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Valorisation of *Mangifera indica* crop biomass residues

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ABSTRACT

Mangifera indica L. is one of the most important commercial plants worldwide in terms of production, marketing and consumption. Although the fruit is widely studied, few works focus other vegetal materials, which could be excellent sources of metabolites with potential application in several industries. Additionally, some *M. indica* varieties remain unstudied. The aim of this work was to explore for the first time the chemical composition and the biological properties of aqueous extracts from *M. indica* var. Nunkourouni leaf and stem bark. Malic and quinic acids were the most abundant organic acids. Mangiferin and gallic acid were the main phenolics in stem bark and leaves, respectively. A concentration-dependent activity was noticed against several reactive species, stem bark displaying stronger antioxidant capacity. The two materials also inhibit α-glucosidase and α-amylase, leaves being more potent. The cytotoxic effects on AGS cells were also approached, leaves being the most active material. The results suggest that *M. indica* leaf and stem bark could be valuable sources of bioactive compounds, contributing to the valorisation of these materials and their further application in high prevalence diseases.

1. Introduction

The genus *Mangifera* belongs to the Anacardiaceae family, order Sapindales, comprising 73 genera and about 850 species (Tharanathan et al., 2006). *Mangifera indica* L., commonly known as mango, is a long living large evergreen tree native from tropical Asia, being now found naturalized in many tropical and sub-tropical regions (Wauthoz et al., 2007). Actually, this tree is cultivated on an area of approximately 3.7 million ha worldwide and its fruit is one of the most important fruit crops, having socio-economic significance in many countries (Tharanathan et al., 2006; Jahurul et al., 2015). In West Africa mango varieties are grouped into four categories: the local or polyembryonic mangos (Nunkourouni and Number One), which are used as graft support for producing non-fibrous fruits intended for marketing; the first monoembryonic varieties propagated by grafting (Amélie, Julie, Sabot, Djibelor and Cuisse Madame); the Floridian varieties, also monoembryonic and propagated by grafting, introduced later and used for export (Kent, Keitt, Palmer, Zill, Valencia, Smith, Irwin and Haden); varieties used for the regional markets (Brooks, Davis-Haden, Miami Late, Springfels, Beverly, Eldon and Ruby) (Rey et al., 2004). Nunkourouni variety, also known as Tête de Chat, is widespread throughout West and Central Africa. Nevertheless, to our knowledge, there is no previous study involving this *M. indica* variety.

Besides the use of the fruit for human consumption, mango leaf and stem bark are known to possess several biological properties, including antioxidant, anti-inflammatory and antidiabetic ones (Sanchez et al., 2000; Aderibigbe et al., 2001; Garrido et al., 2004). Actually, the use of mango-derived extracts as herbal drugs is widespread in traditional medicine. For example, mango seeds are used in India as an antidiarrheal agent (Sairam et al., 2003). Fresh mango kernel is consumed in Fiji to treat dysentery and asthma (Singh, 1986). Leaves have been widely used in tropical Africa in infusions due to their antipyretic and antidiarrheal effects, and the stem bark is used to prepare an antihypertensive, antidiarrheal and an antiulcer infusion (Wong, 1976; Lauricella et al., 2017).

Several studies have focused on mango fruit, its by-products (peels and seeds) and stem bark, describing the presence of high levels of health-promoting substances, such as phenolic compounds, carotenoids, tocopherols and sterols (Núñez Sellés et al., 2002; Barreto et al., 2008; Masibo and He, 2008; Kim et al., 2010; Jahurul et al., 2015). Concerning to *M. indica* leaf, there are some works reporting its physicochemical parameters (Romero et al., 2015), its potential to

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scavenge heavy metal pollutants (Laskara et al., 2017) and different techniques to extract its compounds (Kulkarni and Rathod, 2014). Recently, Pan et al. (2018) isolated and identified twenty-two phenolic compounds (5 benzophenones and 17 flavonoids) from the different fractions of mango leaves. Nonetheless, it is widely known that the cultivar, the plant physiological stage and the climatic conditions are parameters that influence the phenolic composition (Scalbert and Williamson, 2000).

Since agricultural industry produces billions of tons of residues in non-edible portions derived from the cultivation and processing of a particular crop, including primary biomass residues (leaves, roots, stems), that cause pollution, management and economic problems (Santana-Méridas et al., 2012), several studies have been developed to use the agricultural residues as a source of high-value products (Figueiredo-González et al., 2017; Zeković et al., 2017). Thus, the main goal of this work was to expand the knowledge on mango leaves and stem bark, valuing the potential application of these residues in the treatment of diseases with high prevalence worldwide. To achieve these purposes, phenolic compounds and organic acids profiles of aqueous extracts from leaves and stem bark of mango from var. Nunkourouni were characterized, for the first time, by high pressure liquid chromatography coupled to a diode-array detector (HPLC-DAD) and high pressure liquid chromatography coupled to an ultraviolet detector (HPLC-UV), respectively, both analytical methods being fully validated. Antioxidant potential, checked against 2,2-diphenyl-1-picrylhydrazyl (DPPH·), superoxide anion ($O_2\cdot^-$) and nitric oxide (·NO) radicals, and lipid peroxidation, was assessed through spectrophotometric assays. Antidiabetic capacity, concerning to α-glucosidase and α-amylase inhibitory capacity, was evaluated by spectrophotometric microassays. The cytotoxic potential of both herbal infusions against human gastric cancer cells (AGS) was approached.

