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Metabolic profile and cytotoxicity of non-polar extracts of pineapple leaves and chemometric analysis of different pineapple cultivars

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ABSTRACT

The use of co-products from the fruit agroindustry as a source of bioactive molecules presents economic advantages, since these compounds are highly available and inexpensive. Therefore, the present study aimed to evaluate their qualitative chemical composition followed by multivariate analysis, and to correlate the results with those of the cytotoxicity tests. The non-polar metabolic profiles of the leaves of seven commercial pineapple (*Ananas comosus* (L.) Merr.) cultivars were investigated by using GC–MS. Twenty-seven metabolites were identified from the seven hexanic extracts. Multivariate analysis (Hierarchical Cluster Analysis, Principal Component Analysis and Partial Least Squares) revealed significant differences in the metabolic fingerprint of the different cultivars. Overall, the hexanic extracts of the seven cultivars of pineapple leaves showed promising antitumor activity against the six tumor lines tested (colon, leukemia, prostate, astrocytoma, breast and cervix). Correlation of the chemometric data, qualitative analysis, and cytotoxic tests allowed us to determine the possible biomarkers responsible for each specific antitumor activity. This study demonstrates a great valuation potential of a co-product little explored in the agroindustry of foods through the prospection of biologically active compounds.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr., Bromeliaceae family) is one of the most widely consumed tropical fruits in the world due to its pleasing aroma and flavor. Brazil is the main center of origin and diversity of the genus *Ananas* and has important genetic variability to explore.

In addition to its food and nutritional value, pineapple has many other potential uses. It can be used for ornamental purposes (Souza et al., 2014, 2012), as reinforcing fiber for a variety of products (Sena Neto et al., 2015, 2013), and as paper fiber. It also has enzymes with proteolytic action and secondary metabolites (Manetti et al., 2009; Marques et al., 2007; Yusof et al., 2011), and is used as animal feed (Fagundes and Fagundes, 2010; Santos et al., 2008).

Pineapple has well-known and wide-ranging therapeutic properties. In recent years, studies have sought to consolidate and systematize the application of these therapeutic properties of pineapple, including its cytotoxic activity. In addition, the literature describes other therapeutic properties associated with pineapple, including antidiabetic, antihyperlipidemic, and antioxidant effects (Xie et al., 2005). Some previous studies have reported that pineapple can improve insulin sensitivity in type 2 diabetes (Xie et al., 2006).

In vitro studies have shown the activity of various fruit extracts against different stages of tumor formation and indicate that diets rich in fruits and vegetables may have protective effects against cancer. Epidemiological studies suggest that a diet rich in fruits and vegetables, with a high concentration of bioactive compounds, contributes to a reduction in risk of certain types of cancers in humans (Veer et al.,

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2000).

Research on the antioxidant and anti-inflammatory properties of fruits has been performed, particularly using *in vivo* assays, in order to establish solutions to prevent and assist in the treatment of cancer (Kalra et al., 2008; Landete, 2013). Some compounds in the hydro-alcoholic extract of pineapple leaves have previously been determined (Ma et al., 2007). However, there is still little information that relates anticancer activity to the bioactive compounds found in the leaves of cultivars pineapple.

It is known that this agroindustrial residue (pineapple leaves) are produced in large quantity and are currently discarded in the environment. Therefore, it is necessary to establish studies that may contribute to the valuation of these residues that could potentially become inputs of the pharmaceutical industry. Despite the numerous medicinal uses of pineapple, phytochemical investigations on this species are scarce. In relation to its non-polar composition, the literature is even scarcer, in terms of both the chemical profile of the pineapple leaves and the association of compounds with specific biological activities.

It is important to note that comparisons of chemical profiles of extracts with different biological activities can be used to indicate hit compounds related to the observed activity (Silva et al., 2016). The correlation between chemical compounds and biological activity can be determined through the use of chemometric tools such as Hierarchical Cluster Analysis (HCA), Principal Component Analysis (PCA) and regression analysis by Partial Least Squares (PLS).

In this context, the objective of this study was to determine the non-polar profile of pineapple leaves (seven commercial cultivars), by using gas chromatography coupled to mass spectrometry (GC–MS). Furthermore, it aimed to correlate the chemical compounds identified in the pineapple leaves with the results of the cytotoxicity tests, aiming at the prospection of biologically active compounds that will add value to this agroindustrial residue.

