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Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana

Newton K. Amaglo [a], Richard N. Bennett [b,*], Rosario B. Lo Curto [c], Eduardo A.S. Rosa [b], Vincenzo Lo Turco [c], Angela Giuffrida [c], Alberto Lo Curto [c], Francesco Crea [d], Gladys M. Timpo [a]

[a] *Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana*
[b] *CITAB-Departamento de Fitotecnia e Engenharia Rural, Edificio Ciências Agrárias, Universidade de Trás-os-Montes e Alto Douro (UTAD), Apartado 1013, 5001-801 Vila Real, Portugal*
[c] *Department of Food and Environmental Sciences, University of Messina, Salita Sperone, 31, 98166 Messina, Italy*
[d] *Department of Inorganic Chemistry, Analytical Chemistry and Physical Chemistry, University of Messina, Salita Sperone, 31, 98166 Messina, Italy*

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ABSTRACT

The purpose of this new study was to determine the types and levels of major phytochemicals (non-nutrients) and nutrients in the different tissues from vegetative and flowering *Moringa oleifera* L. an important multipurpose crop. Rhamnose and acetyl-rhamnose-substituted glucosinolates were found in all of the *M. oleifera* tissues with different profiles depending on the tissue. In addition the tissues of *M. oleifera* had a relatively complex flavonoid profile consisting of glucosides, rutinosides, malonylglucosides and traces of acetylglucosides of kaempferol, quercetin and isorhamnetin. Fatty acid profiling of the different tissues showed that leaves were rich in palmitic (16:0) and linolenic (18:3) acid whereas seeds were predominated by oleic acid (18:1). Roots were rich in palmitic and oleic acid, whereas stems and twigs predominately contained palmitic acid. Potassium, magnesium and calcium were the predominant minerals in all of the tissues. Low levels of selenium were detected only in whole seeds.

1. Introduction

Moringa species and specifically *M. oleifera*, a.k.a *M. pterygosperma*, from the Capparales family Moringaceae are important multipurpose crops in Africa and India. The species appear to have originated in India and Africa, but are now grown around the world. There are major production sites in Ghana, Senegal, and Malawi, smaller production in New Zealand and Fiji, and more recently production has begun in Nicaragua and Bolivia. *Moringa* species are often important famine foods because of their high tolerance to arid conditions due to the formation of very large tuberous roots (Sena et al., 1998). The uses of *Moringa* species are diverse, including the use of roots, leaves, flowers, green pods, and seeds for human foods, and additionally stems and petioles for animals for animal feed. Seeds are used for the production of cooking oil, biodiesel and as a source of an important water purification agent; proteins in the seeds can be easily extracted and used for treating contaminated water and also as a reliable method for cleaning slurry materials. Older roots and root and tree bark are a good source of tanning agents. Various medicinal compounds are obtained from all parts of the plant. The various food/medicinal and industrial uses of *M. oleifera* have recently been reviewed (Anwar, Latif, Ashraf, & Hassan Gilani, 2007; Bhuptawat, Folkard, & Chaudhari, 2007; Rashid, Anwar, Moser, & Knothe, 2008).

Relating the reported health effects to the composition is a key part of determining beneficial/nutritional and anti-nutritional effects. Another fundamental consideration, when evaluating different plant tissues for their uses, is that they often contain different levels and profiles of nutrients and phytochemicals (non-nutrients), i.e. ontogenic differences, due to innate differences in biosynthesis, transport and catabolism properties of the individual tissues. For example, vegetative tissues can contain different nutrients and phytochemicals compared with reproductive tissues (Bennett et al., 2003 and Bennett et al., 2006). *Moringa* species are rich sources of various phytochemicals including uncommon sugar-modified glucosinolates, although there are only details on quantity and profiles for *M. oleifera*, *M. peregrina* and *M. stenopetala*. (Bennett et al., 2003; Fahey, Zalcmann, & Talalay, 2001). The predominant glucosinolate is 4-*O*-(α-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) and depending on the tissues three mono-acetyl-rhamnose isomers of this glucosinolate have also been detected (Bennett et al., 2003; Kjaer, Malver, El-Menshawi, & Reischt, 1979). There are also reports of various nitriles, thiocarbamates and carbamates, with strong hypotensive and spasmolytic effects, present in leaves of *M. oleifera* (Leuck & Kunz, 1998). It is clear that these are probably derived from the catabolism of the major glucosinolates by the

* Corresponding author.
E-mail address: rnbennett@utad.pt (R.N. Bennett).

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action of myrosinase – primarily forming isothiocyanates and nitriles which can then be catabolised further into the various nitrile, thiocarbamate and carbamate derivatives. However, the levels of these compounds appear to be very low compared with the other phytochemicals. Chlorogenic acids and flavonols have been reported in different tissues of *M. oleifera* and *M. stenopetala* but there is no information for other *Moringa* species (Bennett et al., 2003; Lako et al., 2007; Manguro & Lemmen, 2007; Siddhuraju & Becker, 2003). The predominant core aglycones are flavonols: quercetin > kaempferol > isorhamnetin. However it is clear there are problems with taxonomic identifications since one report on leaf flavonols of *M. oleifera* describes the presence of galloylated flavonoids, which have not been found in any of the previous studies (Bennett et al., 2003; Manguro & Lemmen, 2007; this study). In parallel with the phenolics studies are several reports of antioxidant activities of different leaf extracts (Bajpai, Pande, Tewari, & Prakash, 2005; Iqbal & Bhanger, 2006; Siddhuraju & Becker, 2003).

