http://dx.doi.org/10.5935/0103-5053.20150209

J. Braz. Chem. Soc., Vol. 26, No. 11, 2233-2240, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Essential Oil from Flowers of *Solanum stipulaceum*: Composition, Effects of γ-Radiation, and Antileukemic Activity

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The composition of essential oils from flowers of *Solanum stipulaceum* Roem & Schult collected in May and September was studied for the first time. Effects of γ-radiation on volatile constituents were investigated by gas chromatography (GC) with flame ionization detector (FID). In addition, the antileukemic activity was studied by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay against human cell lines HL-60 and THP-1. The main constituents of the essential oil of flowers collected in May were β-caryophyllene (25.8%), γ-gurjunene (11.9%), and β-gurjunene (8.2%), whereas the essential oil of flowers collected in September was mainly composed of β-caryophyllene (26.5%), caryophyllene oxide (11.0%), and γ-gurjunene (10.0%). The main components of essential oil from flowers collected in September were not significantly affected by γ-radiation at lower doses than 10.0 kGy. No cytotoxic activity in leukemic cell lines was observed for non-irradiated samples. However, irradiated samples exhibited slightly cytotoxic activity.

Keywords: *Solanum stipulaceum*, essential oil, β-caryophyllene, gamma-radiation, antileukemic activity

Introduction

Essential oils are mixtures of volatile natural organic compounds usually obtained from plant material, which usually consist of terpenoids, aromatic, and aliphatic compounds.[1] Essential oils exhibit a large spectrum of biological properties, such as antimicrobial, analgesic, and antiseptic.[2] The pleasant fragrance of the essential oil components makes them a raw material widely employed by cosmetic, pharmaceutical, agricultural, and food industries. In nature, essential oils play an important role in the protection of plants in their ecosystem.[3]

The family Solanaceae includes about 3,000 species distributed in 96 genera, and 1,500 of them belong to the genus *Solanum*. *Solanum* species occur in different regions around the world, with the greatest diversity concentrated in Central and South America.[4] *Solanum stipulaceum* Roem & Schult (popularly known as "caiçarinha") is an endemic and native Brazilian plant, widely distributed in the Cerrado region of the state of Minas Gerais.[5] The polar extract of the stem of *S. stipulaceum* exhibits molluscicidal activity and cardiac-depressant properties.[6,7] Alkaloids, such as solasodine, solaparnaine, and solamargin, were isolated from their fruits, stems and branches.[8,9] Although some studies have been reported for extracts of *S. stipulaceum*, the flowers have not been studied.

γ-Radiation is an efficient method for microbial decontamination and insect disinfestation of vegetal materials. Moreover, radiosterilization is the most widely used method for commercial preparations of medicinal

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plants.[10] However, chemical composition and biological activities of vegetal species can be changed when plant material is exposed to γ-radiation.[11] In the case of volatile compounds, some studies indicated that the γ-radiation effect is negligible,[12-14] however, significant changes in the essential oil composition have been observed for samples exposed at high doses of ionizing radiation.[15-17]

The present work describes for the first time the chemical composition of the essential oil from flowers of *S. stipulaceum*. Seasonal variations are also considered in the analysis of chemical oil composition, and the effects of γ-radiation on essential oil constituents are studied for flowers submitted at different radiation doses. Moreover, antileukemic activity and evaluation of γ-radiation effects on cytotoxicity are investigated for the essential oil from flowers of *S. stipulaceum*.

Experimental

Plant material

Flowers of S*olanum stipulaceum* Roem & Schult were collected in Montes Claros (state of Minas Gerais, Brazil) in May and September 2014 (samples MS and SS, respectively). The botanical identification was made by Maria Olivia Mercadante-Simões (Universidade Estadual de Montes Claros, Minas Gerais, Brazil). The voucher specimen (BHCB 169873) has been deposited in the Herbarium of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais.

Ionizing radiation treatment

Flower samples collected in September 2014 (SS) were irradiated. Five samples (20.0 g) were placed in plastic packages and exposed to γ-radiation at 1.0, 2.5, 5.0, 10.0, and 20.0 kGy at room temperature (22 °C) in the Centro de Desenvolvimento da Tecnologia Nuclear (CDTN, Belo Horizonte, Brazil). The samples were exposed to γ-radiation using a GammaBeam-127 irradiator, model IR-214 (Nordion Inc.) equipped with a cobalt-60 source. The dose rate was 2.81 kGy h^{-1}. The irradiator was calibrated with a Fricke standard dosimeter, and the absorbed doses were controlled by the exposure time of each sample to the source.

