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Journal of the Saudi Society of Agricultural Sciences

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FULL LENGTH ARTICLE

Functional composition, antibacterial and antioxidative properties of oil and phenolics from Moroccan *Pennisetum glaucum* seeds

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Received 20 December 2015; revised 15 April 2016; accepted 20 April 2016

KEYWORDS

Nutritional characteristics;
Antibacterial;
Antioxidant;
Pearl millet;
Fatty acids;
Phenolics

Abstract Pearl millet (PM) is the fifth most important cereal crop in the world after rice, wheat, maize, and sorghum. To date no previous studies have evaluated the nutritional characteristics and health promoting activity of the Moroccan variety. In this focus, this study aims at characterizing Moroccan *Pennisetum glaucum* seeds for their chemical composition (fibers, oil, proteins, fatty acids, minerals and phenolics), and antioxidative and antibacterial properties of polar and apolar fractions. The Moroccan variety contains considerable amount of proteins ($10.84 \pm 0.22\%$), Oil ($6.45 \pm 0.12\%$), Calcium (Ca) (211.01 ± 5.12 mg/100 g) and Magnesium (Mg) (174.04 ± 3.12 mg/100 g). Manifestly, PM is a good source of lipids since the major fatty acids found in PM oil were oleic (38.39%) and linoleic (36.61%). Moreover, phenolic and flavonoid content of PM fractions varied from 4.19 ± 0.21 to 22.78 ± 0.42 mg GAE/g fdw, and from 0.75 ± 0.30 to 15.60 ± 2.74 mg RE/g fdw, respectively. Antioxidant evaluation resulted in higher activity for the ethanolic fraction (208.01 ± 2.54 mg TE/g fdw (DPPH)/ 8.29 ± 0.11 mg TE/g fdw (TEAC)/ 21.20 ± 0.57 mg AAE/g fdw (FRAP)). Furthermore, the antibacterial activities of the obtained fractions on gram negative and positive bacteria (*Escherichia coli*, *Citrobacter freundii*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus* sp., *Bacillus cereus* and *Listeria ivanovii*) were eval-

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Peer review under responsibility of King Saud University.



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<http://dx.doi.org/10.1016/j.jssas.2016.04.007>

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Please cite this article in press as: Marmouzi, I. et al., Functional composition, antibacterial and antioxidative properties of oil and phenolics from Moroccan *Pennisetum glaucum* seeds. *Journal of the Saudi Society of Agricultural Sciences* (2016), <http://dx.doi.org/10.1016/j.jssas.2016.04.007>

uated using disk diffusion method. The results show that among all fractions, only the ethanolic fraction exhibited an antibiotic effect on all tested bacteria.

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1. Introduction

Pennisetum glaucum or pearl millet (PM) also known as “illan” in Morocco is one of the most important cereals grown in tropical semi-arid regions such as Africa and Asia. Pearl millet is known for many biological activities (Shahidi and Asekar, 2013). However, to our knowledge no previous studies have compared the antibacterial activity of polar and apolar fractions from Moroccan PM seeds. Recently, the antimicrobial properties of lipids have become increasingly recognized (Parsons and Rock, 2011). Obviously, bacteria ability to evade any form of existing therapy is evident. Consequently, pathogens resistant to one or more antibiotics are emerging and spreading worldwide. Therefore, finding an adequate natural alternative treatment and options for infectious disease has become increasingly important (Nussbaum et al., 2006). Manifestly, many of the antibacterial agents were natural products or potent semisynthetic variations (Newman et al., 2003). Moreover, factors such as polarity may influence extracts and compound effectiveness. Accordingly, a previous study has described the correlation between polarity, phytochemical compositions and bioactivities (Marmouzi et al., 2015). Hence the chemical composition may change following the extraction method and solvent polarity, contributing to enhance the antibacterial potential of plants extracts. In our ongoing research focusing on food chemistry and bioactivities (Marmouzi et al., 2015, 2016; Gharby et al., 2011, 2015a,b; Harhar et al., 2011, 2014) the present study aims to evaluate the chemical composition (fatty acids, minerals, proteins, fibers, phenolics) and pharmaceutical potential of polar and apolar fractions from Pearl millet cultivars based on antioxidative potential and antimicrobial effects.

2. Materials and methods

2.1. Plant material and extraction

Three different solvents (Petroleum ether, Ethyl acetate and Ethanol) were used to fractionate the soluble compounds from Pearl Millet (PM) in ascending polarity. PM seed powders (250 g) were extracted by using a soxhlet extractor for 6 h with 400 mL of extractant under reflux conditions. The organic solvent in the extracts was removed using a rotary evaporator to yield three fractions: Petroleum ether (MPE), Ethyl acetate (MEA) and Ethanol (MET).

2.2. Proximate analysis

The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25. All methodologies followed the recommendations of AOAC (1990) and all measurements were done in triplicate. Crude fat was determined

by extracting a known aliquot of sample (100 g) with petroleum ether, using a Soxhlet apparatus. Results for each parameter were expressed in percentage (%). Acid detergent fiber (ADF) and lignin content (ADL) were determined using the method described by Van Soest (1963). Neutral detergent fiber (NDF) was determined according to Van Soest and Wine (1967). The amount of cellulose in samples was estimated according to AOAC methods.

2.3. Mineral composition

The mineral composition (Ca, Mg, Fe and Zn) was determined using an inductively coupled plasma atomic emission spectroscopy (ICP AES, Jobin Yvon Ultima 2), as previously described (Marmouzi et al., 2015,2016).

2.4. Fatty acid analysis

PM Oil has been prepared according to a previously described method (Gharby et al., 2011). Aliquots (1 μ L) were injected into a gas chromatograph (Varian CP-3800, Varian Inc.) equipped with a FID. The column used was a CP-Wax 52CB column (30 m \times 0.25 mm i.d.; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium, with a total gas flow rate of 1 mL/min. The initial column temperature was 170 $^{\circ}$ C, the final temperature was 230 $^{\circ}$ C, and the temperature was increased by steps of 4 $^{\circ}$ C/min. The injector and detector temperature were 230 $^{\circ}$ C. Data were processed using Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

2.5. Phenolic contents

The amount of phenolic contents (PC) was determined according to the Folin–Ciocalteu method as described by Spanos and Wrolstad (1990), and modified by Lister and Wilson (2001). The phenolic content was determined as mg of gallic acid (mg GAE/g fdw) equivalent per g of fraction dry weight.

2.6. Flavonoid content

The flavonoid contents (FC) in the fractions were determined using a colorimetric method (Dewanto et al., 2002). The absorbance was recorded against a blank at 510 nm. The flavonoid content was determined as mg of rutin (mg RE/g fdw) equivalent per g of fraction dry weight.

2.7. Trolox scavenging equivalent activity (DPPH)

The free radical scavenging activity of the PM fractions was measured by 2,2'-Diphenyl-1-picrylhydrazyl hydrate (DPPH) (Huang et al., 2011), with some modifications. The radical-scavenging activity was calculated as a percentage of DPPH discoloration and represented as trolox equivalent from the standard curve.

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