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Optimization of extraction method to obtain a phenolic compounds-rich extract from *Moringa oleifera* Lam leaves

C. Rodríguez-Pérez[a,b], R. Quirantes-Piné[b,*], A. Fernández-Gutiérrez[a,b], A. Segura-Carretero[a,b]

[a] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avenida Fuentenueva s/n, 18071 Granada, Spain
[b] Research and Development Functional Food Centre (CIDAF), Health Science Technological Park, Avenida del Conocimiento s/n, Edificio BioRegión, 18016 Granada, Spain

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ABSTRACT

Moringa oleifera Lam is considered one of the most useful tree in the world because every part of the *Moringa* tree can be used such as nutritional supplement, for medication, and industrial purposes.

Conventional solid–liquid extraction and ultrasound-assisted extraction (UAE) were performed using different solvents and mixtures of solvents with water. The total phenolic content was determined using Folin–Ciocalteu assay. UAE using ethanol:water (50:50) was the best extraction procedure, which allowed 47 ± 4 mg gallic acid equivalents (GAE)/g dry leaf to be obtained. In addition, high-performance liquid chromatography coupled to electrospray ionization quadropole-time of flight mass spectrometry (HPLC–ESI–QTOF–MS) was used to characterize the bioactive compounds in the resulting extract. Consequently, 59 compounds were tentatively characterized, phenolic acid derivatives and flavonoids being the most abundant. Furthermore, 30 of these compounds were tentatively identified for the first time in *M. oleifera* leaves.

This study shows that leaves from *M. oleifera* are a good nutritional resource used as a nutritional supplement and may carry additional opportunities for food ingredient innovations, pharmaceutical and cosmetics products.

1. Introduction

Moringa oleifera (Moringaceae), also known as "the tree of life", is mainly native to India and Africa. It is considered one of the most useful trees in the world because every part of the *Moringa* tree can be used for food, medication and industrial purposes (Moyo et al., 2011). In particular, the leaves can be eaten fresh in salad, cooked, or stored as dried powder for many months without loss of nutritional value. For this reason, in some areas of Africa, various relief organizations are promoting consumption of its leaves as a nutritional supplement together with rice and other foods to prevent malnutrition in the poorest countries. Apart from treating malnutrition, in rural areas of Uganda, its leaves are used to treat a wide range of medical conditions such as HIV/AIDS-related symptoms, bronchitis, ulcers, malaria and fever, among others (Kasolo et al., 2010).

Its benefits could be attributed to their composition of phenolic compounds. The search for new antioxidants and phenolics from herbal sources has garnered great attention in the last decade. In this regard, leaf extracts of *M. oleifera* have been reported to exhibit antioxidant activity both *in vitro* and *in vivo* due to their abundance of phenolic acids and flavonoids (Vongsak et al., 2013). This fact, together with the possibility of the phenolic compounds interacting with other plant components, makes the extraction probably the most important step in sample pretreatment. The extraction of polyphenols depends greatly on the solvent's polarity, method and extraction time, which determine both the quantitative and qualitative composition of those compounds. The polarities of phenolic compounds vary significantly and it is difficult to develop a single method for optimal extraction of all phenolic compounds (Garcia-Salas et al., 2010). The total polyphenol content determined from the same plant and its corresponding antioxidant activity may vary widely depending on the extraction conditions applied. Due to the aforementioned, the necessity of optimizing the extraction method for each sample prior to carrying out the analytical characteriza-

* Corresponding author at: Research and Development Functional Food Centre (CIDAF), Health Science Technological Park, Avenida del Conocimiento s/n, Edificio BioRegión, 18017 Granada, Spain. Tel.: +34 685 814310; fax: +34 958 637083.
E-mail address: rquirantes@ugr.es (R. Quirantes-Piné).

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tion seems indispensable. The optimal extraction method should be simple, rapid and economical.

Another point to keep in mind is that there are considerable variations in the nutritional value of *M. oleifera*, which depend on several factors like genetic background, environment and cultivation methods, as reported by Moyo et al. (2011).

Previous phytochemical analyses of *M. oleifera* from different countries have shown that the leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as known antioxidants such as β-carotene, vitamin C, and flavonoids (Mbikay, 2012). However, *M. oleifera* from Madagascar (South Africa) has been little studied. Authors such as Moyo et al. (2011) have proclaimed the necessity of studying the nutritional value of *M. oleifera* of the South African ecotype (Moyo et al., 2011).

For this reason, the purposes of this study were: (1) to determine the best way to extract the greatest amount of phenolics and other bioactive compounds from the leaves of *M. oleifera*; (2) to characterize the bioactive compounds using HPLC–ESI–QTOF–MS in order to provide an exhaustive compositional information.

