Original Research Article

PHYTOCHEMICAL CHARACTERIZATION OF SEED KERNEL FROM *JATROPHA CURCAS*

ABSTRACT

The objective of this study was to characterize the naturalized Sudanese jatropha *(Jatropha curcas)* seed kernel for its proximate composition, minerals, phenolics and fatty acids properties. The results showed that crude fat content in jatropha seed kernel was 52% and crude protein reached 24%. Jatropha seed kernel fatty acid wass classified as oleic acid (45 %) and linoleic acid (35%) group. Jatropha showed total soluble phenolics of about 625 mg GAE/100 g DW and was characterized most 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 our 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.

Key words: *Jatropha curcas*, Proximate Composition, Minerals, Phenolics, Fatty Acids

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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 ElGhazal and Bahr ElJebel [1] and became a naturalized plant spreading in the wild. Jatropha is a multipurpose plant with numerous environmental, medicinal and industrial advantages [2, 3]. Jatropha is adapted to a wide range of edaphic and climate conditions and has an ability to grow on marginal lands and control soil erosion and thus retain degraded soil. Industrially *Jatropha curcas* is a rich oil seed producing plant that can produce seeds from 9–12 months however the best yield starts after 2 – 3 years. Under optimum conditions jatropha tree can produce 4-5 kg per year starting from the fifth year. Jatropha tree can continue seed production up to 50 years. It is reported that jatropha seed production can reach 5 tons per ha giving about 1.85 tons of oil per year and seeds contain about 30 to 40% of oil [4]. *Jatropha curcas L.* oil was classified as a non-edible oil [5], because of presence of some anti-nutritional factors [6], suitable as a source of environmentally friendly bio-diesel oil. The oil is also used for making soap and candles. The by-product of the seed oil is used as organic fertilizers and pesticides [2]. In addition to industrial advantages several parts of jatropha were reported to have uses in folk medicine [7]. Makkar *et al*. [8] reported the possibility of using the protein from jatropha seed cake for animal feeds. Pandey *et al*. [9] 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 increasing positive impacts on farmers' livelihood. Cost-benefit analysis of jatropha large scale plantations in India revealed economic feasibility of the crop [10].

In Sudan the plant is still having very limited socio-economic value. Also limited studies have been done to evaluate the Sudanese naturalized *Jatropha curcas L.* for its phytochemical properties which might expand the socio-economic potential of the plant. This study, therefore, was undertaken to characterize *Jatropha curcas L.* seed kernel for its phytochemical properties including proximate composition, minerals, total soluble phenolics and identification and quantification of phenolics and fatty acids constituents.

2. MATERIALS AND METHODS

2.1 Plant Material

Seeds of *Jatropha curcas L.*, mature and free from diseases, obtained from National Tree Seeds Centre – Forestry and Gum Arabic Research Centre. The seeds were collected 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 belongs to the low rainfall woodland savanna [11] where the mean annual temperature is about 29.9 °C and the mean annual rainfall is 542 mm.

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

2.2 Chemicals and Reagents

All 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. Milli-Qplus 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 fiber were determined according to AOAC methods [12]. Carbohydrates were calculated by difference.

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 fatty acid was methylated as described by Christie [13] and Emanuel *et al*. [14] The condition of gas chromatograhy described by Emanuel *et al*. [14] was applied.

2.5 Mineral Elements

Mineral elements were determined according to the methods described by Gul and Safdar [15] with some modifications. Briefly, one g of sample was subjected to an overnight cold digestion with 10 mL of 16N HNO_3 followed with a hot digestion until appearance of white fumes and let to cool to ambient temperature. The aliquot volume was diluted with 1.0N HNO_3 and filtered. Mineral elements were determined using atomic absorption spectrometer (AAnalyst 700 atomic absorption spectrometer, Perkin Elmer, Massachussets, USA).

2.6 Extraction and Measurement of Total Soluble Phenolics

Total soluble phenolics was extracted and measured as described by Yahia *et al*. [16]. The extraction was done using acetone (80%) and the determination was performed using Folin-Ciocalteu reagent assay. The absorbances were 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*. [16] using HP 1100 series HPLC (Hewlett-Packa GmbH, Waldbronn, Germany), equipped with a diode-array detector DAD, at 280 and 320 nm**.** A 250 × 4.6 mm i.d.,

5 µm, X-terra RP 18 column (Waters, Ireland) was employed. 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 acids (hydroxybenzoic acids); caffeic acid, chlorogenic acid, cinnamic acid, p-coumaric acid, ferulic acid, 2-hydroxycinnamic acid and sinapic acids (hydroxycinnamic acids); kaempferol and quercetin (flavonols) and catechin and epicatechin (flavan-3-ols). Standards 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 Kernel

Table 1 shows the result of proximate composition and mineral concentrations of jatropha seed kernel. Jatropha seed kernel was characterized by presence of high contents of crude fat (52 %) and crude protein (24 %). The concentrations of mineral elements measured for jatropha seed kernel showed high K (2938 mg /100 g DW), Mg (642 mg/100 g DW) and Ca (61.58 mg/100 g DW) concentrations.

