Industrial Crops and Products 91 (2016) 179–185

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Compositional features and bioactive properties of whole fraction from *Aloe vera* processing

María L. Flores-López [a], Aloia Romaní [a], Miguel A. Cerqueira [a,b,*], Raúl Rodríguez-García [c], Diana Jasso de Rodríguez [c,**], António A. Vicente [a]

[a] Centre of Biological Engineering, Universidad do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[b] International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga s/n, 4715-330 Braga, Portugal
[c] Universidad Autonoma Agraria Antonio Narro, Calzada Antonio Narro No. 1923, Colonia Buenavista, Saltillo, 25315 Coahuila, Mexico

ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 7 July 2016
Accepted 11 July 2016
Available online 19 July 2016

Keywords:
Organic acids
Phenolic compounds
Antioxidant activity
Antifungal activity

ABSTRACT

Aloe vera processing leaves generates a liquid and bagasse fraction. The resulting bagasse is mostly discarded as waste. Both the bagasse and liquid fraction can have interesting metabolites with biological activities for pharmaceutical and agro-food industries. The main objectives of the present work were: (1) to characterize the gel, liquid fraction and bagasse of *A. vera*; (2) to obtain extracts from bagasse (ethanolic extract, EE-B and aqueous extract, AE-B); and (3) to evaluate biological activity of gel, liquid and bagasse extracts in terms of the antifungal effect on phytopathogenic fungi and antioxidant activity by the DPPH radical scavenging method. The carbohydrates were the major component of *A. vera* fractions corresponding to 57.45, 40.09 and 58.47 g of carbohydrates/100 g of gel, liquid fraction, and bagasse respectively. Uronic acids and malic acid were hallmarks of gel (15.80% and 18.17%, respectively); whilst for bagasse the occurrence of lignin is to be highlighted. The total phenolic content of the liquid fraction was 43.30 mg aloin g^{-1} extract, whereas the value of IC_{50} was 7.66 mg mL^{-1}; the first was significantly higher and the second was lower when compared to the corresponding values for the gel (19.11 mg aloin g^{-1} and 17.01 mg mL^{-1}, respectively). EE-B presented a greater antioxidant activity, higher total phenolic content and better antifungal activity than AE-B. In all the treatments, the antifungal effect was concentration-dependent and varied according to the fungus genera used in the experiments. *A. vera* gel and liquid fraction as well as EE-B are interesting natural alternatives to control phytopathogenic fungi in industrial crops during pre- and postharvest stages.

1. Introduction

For many years, plants from different ecosystems have been collected and studied as a source of new bioactive compounds for a huge range of applications, such as antioxidants (Kuppusamy et al., 2016), drugs (Zengin et al., 2015), pesticides (Jasso de Rodríguez et al., 2011), among others.

Postharvest damages of fruits and vegetables are often caused by colonization of various microorganisms, reducing their shelf life as well as their market value. In developing countries postharvest losses reach more than 40%, being these losses even higher in the storage stage than those occurring in the field (Flores-López et al., 2015). The use of synthetic chemicals, such as pesticides, is the most common approach for disease control in different crops; however, the application of such chemicals has caused severe damage to the health and environment, and frequently their application is only allowed during preharvest (Jasso de Rodríguez et al., 2011). Their indiscriminate use has developed microorganism resistance to the most widely used synthetic pesticides, causing their exit of the market (Flores-López et al., 2015). Thence, the need for new pesticides with enhanced performance and having a low impact on the environment. Natural products represent an eco-friendly alternative to the use of chemicals for the management of diseases of fruit and vegetables.

* Corresponding author at: International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330 Braga, Portugal. Tel.: +351 253 140 112; fax: +351 253 140 119.
** Corresponding author at: Universidad Autonoma Agraria Antonio Narro, Calzada Antonio Narro No. 1923, Colonia Buenavista, Saltillo, 25315 Coahuila, Mexico. Tel.: +52 844 4110296; fax: +52 844 411 02 11.
E-mail addresses: miguelribeirocerqueira@gmail.com, miguel.cerqueira@inl.int (M.A. Cerqueira), dianajassocantu@yahoo.com.mx (D. Jasso de Rodríguez).

http://dx.doi.org/10.1016/j.indcrop.2016.07.011
0926-6690/

Aloe vera, from the Liliaceae family, is a perennial plant with lance shaped leaves formed by a thick epidermis (skin). It has traditionally been consumed as whole leaf in folk medicine for its beneficial health effects (Grindland and Reynolds, 1986). Its biological activity is broadly accepted and it is used for several medical, nutraceutical and cosmetic applications (Boudreau and Beland, 2006). The plant is divided in two components: a colourless mucilaginous pulp (gel) and a bitter yellow sap (exudate) (Grindland and Reynolds, 1986). The gel is the most studied and used part of *A. vera* due to its complex chemical composition. It is composed by carbohydrates being mostly acemannans polysaccharides (Lee et al., 2001), but also soluble sugars, organic acids, proteins, phenolic compounds, vitamins, minerals and aminoacids are present (Boudreau and Beland, 2006). The effectiveness of *A. vera* gel to control fungal growth has been extensively proven against *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea*, and *Alternaria alternata*, among others (Castillo et al., 2010). Also, it has been incorporated into edible coatings (neat or in combination with other components) to extend the postharvest storage of strawberries (Sogvar et al., 2016) and apple slices (Chauhan et al., 2011).

