J. of Supercritical Fluids 122 (2017) 63–69

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

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

Bioactivity of extracts of *Musa paradisiaca* L. obtained with compressed propane and supercritical CO_2

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ARTICLE INFO

Article history:
Received 1 November 2016
Received in revised form
12 December 2016
Accepted 13 December 2016
Available online 14 December 2016

Keywords:
Antioxidant activity
Antibacterial activity
Anti-complement activity
Anti-inflammatory
Banana

ABSTRACT

This paper reports the assessment of bioactivity of inflorescences extracts of *Musa paradisiaca* L. obtained using supercritical CO_2 ($scCO_2$) and compressed propane as solvents. The effects of extraction conditions over the antioxidant and antibacterial activity were evaluated. The highest antioxidant activity (3576.86 ± 18.36 mg of α-tocopherol/g extract) was found for extracts obtained using $scCO_2$ as solvent at 353.15 K and 25.0 MPa. The extracts did not showed inhibition for the bacteria tested. The immunomodulatory effects of the extracts obtained by the extraction with 308.15 K and 3.0 MPa showed a significant inhibitory effect on the alternative pathway (AP) in all concentrations tested (333–5.2 $\mu g\,mL^{-1}$), with at least 70% reduction in the AP-induced haemolysis when compared with the 100% haemolysis control without the use of the extract.

1. Introduction

Extracts of natural plants that present biologically active substances in their composition can be used in a variety of products such as food, cosmetic and drugs formulations [1,2]. The growing interest and the widely application possibilities have stimulated the interest of various industrial sectors to explore and obtain extracts of new plant species, as well as optimize the extraction methods and evaluations of chemical composition, antioxidant and antimicrobial extracts assessments [3]. The demand for natural additives in industrialized foods to replace the synthetics, has led to the expansion of interest towards plants-based antibacterial and antioxidants. However, the sustainability of these natural antibacterial and antioxidants depend significantly on the abundance and the availability of the raw materials [4]. In addition, extracts of medicinal plants with ability in inhibiting the alternative pathways (AP) of the complement system (CS) is promising for the treatment of chronic autoimmune and inflammatory diseases, especially because the AP is responsible for about 80–90% of complement activation with tremendous effects on the inflammatory process [5].

Musa paradisiaca L. belongs to the family *Musaceae* and its fruit is the second most produced fruit in the world and one of the most important food source along with rice, wheat and corn [6]. In Brazil, banana is the second most widely produced fruit, placing the country as the fifth producer in the world (7 million tons produced in 2013). Concerning the babana chain production, it is estimated that about 105 million tons of waste are generated very year [7] that are mainly leaves, pseudostems, stalks and the inflorescences, which may represent an economic opportunity for the producers. From the environmental point of view, it is essential to reuse these wastes because it has been improperly discarded in landfills, contributing to the generation of environmental problems or it is left in the plantations where it is used as fertilizer for the soil [8].

The extraction of bioactive compounds from the waste of banana cultivation can be an interesting way to improve the profit in this chain by using the extracts and/or its components in food and pharmaceutical formulations.

Different parts of the banana plant are commonly used as food and popular medicine in many Asian countries and in Brazil [9]. Furthermore, the parts of banana plant, such as flower, pseudostem and rhizome have been studied for treating diarrhea and dysen-

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http://dx.doi.org/10.1016/j.supflu.2016.12.004
0896-8446/

Table 1
Experimental conditions for extraction yields for *Musa paradisiaca* L. extraction with $scCO_2$ and compressed propane as solvents [33].

Run	Solvent	T (K)	P(MPa)	Extraction yield[a] (wt%)	Time of extraction (min)
1	CO_2	313.15	15.0	1.74	180
2	CO_2	353.15	15.0	0.43	180
3	CO_2	313.15	25.0	3.60	180
4	CO_2	353.15	25.0	2.54	180
5	CO_2[b]	323.15	20.0	1.84 ± 0.12	180
6	Propane	308.15	3.0	2.57	180
7	Propane	338.15	3.0	2.91	180
8	Propane	308.15	10.0	2.50	180
9	Propane	338.15	10.0	3.14	180
10	Propane[b]	323.15	6.5	2.85 ± 0.20	180

[a] Mass of extract by the mass of dried material used × 100.
[b] Average value and standard deviation of triplicate runs.

tery, intestinal colitis [10], antilithic [11], inflammation, pain and snakebite [12–14], and protein metabolic disorders [15]. In addition, its potential as antimicrobial agent [16], antiulcerogenic [17], antihelmintic [18], hypoglycemic [19–21], antioxidant [22,23] and hypocholesterolaemic [24] has also been demonstrated over last decades.