2. Materials and methods

2.1. Samples, reagents, and chemicals

Seven cultivars found at the Active Germplasm Bank of Pineapple (AGB Pineapple) or at the Experimental Fields of Embrapa Cassava and Fruits (Embrapa Cassava & Fruits, Cruz das Almas, Bahia, Brazil) were investigated. Twenty healthy leaves from 10 different plants (two leaves per plant) belonging to each cultivar were harvested. The samples were dried in a circulating air oven for three days at 50 °C. Subsequently, the dry plant material was ground and packed in sealed and transparent plastic bags, properly identified, and protected from excessive heat, moisture, and light. Table 1 presents information of interest about the cultivars selected for this work.

Water was purified using a Milli-Q Integral Water Purification System (Millipore, Bedford, MA, USA). Ethanol (96%) and hexane (95%) used for the extraction were purchased from Tedia (Rio de Janeiro, RJ, Brazil). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Life Science). The n-alkane homologous series of C8-C30, pyridine, and MSTFA (N-trimethylsilyl-*N*-methyl trifluoroacetamide) were purchased from Sigma-Aldrich (Supelco Solutions).

2.2. Extraction and derivatization

Initially, 500 mg of dried plant leaf samples was weighed and transferred into a test tube. Next, 4 mL of hexane was added, and the mixture was vortexed for 1 min. The extraction of non-polar compounds was performed in an ultrasonic bath for 20 min at a fixed power of 135 W. Then, the test tubes were centrifuged to decant suspended plant material at 3000 rpm for 10 min. Finally, a 3 mL aliquot of the hexane phase was collected and dried in a rotary evaporator (Chagas-Paula et al., 2015; Nehme et al., 2008). The extraction procedure was performed in quadruplicate for each of the seven cultivars of pineapple leaves.

The dry hexane extracts (10 mg each) were transferred to vials and dissolved in 200 µL of pyridine. Next, 200 µL of MSTFA was added, and the final solution was placed in a water bath at 37 °C for 30 min (Silva et al., 2016). Subsequently, the samples were filtered (0.20 µm PTFE) and stored in 2 mL vials for 24 h at 4 °C before GC–MS analysis.

2.3. Identification of compounds of Ananas spp. Leaf extracts by GC–MS

Samples were analyzed by GC–MS 7890B/MSD-5977 A (Agilent, California, USA). Chromatographic separations were performed using a 5% phenyl-methyl column (HP-5MS 30 m x 0.25 mm x 1.0 µm; Agilent Technologies). The carrier gas was helium (1 mL min^{-1}), and the injection volume was 1 µL in split mode (1:10) at 260 °C. The oven temperature was kept at 120 °C for 3 min and then programmed to 320 °C at 3 °C min^{-1}. The mass spectrometer operated in EI mode (70 eV) with a scan mass range of 40 to 660 *m/z*. The total time of the analysis was 79.67 min. The trimethylsilane (TMS) derivatives were tentatively identified by comparison of their mass spectra with those available from the National Institute of Standards and Technology (NIST) from the Spectrometer Database (NIST 2.0) and by comparing their linear retention indexes (LRI) with a C8-C30 n-alkanes series (van Den Dool and Kratz, 1963).

Table 1
Cultivars from the active germplasm bank of pineapple (AGB Pineapple) or from the experimental fields of Embrapa Cassava and Fruits.

Cultivars	Category/ origin / sample collection site	Cultivation status
Perolera (PE) BGA 049	Local cultivar / Venezuela / AGB pineapple of Embrapa	This cultivar is adapted to high altitudes up to 1500 m, and is cultivated in Colombia and Venezuela
Perola (BGA 001)	Cultivar / Selection from Brazil / AGB pineapple of Embrapa	Widely cultivated in Brazil
Golden or MD2	Cultivar /Selection from Central America / Experimental fields of Embrapa	This cultivar was selected in Central America and is widely cultivated in several pineapple-producing regions.
Smooth Cayenne (SC)	Cultivar / Selection from Venezuela / Experimental fields of Embrapa	This cultivar covers 70% of the area planted with pineapple worldwide
Imperial	HYBRID of PE x SC /Breeding Program - Brazil / Experimental fields of Embrapa	Small-scale plantings in Brazil
Vitoria	HYBRID of PE x SC /Breeding Program - Brazil / Experimental fields of Embrapa	Expanding cultivated areas
Ajuba	HYBRID of PE x SC /Breeding program - Brazil / Experimental fields of Embrapa	Expanding cultivated areas