Journal of Medicinal Plants Research Vol. 7(9), pp. 504-508, 3 March, 2013
Available online at http://www.academicjournals.org/JMPR
DOI:10.5897/JMPR012.1113
ISSN 1996-0875 ©2013 Academic Journals

Full Length Research Paper

Intraspecific variation and cytotoxic evaluation of the essential oils from *Oxandra sessiliflora* R. E. Fries

Armenio A. de Carvalho A. da Silva[1], Elcilene A. de Sousa[1], Alisson L. Matsuo[2], João Henrique G. Lago[3] and Mariana H. Chaves[1]*

[1]Department of Chemistry, Universidade Federal do Piauí, Piauí, Brazil.
[2]Discipline of Cell Biology, Department of Micro, Immuno and Parasitology, Universidade Federal de São Paulo, São Paulo, Brazil.
[3]Institute of Environmental, Chemical and Pharmaceutical Sciences, Universidade Federal de São Paulo, São Paulo, Brazil.

Accepted 26 November, 2012

For the first time, the chemical composition and cytotoxic evaluation of essential oils from leaves of *Oxandra sessiliflora* R. E. Fries (Annonaceae) has been reported. Leaves of *O. sessiflora* were collected in four different periods during the years 2010 (September and December) and 2011 (March and June). The essential oils, obtained by hydrodistillation procedures, were analyzed by Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS). Chemically, the predominance of hydrocarbon sesquiterpenes was detected (57.54 to 75.06%), with germacrene D (17.12 to 32.20%) and biclyclogermacrene (7.95 to 18.91%) as the main compounds. These oils were also composed of monoterpenes (9.65 to 17.02%) and oxygenated sesquiterpenes (4.42 to 11.43%). Additionally, the essential oils obtained from leaves collected in September, 2010 and March, 2011 were tested *in vitro* against murine (B16F10-Nex2) and human melanoma (A2058), breast adenocarcinoma (MCF7), leukemia (HL-60), and cervical carcinoma (HeLa) cell lines. Since values of IC_{50} to HL-60 cells were determined as approximately 6 µg/mL in both tested oils, the results suggested that the essential oils and their compounds could be used as prototypes for the development of new medicines for the treatment of human leukemia.

Key words: *Oxandra sessiliflora*, essential oil composition, intraspecific variation, *in vitro* cytotoxic potential.

INTRODUCTION

Oxandra is a genus from Annonaceae which includes twenty-two species of trees from tropical regions of South America (Lobão et al., 2005). In Brazil were found 14 species, mainly in the Amazon, Cerrado, and Atlantic Forest (Maas et al., 2010). Despite the occurrence of these species in the Brazilian biomes, there are few articles which report phytochemical and pharmacological studies on *Oxandra*. Alkaloids, triterpenes, monoterpenes, and steroids were isolated from *O. xylopioides* (El-Shanawany, 1985; El-Shanawany et al., 1985; Zhang et al., 1987; Arango et al., 1987a, b; Guinaudeau et al., 1988), which displayed anti-inflammatory and antioxidant activities, while anti-leishmanial and anti-chagasic monoterpenes were obtained from *O. espintana* (Hocquemiller et al., 1991). Alkaloids, sesquiterpenes and triterpenes were isolated from *O. asbeckii* but no pharmacological aspects were described (Tinto et al., 1992).

*Corresponding author. E-mail: mariana@ufpi.edu.br.

O. sessiliflora R.E. Fries is known in Brazil with vernacular name “conduru-preto” and has been geographically described in the Amazonian and Cerrado regions, mainly in the Pará, Amazonas, Tocantins, Maranhão, Ceará, and Piauí States (Abreu and Castro, 2004; Mesquita and Castro, 2007). Since there are no previous phytochemical or pharmacological investigations carried out on this species, and based on the course of our studies with volatiles from Brazilian species (Sartorelli et al., 2012; Santana et al., 2012), this study reports the chemical composition and the intraspecific variation of the essential oils from leaves of *O. sessiliflora* R. E. Fries (Annonaceae). Additionally, the *in vitro* cytotoxic potential of crude oils was evaluated against murine and human cell lines.

