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Changes in the essential oil composition of leaves of *Echinodorus macrophyllus* exposed to γ-radiation

Thiago M. Silva,*[*,1] Roqueline R. S. Miranda,[1] Vany P. Ferraz,[1] Márcio T. Pereira,[2] Ezequias P. de Siqueira,[3] Antônio F. C. Alcântara[1]

[1]*Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Brazil,*
[2]*Centro de Desenvolvimento da Tecnologia Nuclear, Belo Horizonte-MG, Brazil,*
[3]*Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte-MG, Brazil.*

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Abstract: Leaves of *Echinodorus macrophyllus* (Kunth) Micheli, Alismataceae, were exposed to different doses of γ-radiation (0.00, 1.00, 3.00, 5.00, 10.00, and 20.00 kGy) and the chemical composition of their essential oils was investigated. The extractive process of the essential oil was more favored when the leaves were irradiated. The essential oil components were identified by correlation between GC-FID data and retention parameters obtained from the Kováts method. Moreover, GC-MS analyses of the essential oils were correlated with fragmentation profiles in the NIST standard mass fragmentation data bank. The essential oil of *E. macrophyllus* contains biologically active constituents of different chemical classes. Acyclic monoterpenes and sesquiterpenes showed increase in concentration when the leaves were exposed to γ-radiation. On the other hand, the component concentrations of some chemical classes were lightly decreased, *i.e.*, for bicyclic monoterpenes, diterpenes, triterpenes, carboxylic esters, and carotenoid derivatives.

Introduction

The γ-radiation is the safest and the most efficient method of food preservation and microbiological decontamination of plant after harvest (Chatterjee et al., 2012). This method causes structural damage to the DNA molecules and affects the reproducibility of microorganisms, and as a consequence the total population of mycoflora and quantity of mycotoxins are decreased in the irradiated material (Caillet et al., 2009). The reduction of microorganisms in the irradiated material is usually observed at low doses (1.5-3.5 kGy). Some studies report that irradiated materials at doses higher than 10 kGy are microbiologically decontaminated without compromising their nutritional capacities and pharmacological properties (Hanis et al., 1988; Onyenekwe et al., 1997; Migdal & Owczarczyk, 1998; Owczarczyk et al., 2000; Aziz & Moussa, 2002).

The γ-radiation also causes damage to plant cell membranes and usually promotes better extraction of their constituents (Byun et al., 1999). The change in concentration of compounds containing sulfuric, acid, alcohol, aldehyde, ester, furan, or ketone groups is observed in plant extracts which were submitted to different doses of γ-radiation. The concentration of phenolic compounds usually decreases in extracts exposed to γ-radiation, due to radioprotective property of these compounds (Gyawali et al., 2006; Silva et al., 2012)

The essential oils (EO) extracted from vegetal species exhibit biological activities against fungi and bacteria and shows important ecological role in plant-insect interactions. Other studies also report the use of EO in food flavoring and perfumery (Perrucci et al., 1995; Lahlou, 2004). The relative proportion of the EO constituents of *Curcuma longa, Thymus vulgaris thymoliferum, Eucalyptus radiata,* and *Lavandula angustifolia* did not change when these plants were exposed to γ-radiation (Haddad et al., 2007; Dhanya et al., 2011). However, qualitative and quantitative changes in concentration were observed for EO constituents of some plant species, such as *Turnera diffusa* (Camargo et al., 2008).

Echinodorus macrophyllus (Kunth) Micheli, Alismataceae, popularly known in Brazil as "chapéu-de-couro", is very used as medicinal plant and exhibits some pharmacological activities, such as astringent, diuretic, antiarrhythmic, anti-inflammatory, and anti-rheumatic agents, in the treatment of atherosclerosis, skin, liver, and urinary tract (lithiasis and nephritis) diseases, and immunosuppressive and cytotoxic effects (Leite et al.,

2007; Pinto et al., 2007; Silva et al., 2012). There are few phytochemical reports about *E. macrophyllus* (Silva et al., 2012) and its EO has not yet been extensively studied (Tanus-Rangel et al., 2010). So, this work describes the identification of the EO components and investigation of the chemical integrity of the leaves of *E. macrophyllus* when submitted to γ-radiation. The components and composition of the EOs were analyzed by gas chromatography with a flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) methods.

