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SHORT COMMUNICATION



Cytotoxicity and anti-inflammatory activities of *Gallesia integrifolia* (Phytolaccaceae) fruit essential oil

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ABSTRACT

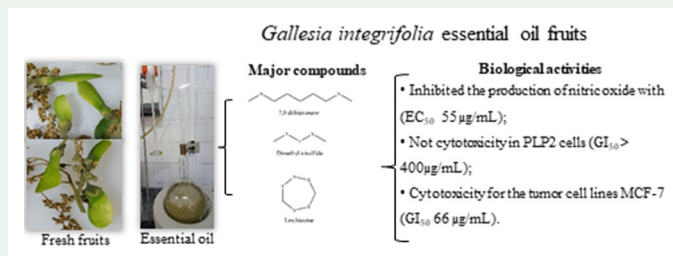
The chemical composition of *Gallesia integrifolia* fruits essential oil was obtained by hydrodistillation using a modified Clevenger apparatus was investigated by gas chromatography coupled to mass spectrometry (GC/MS). In addition, the cytotoxicity activity against human tumor cell lines MCF-7 (breast adenocarcinoma), NCI-H460 (large cell lung carcinoma), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma), and non-tumor PLP2 (porcine liver primary cells) as well as the anti-inflammatory action was proposed. The compounds were predominantly organosulfates (2,8-dithianonane, dimethyl trisulfide, and lenthionine). Anti-inflammatory activity that provides 50% inhibition of nitric oxide production (55 µg/mL) of essential oil of the fruits. It also presents cytotoxic activity against MCF-7 (GI₅₀ = 66 µg/mL), NCI-H-460 (GI₅₀ = 147 µg/mL), HeLa (GI₅₀ = 182 µg/mL) and HepG2 (GI₅₀ = 240 µg/mL). The essential oil is more active in tumor cells than in non-tumor cells and the GI₅₀ values for essential oil reported in our work support future studies.

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1. Introduction

Gallesia integrifolia (Spreng.) Harms (Phytolaccaceae) is a tree native to the Atlantic forest of Brazil. This species is popularly known as *pau d'alho* in Brazil due to its characteristic alliaceous odor. In popular medicine, it is utilized by *quilombo* communities and rural settlements in the northern region of Rio de Janeiro state in Brazil to treat the flu, bronchitis, inflammations, memory disorders, mycosis, and as a sedative (Neves 2012). In addition, it is used to treat pneumonia, vermin, gonorrhea, prostate tumors, and rheumatism (Lorenzi 2002).

Several classes of phytochemicals from different parts of *G. integrifolia* have been reported, including terpenes, porphyrins, branched alcohols, phenols, aromatic ketones, and sulfur-containing natural substances. In the essential oil were reported sulfur compounds in the leaves (97.00%), flowers (93.33%) and fruits (86.74%). In the fruits the main compounds were 2,3,5-trithiahexane, 6-dithiaoctan-1,8-diol, Methanethiol (Raimundo et al. 2017), 2,8-Dithianonane, Dimethyl trisulfide and Lenthionine with antimicrobial potential (Raimundo et al. 2018). For the crude extract of the fruits also, the main compounds were sulfur (9.11%) as 2,4-dithiapentane (2.26%), 2,1,2,4-trithiolane (1.87%) and 2,3,5-trithiahexane (4.98%). The extracts and essential oil of *G. integrifolia* are reported to have larvicidal, antifungal, acaricidal, anti-ulcer, antinociceptive, anti-inflammatory and antiviral activities (Silva Júnior et al. 2013; Arunachalam et al. 2017; Raimundo et al. 2017; Raimundo et al. 2018). However, studies on cytotoxic activity of *G. integrifolia* fruit essential oil have not been found. Thus, this study aimed to evaluate the chemical composition and the cytotoxic and anti-inflammatory activities of *G. integrifolia* fruit essential oil.