MATERIALS AND METHODS

Collection of samples

Leaves of *O. sessiliflora* were collected at Parque Ambiental de Teresina, Piaui State, Brazil on the 15th September and December, 2010 as well as 15th March and June, 2011. The voucher specimen has been deposited at Herbario Graziela Barroso do Amaral (UFPI) under number TEPB 27870.

Extraction of the essential oils

To each collection, fresh leaves (300 g) were subjected to hydrodistillation in a clevenger type apparatus for 4 h. After extraction, the essential oils were dried over anhydrous Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure (100 mmHg). Chemical analyses of the obtained crude oils were performed immediately.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses

GC analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with a DB-5 column (30 m × 0.25 mm internal diameter and 0.1 µm film thickness), using helium as carrier gas. Temperature programming was performed as follows: 60 to 240°C at 3°C/min. The injector and interface temperatures were established as 220 and 240°C, respectively. Component concentrations were calculated from electronic integration of Flame ionization detector (FID) peak areas without the use of response factor correction. GC-MS analyses were carried out in a Shimadzu GC-17A chromatograph interfaced with a MS-QP-5050A mass spectrometer, using the same conditions described above. The MS operating conditions were: ionization voltage 70 eV ion source at 230°C.

Compounds identification

The essential oils were obtained by steam distillation and analyzed by GC and GC-MS. The identification of the components was based on the comparison of their mass spectra with those in the spectrometer data base (Willey 229 library) and confirmed by determination of their Kovats indexes which were determined relatively to the retention times of a series of n-alkanes (Adams, 2008).

Cell lines

The murine melanoma cell line B16F10 was originally obtained from the Ludwig Institute for Cancer Research (São Paulo, Brazil). The melanotic B16F10-Nex2 subline, characterized at the Experimental Oncology Unit (UNIFESP - Federal University of São Paulo), was characterized by low immunogenicity and moderate virulence. Human melanoma cell line (A2058), breast adenocarcinoma (MCF7) and leukemia (HL-60) cell lines were obtained from the Ludwig Institute for Cancer Research. Human cervical carcinoma (HeLa) was acquired from Dr. Hugo Pequeno Monteiro, UNIFESP. All cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2, in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10 mM N-2-hydroxyethylpiperazine-N_2-ethanesulfonic acid (Hepes) (Sigma, St. Louis, MO), 24 mM sodium bicarbonate (Sigma), 40 mg/L gentamycin (Schering-Plough, São Paulo, Brazil), pH 7.2, and 10% fetal calf serum (Invitrogen).

In vitro cytotoxic activity

The essential oils extracted from *O. sessiliflora* were re-suspended in dimethyl sulfoxide (DMSO) at final concentration of 10 mg/mL, diluted in RPMI medium containing 10% fetal calf serum ranging from 100 to 0 µg/mL and incubated with 1×10^4 cells in a 96-well plate. After 24 h of incubation, cell viability was measured using the Cell Proliferation Kit I (MTT) (SIGMA), an MTT-based colorimetric assay (Mosmann, 1983). Readings were made in a plate reader at 570 nm, with a reference of 650 nm. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

The chemical composition and the relative percentages of constituents identified in the essential oils obtained from leaves of *O. sessiliflora* in September and December, 2010 (Samples A and B) and March and July, 2011 (Samples C and D) are shown in Table 1. This table also shows the yields of each essential oil, which were calculated on basis of weight of fresh leaves. Comparatively, the essential oil from leaves of *O. lanceolata* from Cuba (Pino et al., 2005) was previously analyzed and the yield of this oil was calculated as 0.7%, higher than that found in *O. sessiliflora* (Samples A: 0.25%, B: 0.28%, C: 0.23%, and D: 0.20%).