2. Materials and methods

2.1. Standards and reagents

Oxalic, aconitic, citric, ascorbic, tartaric, malic, quinic, shikimic, fumaric, gallic and linoleic acids were from Sigma-Aldrich (St. Louis, MO, USA); ellagic acid, mangiferin and quercetin-3-*O*-glucoside were from Extrasynthèse (Genay, France). Sodium nitroprusside dihydrate was from Riedel-de Haën (St. Louis, MO). *N*-(1-Naphthyl)ethylenediamine dihydrochloride, phosphoric acid, iron (II) sulphate ($FeSO_4{\cdot}7H_2O$) and methanol were from Merck (Darmstadt, Germany). Sulphanilamide, β-nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulphate (PMS), DPPH, acarbose, α-glucosidase (from *Saccharomyces cerevisiae*), α-amylase (from porcine pancreas), 4-nitrophenyl-α-D-glucopyranoside (PNP-G), dinitrosalicylic acid, soluble starch and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. Ethanol and diethyl ether were from Panreac (Barcelona, Spain) and Fisher Scientific (Loughborough, UK), respectively. The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA). The Chromabond C18 SPE columns (70 mL/10,000 mg) were purchased from Macherey-Nagel (Duren, Germany). Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's phosphate buffered saline, heat inactivated foetal bovine serum (FBS), Pen Strep solution (penicillin 5000 units/mL and streptomycin 5000 µg/mL) and 0.25% trypsin-EDTA (1X) were purchased from Gibco, InvitrogenTM (Grand Island, NY, USA).

2.2. Plant material

The young leaves of *M. indica* and stem bark were collected in Bondoukou (North East of Ivory Coast), in July 2016. Plant material was botanically identified by Dr. Vangha Madeleine from the Laboratory of Physiology and Botanical of University of Nangui Abrogoua (Ivory Coast). In order to obtain a representative sample, leaves and stem bark were collected from different trees. Samples were collected to sterile plastic bags and immediately transported to the laboratory in insulated sealed ice-boxes wash under running water and dehydrated at 20 °C for 20 days, protected from light. The plant material was then powdered (mean particle size lower than 910 µm). Voucher specimens were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Porto University (MIL072016 and MISB072016).

2.3. Extraction

In order to simulate the usual form of human consumption (Wong, 1976), an aqueous extract of each material was prepared by boiling ca. 5 g of powdered material for 30 min, in 500 mL of distilled water. The resulting extracts were filtered using a Büchner funnel and then freeze-dried in a Virtis SP Scientific Sentry 2.0 apparatus (Gardiner, NY 12525, USA). The lyophilized extracts were kept in a desiccator in the dark until analysis. The extraction yields of the aqueous extract from leaves and stem bark were 28.72% and 26.48%, respectively.

2.4. HPLC-DAD for phenolic compounds analysis

Each lyophilized extract was redissolved in water, filtered and 20 µL were analysed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 (25.0 × 0.46 cm; 5 µm, particle size) column, according to a described procedure (Oliveira et al., 2009). The solvent system used was a gradient of water-formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min and 80% B at 60 min, at a solvent flow rate of 0.9 mL/min. Detection was achieved with a Gilson Diode Array Detector (DAD). Spectral data from all peaks were accumulated in the range 200–400 nm, and chromatograms were recorded at 255, 280, 320 and 350 nm. The data were processed on a Clarity Software system. Peak purity was checked by the software contrast facilities. The compounds in each extract were identified by comparing their retention times and UV–vis spectra in the 200–400 nm range with authentic standards, and their quantification was achieved by the absorbance recorded in the chromatograms relative to external standards. Ellagic acid and quercetin-3-*O*-glucoside were determined at 255 and 350 nm, respectively. Gallic acid derivative was determined as gallic acid at 280 nm, and mangiferin derivative as mangiferin at 320 nm. Each extract was analysed in triplicate.

2.5. HPLC-UV for organic acids analysis

Before HPLC analysis, each lyophilized extract was dissolved in acid water (pH 2 with HCl). The solution obtained was passed through a SPE C18 column, previously conditioned with 30 mL of methanol and 70 mL of acid water. The aqueous solution was then evaporated to dryness under reduced pressure (40 °C), redissolved in sulphuric acid 0.01 N (1 mL) and 20 µL were analysed on an analytical HPLC unit (Gilson), using an ion exclusion column Nucleogel® Ion 300 OA (300 × 7.7 mm), in conjunction with a column heating device set at 30 °C, according to a described procedure (Oliveira et al., 2009). Elution was carried out at a solvent flow rate of 0.2 mL/min, isocratically, with sulphuric acid 0.01 N as the mobile phase. Detection was performed with a Gilson UV detector set at 214 nm. The data were processed on a Clarity Software system (Data Apex, Prague, Czech Republic). Organic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards.