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Phytochemical characterization, antioxidant, antimicrobial and pharmacological activities of *Feijoa sellowiana* leaves growing in Tunisia

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ABSTRACT

In this study, headspace solid-phase microextraction coupled to GC–MS was applied to identify the volatile bioactive compounds in the leaves of *Feijoa sellowiana* growing in Tunisia. Thirty-one components were identified, representing 99.9% of the total volatiles. The major constituents were limonene (36.2%), β-caryophyllene (27.8%), aromadendrene (12.5%), and α-copaene (6.6%). Also, the *F. sellowiana* leaves extract (FSLE) was phytochemically characterized. Antioxidant activity was estimated by different *in vitro* assays, such as ABTS cation radicals scavenging, iron-chelating capability, ability to inhibit lipid peroxidation, superoxide inhibition and DNA protection assay. The antibacterial and antifungal activities of FSLE were also investigated by the disc diffusion and microdilution methods.

In vitro inhibition of diabetes key enzymes (α-glucosidase and α-amylase) was evaluated. The study of kinetics inhibition showed that the FSLE demonstrated a strong inhibition of both α-glucosidase (IC_{50} = 8.0 ± 0.2 µg/mL) and α-amylase (IC_{50} = 70.20 ± 0.8 µg/mL) in non-competitive manner. The acute toxicity of FSLE on *Wistar* rats at the doses of 200, 500 and 2000 mg/kg body weight (BW) was investigated. Our findings revealed that leaves extract at such doses as up to 2000 mg/kg did not cause any signs of toxicity or deaths in rats. Based on hematological and biochemical analyses of hepato-biliary and renal functions, we concluded that the FSLE is tolerated by rats. The analgesic effect of FSLE was assayed using the acetic acid writhing test in mice. At 100 mg/kg, the FSLE showed a higher analgesic activity (88.08 ± 0.73%) than that of acetylsalicylic acid (ASL) (62.69 ± 0.26%) used as positive control.

1. Introduction

The feijoa, *Feijoa sellowiana* (O. Berg.) (synonym, *Acca sellowiana*) belongs to the Myrtaceae family (Landrum, 1986). It is a shrub native to the South America (Paraguay, Uruguay, Brazil and Argentina), where it is widely distributed. Actually, it is abundantly cultivated in many countries, owing to its easy adaptability to subtropical regions climate (Giuseppe and Corrado, 2004). Presently, *F. sellowiana* grows throughout the Mediterranean area, where it is easily acclimatized and is introduced in various human food. Approximately, 20 varieties of feijoa were described in literature. This tree appears as an olive tree and grows in similar conditions and to a similar size (Giuseppe and Corrado, 2004).

It is characterized by hermaphroditic flowers (Dettori and Di Gaetano, 1991). The fruit which ripens on the tree to the size of a small apple appears with a smooth green skin and a soft white flesh (Di Cesare et al., 1998). The fruit of feijoa is characterized by the very aromatic flavor and a juicy pulp around the seeds.

In the Australian area, the plant is introduced in various industrial products, particularly in the form of syrups, jams, liqueurs and crystallized fruits. Several researchers showed that the fruit of feijoa contains high amounts of volatile compounds, including both methyl- and ethyl-benzoate, which are responsible for the strong feijoa-like character of the fruit, together with tannins, quinones, steroidal saponins

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and flavonoids (Shaw et al., 1990). Romero-Rodriguez et al. (1994) showed that *F. sellowiana,* similarly to other exotic fruits, was characterized by high levels of carbohydrates, ascorbic acid, minerals and iodine (Romero-Rodriguez et al., 1994). The feijoa fruit extract exhibited a powerful antimicrobial and antifungal activity (Motohashi et al., 2000; Vuotto et al., 2000). Furthermore, anti-cancer activities of the full feijoa extract have been described (Bontempo et al., 2007; Nakashima, 2001).

Despite all these researches on the fruit and peel of feijoa, little information is available about the pharmacological activities of its leaves. For this reason, we examined the phytochemical composition, volatile components, antioxidant and antimicrobial activities of feijoa leaves obtained from plants cultivated in Tunisia. In order to discover the usefulness of this plant in medicine, *in vitro* diabetes key enzymes inhibition, acute toxicity, analgesic and cytotoxic activities were also performed.

2. Material and methods

2.1. Chemicals reagents and enzymes

2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), trichloroacetic acid (TCA), iron (II) sulfate ($FeSO_4$), iron (II) chloride ($FeCl_2$), iron (III) chloride ($FeCl_3$), Folin–Ciocalteu reagent (FC reagent), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), acetic anhydride, sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$), ethylenediaminetetraacetic acid (EDTA), ascorbic acid, ferrozine, *Saccharomyces cerevisae* α-glucosidase, *p*-nitrophenyl-α-D-glucopyranoside (pNPG), acarbose, *p*-nitrophenyl phosphate (pNPP) and porcine pancreatic α-amylase and all other chemicals used for the analyses were of analytical grade and were purchased from Sigma-Aldrich.