Materials and Methods

Plant material

The leaves of *Echinodorus macrophyllus* (Kunth) Micheli, Alismataceae, were obtained in April 2011 in the city of Belo Horizonte, Minas Gerais (Brazil). A voucher specimen was deposited in the herbarium of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais, under the code: BHCB 28,557.

Ionizing radiation treatment

Six samples of dried and powdered leaves of *E. macrophyllus* weighting 250 g each were placed in polyethylene packages. Five samples were submitted to different doses of γ-radiation (1.00, 3.00, 5.00, 10.00, and 20.00 kGy), named samples S_1, S_3, S_5, S_{10}, and S_{20}, respectively. One sample was not exposed to γ-radiation (control sample, named sample S_0). All samples were stored at -18 °C until required for experiments. The samples were submitted to γ-radiation apparatus in Gammacell with source ^{60}Co. The dose rate was 2.50 kGy/h and a dose rate error of ±0.02 kGy.

Isolation of the essential oils

The six samples of *E. macrophyllus* were submitted to hydrodistillation for 5 h using a Clevenger-type apparatus. The aqueous emulsion was concentrated and submitted to extraction with dichloromethane. The solvent was evaporated at room temperature, providing the yellowish viscous essential oils EOS_0 (0.0695 g), EOS_1 (0.1672 g), EOS_3 (0.1159 g), EOS_5 (0.1800 g), EOS_{10} (0.1453 g), and EOS_{20} (0.1045 g) from S_0, S_1, S_3, S_5, S_{10}, and S_{20}, respectively. The EO were immediately analyzed.

TLC analyses

Thin Layer Chromatography was developed using silica gel HF_{254} glass plate (Merck). The chromatographic profiles were obtained by elution of the plates using pure *n*-hexane and dichloromethane. The spots were firstly revealed by UV light at 254 and 366 nm and after with a solution of vanillin/sulfuric acid (10%) and plates heated at 100 °C.

GC-FID analyses

The GC-FID analyses were performed on a HP Gas Chromatograph (HP 5890) with FID detector. The separations were carried out with a capillary column (Equity-5; 30 m x 0.25 mm with a 0.25 µm film thickness), which was purchased from Supelco Analytical. Hydrogen was used as carrier gas at flow rate of 2 mL/min. Exactly 1 µL of sample was injected in the equipment at a temperature programming from 60 to 300 °C (3 °C/min). The injector and detector temperatures were set at 270 and 300 °C, respectively (Pimenta et al., 2006; Radulovic et al., 2010).

The samples were diluted in chloroform (1% p/v) and the EO components were identified using retention parameters in gas chromatography based on the Kováts method, *i.e.*, by comparison of their linear retention indexes with the corresponding value of standards (C_{10}-C_{18}, alkanes).

GC-MS analyses

The GC-MS analyses were carried out on equipment Shimadzu GC-MS (QP5050A) equipped with a capillary column (DB-5; 30 m x 0.25 mm with a 0.25 µm film thickness). Helium was used as carrier gas at flow rate of 2 mL/min, using the same conditions described above for GC-FID analyses.

The spectrometric data were manipulated using the AMDIS (Automated Mass Spectral Deconvolution and Identification System) software. The EO constituents were identified through comparison of the mass spectral fragmentation profile of the sample with the correspondent one available in the NIST standard mass fragmentation data bank (Nist Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, version 2002, upgraded to 2006).

Results and Discussion

Chemical analyses of the essential oils

EOS_0 was qualitatively analysed by TLC. The elution of EOS_0 with dichloromethane afforded seven spots revealed with sulphuric vanillin (R_f 0.43, 0.46, 0.67, 0.80, 0.84, 0.87, and 0.99). The TLC analysis indicated that the EO of *E. macrophyllus* contains constituents of different polarities.