The conventional methods for the extraction of *A. vera* gel are: (1) the traditional hand filleted pulp method, in which the entire gel is blended; and (2) the mechanical procedure characterized by a mechanical filleting followed by pressing, where the resulting gel can also be liquidized and filtered. The mechanical procedure also allows obtaining a liquid fraction (Jasso de Rodríguez et al., 2005). Recently, the interest for the liquid fraction has arisen, since it has shown to possess antifungal activity (Jasso de Rodríguez et al., 2005) and beneficial effects such as increasing the shelf life of blueberries has been reported (Vieira et al., 2016). However, there is limited information about the chemical composition and biological activities of *A. vera* liquid fraction.

The production process of *A. vera* fractions generates a large amount of solid wastes. These residues (bagasse) include the spikes, bases and tips removed from the leaves, and the skin resulting from the separation of the gel. Thus far, the bagasse has not been given any added value. Bioactive compounds can be extracted from the bagasse using organic solvents which are safe/less toxic (Cann, 2009), allowing an integral exploitation of *A. vera*. Therefore, the aims of this work were to (1) characterize the gel, liquid and bagasse of *A. vera*, (2) obtain extracts from bagasse, and (3) evaluate biological activity of gel, liquid and bagasse extracts in terms of antioxidant and antifungal activities on phytopathogenic fungi.

2. Materials and methods

2.1. Materials

Malic acid was supplied from Riedel-de Haën (Germany), citric acid anhydrous from J. T. Baker (USA), formic acid from Merck (Sweden), acetic acid from Sigma (USA) and lactic acid was supplied by Acros organics (USA). Galactose and mannose were obtained from Acros organics (USA), glucose from Fisher Scientific (USA), arabinose from Sigma (USA), galacturonic acid and xylose were supplied from Fluka (Slovakia). Sulfuric acid (95–98%) and barium carbonate were purchased from Sigma (USA). Aloin of purity >97% from *Aloe barbadensis* Miller leaves, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), buthylatedhydroxyanisole (BHA), sodium carbonate (Na_2CO_3) and Folin-Ciocalteau (FC) reagent were purchased from Sigma (USA). Ethanol absolute and methanol were obtained from Fisher chemical (UK). Potato dextrose agar (PDA) was purchased from Difco (France) and potato dextrose broth (PDB) from Liofilchem (Italy). All samples, standards and eluents were prepared using demineralized Milli-Q water from Millipore, USA.

2.2. Plant material and sample preparation

Fresh whole *Aloe vera* leaves (four years old), supplied by Aloe Vera Ecológico (Alicante, Spain), were washed with water, immersed in a 2.0% sodium hypochlorite solution, and rinsed with distilled water. The leaves were weighed (g), and measured for their length (cm), thickness (cm) and width (cm). For each leaf the spikes, inferior and superior parts were removed before longitudinally slicing to separate the epidermis from the parenchyma (fillet). The fillet was pressed by means of a laboratory manual roll processor and filtered in order to separate the liquid fraction from the gel and the bagasse. The yields were determined and expressed as percentage of either the obtained gel or liquid fractions with respect to the entire leaf weight. The gel and liquid fractions were pasteurized by heating at 65 °C for 30 min and cooled immediately; this step was repeated three times (Jasso de Rodríguez et al., 2005). Afterwards, one part of the samples was lyophilized and another was stored at −20 °C until further analyses were performed.

2.2.1. Preparation of the bagasse extracts

The bagasse resulting from the separation of the gel and liquid fraction was dried at 40 °C, then ground to a particle size equivalent to mesh No. 50 prior to extraction. Approximately 5 g of dried bagasse was thoroughly extracted in a Soxhlet apparatus during 48 h with absolute ethanol or distilled water (ratio 1:20) at 99.4 °C and 78.4 °C for aqueous (AE-B) and ethanolic extract (EE-B), respectively. The crude extracts were subsequently filtered (N°1 Whatman filter paper) and concentrated in a rotary evaporator. The extracts were stored in the dark at 5 °C until further use.

2.3. Physico-chemical characterization of Aloe vera fractions

All methodologies were conducted following the recommendations of the Official Method of Analysis (AOAC, 1990). The lipid content was determined gravimetrically by means of Soxhlet extraction (AOAC 960.39). The crude protein level was calculated by the Kjeldahl method with a conversion factor of 6.25 (AOAC 960.52). The ash content was evaluated by incineration in a muffle at 550 °C (AOAC 923.03). Moisture content was determined using the method AOAC 934.06. The pH value was determined using a pH meter (Metrohm, Swiss). All measurements were carried out in triplicate.

2.3.1. Organic acid analysis

The extraction of organic acids from lyophilized gel and liquid fraction was carried out with water (30 min at 60 °C), following the method described by Bozzi et al. (2007). After the extraction process, solutions were filtered through a 0.45 μm cellulose acetate membrane and organic acids (malic, citric, acetic and lactic acid) were determined by High-Performance Liquid Chromatography (HPLC). Chromatographic separation was performed using a Metacarb 87 H column (300 × 7.8 mm, Varian, USA) under the following conditions: mobile phase 0.005 mol L^{-1} H_2SO_4, flow rate 0.7 mL min^{-1}, and column temperature 60 °C. The equipment used was a UV detector set at 210 nm (Jasco, Tokyo, Japan) and a Jasco AS-2057 Plus intelligent auto sampler (Jasco, Tokyo, Japan). The volume injected was 20 μL per sample. The peaks obtained from each sample were identified and quantified through standard calibration curves.

2.3.2. Polysaccharide analysis after hydrolysis

Bagasse and lyophilized gel and liquid fraction were hydrolyzed via a two-step acid hydrolysis for polysaccharides quantification. Samples (100 mg) were pre-hydrolyzed in H_2SO_4 72% by continuously stirring at 30 °C during 1 h; then post-hydrolysis was