2.2. Plant material

The leaves of *Feijoa sellowiana* were collected in October-November 2016 from the M'Saken (Sousse-Tunisia) area. The plant material was identified by Pr. Fethia Harzallah Skhiri (High Institute of Biotechnology of Monastir, Tunisia). A voucher specimen (N. FS16-17) has been deposited in the Herbarium of the Laboratory of Bioressources: Biologie Integrative and Valorization, High Institute of Biotechnology of Monastir, University of Monastir, Tunisia.

2.3. Volatile compound analyses

The volatile components of the *F. sellowiana* leaves were determined using headspace solid-phase microextraction (HS-SPME), a technique introduced for the first time by Pawliszyn (Arthur and Pawliszyn, 1990). This is an inexpensive, rapid, and solvent-free technique for the headspace analysis of volatile compounds from different samples (Wardencki et al., 2004). Even though the HS-SPME/GC–MS technique was already applied by Chinese researchers (Bai et al., 2016) to study the volatile constituents from *Feijoa sellowiana* leaves, this work is the first study of the volatile compounds emitted by the Tunisian *F. sellowiana* leaves using the same technique.

Supelco SPME devices coated with polydimethylsiloxane (PDMS, 100 μm) were used to sampling the headspace of leaves inserted into a 50 mL glass flask and allowed to equilibrate for 30 min. SPME sampling was performed using the same new fiber, preconditioned according to the manufacturer instructions, for all the analyses. Sampling was accomplished at 24 ± 1 °C. After the equilibration time, the fiber was exposed to the headspace for 15 min. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC–MS system.

GC-EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; and splitless injection. Identification of the constituents was based on the comparison of the retention times with those of authentic samples. In fact, the comparison was carried out between their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 2014 and ADAMS 2007) and home-made library mass spectra built up from pure substances and components of known mixtures and MS literature data (Swigar and Silverstein, 1981; Davies, 1990; Adams, 2007; Joulain and König, 1998).

2.4. Extraction

For extraction purpose, the *F. sellowiana* leaves were dried by active ventilation and ground in an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany) using 0.2 mm hole size sieves. An aliquot (1 g powder) of each sample was extracted with 40 mL of ethanol under slow stirring at 25 °C for 1 h and then filtered using Whatman N° 4 filter paper. The obtained residue was subjected to a second extraction with 40 mL of ethanol. The recovered ethanol extracts were combined and evaporated at 40 °C using rotary evaporator (Büchi R-210, Flawil, Switzerland). The final residue was re-dissolved in water using ultrasonic bath to obtain a final concentration of 20 mg/mL, and stored at 4 °C.

2.5. Phytochemical composition

The total phenolic contents (TPC) in FSLE was estimated by the Folin–Ciocalteu procedure (Wolfe et al., 2003). Gallic acid was used as standard and the results were expressed as mg of gallic acid equivalents (GAE) per g of FSLE.

For total flavonoids content (TFC) determination, the method of Jia et al. (1999) was applied. Catechin was used to calculate the standard curve and the results were expressed as mg of catechin equivalents (CE) per g of FSLE.

Furthermore, the content of flavonols was determined according to the method proposed by Romani et al. (1996). The results were expressed as mg rutin equivalents (RE) per g of FSLE.

However, the concentration of *ortho*-benzenediol contents (OBC) in the FSLE was measured according to Mekni et al. (2013) procedure. The *o-diphenols* were expressed as mg hydroxytyrosol equivalents (HE) per g of FSLE.

Finally, the condensed tannins contents (CTC) were determined according to the Julkunen-Tiitto (1985) method. The results were expressed as mg CE g/extract. Values presented are the average of three measurements.

2.6. Evaluation of the antioxidant activity

2.6.1. ABTS radical scavenging activity assay

The antiradical activity of FSLE was tested using the $ABTS^{\cdot+}$ free radical discoloration assay described by Re et al. (1999) with some modifications. A stock solution of 7 mmol/L of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) aqueous solution was prepared. The ABTS radical cations ($ABTS^{\cdot+}$) were produced by the reaction of the ABTS stock solution with 2.45 mmol/L $K_2S_2O_8$. The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain an absorbance of 0.7 ± 0.02 units at 734 nm. Samples were separately dissolved in ethanol to yield the following concentrations: 0.031, 0.062, 0.125, 0.25, 0.5 and 1 mg/mL. In order to measure the FSLE antioxidant activity, 10 μL of each sample were added to 990 μL of diluted $ABTS^{\cdot+}$ at various concentrations. The absorbance was read after 20 min. The antioxidant effect of each sample was expressed as the inhibition percentage (%). The $ABTS^{+}$ scavenging percentage was calculated as follows: