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SHORT COMMUNICATION

Bixa orellana L. by-products' fractions from an industrial process: antiproliferative activity on tumor cells and chemical profile

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ABSTRACT

This study evaluated the phytochemical characterization of *Bixa orellana* (BO extract) unsaponifiable extract and resulting fractions (F fraction - FF, Geranyl fraction – GF and R fraction- RF) obtained as by-products of an industrial process investigating *in vitro* antiproliferative activities in human tumoral cells. The main compounds identified by GC-MS for BO extract were Geranylgeraniol (61.51%); for FF: Geranylgeraniol (70.23%); for GF: Geranylgeraniol (78.92%) and for RF: β-cubebene (27.75%). Quantifications of geranylgeraniol by GC-FID presented the percentage content: BO 27.52%; FF 38.52%; GF 51.44% and RF 1.81%. BO extract showed a significant antiproliferative activity, with GI_{50} up to 4 μg/mL. All fractions had a remarkably similar antiproliferative activity profile (GI_{50} 27-47 μg/mL). Data reported herein showed an important cytostatic effect for BO extract, nevertheless this activity is not attributed exclusively to geranylgeraniol. In conclusion, this by-product becomes of great value, being a potential candidate for development of new anti-tumor ingredients.

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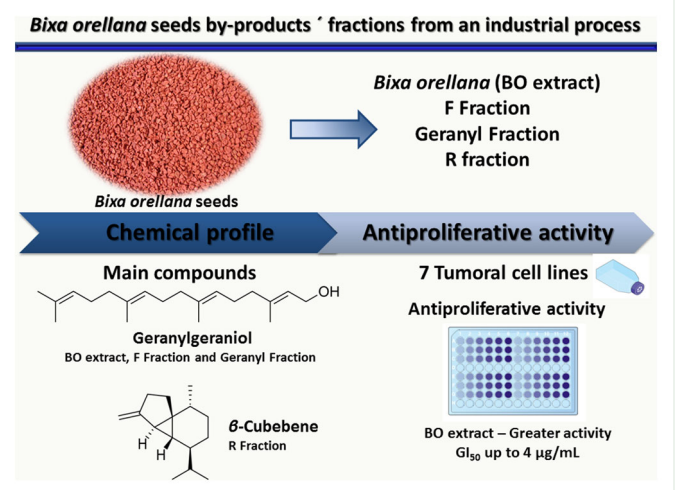

1. Introduction

Bixa orellana Linn. (Bixaceae), known as annatto in English, achiote in Spanish or urucum in Portuguese (Brazil), is a shrub native to tropical South America (Teixeira da Silva et al. 2018). This specie has been used in folk medicine for treatment and prevention of many health disorders such as fevers, asthma, allergy, ulcers, diarrhea, diabetes, and skin problems (Raddatz-Mota et al. 2017).

The plant's seeds are used worldwide in food and cosmetic industries, being one of the mostly used natural dyes due to high carotenoid compounds content. Bixin, a red color carotenoid, is responsible for the dyeing characteristics of *Bixa orellana* (BO) seeds. Previous reports demonstrated the antiproliferative activities of bixin on *in vitro* studies, through mechanisms involving apoptosis and interruption of cell cycle (Anantharaman et al. 2016; Santos et al. 2016; de Oliveira Júnior et al. 2019). Alongside the carotenoid content, the species seeds' have been reported to yield high levels of terpenes, among them geranylgeraniol, which can attain approximately 57% of total terpene content in the dry seed matter (Lourido Perez and Martinez Sanchez 2010; Raddatz-Mota et al. 2017).

During the industrial process of extracting pigments from BO seeds, other fractions are generated as by-products. Among them is an unsaponifiable non-polar seed extract that by vacuum distillation provide fractions containing high geranylgeraniol yields (Carvalho 2015).

To our knowledge, few studies report the antiproliferative activity of *Bixa orellana* L. extract, with data only described with the isolated compound bixin. In this context, given *Bixa orellana* industrial importance and the resulting by-products generated, herein we describe the phytochemical characterization of *Bixa orellana* (BO extract) unsaponifiable extract and the resulting fractions (F fraction - FF, Geranyl fraction - GF

Table 1. Concentration of unsaponifiable extract of *Bixa orellana* (BO), F fraction (FF), Geranyl fraction (GF), R fraction (RF) and Doxorubicin (DOX) necessary to cause 50% growth inhibition (GI_{50} values), in μg/mL on human tumor cell lines, after 48 h of treatment.

	GI_{50} (μg/mL)[a]							
	U251	MCF-7	NCI/ADR-RES	786-0	NCI-H460	PC-3	HT29	HaCaT*
DOX[#]	0.036	<0.025	0.07	<0.025	<0.025	0.127	0.03	<0.025
BO	3.9	3.1	2.5	23.3	3.3	2.8	3.3	2.85
FF	34.9	29.4	27.5	29.2	33.8	27.7	30.3	29.4
GF	34.3	30.0	27.5	30.5	112.3	28.5	33.7	47.05
RF	28.3	28.1	28.1	28.5	31.4	28.0	31.0	27.1

Notes: U251 (glioma); MCF-7 (breast); NCI/ADR-RES (ovary with multiple drug resistance phenotype); 786-0 (kidney); NCI-H460 (lung); PC-3 (prostate) and HT29 (colon).
*non-tumor cell line HaCaT (immortalized human keratinocyte).
[a]GI_{50}: Growth Inhibition 50 – concentration required to inhibit 50% of cell growth.

and R fraction - RF) produced as by-products through an industrial process investigating the *in vitro* antiproliferative activities on seven human cancer cells lines.

