



Phytochemical Characterization of Naturalized Sudanese *Jatropha curcas* Seed Kernels

Nagwa Kamal-Eldin M. Salih^{1*} and Elhadi M. Yahia²

¹Forestry Research Centre, Agricultural Research Corporation, P.O.Box 7089, Khartoum, Sudan.

²Laboratorio de Fitoquímicos y Nutrición, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Campus Juriquilla, Avenida de las Ciencias, 76230 Querétaro, Mexico.

Authors' contributions

This work was carried out in collaboration between both authors. Collection of samples was done by the author NKEMS whereas all the other activities were distributed equally between the two authors. Both authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to characterize the naturalized Sudanese *Jatropha curcas* L) seed kernel for its proximate composition, minerals, phenolics and fatty acids properties. The results showed 52% crude fat and 24% crude protein in *Jatropha* seed kernel. Also fatty acid classified as oleic acid (45%) and linoleic acid (35%) group was found in *Jatropha* kernel. *Jatropha* kernel had total soluble phenolics of about 625 mg GAE/100 g DW and was characterized by the presence of vanillic acid (112 mg/100 g DW), cinnamic acid (64 mg/100 g DW) and gallic acid (44 mg/100 g DW). These characterizations might expand the socio-economic potential and value of Sudanese *Jatropha curcas* L. as a medicinal plant in addition to its oil production. The edibility of seeds depends on the quality and quantity of anti-nutritional factors and the possibility of detoxification which need further investigation.

Keywords: *Fatty acids; Jatropha curcas; minerals; phenolics; proximate composition.*

*Corresponding author: Email: nagwa.salih@yahoo.com;

1. INTRODUCTION

Jatropha curcas L. is a perennial shrub or small tree which can reach a height of 6 m. The plant belongs to the Family *Euphorbiaceae* and commonly known as physic nut or jatropha. The genus name *Jatropha* was derived from the Greek words *jatros*, which means doctor, and *trophe*, which means food. That might mean a traditional use of the plant as a source of medicine and food. *Jatropha* is native to North America and has a wide distribution, as a naturalized plant, in several regions across the tropics and subtropics in Africa and Asia. *Jatropha* was introduced to Sudan as a hedge plant in Kordofan, White Nile, Bahr El-Ghazal and Bahr El-Jebel [1] and subsequently became naturalized. *Jatropha* is a multipurpose plant with numerous environmental, medicinal and industrial advantages [2,3]. *Jatropha* is adapted to a wide range of edaphic and climatic conditions and can grow on marginal land. It can control soil erosion and thus retain degraded soil. Industrially, *Jatropha curcas* is a rich source of seed oil with oil content between 30 to 40% of seed weight [4]. *Jatropha* can produce seeds at age 9-12 months, however, the best yield starts after 2-3 years [5]. Under optimum conditions, *jatropha* tree can produce 4-5 kg seed per year starting from the fifth year and seed production can continue for up to 50 years [6]. It is reported that *jatropha* seed production can reach 5 tons per ha with about 1.85 tons of oil per year. *Jatropha curcas* L. oil is classified as non-edible oil [7], because of the presence of some anti-nutritional factors [8], but suitable as a source of environmentally friendly bio-diesel oil. Additionally, *jatropha* oil is used for making soap and candles [9] and the by-product of the seed oil is used as organic fertilizers and pesticides [2]. Moreover, several parts of *jatropha* were reported to have uses in folk medicine [10]. Zhao et al. [11] reported the possibility of using the protein from *jatropha* seed cake for animal feeds. Pandey et al. [12] reviewed the potential of *Jatropha curcas* L. for numerous environmental, industrial and medicinal uses. With the multiple potentials of *jatropha* and increasing demand for biofuel, *Jatropha curcas* plantations are expected to expand and have a positive impact on farmers' livelihood. Cost-benefit analysis of *jatropha* large scale plantations in India revealed economic feasibility of the crop [13].

In Sudan, the plant has limited socio-economic value. Also limited studies were conducted to evaluate the Sudanese naturalized *Jatropha*

curcas L. plant for its phytochemical properties, which might improve the socio-economic potential of the plant. This study was therefore undertaken to characterize *Jatropha curcas* L. seed kernel for its phytochemical properties, including proximate composition, minerals, total soluble phenolics as well as the identification and quantification of phenolics and fatty acid constituents.

2. MATERIALS AND METHODS

2.1 Plant Material

Mature, at harvesting stage, and disease-free *Jatropha curcas* L. seeds were obtained from the National Tree Seeds Centre of Forestry and Gum Arabic Research Centre. The National Tree Seeds Centre collected the seeds from El Rashad district (lat. 11° 40' – 11° 55' N and long. 30° 45' – 31° 25' E), Eastern Nuba Mountains, Southern Kordofan state, Sudan. The area of collection is classified as a low rainfall woodland savanna [14] with a mean annual temperature of about 29.9°C and a mean annual rainfall of 542 mm.

Jatropha seeds were air-dried in shade and separated by hand into shells and kernels. Composite kernel samples were milled using an M20 universal grinding mill (IKA work) and stored in brown containers.