2. Results and discussion

The yield of *G. integrifolia* fruit colorless essential oil was 0.5 mL/kg ($0.05 \pm 0.006\%$) with density of 1.48 g/mL and refraction index of 1620. The results for the fruit essential oil have density and refractive index in accordance to Raimundo et al. (2017). The chemical composition analysis of the essential oil identified 29 constituents with the predominance of organosulfurate compounds (Table S1). The major compounds were 2,8-dithianonane (52.9%), dimethyl trisulfide (15.2%), and lenthionine (14.8%) (Table S1; Figures S1–S4). The presence of sulfureted compounds in the essential oil is related to the characteristic alliaceous odor (Akisue et al. 1986). The results found in the present study are in agreement with Raimundo et al. (2018). Among the 29 identified compounds, five of them such as 4-methyl-2-thianonane, 1-oxa-4,7-dithiononane disulfide, bis (2-sulfhydryl ethyl), 2-(octylthio) ethanol, and propane, 1,1'-thiobis [3-(methylthio)] have not yet been reported for plants. Thus, we suggest that these five compounds might be artifacts produced during the extraction process (Venditti 2018) or that they are unique compounds from *G. integrifolia* and, in that case, they need further studies (Table S4). The remaining 24 compounds have already been reported for *G. integrifolia* and other plants in a diversity of botanical families (Table S4).

Organosulfurates are secondary metabolites characterized by a sulfur atom linked to the cyanate group, produced by vegetables, and based on sulfureted amino acids such as methionine and cysteine, which provide the synthesis of organosulfur

compounds through thermal or enzymatic processes (Kyung and Lee 2001; Dewick 2002); therefore, they are able to direct the synthesis of organosulfur compounds.

Therefore, methionine, an amino acid found in Phytolaccaceae species (Dewick 2002), degrades by thermal process and produces methional, which in turn degrades itself and originates sulfur compounds. One of these products is methanethiol which can be transformed into dimethyl disulfide in the presence of oxygen (Schutte and Teranishi 1974) and, through a transfer reaction of the methyl group, it can also form dimethyl sulfide (Lomans et al. 2002).

Sulfur-containing defense compounds are crucial for plant survival during biotic and abiotic stress such as temperature (heat or chilling), water (drought or flooding), salinity, proton toxicity, heavy metals, overexposure to ultraviolet rays, ozone, and others. For that, the plant produces elemental sulfur, hydrogen sulfide, glutathione, phytochelatins, sulfur rich proteins and various secondary metabolites (Capaldi et al. 2015). The formation of these compounds in plants is closely related to the supply, demand, uptake, and assimilation of sulfur (Anjum et al. 2015; Putnik et al. 2019).

The fruit essential oil inhibited the production of nitric oxide with EC_{50} of 55 $\mu\text{g/mL}$, a value that is 3.4-fold higher than the positive control dexamethasone (16 $\mu\text{g/mL}$) (Table S2). However, although the fruit essential was less effective, it is important to consider that dexamethasone is an isolated compound, and the essential oil is a mixture of chemical compounds. The anti-inflammatory activity of the fruit essential oil found in our study supports the popular use of this species to treat inflammations (Neves 2012).

The anti-inflammatory potential of the fruit essential oil from *G. integrifolia* can also be related to the presence of sulfur compounds, as the molecules that donate hydrogen sulfide (H_2S) have anti-inflammatory activity (Bátaí et al. 2018). Hydrogen sulfide activates T-type CaV 3.2 channels that modulate the feeling of pain in sensory neurons, inhibit voltage-dependent K^+ channels contributing to the depolarization of peptidergic sensory neurons and somatostatin release of these cells, have anti-proliferative effect in T cells, and induce polymorphonuclear cell apoptosis. However, high concentrations of H_2S have pro-inflammatory effects (Wang 2012; Bátaí et al. 2018).