Essential oils isolation and analysis

Irradiated and non-irradiated flowers of *S. stipulaceum* were submitted to hydrodistillation for 5 h on a Clevenger-type apparatus. After distillation, the essential oils were extracted three times with dichloromethane, dried over anhydrous sodium sulfate and filtered. After solvent evaporation, volatile oils were stored at 4 °C in the dark until analysis.[18] Solutions of essential oils at 1% in chloroform were prepared for gas chromatography (GC) analysis. All reagents and organic solvents were purchased from Sigma-Aldrich Co. GC with flame ionization detector (FID) analyses were carried out using an Agilent HP 7820A GC system. An Agilent HP5 column (30 m × 0.32 mm × 0.25 μm) was used with hydrogen as the carrier gas (3 mL min^{-1}). The GC oven temperature was programmed from 70-250 °C at 3 °C min^{-1}, with injector temperature at 250 °C, injection volume 1 μL, split ratio adjusted at 30:1, and FID detector temperature at 250 °C. Percentages of separated compounds were calculated from GC-FID peak areas using EZChrom Elite Compact software. GC/mass spectrometry (MS) analyses were performed using a Shimadzu QP2010 ULTRA GC/MS system. An Rxi-1MS column (30 m × 0.25 mm × 0.25 μm) was used with helium as carrier gas (1.5 mL min^{-1}). GC oven temperature was kept at 70 °C for 2 min and programmed to 250 °C at 5 °C min^{-1}. The injector temperature was 250 °C, and the injection volume was 1 μL. The split ratio was adjusted at 10:1. MS interface and the detector temperature was 250 °C. Electron ionization (EI) MS were recorded at 70 eV. Data acquisition was performed and analyzed by GCMSsolution software. Identification of essential oil components was carried out after comparison with those available in the computer library (NIST11) and by comparison of their Kováts retention index with a series of *n*-alkanes.

Antileukemic activity

The cytotoxicity of essential oils was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) colorimetric assay. The evaluation of the cytotoxic activity was performed with human acute promyelocytic leukemia cell line American Type Culture Collection (ATCC)# CCL-240 (HL-60), acute monocytic leukemia cells ATCC# TIB-202 (THP-1) and lung fibroblast ATCC# CCL-95.1 (Wi-26VA4) cell lines. Cells were plated in 96-well plates (2×10^5 cells *per* well) and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. After 24 h, the wells were washed with culture medium (RPMI-1640 + 20% inactivated fetal bovine serum + 2 mmol L^{-1} *L*-glutamine) and incubated with samples at concentrations from 0.10 to 100 μg mL^{-1}. After 48 h of incubation, the plates were treated with MTT (5 mg mL^{-1}). Colorimetric measurements were performed at 550 nm using the microplate reader Spectramax M5e.

All experiments were performed in triplicate. Cytotoxicity was scored as the percent of reduction in absorbance *vs.* untreated control cultures. The results were expressed by the IC_{50} values (concentration of the drug that reduced cell viability by 50%). IC_{50} values were calculated using OriginPro 8.0 software.

Results and Discussion

Hydrodistillation of non-irradiated samples collected in May and September provided pale yellowish oils with a pleasant aroma, yielding 0.08 and 0.03% (m/m), respectively, from flower extracts. Figure 1 shows the GC-FID chromatograms of essential oils from samples MS and SS. Twenty-four constituents were identified in the essential oil from MS, corresponding to 88.2% of the overall oil composition. In turn, essential oil from SS provided only fourteen constituents with chemical structures identified, corresponding to 90.3% of the overall oil composition. The compound name, Kováts retention index, and percentage of the volatile constituents of both samples are given in Table 1.

