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Phenolic composition and antioxidant activity of kenaf leaves

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ABSTRACT

Kenaf (*Hibiscus cannabinus* L.) is a short day, annual, herbaceous plant producing high quality cellulose. Kenaf is a valuable fiber crop that is cultivated for its fibrous stem. Recently, seeds and leaves have been also considered as a source of industrial products, such as biopharmaceuticals. However, their pharmacological effects and chemical composition are still poorly studied.

In this context, the aim of this work was to identify the phenolic compounds and to evaluate the antioxidant activity of kenaf leaves extracts, comparing two varieties, Everglades 41 and Tainung 2, produced in Portugal. The identification of the phenolic compounds in the leaves was done using advanced analytical techniques such as high-performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry (HPLC–ESI–QTOF–MS). Parallel to this, in vitro antioxidant activity and total phenolic content of extracts were also determined. The antioxidant activity was evaluated with the use of two assays (oxygen radical absorbance capacity assay—ORAC and DPPH assay) and the total phenolic content by the Folin–Ciocalteu method.

HPLC–ESI–QTOF–MS analyses enabled to tentatively identify 29 compounds in both varieties, mainly chlorogenic acids and quercetin and kaempferol derivatives. Some of the compounds found in the kenaf leaves were related before to the *Hibiscus* genus. Results of Folin–Ciocalteu, DPPH and ORAC assays showed that Everglades 41 leaves were richer in phenolic compounds presenting a higher antioxidant potential than Tainung 2 leaves. This study indicates that kenaf leaves can be considered as potentially new source of antioxidants but its future use in food and nutraceutical matrices still need to be tested and evaluated.

1. Introduction

Kenaf (*Hibiscus cannabinus* L.) is an annual dicotyledonous herbaceous plant (Maganha et al., 2010) similar to cotton, okra, hollyhock and roselle (Alexopoulou et al., 2013b). Kenaf belongs to the Malvaceae family and originated from Africa being disseminated in the 20th century in Asia and USA. India and China are the top producers, representing ca. 70% of world total kenaf production (FAO, 2013). In Europe the production is not yet extensive (Alexopoulou et al., 2013a). The crop is resistant in semi-arid conditions like those found in Mediterranean region, reaching significant levels of aboveground production only with a range of 250–400 mm of water, much less than those required for traditional crops such as maize, sugar beet or alfalfa (Alexopoulou et al., 2009). According to Patanè et al. (2009), 14–16 Mg per ha of aboveground dry biomass (Tainung 2) can be obtained in Sicily with 330–340 mm water distribution (corresponding to 50% evapotranspiration restoration). In Central Greece, Alexopoulou et al. (2000) reported aboveground yields of 16–24 Mg per ha (Tainung 2 and Everglades 41) with only ca. 330 mm of water added by irrigation from May to September (water from rainfall was negligible). In Caparica pilot fields, 14–18 Mg per ha of aboveground dry biomass (Tainung 2) can be achieved with 420 mm water distribution (corresponding to 25% evapotranspiration restoration) (Fernando et al., 2007). Therefore, it could be used as an important alternative crop in areas with poor or moderate water availability (Bañuelos et al., 2002; Patanè and Sortino, 2010). Being an annual crop it can be easily accepted by the farmers, and can be grown in rotation systems with traditional food crops

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improving biodiversity, internal nutrient recycling, maintenance of the long-term productivity of the land, avoidance of accumulation of diseases and pests associated with mono-cropping and increasing crop yields (Fernando, 2013; Zegada-Lizarazu and Monti, 2011).