2.2 Chemicals and Reagents

Chemicals and reagents used in this study were Sigma-Aldrich products (St. Louis, MO). The purity of fatty acids and phenolic acids standards were > 99%. All the other chemicals were obtained from J.T. Baker (Baker Mallinckrodt, Mexico) and were High-Performance Liquid Chromatography (HPLC) grade. A Milli-Q plus water purification system (Millipore Corporation, Bedford, MA) was used to prepare HPLC grade water.

2.3 Proximate Composition

Moisture, crude fat, crude protein, ash and crude fibre were determined according to AOAC methods [15].

2.4 Determination of Fatty Acids

The sample was prepared for FAME (Fatty Acid Methyl Ester) analysis using 100 µL of the oil extracted for determination of fat content

mentioned above. The condition of liquid chromatography and fatty acid methylation were prepared as described by Emanuel et al. [16]. Carbohydrates were calculated by differences.

2.5 Mineral Elements

Mineral elements were determined according to the methods described by Gul and Safdar [17] with some modifications. Briefly, one gram of sample was subjected to overnight cold digestion with 10 mL of 16N HNO₃ followed by hot digestion until the appearance of white fumes. The suspension was cooled to ambient temperature. The aliquot volume was diluted with 1.0N HNO₃ and filtered. Mineral elements were determined using atomic absorption spectrometer (AAAnalyst 700 atomic absorption spectrometer, Perkin Elmer, Massachusetts, USA).

2.6 Extraction and Measurement of Total Soluble Phenolics

Total soluble phenolics were extracted and measured as described by Yahia et al. [18]. The extraction was performed using 80% acetone and the determination was done using Folin-Ciocalteu reagent assay. The absorbance was measured at 630 nm using a Dynex MRX microplate reader spectrophotometer (Dynex Technol. Chantilly, VA). Total soluble phenolics content was expressed as mg GAE/ 100 g DW (milligrams of Gallic Acid Equivalents per 100 g Dry Weight).

2.7 Identification and Quantification of Phenolic Constituents

Phenolic constituents were identified and quantified as described by Yahia et al. [18] using HP 1100 series HPLC (Hewlett-Packard GmbH, Waldbronn, Germany), equipped with a diode-array detector (DAD), at 280 nm and 320 nm. A 250 × 4.6 mm i.d., 5 μm, X-terra RP C18 column (Waters, Ireland). For the mobile phase, 1% formic acid/acetonitrile in a ratio 98:2 (v:v) at a flow rate of 0.5 mL/min was used. The phenolic compounds of interest in this study were gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid and vanillic acid (hydroxybenzoic acids); caffeic acid, chlorogenic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, 2-hydroxycinnamic acid and sinapic acid (hydroxycinnamic acids); kaempferol and quercetin (flavonols) and (+)-catechin and (-)-epicatechin (flavan-3-ols). Standard calibration curves were prepared for quantification.

2.8 Statistical Analysis

Statistical analysis was done using StatView statistical program. Results were represented as mean ± standard deviation of observations of six replicates.

3. RESULTS

3.1 Proximate Composition and Mineral Concentrations of *Jatropha curcas* Seed Kernels

Table 1 shows the result of proximate composition and mineral concentrations of jatropha seed kernels. The kernels were found to contain 52% crude fat and 24% crude protein. As to mineral elements concentration, jatropha seed kernels were characterized by presence of K (2938 mg /100 g DW), Mg (642 mg/100 g DW) and Ca (61.58 mg/100 g DW).

Table 1. Proximate composition (%) and mineral concentrations (mg/100 g DW) of *Jatropha curcas* seed kernel

Parameters	Proximate composition
Moisture content	4.7± 0.18
Crude fat	52.30± 2.35
Crude fibre	6.16± 0.25
Crude protein	24.35± 1.24
Ash	5.23± 0.98
Carbohydrates	7.26 ± 1.02
Mineral elements	Concentrations
Ca	61.58 ± 3.12
Na	7.13 ± 0.05
Fe	1.42 ± 0.00
Cu	0.96 ± 0.01
Zn	1.06 ± 0.00
Mn	0.40 ± 0.00
Al	0.32 ± 0.00
K	2938.50 ±12.32
Mg	642.59 ± 6.49

3.2 Fatty Acid Composition of *Jatropha curcas* Seed Kernels

Table 2 lists the fatty acids composition detected in jatropha kernel oil. Analysis of fats revealed four major fatty acids including two saturated fatty acids, namely palmitic (C16:0) and stearic (C18:0), and two unsaturated fatty acids, namely oleic (C18:1) and linoleic (C18:2). Saturated fatty acids represent 19% and unsaturated fatty acids represent 80% of the total fatty acids measured in jatropha seed kernels. Oleic acid

(monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid) were 45% and 35% of total fatty acids, respectively. No linolenic acid (omega-3) was detected in the analyzed sample. According to the result, jatropha kernel oil is classified as an oleic – linoleic acid group.