Essential oil from MS provided a high content of sesquiterpene hydrocarbons (76.2%). The most abundant component was β-caryophyllene (25.8%), followed by γ-gurjunene (11.9%), β-gurjunene (8.2%), α-selinene (5.3%), *D*-germacrene (3.6%), δ-cadinene (3.6%), aromandendrene (3.5%), δ-elemene (2.8%), α-humulene (2.7%), β-copaene (2.4%) γ-muurolene (1.8%), α-copaene (1.2%), α-neoclovene (0.8%), and α-gurjunene (0.7%). Sesquiterpene epoxides (6.9%) and sesquiterpenols (3.8%) were found in relatively smaller amounts, specifically, caryophyllene oxide (3.6%), alloaromadendrene oxide-(2) (2.2%), isoaromadendrene epoxide (1.1%), β-spathulenol (2.4%), cubenol (1.0%), and viridiflorol (0.5%). Pentadecanoic acid (1.3%) was also detected in MS essential oil. On the other hand, essential oil from SS contains fewer volatile constituents than MS, exhibiting only fourteen constituents (Figure 1 and Table 1). Essential oil from SS contains a lower content of sesquiterpenes (65.9%) and a higher content of sesquiterpene epoxides (14.6%) in relation to oil from MS (76.2 and 6.9%, respectively). Sesquiterpenols were not detected in SS. The volatile compounds α-gurjunene, β-gurjunene, β-copaene, α-neoclovene, γ-muurolene, valencene, isoaromadendrene epoxide, and pentadecanoic acid were not detected in the essential oil of SS. On the other hand, essential oil of SS contains a high content of palmitic acid (9.9%). However, this carboxylic acid was not detected in the essential oil of MS.

Essential oils from flowers of other species of *Solanum* contain many sesquiterpenes, which were also identified in the present work. β-Caryophyllene, α-copaene, β-elemene, γ-muurolene, *D*-germacrene, and δ-cadinene were reported in the essential oil from flowers of *S. stuckeflii*,[20] whereas β-caryophyllene and β-selinene were the predominant sesquiterpenes in the essential oil from flowers of *S. incisurm.*[20]

The differences observed in the composition of essential oils from MS and SS may be related to

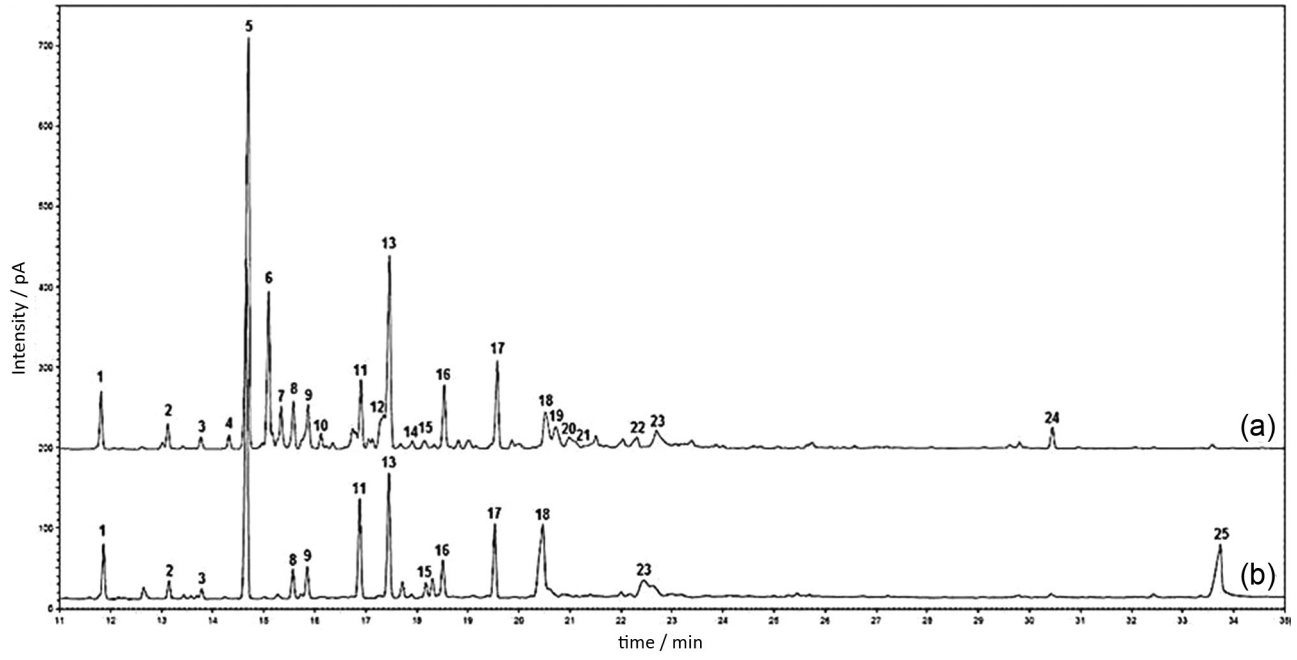

Figure 1. GC-FID chromatograms of essential oils from flowers of *Solanum stipulaceum* for samples collected in (a) May (MS) and (b) September (SS).