2. Results and discussion

The extract and the resulting fractions were analyzed by GC-MS to identify the major peaks by comparison with the mass spectral fragmentation data from NIST 05 library, retention index, and literature. The relative percentages of volatile compounds found: for unsaponifiable extract of *Bixa orellana* (BO) the main components identified were Geranylgeraniol (61.51%) and 38.49% (not identified) (Figure S1 – Supplementary materials); for F Fraction (FF): Ocimene (14.61%), β-cubebene (7.72%), (-)-Spathulenol (7.41%) and Geranylgeraniol (70.23%) (Figure S2 – Supplementary materials); for Geranyl Fraction (GF): Geranylgeraniol (78.92%) and 20.88% (not identified) (Figure S3 – Supplementary materials); and for R Fraction (RF): Ocimene (3.46%), α-cubebene (1.65%), (-) isoledene (3.17%), β-cubebene (27.75%), β-ciclogermacrene (5.32%), Trans-α-bergamotene (2.16%) and (-)-Spathulenol (7.15%), and 49.34% not identified (Figure S4 – Supplementary materials). Since the extract and fractions, object of this study, are by-products of an industrial process of carotenoid enriched fractions, no traces of bixin, isobixin and norbixina were detected in these samples, as reported in previous studies with annatto seeds (Cardarelli et al. 2008; Shahid-Ul-Islam et al. 2016; Raddatz-Mota et al. 2017).

For geranylgeraniol quantification purposes, the peak area calculated from GC-FID chromatograms was correlated linearly with standard geranylgeraniol (85% Sigma-Aldrich) in the 19-1230 μg/mL range ($R^2 = 0.9995$) in triplicate. The percentage amount of geranylgeraniol on % weight/weight (mean ± standard desviation) calculated on BO was 27.52 ± 0.65; for FF 38.52 ± 0.19; for GF 51.44 ± 0.15 and for RF 1.81 ± 0.01.

The antiproliferative activity was evaluated on seven human tumor cells lines and on a non-tumor human cell. The results are described as 50% growth inhibition (GI_{50}) parameter presented in Table 1.

BO extract showed a significant antiproliferative activity in all cell lines tested, with a GI_{50} value up to 4 μg/mL, except for 786-0 cell line ($GI_{50} = 23.3$ μg/mL). Comparing the BO extract with positive control, the GI_{50} values for the extract are approximately 35 to 100 times greater than that demonstrated for doxorubicin for most cell lines

with exception for PC-3 cell line (prostate) that BO extract presented 2 fold GI_{50} value when compared to doxorubicin.

Doxorubicin is a chemotherapeutic drug used as positive control due to well-known cytostatic characteristic (Lüpertz et al. 2010) and is used to treat various types of solid tumors (Anders et al. 2013). However, the use of Doxorubicin is limited due to several side effects, the most dangerous being the cumulative dose-dependent cardiotoxicity (Taymaz-Nikerel et al. 2018). The long use of Doxorubicin also leads to drug-resistant cancer cells, increasing the compound's cytotoxicity (Ferreira et al. 2017). In this sense, BO extract can represent a new prototype for the development of a new chemotherapeutic treatment. Furthermore, our extract is an industrial by-product that could benefit the development of future products. Previous reports have demonstrated no significant or serious side effects in a 6-month study of 750 mg *Bixa orellana* leaf powder daily consumption in humans (Zegarra et al. 2007). However, additional studies are needed to elucidate extract's safety and efficacy issues.

The three fractions demonstrated a remarkably similar antiproliferative activity profile, with GI_{50} values between 27 to 47 μg/mL, except for GF fraction that showed less selectivity for NCI-H460 ($GI_{50} = 112.3$ μg/mL). Regarding the phytochemical profile, the three fractions presented different concentrations of geranylgeraniol. The highest geranylgeraniol content found was in GF (51%), an intermediate concentration in FF (38%) and only 1.8% in RF. Despite these variations, the antiproliferative profile was very similar for the three fractions in almost all cell lines tested, which suggests that geranylgeraniol, may not be the major compound involved in cell proliferation inhibition.

Comparing all the values of concentration required to inhibit 50% of cell growth, BO extract presented GI_{50} values approximately 10 times lower than that observed for the fractions. This greater activity suggest a synergistic effects of geranylgeraniol with other components of the extract needing further studies to identify these active compounds highlighting these interactions. Previous studies reported an anti-leukemic activity of a terpenic subfraction from *Pterodon pubescens*, which are rich in geranylgeraniol, farnesol, and vouacapan compounds (Pereira et al. 2012). Other reports suggests that terpenoids, the main class of compounds found in all fractions and extract studied, can be helpful to decrease proliferation of many tumoral cells, as skin, lung, colon and prostate carcinomas cells (Liby et al. 2007; Rabi and Bishayee 2009).

3. Experimental

See Supplementary materials.

4. Conclusions

The present study showed an important antiproliferative effect of by-products generated in an industrial process for dye compounds from *Bixa orellana* production. Our findings indicated an important cytostatic effect with low GI_{50} values observed for all cell lines treated with BO extract, and this activity is not attributed exclusively to geranylgeraniol compound. These results support the importance of future studies to elucidate the cellular mechanism of action involved in this antiproliferative effect with

further phytochemical investigation to elucidate BO extract content. In conclusion, this by-product becomes of great value, being a potential candidate for development of new anti-tumor ingredients.

Disclosure statement

No potential conflict of interest was reported by the authors.

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