The GC-FID and GC-MS chromatograms of EOS_0 show twenty relatively intense peaks, registered at 9.25 to 67.67 min (Figure 1). The chemical attribution

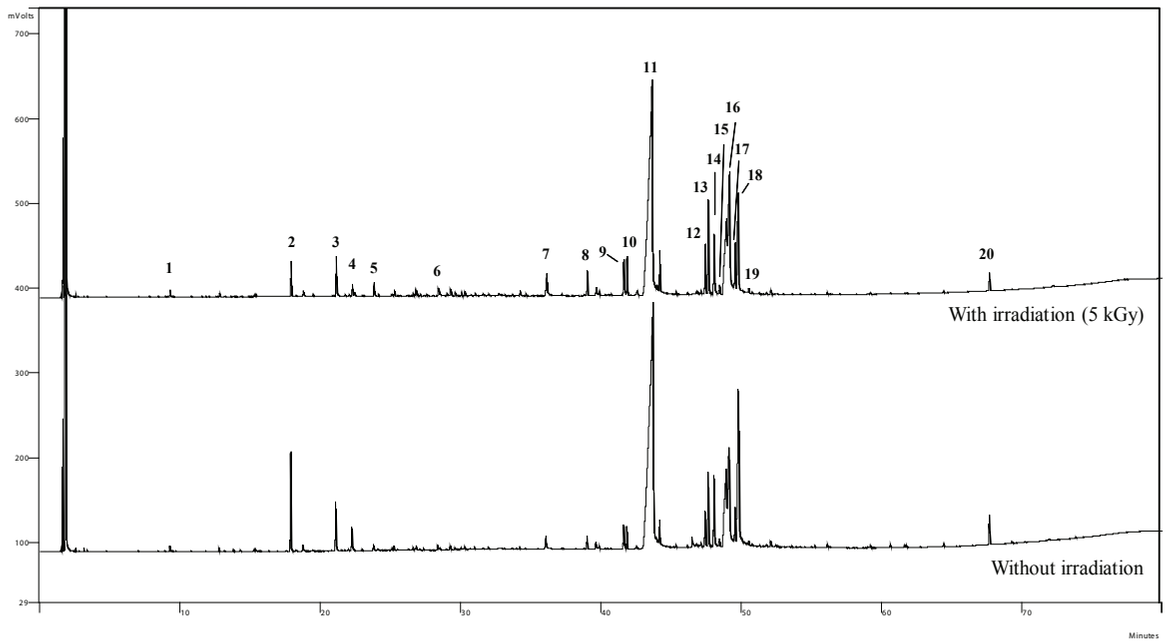

Figure 1. Chromatograms of GC-FID of essential oil isolated from the leaves of *Echinodorus macrophyllus*: without irradiation (bottom) and irradiated at 5.00 kGy (upper). The indicated compounds in the chromatograms are shown in Table 1.

of the GC-FID peaks of EOS_0 was based on the Kováts method. Moreover, the GC-MS peaks of EOS_0 were also chemically attributed through standard mass fragmentation data bank from NIST.

Table 1 shows the chemical class and GC data of the constituents identified in EOS_0. The calculated Kováts retention indexes (CKRI) are very close to the corresponding Kováts retention indexes obtained from the literature (LKRI), principally those peaks registered with retention time between 9.25 and 44.16 min, attributed to the compounds 1 to 11, respectively. The peaks on the chromatogram of EOS_0 correspond to one acyclic monoterpene (AM; compound 1), three carotenoid derivatives (CD; compounds 2, 8, and 9), one bicyclic monoterpene (BM; compound 3), four sesquiterpenes (ST; compounds 4-7), nine carboxylic esters (CE; compounds 10-13 and 15-19), one diterpene (DT; compound 14), and one triterpene (TT; compound 20). The relative percentage of the GC chromatogram peak areas (PA) of EOS_0 indicates that the EO of the leaves of *E. macrophyllus* is rich in carboxylic esters, mainly 10 (44.28%), 18 (12.67%), 16 (7.94%), and 15 (7.85%). The carotenoid derivative 2 (2.93%), diterpene 14 (2.93%), and bicyclic monoterpene 3 (1.68%) are other important chemical constituents found in the EO obtained from the leaves of the plant.

Some biological activities which are described for the individual EO components of *E. macrophyllus* (Chart 1) can also be observed in the species *E. macrophyllus*. For example, EO containing linalool (compound 1) exhibit anti-inflammatory and anticancer properties (Kamatou & Viljoen, 2008), while EO containing dihydroedulan (compound 2) exhibits cytotoxic activity (Skaltsa et al., 2003; Sarikurkcu et al., 2008; Conforti et al., 2009; Stojković et al., 2011). A detailed information about each of the EO components of *E. macrophyllus* is listed in Chart 1.