Table 1. Chemical composition of essential oils from flowers of *S. stipulaceum* collected in May (MS) and September (SS)

Peak No.	Compound[a]	RI[b]	RI[c]	Composition / %	
				MS	SS
1	δ-elemene	1331	1337	2.8	4.2
2	α-copaene	1368	1375	1.2	1.4
3	β-elemene	1386	1389	0.6	0.8
4	α-gurjunene	1401	1408	0.7	ND[d]
5	β-caryophyllene	1411	1418	25.8	26.5
6	β-gurjunene	1423	1425	8.2	ND[d]
7	β-copaene	1429	1430	2.4	ND[d]
8	α-humulene	1436	1442	2.7	2.2
9	aromandendrene	1444	1441	3.5	2.5
10	α-neoclovene	1451	1454	0.8	ND[d]
11	*D*-germacrene	1472	1480	3.6	7.9
12	γ-muurolene	1484	1485	1.8	ND[d]
13	γ-gurjunene	1487	1489	11.9	10.0
14	valencene	1500	1499	0.5	ND[d]
15	7-*epi*-α-cadinene	1506	1507	0.6	1.3
16	δ-cadinene	1517	1521	3.6	3.3
17	α-selinene	1545	1530	5.3	5.7
18	caryophyllene oxide	1571	1573	3.6	11.0
19	β-spathulenol	1576	1578	2.4	ND[d]
20	isoaromadendrene epoxide	1584	1584	1.1	ND[d]
21	viridiflorol	1586	1587	0.5	ND[d]
22	cubenol	1621	1625	1.0	ND[d]
23	alloaromadendrene oxide-(2)	1633	1625	2.2	3.7
24	pentadecanoic acid	1863	1869	1.2	ND[d]
25	palmitic acid	1970	1970	ND[d]	9.9
	Total identified components / %			88.2	90.3

[a]The compounds are listed in order of their elution on HP-5 column; [b]calculated Kováts retention index; [c]Kováts retention index reported in literature[19] for the HP-5 column; [d]not detected.

environmental factors, such as variation in temperature, photoperiod, and humidity conditions and pollinators.[21] These biotic and abiotic factors influence the production of secondary metabolites in plants which can explain the greater variety of constituents in the essential oil from MS in relation to SS.

The effects of γ-radiation on essential oils from flowers of *S. stipulaceum* were observed in the extraction yield and their chemical composition. The yield of volatile oil obtained from non-irradiated flowers collected in September (0.03%) was slightly increased by gamma radiation. Extraction yield was 0.04% for irradiated material at 1.0, 2.5, and 5.0 kGy. Additionally, the extraction yield was 0.05% for irradiated material at higher doses (10.0 and 20.0 kGy). This increase of the extraction yields is due to damage to plant tissues caused by radiation.[22]

Figure 2 shows the GC-FID chromatograms of essential oils from irradiated flowers of *S. stipulaceum* collected in September, and their chemical composition is shown in Table 2. Essential oils from irradiated material exhibited a higher content of caryophyllene oxide, δ-cadinene, and alloaromadendrene oxide-(2) at all radiation doses than the corresponding non-irradiated sample. The content of some constituents increased at a dose of 1.0 kGy, mainly β-caryophyllene, aromadendrene, and caryophyllene oxide, probably as a result of the increase of their extractability.[16] On the other hand, the volatile compounds *D*-germacrene, γ-gurjunene, α-selinene, 7-*epi*-α-cadinene, and palmitic acid decreased after irradiation at 1.0 kGy, which could be due to their radiation sensitivity (Figure 3).[23] An increase of the content of α-copaene, β-elemene, β-caryophyllene, α-humulene, and aromadendrene was observed for

essential oil from flowers exposed to γ-radiation at doses below 10.0 kGy. However, the content of these compounds decreased for samples exposed at a dose of 20.0 kGy. On the other hand, the content of δ-elemene, *D*-germacreme, γ-gurjunene, 7-*epi*-α-cadinene, and α-selinene decreased when flowers were exposed to γ-radiation at different doses. The content of palmitic acid decreased when flowers were irradiated at doses from 1.0 to 10.0 kGy and its content increased at a dose of 20.0 kGy. The content of δ-cadinene, caryophyllene oxide, and alloaromadendrene oxide-(2) also increased at 20.0 kGy (Table 2 and Figure 3). Four new peaks (between 27 and 33 min) were observed in the chromatogram for samples irradiated at 20.0 kGy (Figure 2). Compounds corresponding to these peaks were not identified. Therefore, they were attributed to radiolytic products and correspond to 8.1% of the total oil composition. As a result, γ-radiation induces irregular changes in the composition of the essential oil from flowers of *S. stipulaceum*. However, a direct relation between radiation dose and changes in the essential oil composition was not observed. These results are in agreement with those reported in the literature for essential oils from *Angelica gigas* Nakai, in which the content of sesquiterpene hydrocarbons and oxygenated sesquiterpenes increased without any correlation with γ-radiation dose.[23] Yalcin *et al*.[15] also reported irregular changes in the volatile profile of linseed exposed to γ-radiation, in addition to significant decreases in the content of linseed volatile compounds induced by higher doses of γ-radiation.

Oxidation of β-caryophyllene promoted by γ-radiation or action of free radicals generated in the ionization of water can explain the increase in the content of caryophyllene oxide when flower samples were irradiated at 20.0 kGy. Similar results were also observed for the essential oil from *Piper nigrum L.* after radiation at a dose of 30 kGy. An increase in the content of caryophyllene oxide is matched by a decrease of β-caryophyllene.[17] Hydroxyl radicals (HO•) are generated from water molecules by exposure to γ-radiation. This radical can initiate oxidation of β-caryophyllene by radical addition to the double internal bond, followed by the addition of molecular oxygen to produce peroxyl radicals (RO_2•). The reaction of the formed peroxyl radicals with another organic peroxyl radical (RO_2•) formed *in situ* produces β-hydroxyalkoxyl (RO•) radicals which undergo a ring-retaining reaction to obtain caryophyllene oxide.[24] This oxidation process can occur with other sesquiterpenes of the essential oil, justifying the decrease in the composition of major constituents of the essential oil (Figure 2).

The essential oil from flowers of *S. stipulaceum* contains some active components that have been reported to exhibit cytotoxic activity. β-Caryophyllene, the major constituent identified in this essential oil, has been reported as a cytotoxic agent against human breast and colorectal adenocarcinoma cells.[25] Moreover, the volatile compounds α-humulene, β-elemene, δ-elemene, and caryophyllene oxide exhibit cytotoxicity against different human cancer cells.[25-28]

Some cytotoxicity studies on essential oils from *Solanum* sp. have been reported in the literature. The essential oil from leaves of *S. erianthum* exhibited 98.85 and 97.94% of cell lethality against breast and prostate

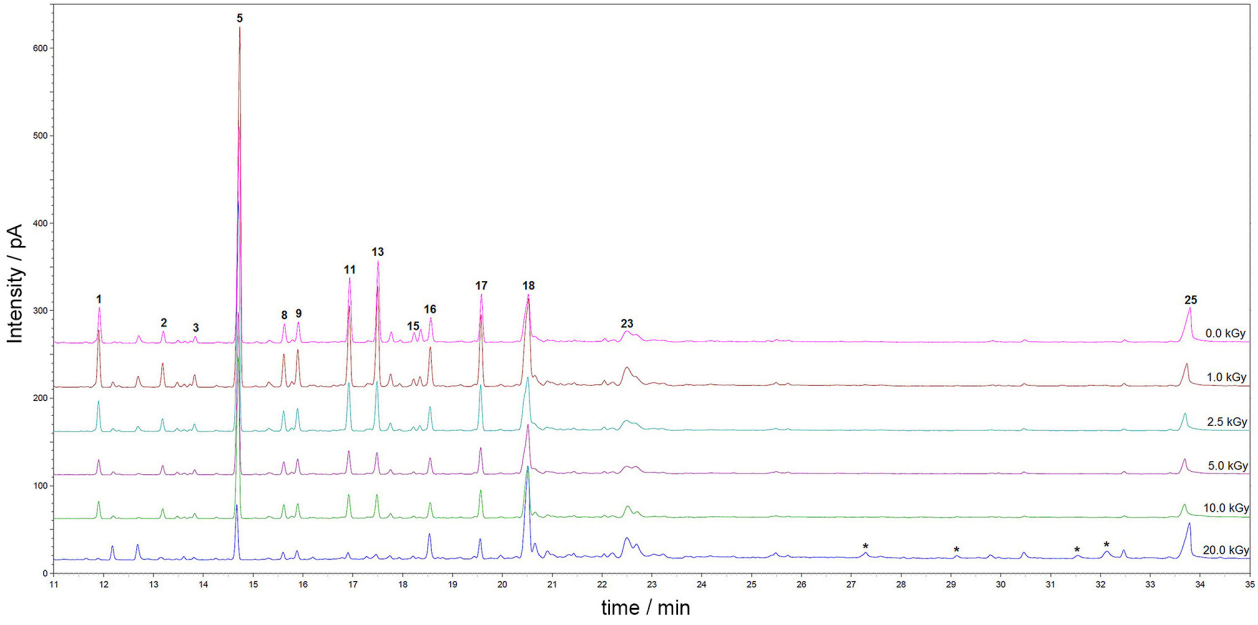

Figure 2. GC-FID chromatograms of essential oils from non-irradiated and irradiated flowers of *S. stipulaceum* collected in September (*: new peaks detected).

Table 2. Composition of volatile oils from irradiated flowers of *S. stipulaceum* collected in September

Compound	Composition / %					
	γ-Radiation dose / kGy					
	0.0	1.0	2.5	5.0	10.0	20.0
δ-Elemene	4.2	4.3	3.8	2.8	3.4	ND[a]
α-Copaene	1.4	1.9	1.7	1.9	2.0	ND[a]
β-Elemene	0.8	1.0	1.0	1.0	1.1	0.4
β-Caryophyllene	26.5	32.3	31.4	31.6	32.2	9.0
α-Humulene	2.2	2.6	2.6	2.5	2.7	1.3
Aromandendrene	2.5	3.0	3.0	3.2	3.0	1.6
D-Germacrene	7.9	6.5	6.4	4.6	4.9	0.9
γ-Gurjunene	10.0	8.0	6.6	4.8	5.2	0.9
7-*epi*-α-Cadinene	1.3	0.7	0.6	0.6	0.5	0.3
δ-Cadinene	3.3	3.4	3.5	3.6	3.4	4.8
α-Selinene	5.7	6.0	6.1	5.1	5.5	3.5
Caryophyllene oxide	11.0	13.2	14.3	17.2	17.4	26.0
Alloaromadendrene oxide-(2)	3.7	4.3	3.8	4.0	5.6	9.2
Palmitic acid	9.9	4.0	5.3	6.7	4.7	13.9

[a]Not detected.