Moreover, the fruit essential oil inhibited the growth of four human tumor cell lines with GI_{50} value of 66 $\mu\text{g/mL}$ for MCF-7, 147 $\mu\text{g/mL}$ for NCI-H-460, 182 $\mu\text{g/mL}$ for HeLa, and 240 $\mu\text{g/mL}$ for HepG2 (Table S3). This is very promising and desirable in the treatment of neoplasms. According to Suffness and Pezzuto (1990), and Boik (2001) essential oils with $GI_{50} < 30 \mu\text{g/mL}$ and pure compounds with $GI_{50} < 4 \mu\text{g/mL}$ are considered promising values. So, the GI_{50} values for *G. integrifolia* fruit essential reported in our work could support future studies.

Our results made evident that the fruit essential oil from *G. integrifolia* had moderate cytotoxic activity against human tumor cell lines, but no cytotoxic activity against porcine non-tumor cells. The SI of the fruit essential oil – which is the relation between GI_{50} value of non-tumor cells divided by tumor cells (Almeida et al. 2014) – was over 6.06. Thus, this essential oil is more active in tumor cells than in non-tumor cells and, therefore, more selective and appropriate for the use against neoplastic cells. The cytotoxicity activity of the essential oil of the fruits of *G. integrifolia* is described for the first time in the literature.

The 2,8-dithianonane (52.9%) compound (Figure S2), a major compound in the fruit essential oil of our study has been synthesized since 1980 and its molecular structure ($C_7H_{16}S_2$) has two sulfur atoms (Golovnya et al. 1980). Only Raimundo et al. (2018) reported this compound as the major one (52.6%) in *G. integrifolia* fruit essential oil. Dimethyl trisulfide (15.2%), which is a hydrogen sulfide (H_2S) donor, was the second most abundant compound in the fruit essential oil of our study. It is a compound belonging to the polysulfide group, utilized as food additive and as antidote for cyanide poisoning. Furthermore, it has been reported to have anti-inflammatory and anti-nociceptive activities in an animal model (Bátaí et al. 2018). Lenthionine (14.8%), the third major compound in the fruit essential oil of our study, was reported in the mushroom aqueous extract of *Lentinus edodes* (Berk.) Singer (current name *Lentinula edodes* (Berk.) Pegler) with anti-inflammatory activity similar to prednisone ($1\text{ }\mu\text{mol/L}$) (Kupcová et al. 2018). However, this compound has low antitumor activity in relatively high doses ($10\text{--}20\text{ }\mu\text{g/mL}$) against cell lines in human liver carcinoma, human cervical carcinoma, and human pancreas carcinoma (Kupcová et al. 2018).

There are no reports in the literature on the cytotoxicity of the fruit essential oil from *G. integrifolia*; however, dimethyl trisulfide (15.2%) (Figure S3) found in the fruit essential oil of our study induced cytoprotection against cell death and aging, caused by oxidative stress by a catalytic mechanism medium of mitochondrial peptide methionine sulfoxide reductase protein (Guan et al. 2017). Compounds containing sulfur, mainly from *Allium* sp. and *Brassica* sp., have shown protective effect against different types of neoplasia by apoptosis (De Gianni and Fimognari 2015). Our results indicate that *G. integrifolia* fruit essential oil was selectively cytotoxic for all tested tumor cells and that the possible mechanism of action can be related to the presence of sulfur compounds. Future studies are needed to evaluate the mechanism of action and the compounds responsible for biological activity.

3. Experimental

Supplementary material related to this paper is available online, alongside Tables S1–S4 and Figures S1–S2.

4. Conclusion

The fruit essential oil from *G. integrifolia* has a predominance of organosulfurate compounds and the major compounds are 2,8-dithianonane, dimethyl trisulfide, and lenthionine. The essential oil is more active in tumor cells than in non-tumor cells and the GI_{50} values for essential oil reported in our work support future studies. This is the first study reported on the cytotoxicity of *G. integrifolia* fruit essential oil.

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Disclosure statement

The authors declare no conflicts of interest.

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