The combination among biological properties of *E. macrophyllus* and biological activities of the majority components of its EO suggests that the anti-inflammatory and anti-rheumatic activities of the plant is mainly related to compound 15, followed by compounds 1, 4, 5, 12, 13, and 14. The anticancer activity of the plant is mainly related to compounds 1, 2, 4, 5, 7, 14, 17, and 20. Its diuretic property is mainly related to compounds 14 and 20. All EO components exhibit antioxidant and antimicrobial activities. However, the literature does not report these properties for the plant.

Effect of γ-radiation in the essential oils

The quantities of the EO obtained from the hydrodistillation of the irradiated leaves (EOS_1, EOS_3, EOS_5, EOS_{10}, and EOS_{20}) were higher than EOS_0 (see section Isolation of the essential oils). These results confirm that γ-radiation causes damage to the leaf cell membranes (Dhanya et al., 2011), and as a consequence the extractive process of the EO has become more favored when the plant material was submitted to radiation. However, the quantity of EOS_1 (0.1673 g) is higher than the quantity of EOS_3 (0.1159 g) and lower than EOS_5 (0.1800 g), *i.e.* the

Table 1. Chemical class and GC data of EOS_0 components: retention time (RT), calculated Kováts retention index (CKRI); literature Kováts retention index (LKRI), and relative percentage of the peak area (PA).

Component	Class	RT (min)	CKRI	LKRI	PA (%)
linalool (1)	AM	9.25	1092	1098	0.11
dihydroedulan (2)	CD	17.88	1304	1300	2.93
10-(acetylmethyl)-(+)-3-carene (3)	BM	21.08	1382	1387	1.68
β-caryophyllene (4)	ST	22.23	1410	1418	0.83
α-caryophyllene (5)	ST	23.77	1448	1454	0.22
(*E*)-nerolidol (6)	ST	28.35	1560	1564	0.19
drimenol (7)	ST	36.06	1749	1759	0.66
hexahydrofarnesyl acetone (8)	CD	38.99	1821	1835	0.40
(*E,E*)-farnesyl acetone (9)	CD	41.60	1885	1918	0.93
methyl hexadecanoate (methyl palmitate; 10)	CE	41.83	1891	1921	44.28
ethyl hexadecanoate (ethyl palmitate; 11)	CE	44.16	1948	1993	1.22
methyl (*Z,Z*)-9,12-octadecadienoate (methyl linolate; 12)	CE	47.41	2027	2092	1.34
methyl (*Z,Z,Z*)-9,12,15-octadecatrienoate (methyl linolenate; 13)	CE	47.63	2033	2098	2.58
(*E*)-phytol (14)	DT	48.04	2043	2114	2.93
methyl octadenoate (methyl stearate; 15)	CE	48.42	2052	2112	7.85
methyl (*E,E,E*)-11,14,17-eicosatrienoate (16)	CE	49.12	2069	2061	7.94
ethyl (*Z,Z*)-9,12-octadecadienoate (ethyl linoleate; 17)	CE	49.54	2080	2155	1.70
ethyl (*Z,Z,Z*)-9,12,15-octadecatrienoate (ethyl linoleate; 18)	CE	49.77	2085	2214	12.67
ethyl octadecanoate (ethyl stearate; 19)	CE	50.52	2104	2194	0.11
squalene (20)	TT	67.67	2524	2790	0.99

AM: acyclic monoterpene; CD: carotenoid derivative; BM: bicyclic monoterpene; ST: sesquiterpene; CE: carboxylic ester; DT: diterpene; TT: triterpene.

Chart 1. Biological activity of EO components extracted from *Echinodorus macrophyllus*.