2. Materials and methods

2.1. Chemicals

HPLC–MS acetonitrile was purchased from Fisher (Thermo Fisher Scientific UK, Bishop Meadow Road, Loughborough, Leicestershire, UK). Acetic acid of analytical grade (purity > 99.5%) was acquired from Fluka (Switzerland). Methanol and acetone used to extract the phenolic compounds from *M. oleifera* were purchased from Panreac (Barcelona, Spain). Ethanol, gallic acid and Folin–Ciocalteu reagent were from Sigma–Aldrich (Steinheim, Germany). Ultrapure water with a resistivity value of 18.2 MΩ was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Plant material

The leaves of *M. oleifera* collected from Madagascar in 2010 were identified by the National Center for environmental research (CNRE), Antanarivo, (Madagascar, Africa) where the voucher specimen was deposited. Branches from the tree were plucked by hand and laid on shelves 3 m long, 30 cm wide and 50 cm apart in a dry place with good ventilation and in darkness. Two weeks later, the leaves were crushed with a stone mortar and the dust obtained was stored in darkness in a dry, fresh place until their treatment.

2.3. Methods for extracting phenolic compounds from M. oleifera leaves

Several extraction methods were performed using different percentages of different solvents and mixtures of solvent with water as shown in Fig. 1. Conventional solid–liquid extraction (maceration) and ultrasound-assisted extraction (UAE) were tested as extraction methods.

2.3.1. Conventional solid–liquid extraction

First, 0.5 g of plant material was extracted with 25 mL methanol:water (50:50, v:v). Maceration was carried out at different times (1, 3, 6 and 12 h) at room temperature in order to know the time frame in which most of the compounds were extracted. There were no significant differences among the times of extraction with maceration. For this reason, the time selected for the other macerations was 1 h.

After that, 0.5 g of plant material was extracted with 25 mL of the corresponding solvent for 1 h on a stir plate at room temperature.

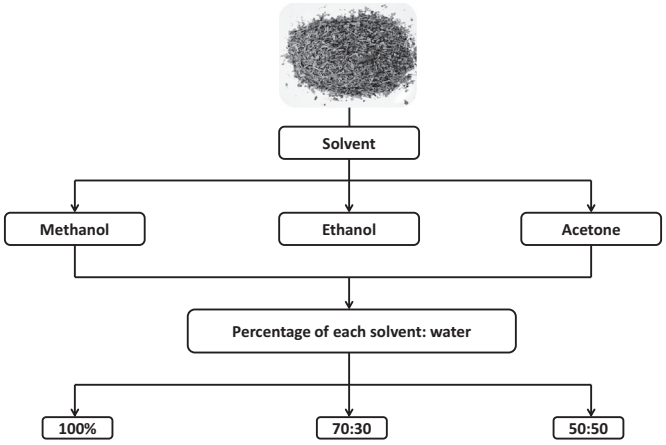

Fig. 1. Percentage of different solvents and mixtures of solvents with water used for each method of extraction.

Then, samples were centrifuged for 10 min at 984 × *g* using a centrifuge to remove solids. Next, the solvent was evaporated using a rotary evaporator under vacuum at 40 °C, and the dried residue was redissolved in 2 mL of the corresponding solvent. These solutions were filtered through a 0.45-μm syringe filter and kept at −20 °C in amber bottles to avoid degradation until analysis. Each extraction was done in triplicate.

2.3.2. Ultrasound-assisted extraction (UAE)

Afterwards, ultrasound-assisted extraction was carried out as described by (Rodríguez-Pérez et al., 2013b) with some modifications. First, 0.5 g of each *M. oleifera* extract was extracted using 25 mL of different solvents in a sonicator Branson B3510 for 15 min at room temperature. Then, the samples were centrifuged for 10 min at 984 × *g* using a centrifuge to remove solids. After centrifugation, the pellets were extracted with fresh solvent under the same conditions in such a way that four consecutive extractions were made. The supernatants were dried in a rotary evaporator under vacuum at 40 °C, and the dried residue was redissolved in 2 mL of methanol, ethanol or acetone. These solutions were filtered through a 0.45-μm syringe filter and kept at −20 °C in amber bottles to avoid degradation until analysis. Each extraction was done in triplicate.

2.4. Determination of total phenolic content

The total phenolic content of the obtained extracts was determined using the Folin–Ciocalteu assay (Herrero et al., 2011) with some modifications. The total volume of the reaction mixture was reduced to 1 mL. 600 μL of water and 10 μL of sample were mixed, to which 50 μL of undiluted Folin–Ciocalteu reagent was subsequently added. After 10 min, 150 μL of 2% (w/v) Na_2CO_3 were added and the volume was made up to 1.0 mL with water. After 2 h of incubation at room temperature in darkness, 200 μL of the mixture was transferred into a well of a microplate. The absorbance was measured at 760 nm using a Synergy Mx Monochromator-Based Multi-Mode Micro plate reader, by Bio-Tek Instruments Inc. (Winooski, VT) and compared to a gallic acid calibration curve (5 to 150 μg/mL) elaborated in the same manner. The total phenolic content was calculated as mean ± SD ($n = 6$) and expressed as mg of gallic acid per g of dry leaves.

2.5. Chromatographic separation

HPLC analyses were carried out using an Agilent 1200 Series Rapid Resolution LC system (Agilent Technologies, Palo Alto, CA,