In terms of proximate composition there have been a few studies on nutritional and phytochemical components of leaves of *M. oleifera* (Barminas, Charles, & Emmanuel, 1998; Freiberger et al., 1998; Makkar & Becker, 1996; Sena et al., 1998). Different tissues of *M. oleifera* are good sources of tocopherols (Sánchez-Machado, López-Cervantes, & Ríos Vásquez, 2006). There is data on the physical and chemical properties, including fatty acid profiles, of the seed oils from *M. oleifera* grown in different countries and also different *Moringa* species (Anwar & Bhanger, 2003; Kleiman, Ashley, & Brown, 2008; Lalas & Tsaknis, 2002; Tsaknis, Lalas, Gergis, Dourtoglou, & Spiliotis, 1999). The leaves of *M. oleifera* are also a rich source of β-carotene (pro-vitamin A) and are being used as a low cost food to improve human health in vitamin A-deficient diets (Babu, 2000). Various nutritionally important macro and micro minerals have also been analysed in *M. oleifera* leaves (Barminas et al., 1998; Freiberger et al., 1998; Sena et al., 1998). A more recently considered important micro mineral in foods is selenium, which is present in food matrices in many biological forms such as selenite, selenate, elemental selenium, selenocysteine, selenomethionine, and selenoproteins (Ganther & Lawrence, 1997; Rayman, 2000).

The purpose of the present study was to determine the types and levels of the selected phytochemicals and nutrients in the different tissues; many of these tissues, although consumed by humans and animals, have not been previously analysed. Selected major phytochemicals (glucosinolates, phenolics and flavonoids) and nutrients (total protein, crude fat, fatty acids and minerals) were determined for the different tissues of *Moringa oleifera*, grown in Ghana, at two distinct developmental stages – vegetative plants and plants post-flowering/pod production. This data will be used as the first step in evaluating the composition in relation to the reported health and other effects.

2. Materials and methods

2.1. Plant material and sample preparation

M. oleifera L. certified seed was obtained from Educational Concern for Hunger Organization (ECHO), Florida, USA. The seeds were grown at the Horticulture Department of the Kwame Nkrumah University of Science and Technology, Kumasi (Latitude 5°, 36 min North; Longitude 0°, 10 min East). Kumasi lies in the semi-deciduous forest zone of Ghana and enjoys a humid tropical climate. The rainfall pattern is bimodal (two wet and two dry seasons). The mean annual rainfall is 1563 mm of which about 55% occurs between March and July and 30% occurs between September and November. There is usually a short dry season in August and a long one from December to March. A considerable variation exits in the duration of the rainy season and intensity of rainfall. Monthly temperature averages range from 27 °C to 29 °C in the year with February, March and April usually being the hottest months.

Seeds were nursed and transplanted in May 2004. Eight weeks old seedlings were set out on the field at a spacing of 2×2 m^2. The root, stem, petiole and leaf samples from vegetative plants were collected when plants were 100 days old. Samples were again collected from plants that had both young and mature pods and flowers, when plants were 320 days old. Seeds were taken from fully mature pods of plants that were 380 days old. All fresh weights and dry weights (post air-drying and post-freeze-drying) were recorded. All samples were initially air-dried in a hot dry electric oven at 45 °C for 72 h in Ghana and the samples sent to UTAD for further processing (freeze-drying followed by powdering).

2.2. Reagents and reference compounds

A sample of purified 4-*O*-(α-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin), purity confirmed at UTAD by HPLC, was kindly donated by Prof. Renato Iori (Research Centre for Industrial Crops, Agriculture Research Council [CRA-CIN], Bologna, Italy). All chemicals and reagents were of analytical grade and were obtained from various commercial sources (Sigma/Aldrich and Merck). All solvents were of high-performance liquid chromatography (HPLC) grade, and all water was ultrapure. Sinigrin (2-propenylglucosinolate; internal standard), chlorogenic acid (3-caffeoylquinic acid) and rutin (quercetin 3-*O*-rutinoside) were obtained from Sigma–Aldrich. Flavonoid glycosides (kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside) and aglycones (kaempferol, quercetin and isorhamnetin) were obtained from Extrasynthese (Genay, France). Fatty acid standards were obtained from Sigma–Aldrich. A partially purified standard of 5-caffeoylquinic acid was isolated from plum fruits (Bennett et al., 2003). Quercetin 3-*O*-(6″-malonylglucoside) and kaempferol 3-O-(6″-malonylglucoside) were purified using a combination of polyamide solid-phase extraction of methanolic extracts and preparative HPLC from lettuce and *M. oleifera* leaves, respectively, using previously reported methods (Bennett et al., 2003).

2.3. Extraction and analyses of phytochemicals

Freeze-dried samples were extracted and analysed by HPLC-DAD-electrospray mass spectrometry (full scan positive and negative ion modes) using a previously reported multi-phytochemical method for the separation, quantification and identification of glucosinolates, phenolic, flavonoids and various other classes of phytochemicals (Bennett, Rosa, Mellon, & Kroon, 2006).

2.4. Extraction and analyses of total protein ($N \times 6.25$)

Moringa samples were stored at +4 °C after receipt; lyophilised overnight at room temperature (25–27 °C) and weighed. Triplicate samples ranging 400–700 mg were digested in a Büchi K-424 Digestion Unit equipped with a B-414 scrubber. Samples were transferred in a Büchi B-324 Distillation unit and the digested samples titrated by a Shott TitroLine automatic titrator with 0.1 N HCl.