The identification of compounds was carried out by interpretation of mass spectra and also by determination of their respective Kovats indexes (Adams, 2008). Chemically, the essential oils from leaves of *O. sessiliflora* was shown to be composed of thirty-three derivatives corresponding to approximately 90% of the total identified volatiles. The oils from December, 2010 (sample B), March and June, 2011 (samples C and D) were composed by a higher diversification of compounds

Table 1. Chemical composition of essential oils from leaves of *O. sessiliflora* in different periods of collections (Samples A to D).

IK	Constituents	Content (%)			
		Sample A	Sample B	Sample C	Sample D
930	α-Pinene	2.21	4.27	1.84	2.76
974	β-Pinene	1.82	4.07	1.80	3.05
975	Sabinene	0.79	-	-	-
990	Myrcene	-	1.05	0.48	0.98
1004	α-Phellandrene	2.12	-	0.37	0.83
1020	*p*-Cymene	-	-	0.69	-
1021	*o*-Cymene	-	2.03	-	1.07
1025	β-Phellandrene	10.08	-	4.47	-
1026	Limonene	-	7.21	-	9.17
1032	*Cis*-β-Ocymene	-	-	-	0.42
1334	δ-Elemene	8.40	10.59	7.42	7.63
1344	α-Cubebene	-	0.47	0.34	0.18
1368	α-Copaene	-	0.90	0.70	0.29
1385	β-Cubebene	-	1.19	0.99	0.44
1386	β-Elemene	1.37	2.82	1.67	1.42
1410	β-Caryophyllene	6.42	9.81	8.08	5.83
1427	Aromadendrene	-	0.63	-	0.31
1442	α-Humulene	1.05	1.52	1.27	0.89
1474	Germacrene D	31.29	17.12	32.20	25.56
1488	Bicyclogermacrene	18.91	7.95	16.72	16.55
1494	Germacrene A	1.04	0.93	1.23	0.85
1502	Valencene	-	-	-	0.20
1505	α-Amorphene	-	0.46	-	-
1517	δ-Cadinene	-	0.52	0.94	0.85
1543	Germacrene B	2.46	2.63	3.50	3.19
1549	Elemol	0.81	-	-	-
1566	Caryophyllene oxide	-	-	-	0.41
1569	Spathulenol	3.61	9.82	3.26	2.20
1571	Globulol	-	-	0.37	-
1577	Viridiflorol	-	-	0.23	-
1581	Guaiol	-	-	0.20	-
1637	δ-Cadinol	-	0.98	0.62	1.37
1645	Torreyol	-	0.63	1.02	0.90
	Essential oil yield/(%)	0.25	0.28	0.23	0.20
	Monoterpenes	17.02	18.63	9.65	18.28
	Sesquiterpenes	75.36	68.97	82.76	69.07
	Hydrocarbon	70.94	57.54	75.06	64.19
	Oxygenated	4.42	11.43	5.70	4.88
	Total	92.38	87.60	90.41	87.35

Sample A: September, 2010; Sample B: December, 2010; Sample C: March, 2011; Sample D: June, 2011.
IK: Kovats retention index

(22 to 25 derivatives identified in each oil) while only 15 compounds were detected in the oil from September, 2010. The characterized compounds were grouped in two main classes of volatiles: monoterpenes (9.65 to 18.63%) and sesquiterpenes (68.97 to 82.76%), being the amount of hydrocarbon (57.54 to 75.06%) higher than the oxygenated C_{15} derivatives (4.42 to 11.43%). Detected qualitative and quantitative variations on the chemical

Table 2. IC_{50} (µg/mL) values of crude essential oil from leaves of *O. sessiliflora* obtained in March and September collections against cell lines.