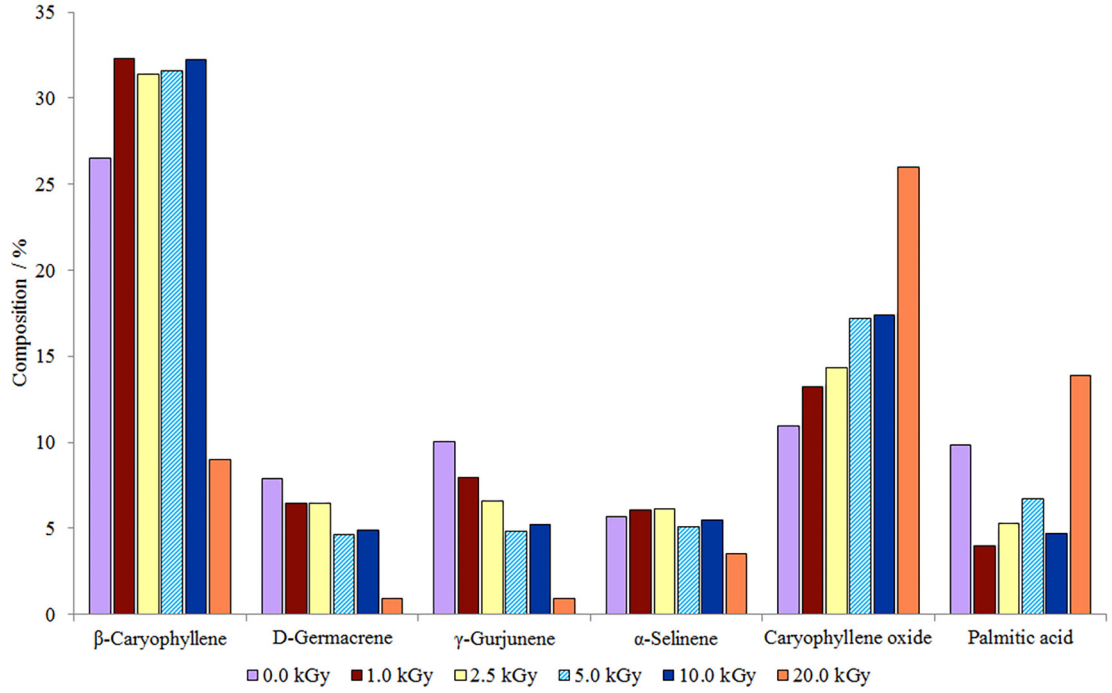

Figure 3. Composition of major constituents of the essential oil from non-irradiated and irradiated flowers of *S. stipulaceum* collected in September.

cancer cells, respectively.[29] Moreover, the essential oil from leaves of *S. macranthum* exhibited 2% lethality against breast cancer cells,[29] whereas the essential oil from leaves of *S. spirale* exhibited significant citotoxicity against breast, oral and lung cancer cells (IC_{50} = 19.69, 26.42 and 24.02 μg mL^{-1}, respectively).[30]

Table 3 shows the results of the cytotoxicity of essential oils from flowers of *S. stipulaceum* collected in September and irradiated. Cytotoxic action of the non-irradiated sample was not observed for an acute myeloid leukemia (AML) model, specifically HL-60 and THP1 cell lines, when compared with the non-selective chemotherapy

control etoposide. Cytotoxic activity was increased for essential oils from irradiated samples, exhibiting a direct relationship with the radiation dose. However, the increase of the cytotoxicity of the samples is not comparable to the corresponding action of etoposide. The essential oil from flowers irradiated at 20.0 kGy was not considered for the cytotoxic assay because the volatile oil composition was significantly affected by γ-radiation.

Table 3. Cytotoxic activity *in vitro* of essential oils from non-irradiated and irradiated flowers of *S. stipulaceum* and etoposide (control) for AML cell lines and control

Sample (radiation dose)	IC_{50}[a] / (μg mL^{-1})		
	HL-60	THP-1	Wi-26VA4
EO_0 (0.0 kGy)	> 100	> 100	> 100
EO_1 (1.0 kGy)	> 100	84.51 ± 4.14	> 100
EO_2 (2.5 kGy)	> 100	73.43 ± 2.19	> 100
EO_5 (5.0 kGy)	96.07 ± 4.98	52.73 ± 2.78	> 100
EO_{10} (10.0 kGy)	89.67 ± 3.78	40.12 ± 1.65	> 100
Etoposide	9.70 ± 1.29	13.80 ± 1.81	7.10 ± 1.03

[a]Values presented as average ± standard deviation. IC_{50}: half-maximal inhibitory concentration; HL-60: human acute promyelocytic leukemia ATCC# CCL-240 cell line; THP-1: acute monocytic leukemia ATCC# TIB-202 cell line; Wi-26VA4: lung fibroblast ATCC# CCL-95.1 cell line.

Some factors may affect the cytotoxic activity of the essential oils from irradiated flowers of *S. stipulaceum*. Concentration of various essential oil constituents, such as caryophylene, a sesquiterpenoid reported as a potential candidate for prevention and treatment of cancer, increased with the γ-irradiation dose.[31] However, since the essential oil is a mixture of volatile compounds, the biological properties can be the result of synergism.[32]

Conclusions

The essential oil composition from flowers of *S. stipulaceum* was here reported for the first time. Twenty-four constituents of the oil were identified in flowers collected in May, and fourteen in the sample collected in September. The major components were β-caryophyllene, γ-gurjunene, and β-gurjunene in the May sample, whereas, in flowers collected in September, β-caryophyllene, caryophyllene oxide, γ-gurjunene, and palmitic acid were identified as the major volatile components.

Essential oils from flowers of *S. stipulaceum* exhibited a decrease in the content of the major volatile components when exposed to γ-radiation at 20.0 kGy, induced by oxidation of their constituents. However, a radiation dose at 10.0 kGy, which is the conventional dose for radiosterilization of plant material, did not significantly affect the content of volatile oil constituents.

The essential oil from non-irradiated flowers of *S. stipulaceum* did not show significant cytotoxic activity against HL-60 and THP-1 cell lines. However, an increase of the antileukemic activity was observed for essential oils from flowers exposed to γ-radiation.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for their financial support.

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Submitted: June 29, 2015
Published online: August 21, 2015