMSO (10 mg/ml)	0.0	0.0	0.0

[a]Human tumor cells lines: NCI-H292 (human pulmonary mucoepidermoid carcinoma); MCF-7 (human breast carcinoma) and HEp-2 (human larynx carcinoma); [b]Positive control (Doxorrubicine - 5 µg/ml); Hx: hexane extract; DCM: dichloromethane extract; CF: chloroform extract; EtOAc: ethyl acetate extract; DMSO: dimethylsulfoxide (Negative control – 10 mg/ml).

MC Johnst exerted toxic effects against cancer cells MCF-7 (adenocarcinoma human breast) (Jacobo-Salcedo et al. 2011); and the ethanol extract showed cytotoxic activity against TC-1 cells (derived from lung epithelium) (Alonso-Castro et al. 2012).

The percentage of inhibition of cell growth in different extracts may be related to the presence of certain groups of compounds such as alkaloids and triterpenes, known for their anticancer properties (Alonso-Castro et al. 2012).

The lupeol has been extensively studied, particularly in research involving the discovery of antitumor compounds (Saleem 2009). Some investigations have been carried out to evaluate the cytotoxic effects of lupeol on tumour cell lines such as Vero (African Green Monkey kidney), B16F10 (low wall melanoma) and HEp-2 (Badami et al. 2003).

The extracts (Hx, DCM, CF and EtOAc) of *P. microphyllum* and *P. mucronatum* showed cell percentages inhibition above 70% in the HEp-2 strain. It was observed that the lupeol was present in all extracts, but in higher percentages in the extracts of *P. microphyllum*.

Indole alkaloids were found in the two species under study. In the DCM, CF and EtOAc extracts of *P. microphyllum* have 2-(1H-indol-3-yl)-*N*,*N*-dimethylethanamine, also called Dimetiltriptamine (DMT). In Hx, DCM, CF and EtOAc extracts from *P. mucronatum* Aspidospermine was found and Aspidospermidine in the latter two extracts.

Alkaloids with amine groups and indole rings have been studied and various activities have been found, among them: antitumor, antiviral, antifungal and anti-inflammatory (Mcnulty et al. 2007, Griffin et al. 2007, Bao et al. 2004, Dassonneville et al. 2000, Xu et al. 2006).

Other research has been carried out with the purpose of showing that the alkaloids have a significant antiproliferative activity for solid tumours. These compounds have also selective cytotoxicity, a fact that stimulates a better investigation of their anticarcinogenic activity (Zhang et al. 2007, Wang et al. 2005).

Any of the extracts as well as the positive control (doxorubicin) presented an inhibition percentage above 75% (considered with high activity) against the MCF-7 line, except the DCM extract of the leaves of *P. mucronatum*. It is known that the breast cancer MCF-7 cells are resistant to chemotherapy, has low susceptibility to conventional drugs such as doxorubicin and cisplatin. This effect can be attributed to aberrant apoptotic pathway (Del Bufalo et al. 2002, Wesierska-Gadek et al. 2003).

CONCLUSIONS

Analysis by GC/MS allowed detection of 51 constituents in extracts of leaves of *P. microphyllum* and *P. mucronatum*. The constituents with higher percentages were lupenone (23.07%), lupeol (18.53%) and dimethyltryptamine (18.36%) for *P. microphyllum* and pentacosane (11.37%), hexacosane (10.72%) and lupenone (10.21%) for *P. mucronatum*. The different extracts of *P. microphyllum* showed cytotoxic activity against cell lines NCI-H292 and Hep-2, while the extracts of the species *P. mucronatum* showed inhibitory action towards the cell lines of NCI-H292, MCF-7, HEp-2. The major cell growth inhibition percentages were observed in the species *P. mucronatum* possibly due to the wide variety of constituents detected.

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