One of the by-products of banana cultivation that has been generally treated as an agro-industrial waste is its inflorescence. It is the unique structure that protrudes out and remains at the terminal part of the fruit until its maturation stage. Some ethnics in the Asian region and Brazil consume the banana influences as part of their daily diet. Xokleng Indians (native people from Ibirama, State of Santa Catarina, Brazil) use the inflorescences in formulation of syrup, which also takes hot water and honey, and it is used as an expectorant to help in the treatment of respiratory diseases [25]. And in last years, some studies have indicated that the extracts of inflorescences *Musa paradisiaca* L. present anti-inflammatory and antioxidant activities for treating respiratory diseases [26], antihyperglycemic activity [27,28] and antimicrobial activity [28].

One of the main challenges for obtaining bioactive compounds from plants is the choice of extraction method because several factors must be considered, such as the solvent polarity, chemical nature of the bioactive compound, yield, selectivity, temperature, solubility, extraction time and costs. Although several methods can be used for obtaining natural plant extracts, the method features essentially define the quality of the final product. Among the extraction techniques, the supercritical extraction and/or the extraction with compressed fluids can be highlighted. In these techniques, a high compressible fluid is used as a solvent at low or middle temperature conditions. The use of supercritical fluids is a promising process for the extraction and fractionation of natural products, particularly for food and pharmaceuticals. The solvent removal facilitated, high selectivity and the use of moderate temperatures in the process are the main advantages of supercritical or compressed fluid extraction [29].

The solvent widely used in supercritical extraction is the CO_2 due to some specific advantages: it is safe, readily available, low cost, non-toxic at lower concentrations in the air, non-inflammable and non-carcinogenic. Furthermore, supercritical operations using CO_2 are performed at relatively low pressures and at near-room temperatures [30,31]. The extracts obtained from supercritical CO_2 ($scCO_2$) can be regarded as all natural, and the products allowed for food applications have the Generally Recognized as Safe (GRAS) status by the Food and Drug Administration (FDA, USA) [32].

A solvent that has been studied for extraction of natural matters and has showed promising results is the compressed propane [33,34]. As mentioned in our previous study [33], the application of compressed propane as solvent has gained attention due to some advantages over other solvents, it requires lower pressures and provides higher solubility of non-polar compounds. Studies suggest that propane can be more effective for obtaining natural extracts when compared to $scCO_2$ in relation to the efficiency of the process [35–39]. Main characteristics of the propane are that it is a relatively low cost solvent, provide solvent-free extracts and non-toxic residues and it is possible to reach high extraction yields over lower pressure conditions when compared to $scCO_2$ [40].

From our best knowledge, studies concerning the bioactivities evaluation of *Musa paradisiaca* L. extracts obtained with $scCO_2$ and compressed propane were not explored in the literature. In this context, this study is focused on the bioactivity of extract of *Musa paradisiaca* L. inflorescences using $scCO_2$ and compressed propane as solvent, in which the antioxidant activity, total antimicrobial activity and the effect extracts on the classical (CP) and alternative pathways (AP) of the complement system (CS) were evaluated.

2. Material and methods

2.1. Obtaining extracts

The inflorescences of *Musa paradisiaca* L. were collected in region of Pinhão (State of Paraná, Brazil). The supercritical fluid extraction procedures were performed as described in our previous study [33]. The extractions were performed with a constant flow rate of $2.0 \pm 0.3\ cm^3\ min^{-1}$. A factorial 2^2 experimental design with a triplicate center point was used. The fixed-bed for supercritical extractions was formed with 20.0 ± 0.2 g of dried and milled inflorescences of *Musa paradisiaca* L. with a moisture content of 8.50 ± 0.51 wt%. The particle size (average particle diameter) was 7.92×10^{-4} m. The experiments were performed at temperatures of 308.15, 323.15 and 338.15 K and 3.0, 6.5 and 10.0 MPa (Table 1).

2.2. Evaluation of antioxidant activity

The total antioxidant activity was determined by the spectrophotometric method involving phosphomolybdenum reduction, as described by Prieto et al. [41]. The method is based on the reduction of Mo^{6+} to Mo^{5+} with subsequent formation of the phosphate-Mo^{5+} complex, which presents maximum absorption at 695 nm. A 0.02 mL aliquot of the solution (0.01 g of extract and 25 mL of ethanol) was combined with 1.5 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The samples were incubated at 368.15 K for 90 min. After the samples were cooled to room temperature they were centrifuged and measured the absorbance of the solution at 695 nm against a blank (1.5 mL of reagent solution and 0.02 mL of ethanol). The results were expressed as mg of α-tocopherol/g of extract.

2.3. Evaluation of antibacterial activity

The antibacterial properties of the extracts obtained were tested by the disk diffusion test [42]. Two Gram-positive bac-