Cell Lines	IC_{50} (µg/mL) essential oils from *O. sessiliflora*	
	Sample A	**Sample C**
B16F10-Nex2	67.5±3.4	46.5±2.3
A2058	> 100	79.8±4.1
HeLa	52.4±2.6	47.4±2.4
MCF7	64.8±3.2	47.4±2.1
HL-60	6.3±0.3	6.2±0.2

Cell lines: B16F10-Nex2: murine melanoma; A2058: human melanoma; MCF7: breast adenocarcinoma, HL-60: leukemia, and HeLa: cervical carcinoma.

constituents of essential oils during the four periods of study are presented in Table 1. The main compounds found in all collections were the hydrocarbon sesquiterpenes δ-elemene (7.42 to 10.59%), β-caryophyllene (5.83 to 9.81%), germacrene D (17.12 to 32.20%), and biclyclogermacrene (7.95 to 18.91%). Beside these compounds, only α- and β-pinenes, β-elemene, α-humulene, germacrenes A and B, and spathulenol were found in the four analyzed oils. Quantitatively, only a small variation was detected in the proportions of δ-elemene, β-elemene, germacrene A, and germacrene B, while the relative amount of β-caryophyllene was higher in December, 2010 (sample B, 9.81%) and March, 2011 (Sample C, 8.08%) in comparison to the other collections (samples A and D, 6.42% and 5.83, respectively).

Similarly, in December, 2010 (sample B), the amounts of δ-elemene (10.59%) and spathulenol (9.82%) were higher than the other periods of collection. Otherwise, in December, 2010 (sample B) the amounts of main derivatives germacrene D (17.12%) and bicycle-germacrene (7.95%) were lower than the other periods of collection. Moreover, the monoterpenes were detected in reduced percentage in the studied oils but with an expressive variation. α- and β-pinenes were detected in the four samples with relative amounts of approximately 2%, except in sample B, where the proportions of both of these compounds were approximately 4%. Otherwise, the amount of β-phelandrene was 10.08% in September, 2010 and 4.47% in March, 2011, being not detected in the other collections. Similarly, limonene was found only in December, 2010 (7.21%) and June, 2011 (9.17%). Based on these evidences, further investigations should be carried out to investigate the influences of some environmental (Gobbo-Neto and Lopes, 2007) and/or microclimatic (Lago et al., 2006) factors in the production/ accumulation of volatiles in *O. sessiliflora*. Finally, based on some evidences that essential oils showed *in vitro* cytotoxicity effects (Santana et al., 2012), the obtained crude oil from leaves of *O. sessiliflora* (Samples A and C) were evaluated against five different tumoral cell lineages. Dose-response curves were generated and IC_{50} values were calculated for this active oil against five cell lines (B16F10-Nex2, A2058, HeLa, MCF7 and HL-60), as shown in Table 2.

Human leukemia cells (HL-60) was the most sensitive to both tested oils, with similar IC_{50} found (approximately 6 µg/mL) B16F10-Nex2, MCF7 and HeLa cells showed moderate potentials with IC_{50} ranging from 46.5 ± 2.3 to 67.5 ± 3.4 µg/mL while A2058 cells was less sensitive with IC_{50} of 79.8 ± 4.1 µg/mL to the oils from sample D and inactive to the oil from sample A. Thus, based on the previous information, the values of IC_{50} lower than 30 µg/mL indicated a promising extract or essential oil (Suffness and Pezzutto, 1991), both oils from samples A and C showed an excellent activity against HL-60. It is important to mention that the different values of IC_{50} observed in the analyzed oils could be directly associated with the presence of different compounds in each oil (Lahlou, 2004) which could be acting individually or synergistically.

ACKNOWLEDGMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support.

REFERENCES

Abreu MC, Castro AAJF (2004). Estudo quantitativo de manchas remanescentes de Cerrado no Parque Ambiental de Teresina, Teresina, Piauí. Publ. Avulsas Ciênc. Amb. 9:1-14.

Adams RP (2008). Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed, Allured Publishig Corporation: Illinois. 804 p.