Compound	Biological activity
1	Antimicrobial, anti-inflammatory, anticancer, antioxidant, insecticidal (Kamatou & Viljoen, 2008), antinociceptive, local anaesthetic, inhibits the cholinesterase, modulates activity in glutamatergic circuits, and modifies the nicotinic receptors (Peana et al., 2004).
2	Antioxidant, antimicrobial, and cytotoxic (Skaltsa et al., 2003; Sarikurkcu et al., 2008; Conforti et al., 2009; Stojković et al., 2011).
3	Anabolic (Jeong et al., 2008), antimalarial, antimicrobial (Kamatou et al., 2005), anti-inflammatory (Olufunke et al., 2009), and inhibits the action of acetylcholinesterase enzyme (Miyazawa & Yamafuji, 2005).
4/5	Anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, insecticidal, antimicrobial, and anaesthetic (Legault & Pichette, 2007; Gertsch et al., 2008; Leandro et al., 2012).
6	Antileishmanial and nematicidal (Arruda et al., 2005; Abdel-Rahman et al., 2013).
7	Antifeedant, antibacterial, antifungal, anticomplemental, cytotoxic, insecticidal, antiallergic, piscicidal, molluscicidal, and regulates the plant growth (Vlad, 2006).
8/9	Antioxidant, antimicrobial (Maua et al., 2003; Sahin et al., 2004), repellent to mosquito (Innocent et al., 2010), and reduces blood cholesterol levels (Sinensky et al., 1994).
10	Antimicrobial, acaricidal, insect repellent, and affects the neuroendocrine system (Wang et al., 2009; Satyal et al., 2012).
11	Actives dismutase, catalase, and peroxidase, antioxidantive enzymes (Dziri & Hosni, 2012), antioxidant, hypocholesterolemic, nematicidal, pesticidal, antiandrogenic, inhibits the action of 5-alpha reductase enzyme (Rajeswari et al., 2012; Jegadeeswari et al., 2012), and produces pancreatitis-like injury in rats (Werner et al., 1997).
12	Antioxidant, antimicrobial, antifungal, and anti-inflammatory (Singh & Majumdar, 1997; Palma & Taylor, 1999).
13	Antioxidant, anti-inflammatory, and mosquito repelling (Tunón et al., 1994; Singh & Majumdar, 1997; Conforti et al., 2007).

14	Antimicrobial, anti-inflammatory, anticancer, and diuretic (Rajeswari et al., 2012).
15	Anti-inflammatory, antimicrobial, and antimalarial (Silva et al., 2004; Hua et al., 2006).
16	Antioxidant, antimicrobial, and antifungal (Huang et al., 2007).
17	Antioxidant, anticancer, and mosquito repelling (Tunón et al., 1994; Ali et al., 2012).
18	Antioxidant, hypoglycemic, nematicidal, and microbial (Tundis et al., 2011; Faizi et al., 2011).
19	Antioxidant and hypoglycemic (Tundis et al., 2011).
20	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemo preventive, inhibits the action of lipoxygenase, pesticide, and diuretic (Pacheco et al., 2009; Abreu et al., 2011; Rajeswari et al., 2012).

Table 2. Constituent relative percentage in the essential oils obtained from the leaves of *Echinodorus macrophyllus* submitted to different γ-radiation doses.

Constituent	γ-Radiation dose/constituent relative percentage					
	0.0 kGy	1.0 kGy	3.0 kGy	5.0 kGy	10.0 kGy	20.0 kGy
1	0.11	0.20	0.18	0.16	0.33	0.38
2	2.93	0.86	0.78	1.13	0.88	1.07
3	1.68	1.32	1.49	1.58	1.79	1.50
4	0.83	0.47	0.57	0.51	0.63	0.53
5	0.22	0.46	0.75	0.57	0.77	0.88
6	0.19	0.35	0.48	0.48	0.29	0.25
7	0.66	1.20	1.53	1.56	1.52	1.65
8	0.40	0.91	0.96	0.93	0.85	1.02
9	0.93	1.44	1.45	1.39	1.36	1.45
10	44.28	43.80	38.08	41.50	36.57	39.84
11	1.22	1.73	1.87	1.65	1.51	2.91
12	1.34	1.78	2.18	1.98	1.70	1.49
13	2.58	3.40	4.17	3.73	3.16	2.69
14	2.93	2.84	2.99	2.85	2.60	2.24
15	7.84	8.20	8.61	8.67	9.12	7.88
16	7.94	11.68	11.29	11.37	12.18	10.04
17	1.70	2.31	2.59	2.27	2.27	1.86
18	12.67	4.70	8.06	6.44	7.60	6.97
19	0.11	0.21	0.21	0.18	0.14	0.12
20	0.99	0.72	0.73	0.70	0.61	0.51
Total	91.55	88.58	88.97	89.65	85.88	85.28
Acyclic monoterpene	0.11	0.20	0.18	0.16	0.33	0.38
Carotenoid derivative	4.26	3.21	3.19	3.45	3.09	3.54
Bicyclic monoterpene	1.68	1.32	1.49	1.58	1.79	1.50
Sequiterpene	1.90	2.48	3.33	3.12	3.21	3.31
Carboxylic ester	79.68	77.81	77.06	77.79	74.25	73.80
Diterpene	2.93	2.84	2.99	2.85	2.60	2.24
Triterpene	0.99	0.72	0.73	0.70	0.61	0.51