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

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Parameters	Composition
Proximate composition	
Moisture content %	4.7± 0.18
Crude Fat %	52.30± 2.35
Crude Fiber %	6.16± 0.25
Crude protein %	24.35± 1.24
Ash %	5.23± 0.98
Mineral Concentrations	
Ca (mg/100 g DW)	61.58 ± 3.12
Na (mg/100 g DW)	7.13 ± 0.05
Fe (mg/100 g DW)	1.42 ± 0.00
Cu (mg/100 g DW)	0.96 ± 0.01
Zn (mg/100 g DW)	1.06 ± 0.00
Mn (mg/100 g DW)	0.40 ± 0.00
Al (mg/100 g DW)	0.32 ± 0.00
K (mg/100 g DW)	2938.50 ±12.32
Mg (mg/100 g DW)	642.59 ± 6.49

3.2 Fatty Acid Composition of *Jatropha Curcas* Seed Kernel

Table 2 shows the fatty acid composition of jatropha kernel oil. Analysis of fats revealed four major fatty acids including two saturated 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 making 19 % and unsaturated fatty acids making 80 % of the total fatty acids was detected in jatropha seed kernel. Oleic acid (monounsaturated fatty acid) and linoleic acid (polyunsaturated fatty acid) made 45% and 35% of total fatty acids, respectively. No linolenic acid (omega-3) was detected in the analyzed sample. According to this result jatropha kernel oil is classified as oleic – linoleic acids 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

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3.3 Total Soluble Phenolics and Phenolics Constituents of *Jatropha Curcas* Seed Kernel

Total soluble phenolics and contents of phenolics constituents in jatropha seed kernel were shown in Table 3. Jatropha kernel showed total soluble phenolics making 625.89 mg GAE/100 g DW. As to phenolic constituents, HPLC analyses revealed that the main phenolics in jatropha seed kernel 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 gallic acid (44.68 mg/100 g DW) and cinnamic acid (64.90 mg/100 g DW). No Flavanol was detected in jatropha seed kernel while the content of epicatechin (flavan-3-ols) was little.

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

Phenolic Constituents	Composition
Hydroxybenzoic acids	
Gallic acid	44.68 ± 2.02
p-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
Flavanols	
Kaempferol	ND
Quercetin	ND
Flavan-3-ols	
Catechin	ND
Epicatechin	1.20 ± 0.00

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TSP	625.89 ± 9.05

ND= not detected

4. DISCUSSION

Our proximate composition values were comparable to what was reported in other studies for jatropha [5, 17, 18, 19].Protein content in jatropha seed was in the range of most legumes and grains which have protein contents ranging from 17 % to 40 % [20]. Although reported toxic [5, 6], Makkar *et al*. [17] mentioned the existence of non-toxic varieties of jatropha in Mexico and the efficiency of roasting as a detoxification method. Also Abou-Arab and Abu-Salem [21] revealed the efficiency of different physical and chemical treatments in lowering the anti-nutrients in jatropha seed to a tolerable level. Abou-Arab and Abu-Salem [21] suggested the high potential of treated jatropha seeds for human consumption as a rich and safe protein source in areas with food shortage, especially. Jatropha mineral values were even higher than the K and Mg values reported for cotton seeds in the study of Özcan [22] which investigated the mineral contents in eighteen oil-bearing Turkish seeds. Lower concentrations of Ca and Na in our jatropha samples compared to the values reported by Nzikou *et al*. [19] could be because of the removal of the seed coat from our jatropha seed samples.

Our jatropha kernel oil had fatty acid composition comparable to many other studies which classified jatropha seed oil as oleic-linoleic acid group and reported 40 - 46% oleic acid and 32 - 37% linoleic acid [5, 19]. The high unsaturated percentage of fatty acid, 80 % of the total fatty acid, detected in our jatropha oil, makes this oil of a medicinal potential. As mentioned above, the utilization of different parts of jatropha, including seed and oil in herbal medicine was reported in many publications [9, 23, 24, 25] to cure eczema and skin diseases, soothe rheumatic pain and has a purgative action. Also the by-product of seed was mentioned to be used as fertilizers, insecticides and pesticides [2].

The importance of phenolic compounds, exist in plant edible oils, for the oxidative stability of the unsaturated fatty acids of these oils and their natural antioxidant activity role in reducing the risks of chronic diseases and providing nutrition and health benefit were revealed [26]. The potential of cinammic acid as cancer chemoprotective bioactive substances was reviewed [27]. Vanillic acid was reported to has a beneficial effect on dextran sulfate sodium -induced ulcerative colitis indicating the usefulness of vanillic acid in regulating chronic intestinal inflammation [28]. Gallic acid was found to has antioxidant activity and cytotoxicity against cancer cells in addition to its beneficial role in treating many other diseases [29]. The presence of appreciable amounts of vanillic, gallic and cinnamic acids in our jatropha kernel might contribute to the potential of the plant as a herbal medicine. However, the possible existence of toxicity of the plant might limit its uses for medicinal and food purposes.

5. CONCLUSION

This study revealed presence of good quantities of oil, protein, minerals, phenolics and fatty acids in our jatropha seed kernel. 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. Also further studies are needed to investigate the biological activities of phytochemicals in jatropha seed kernel.

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Comment [U5]: There are 14 from 29 references not up to date (more than 10 years)

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