Table 2. Fatty acids percentage of *Jatropha curcas* seed kernel

Fatty acid	Composition
Saturated fatty acids	
Palmitic acid 16:0	12.26± 2.04
Stearic acid 18:0	6.84± 0.07
Mono-unsaturated fatty acids	
Oleic acid 18:1 n-9	45.32± 3.10
Poly-unsaturated fatty acids	
Linoleic acid 18:2 n-6	35.57± 4.23
Linolenic acid 18:3 n-3	ND

*ND= not detected

Table 3. Phenolic constituents (mg/100 g DW) and total soluble phenolics (mg GAE/100 g DW) of *Jatropha curcas* seed kernels

Phenolic constituents	Composition
Hydroxybenzoic acids	
Gallic acid	44.68 ± 2.02
<i>p</i> -hydroxybenzoic acid	ND
Vanillic acid	112.34 ± 8.65
Hydroxycinnamic acids	
Caffeic acid	1.35 ± 0.00
Chlorogenic acid	0.96 ± 0.00
Cinnamic acid	64.90 ± 2.89
Flavonols	
Kaempferol	ND
Quercetin	ND
Flavan-3-ols	
(+)-Catechin	ND
(-)-Epicatechin	1.20 ± 0.00
TSP	625.89 ± 9.05

*ND= not detected

3.3 Total Soluble Phenolics and Phenolics Constituents of *Jatropha curcas* Seed Kernels

Total soluble phenolics and phenolics constituents in jatropha seed kernels are listed in Table 3. The total soluble phenolics in jatropha kernels were 625.89 mg GAE/100 g DW. As revealed by HPLC analyses, the main phenolics compounds in jatropha seed kernels corresponded to two hydroxybenzoic acids (vanillic acid and gallic acid) and one hydroxycinnamic acid (cinnamic acid). The vanillic acid was found in the highest concentration (112.34 mg/100 g DW), followed

by cinnamic acid (64.90 mg/100 g DW) and gallic acid (44.68 mg/100 g DW). Flavonols were not detected in jatropha seed kernels, while the content of (-)-epicatechin (flavan-3-ol) was low.

4. DISCUSSION

The proximate composition values in this study were comparable to what was reported in other studies for jatropha [7,19,20]. Protein content in jatropha seed was in the range of most legumes and grains, which have protein contents ranging from 17% to 40% [21]. Although jatropha was reported toxic [7,8], Vandepitte et al. [22] mentioned the existence of non-toxic varieties of jatropha in Mexico. Abou-Arab and Abu-Salem [23] reported the effectiveness of different physical and chemical treatments in lowering the anti-nutrients in jatropha seed to tolerable levels. Abou-Arab and Abu-Salem [23] suggested the potential of detoxified jatropha seeds as a rich and safe protein source for human consumption especially in food shortage areas. Jatropha K and Mg values were higher than the values reported for cotton seeds in the study of Özcan [24], which investigated the mineral contents in eighteen oil-bearing Turkish seeds. Contrary to Ca and Na values reported in this paper, Nzikou et al. [20] found higher concentrations of Ca and Na in jatropha oil. The lower values reported in this paper might be because of the removal of the seed coat from jatropha seeds.

Jatropha kernel oil under investigation in this study had fatty acid composition compared to other studies, which classified jatropha seed oil as an oleic-linoleic acid group with 40 - 46% oleic acid and 32 - 37% linoleic acid [6,7,20]. The high percentage of unsaturated fatty acid (Table 2), i.e. 80% of the total fatty acid, detected in jatropha oil, render the oil as having medicinal potential. The utilization of different parts of jatropha, including seed and oil in herbal medicine, was reported in many publications [9,25,26,27] to cure eczema and skin diseases, soothe rheumatic pain and has purgative action. Also, the by-product of seed was mentioned to be used as fertilizers, insecticides and pesticides [2].

The importance of phenolic compounds in plant edible oils for the oxidative stability of unsaturated fatty acids and their natural antioxidant activity in reducing the risks of chronic diseases and providing nutrition and health benefit were investigated earlier [28]. The potential of cinnamic acid as cancer chemoprotective bioactive substances was

reviewed [29]. Vanillic acid was reported to have a beneficial effect on dextran sulfate sodium-induced ulcerative colitis, indicating the usefulness of vanillic acid in regulating chronic intestinal inflammation [30]. Gallic acid was found to have antioxidant activity and cytotoxicity against cancer cells in addition to its beneficial role in treating many other diseases [31]. The presence of adequate concentrations of vanillic, gallic and cinnamic acids in jatropha kernels might contribute to the potential of the plant as herbal medicine. However, the possible existence of toxicity of the plant might prevent its uses for medicinal and food purposes.

5. CONCLUSION

This study revealed the presence of adequate quantities of oil, protein, minerals, phenolics and fatty acids in jatropha seed kernels. The potential of jatropha seed kernel protein for edible purposes depends on the quality and quantities of anti-nutritional substances and possibilities of detoxification which needs further studies. Additionally, further studies are needed to investigate the biological activities of phytochemicals in jatropha seed kernels.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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