Arango GJ, Cortes D, Cassels BK, Cave A, Merienne C (1987a). Alkaloids of the Annonaceae. Part 80. Azafluorenones from *Oxandra* cf. *major* and biogenetic considerations. Phytochemistry. 26:2093-2098.

Arango G, Cortes D, Cave A (1987b). Alkaloids of the Annonaceae. Part 74. Three bis-dehydroaporphines from *Oxandra* cf. *major*. Phytochemistry. 26:1227-1229.

El-Shanawany MA (1985). A new alkaloid from *Oxandra xylopioides* Diels. Bull. Pharm. Sci. 8:127-143.

El-Shanawany MA, Slatkin DJ, Schiff PL, El-Shabrawy A (1985). Oxylopidine and oxylopinine. New alkaloids from *Oxandra xylopioides* Diels. Bull. Pharm. Sci. 8:172-191.

Gobbo-Neto L, Lopes NP (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim. Nova. 30:374-381.

Guinaudeau H, Leboeuf M, Cavé A (1988) Aporphinoid alkaloids, IV. J. Nat. Prod. 51:389-474.

Hocquemiller R, Cortes D, Arango GJ, Myint SH, Cavé A (1991). Isolement et synthese de l'espintanol, nouveau monoterpene antiparasitaire. J. Nat. Prod. 54:445-452.

Lago JHG, Favero OA, Romoff P. (2006). Microclimatic factors and phenology influences in the chemical composition of the essential oils from *Pittosporum undulatum* Vent. leaves. J. Braz. Chem. Soc.

17:1334-1338.
Lahlou M (2004). Methods to study the phytochemistry and bioactivity of essential oils. Phytother. Res. 18:435-448.
Lobão AQ, Araujo DSD, Kurtz BC (2005). Annonaceae das restingas do estado do Rio de Janeiro, Brasil. Rodriguésia. 56:85-96.
Maas P, Rainer H, Lobão A (2010) Annonaceae in Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro (http://floradobrasil.jbrj.gov.br/2010/FB110491).
Mesquita MR, Castro AAJF (2007). Florística e fitossociologia de uma área de Cerrado Marginal (Cerrado Baixo), Parque Nacional de Sete Cidades, Piauí. Publ. Avulsas Conserv. Ecossist. 15:1-22.
Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65:55-63.
Pino JA, Marbot R, Payo A, Chao D, Herrera P, Marti MP (2005). Leaf oil of *Oxandra lanceolata* (Sw.) Baill. from Cuba. J. Essent. Oil Res. 17:567-568.
Santana JS, Sartorelli P, Guadagnin RC, Matsuo AL, Figueiredo CR, Soares MG, Lago JHG, Silva AM (2012). Essential oil from *Schinus terebinthifolius* leaves chemical composition and *in vitro* cytotoxicity evaluation. Pharm. Biol. 50:1248-1253.
Sartorelli P, Santana JS, Guadagnin RC, Pinto EG, Tempone A, Stefani HÁ, Soares MG, Silva AM, Lago JHG (2012). *In vitro* trypanocidal evaluatioin of pinane derivatives from essential oils of ripe fruits from *Schinus terebinthifolius* Raddi (Anacardiaceae). Quim. Nova. 35:743-747.
Suffness M, Pezzuto JM (1991). Assay Related to Cancer Drug Discovery. In: Hostettmann, K., Ed., Methods in Plant Biochemistry Assays for Bioactivity Vol. 6, Academic Press, USA. Pp. 71 – 133.
Tinto WF, Blair LC, Reynolds WF, McLean S (1992). Terpenoid constituents of *Oxandra asbeckii*. J. Nat. Prod. 55:701-706.
Zhang J, El-Shabrawy AO, El-Shanawany MA, Schiff-Jr PL, Slatkin D (1987). New azafluorene alkaloids from *Oxandra xylopioides*. J. Nat. Prod. 50:800-806.