EO quantity is not directly proportional to the radiation dose.

Table 2 shows the relative proportion of the constituents of EOS_1, EOS_3, EOS_5, EOS_{10}, and EOS_{20} based on CG-FID analyses (see Figure 1). The change in concentration of some essential oil components can be attributed to extraction efficiency and chemical and structural stabilities of these components when the leaves are exposed to γ-radiation.

Compounds 1, 5, 7-9, 11, 12, and 16 show higher relative proportions when the samples were exposed to γ-radiation. On the other hand, compounds 2, 4, 10, 18,

and 20 show lower relative proportions when the samples were exposed to γ-radiation. The samples submitted to γ-radiation did not show significant change in relative proportion for the other compounds (3, 6, 13-15, 17, and 19).

The effect of the γ-radiation on the EO can be also observed when their chemical constituents are grouped in the chemical classes (Table 2). The concentration of acyclic monoterpene and sesquiterpene derivatives is increased when the plant was exposed to γ-radiation. On the other hand, the component concentrations of the other chemical classes were lightly decreased, i.e. for triterpene, diterpene, esters, and carotenoid derivatives. However, the effect of the γ-radiation is not the same for the different compounds in each chemical class. Examples are observed to carboxylic esters: the concentration of the compounds 11 and 16 increases when the radiation dose was higher, while the concentration of the compounds 10 and 18 decreases under the same conditions. As result, the effects of γ-radiation are intrinsic and specific for certain compounds. Similar compounds can exhibit different effects with γ-radiation. Moreover, the change in concentration of the EOs' components is not dependent of γ-radiation dose.

Conclusion

In conclusion, the essential oil of *E. macrophyllys* is rich in carboxylic esters, carotenoid derivatives, and terpenes. These chemical classes are represented on the essential oil by compounds that exhibit a large spectrum of the biological activities. The concentration of some essential oil components is changed when the leaves are exposed to γ-radiation. As a consequence of the use of γ-radiation to the biological decontamination of the leaves of *E. macrophyllus*, some alterations in the biological properties of the plant can certainly occur. Compound 16 is the main component among those that exhibited increase in concentration when leaves of *E. macrophyllus* were subjected to radiation. According to the literature data, this compound exhibits antioxidant, antimicrobial, and cytotoxic properties. As a consequence of changes in its concentration can modify the biological activities of the essential oil.

On the other hand, compounds 2, 10, and 18 are the main components among those that exhibited decrease in concentration when leaves of *E. macrophyllus* were subjected to radiation. These compounds exhibit antioxidant, antimicrobial, antifungal, acaricidal, hypoglycemic, nematicidal, insect repellent, disturbance in the neuroendocrine system, and promotion of the protein synthesis and hypopharyngeal gland development in honey properties and changes in their concentration can also modify the biological activities of the essential oil.

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Authors' contributions

TMS (PhD student) contributed in collecting plant, essential oil isolation, analysis the GC-FID and GC-MS data, and draft the paper. RRSM contributed to critical reading of the manuscript. VPF contributed to chromatographic analysis (GC-FID). MTP contributed in gamma radiation of the samples. EPS contributed to chromatographic analysis (GC-MS). AFCA supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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***Correspondence**

Thiago de Melo Silva
Instituto de Ciências Exatas, Universidade Federal de Minas Gerais.
Av. Antônio Carlos, 6627, 31270-901 Belo Horizonte-MG, Brazil
silvatm@ufmg.br
Tel.: +55 31 3409 5728
Fax: +55 31 3409 5700