Kenaf is a valuable fiber crop and its fibrous stem are used for textiles, paper and pulp production, insulation mats, medium density fiber boards, biocomposites, absorbent materials, animal bedding, etc., (Alexopoulou et al., 2013b). Being cultivated especially for its fibrous stem, leaves (and other fractions of the plant) are crop residues which, in a biorefinery concept, driven by the bioeconomy challenge, should be reused and valorized. Yet, few studies have evaluated these fractions of the plant for industrial applications. According to Ryu et al. (2006), kenaf has long been used as a medicinal plant in Africa. Maganha et al. (2010), in their work, showed that plants of the genus *Hibiscus* are interesting sources of potential bioactive molecules, such as phenolic compounds, triterpene derivatives and phytosteroids with antioxidant, cardioprotective, antihypertensive and antiproliferative activities. Kenaf seeds contain 20–25% oil which is rich in polyunsaturated fatty acids (PUFA's), being edible with no toxins. Linoleic acid (C18:2) is the dominant PUFA, followed by oleic acid (C18:1), considered essential for normal growth and health, and important for reducing cholesterol and heart diseases. Seeds oil contains 4–10% of phospholipids and 0.6–1.2% of sterol (Mohamed et al., 1995). In addition, it was found that aqueous and methanol extracts of kenaf seeds may potentially serve as new sources of antioxidants for many applications (e.g., nutraceutical and functional food, and food preservation) due to its high phenolic content (Yusri et al., 2012). Regarding kenaf leaves, Kobaisy et al. (2001) studied the composition of the essential oil of the leaves and its phytotoxic and fungitoxic activity. Results indicated that the oil was phytotoxic to lettuce and bentgrass and had antifungal activity against *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *and Colletotrichum accutatum*. Other studies performed on kenaf leaves indicate that aqueous extracts demonstrated haematinic activity on haemolytic anaemic rats (Agbor et al., 2005a) and hepatoprotective activity against carbon tetrachloride and paracetamol induced liver damage in rats (Agbor et al., 2005c). Furthermore, the hydroalcoholic extract of *Hibiscus cannabinus* leaves exhibited a potent lipid lowering activity in diet induced hyperlipidemia (Shivali and Kamboj, 2010). There is also evidence that kenaf has a significant immunomodulatory effect in activated macrophages by inducing the expression of a potent cytoprotective molecule (Lee et al., 2007). Nevertheless, there are no relevant studies on the phenolic content and antioxidant activity of kenaf leaves. Thus, this study aimed to investigate the phenolic content and composition and the antioxidant activity of leaf extracts from two kenaf varieties, Everglades 41 and Tainung 2. These two varieties are commercial varieties extensively cultivated in the world thus originating a significant amount of crop residues, enabling its industrial exploitation (Alexopoulou et al., 2013c). Moreover, these two common varieties were also extensively studied in the framework of the Biokenaf project (www.cres.gr/biokenaf), supported by the European Union, and showed good adaptability to the climatic conditions of the Mediterranean region (Alexopoulou et al., 2013a). Additionally, according to Fernando et al. (2007), the amount of leaves potentially harvestable in combination with the cultivation of kenaf for fibre production can reach significant and marketable yields (in the range of 3.4–6.0 Mg per ha), just before plant flowering. In this way, kenaf leaf extracts of these two varieties, produced in Portugal, were analyzed by HPLC–ESI–QTOF–MS to thoroughly characterize their phenolic content, the total phenolic content was determined by the Folin–Ciocalteu method and were subjected to several assays (oxygen radical absorbance capacity assay—ORAC and DPPH assay), to measure their antioxidant activity.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were of analytical reagent grade and used as received. HPLC–MS-grade methanol were purchased from Labscan (Dublin, Ireland). The glacial acetic acid (HPLC grade), the standards utilized and, fluorescein (95%), Trolox (6-hydroxy-2,5,7,8-tetramethylchro-man-2-carboxylic acid) (97%), Folin–Ciocalteu reagent, AAPH (2,2′-azobis-2-methyl-propanimidamine dihydrochloride) (97%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), monobasic sodium phosphate (99%), dibasic sodium phosphate (99%), were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol used for the extraction of the phenolic compounds and sodium acetate (99%), were from Panreac (Barcelona, Spain). The standards of phenolic compounds were from Extrasynthèse (Lyon, France). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Plant samples

The leaves of *H. cannabinus* L. (two varieties, Everglades 41 and Tainung 2) were harvested from the pilot fields of kenaf established in Caparica, near Lisbon, Portugal, in September 2005, before flowering (14th–17th October). The experimental fields are situated in the Peninsula of Setúbal, in the south border of the river Tejo, near the estuary and Atlantic coast (latitude 38°40′03″N, longitude 9°12′28″W, altitude of 50 m), where the climate is warm temperate. During the experimental period, 4th May 2005–5th December 2005, the average minimum temperature was 15 °C and the average maximum temperature was 24 °C, with a total of 210 mm rainfall (2005 autumn was dry in Portugal). The experimental plots were established in a dominantly clay and alkaline soil. Standard basic plots had a surface area of 5×8 m^2 and three replications were used for each variety. Both varieties were grown with the same agronomic techniques and in the same field, being sowed and harvested on the same day. The fields were sowed at 4th May using a row spacing of 0.50 m and a distance between rows of 0.10 m (20 seeds per m^2). P-fertilizer (60 kg P_2O_5.ha^{-1}), K-fertilizer (120 kg K_2O.ha^{-1}) and ½ N-fertilizer (37.5 kg N.ha^{-1}) were applied at the time of sowing. The other ½ N-fertilizer was applied when the plants reached approximately 20 cm height (about 1 month after sowing). All the fields were fully irrigated in order to compensate the water deficit of the soil, and to prevent water stress (*ca.* 840 mm was added to the fields).

At harvest, in each plot, 1 m^2 of area was collected, ca. 17–20 plants. The harvested leaves were then dried at 40 °C in a vacuum oven, ground up manually with a porcelain mortar and pestle and stored in darkness, in a dry place at room temperature until use.

2.3. Leaf extracts

An adaptation of the extraction method used by Agbor et al. (2005b) was applied. For each variety, the extracts were prepared from a mixture of all the dry leaves from the replicated fields. 500 mg of kenaf leaves, weighed by an analytical scale Mettler Toledo AB204, were milled in 15 mL of MeOH by an electric mill, IKA®T18basic ultra-turrax. The mixture was maintained for 2 h in the ultrasonic bath (Branson 3510) at room temperature. Then, samples were centrifuged for 15 min at 4000 rpm using a centrifuge (Labofuge 200, Heraeussepatech), the supernatant was removed, and the extraction was repeated three more times. The supernatants were collected, evaporated in a rotary evaporator at 40 °C, and reconstituted with 2 mL of methanol. Finally, the extracts were filtered through 0.45 μm syringe filters and stored at −18 °C until analysis, to avoid degradation. Three replicated extracts were