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Proximate composition, phenolic content and antioxidant potential of the leaves of four *Jatropha* species

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ABSTRACT

Jatropha is a large euphorbiaceous genus of tropical and subtropical distribution. Several species are used as traditional remedies for mouth sores, diarrhoea and skin diseases. This study aimed to estimate the amount of phenolic components in correlation to the antioxidant potential of the leaves of four *Jatropha* species acclimatised in Egypt. The plant material was subjected to determination of pharmacopoeial standards (ash values, moisture and crude fibre contents) and macronutrients (fat, protein and carbohydrates) for the establishment of reliable quality control criteria. Quantitation of phenolic compounds was performed spectrophotometrically and DPPH radical scavenging assay applied for *in-vitro* assessment of anti-oxidant activity. Quality control parameters differed among the analysed samples; total and water-soluble ash values were distinctly higher in *Jatropha curcas* and crude fibres in *J. gossypifolia* and *J. multifida*. Total phenolic, flavonoid and tannin contents were present in significant amounts in *J. curcas*, *J. gossypifolia* and *J. multifida* leaves; this could be partially correlated to their relatively moderate to appreciable anti-oxidant activities (IC₅₀s 1.03, 1.7 and 1.26mg/mL, respectively). Meanwhile, the least amounts of phenolic compounds and lowest anti-oxidant potential were recorded for those of *Jatropha integerrima*. Results of proximate and macronutrient analyses could serve as suitable criteria for quality control of the investigated *Jatropha* species. The appreciable amounts of polyphenols detected in the leaves recommend intensive chemical and biological investigation of their component metabolites for further implementation in pharmaceutical products.



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INTRODUCTION

Jatropha, a genus of Euphorbiaceae (or spurge family), comprises around 170 species (Kumar and Sharma, 2008) of trees, shrubs, subshrubs and herbs that are native to tropical and subtropical America and Africa (Ma and Cheng, 1997). The genus is subdivided into two subgenera; subgenus *Jatropha* originating mainly from Africa, India, South and North America, and subgenus *Curcas* that is indigenous to Mexico, Texas and Arizona (Dehgan, 1982). Among members of the genus successfully acclimatised in Egypt are *Jatropha curcas* L., *Jatropha gossypifolia* L., *Jatropha integerrima*

Jacq. and *Jatropha multifida* L., which were selected for the current investigation.

On the economic scale, the most reputed among the designated species is *Jatropha curcas* L. owing to the globally increasing use of its seed oil for production of biodiesel. In this respect, it has been claimed by the Planning Commission of India as main biodiesel source due to the ease of its conversion to a product with very close properties to crude diesel (commission, 2003). Moreover, these species are used as folk remedies in many countries. The decoction of *Jatropha curcas* leaves is indicated for curing mouth sores in Ghana (Asase *et al.*, 2005), and that of *Jatropha gossypifolia* in the treatment of diarrhoea in India and Brazil (Dash and Padhy, 2006). Fruits of *Jatropha multifida* are used for skin diseases in Cambodia (Kirtikar and Basu, 1980). Leaves of *Jatropha integerrima* are commonly utilized as purgative (Mongkolvisut *et al.*, 2006). These traditional applications have been scientifically supported by several research articles (Sabandar *et al.*, 2013). Thus, the methanol extract of the leaves of *Jatropha curcas* (Mujumdar and Misar, 2004), as well as the methanol and petroleum ether extracts of those of *Jatropha gossypifolia* (Panda *et al.*, 2009), produced an effective anti-inflammatory action. Besides, the leaf extract of *Jatropha gossypifolia* (Oduola *et al.*, 2005) and latex of *Jatropha curcas* (Osoniyi and Onajobi, 2003) exerted a potent anticoagulant effect. In addition, different extracts of *Jatropha curcas* (Igbinosa *et al.*, 2009), *Jatropha gossypifolia* (Ogundare, 2007) and *Jatropha multifida* (Aiyelaagbe, 2001) displayed a moderate antibacterial efficiency. Moreover, the antiulcer, cytotoxic, hepatoprotective, wound healing and antioxidant activities of the different species were experimentally ascertained (Sabandar *et al.*, 2013).

Beside their medicinal benefits, *Jatropha* species are considered as a wealthy source of different types of secondary metabolites especially diterpenoids, peptides and polyphenols (Aiyelaagbe *et al.*, 2007). The latter are commonly found in both edible and non-edible plants and are reported to exert multiple biological effects, including antioxidant activity (Kähkönen *et al.*, 1999). Accumulated evidence indicates that reactive oxygen species (ROS) are involved in the pathophysiology of ageing and a multitude of health disorders including cardiovascular and neurodegenerative diseases, diabetes and cancer. Antioxidants such as phenolic compounds can neutralize free radicals and may be of great importance in the prevention of these diseases (Lopez *et al.*, 2003).

The current study aimed to evaluate the antioxidant potential of the leaves of the four selected species in correlation to their phenolic content to

assess the possibility of their use as herbal medicines. This necessitates an accurate determination of the proximate and macronutrient composition of the plant material prior manipulation (Hussain *et al.*, 2009).

MATERIAL AND METHODS

Plant Material

Fresh leaves of *Jatropha curcas* L., *Jatropha gossypifolia* L., *Jatropha integerrima* Jacq. and *Jatropha multifida* L. were collected from plants cultivated at the Medicinal, Aromatic and Poisonous Plants Experimental Station of the Faculty of Pharmacy, Cairo University (Giza, Egypt). Samples identity was confirmed by Mrs Therese Labib, consultant of Plant Taxonomy at the Ministry of Agriculture and former director of Orman Botanical Garden (Giza, Egypt). Vouchered herbarium specimens of the selected species (code numbers: 582018I, 582018II, 582018III and 582018IV, respectively) are kept at the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Leaves were air-dried, in the shade, finely powdered and carefully stored until use.

Preparation of Plant Extracts

Samples (200g, each) of the powdered leaves of the selected *Jatropha* species were extracted by cold maceration in absolute ethanol (1L×3), until exhaustion. The solvent was, in each case, evaporated to dryness under vacuum (at 40°C) using a rotary evaporator (Buchi, G. Switzerland). The dark green residues obtained were saved in tightly closed containers, at -4°C, for further analysis.

Determination of Proximate and Macronutrient Composition

Proximate analysis of the examined leaves samples was performed by adopting the method of the Association of Official Analytical Chemists (A.O.A.C., 2000). The analytical standards determined included total ash, acid-insoluble ash, water-soluble ash, moisture and crude fibre contents; besides the amounts of macronutrients viz., total fat, protein and carbohydrate contents were estimated. All experiments were performed in triplicates. Results, recorded in Table 1 and illustrated in Figures 1 & 2, are expressed in g per 100g fresh plant material.

Determination of Phenolic Components of the Ethanol Extracts

This was performed by application of published spectrophotometric procedures for estimation of total phenolic, flavonoid and tannin contents of the ethanol extracts of the leaves (Ivanova *et al.*, 2010, Geissman, 1962, Price *et al.*, 1978). Standard gallic acid, quercetin and tannic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used

to establish the calibration curves for estimation of total phenolics, flavonoids and tannins, respectively. Measurements were carried out, at the specified wavelength for each assay, by the aid of a UV/Visible spectrophotometer (Shimadzu, UV-1650 PC, Kyoto, Japan).

Determination of total phenolic content

Spectrophotometric determination of the total phenolic content was carried out using the Folin-Ciocalteu colourimetric method by measuring the absorbance of the colour produced at 730nm (Ivanova *et al.*, 2010). The total phenolic contents, expressed as mg gallic acid equivalent (GAE)/g of dry plant powder were deduced from the standard gallic acid pre-established calibration curve equation: $Y = 0.0086 X - 0.0045$, $R^2 = 0.9989$; where Y=absorbance of the sample, X=corresponding concentration and R^2 =correlation coefficient.

Determination of flavonoid content

Total flavonoid content was determined by measuring the yellow colour developed upon reacting flavonoids with aluminium chloride reagent, at 420nm (Geissman, 1962). Flavonoid contents, expressed in mg quercetin equivalent (QE)/g of dry plant powder, were calculated from the standard quercetin pre-established calibration curve equation as follows:

$$Y = 1.5625 X - 0.0034, R^2 = 0.9984.$$

Determination of tannin content

Spectrophotometric determination of tannin content was carried out using the vanillin/hydrochloric acid colourimetric method by measuring the absorbance (Y) of the developed colour at 500nm (Price *et al.*, 1978). Tannin concentrations of the tested samples (X), expressed as mg tannic acid equivalent (TAE)/g of dry plant powder, were deduced from the standard tannic acid calibration curve equation: $Y = 0.148 X - 0.0194$, $R^2 = 0.9998$.

All experiments for phenolic compounds determination were carried out in triplicates. The calculated averages were converted into mg of corresponding reference compound equivalents per 100g dry plant material. Results are presented in Table 1 and Figure 3.

Evaluation of Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential of the different samples, estimated according to a reported procedure (Amić *et al.*, 2003), was taken as a measure of anti-oxidant activity. A 0.004% solution of DPPH radical in methanol was prepared and stored in the dark until needed. Serial dilutions (0.61µg/mL to 10mg/mL) of each of the dry ethanol extracts under investigation were prepared in methanol. The

assay was carried out in a 96-well microplate. Aliquots (20µL, each) of the tested samples solution were separately added to 180µL of DPPH solution. The resulting reaction mixtures were kept at room temperature, for 30min. The absorbance of each sample was measured, at 520nm, by means of the UV/Visible spectrophotometer. Pure methanol was used as a blank, an extract-free DPPH solution as control and standard ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) as reference antioxidant drug for comparison. The radical scavenging activities of the tested samples, expressed as percentages, were calculated from the following equation:

$$\text{Scavenging activity (\%)} = (A_1 - A_0 / A_0) \times 100$$

Where A_0 is the absorbance of the control experiment, and A_1 is that of the extract/reference standard. Median inhibitory concentration (IC_{50}), or amount of extract/standard required to scavenge 50% of DPPH free radicals, was deduced from the graph obtained by plotting the percentage inhibition produced by the sample against its respective concentration. Results, as depicted in Figure 4, were obtained by means of a GraphPad Prism Software version 5.0.

RESULTS AND DISCUSSION

Proximate and Macronutrient Composition

A variability in proximate and macronutrient composition was observed among the leaves of the investigated species (Table 1, Figures 1 & 2). Thus, the total and water-soluble ash contents were the highest in *J. curcas* and that of acid-insoluble ash in *J. gossypifolia*. Meanwhile, the moisture content of *J. multifida* leaves exceeded those of other leaves, and the crude fibre contents of both *J. gossypifolia* and *J. multifida* were nearly equivalent and more than twice those in the other two samples. As far as macronutrients are concerned, the protein and carbohydrate contents of all analysed samples were almost similar, whereas the fat content was the highest in *J. gossypifolia*.

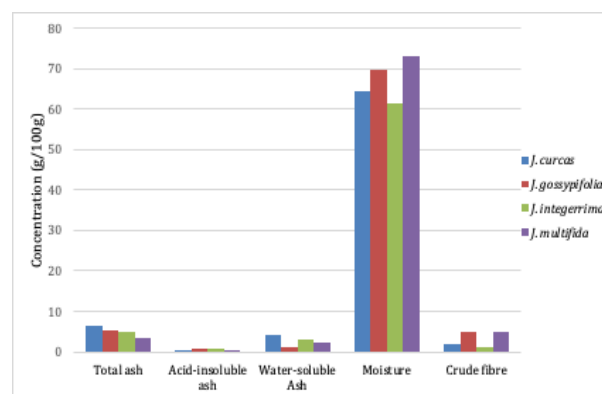


Figure 1: Bar chart representing the results of proximate analysis of the leaves of the selected *Jatropha* species (calculated on fresh weight basis)

Table 1: Results of proximate, macronutrient and phenolic analyses of the leaves of the four selected *Jatropha* species

Estimated content		Leaves samples (100g)			
		<i>J. curcas</i>	<i>J. gossypifolia</i>	<i>J. integerrima</i>	<i>J. multifida</i>
Phenol- ics & Macronutrients	Total ash*	6.47	5.2	4.93	3.61
	Acid-insoluble ash*	0.512	0.73	0.61	0.58
	Water-soluble Ash*	4.13	1.31	2.9	2.42
	Moisture*	64.45	69.53	61.57	73.21
	Crude fiber*	1.83	5.1	1.16	5.07
	Fat*	0.256	0.61	0.5	0.18
	Protein*	4.9	5.6	4.56	4.88
	Carbohydrate*	88.37	88.59	90.01	91.33
	Total phenolic**	1965.7	2193.6	740.1	1449.4
	Flavonoid***	219.8	503.3	107.8	98.8
	Tannin****	5654.0	7384.0	1573.0	2938.0

Results are the averages of three determinations. *expressed in g; **expressed in mg GAE; ***expressed in mg QE; ****expressed in mg TAE

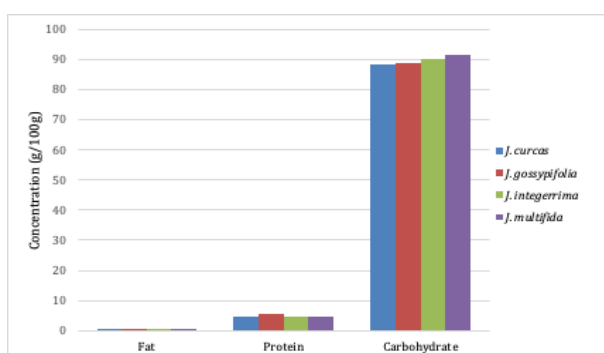
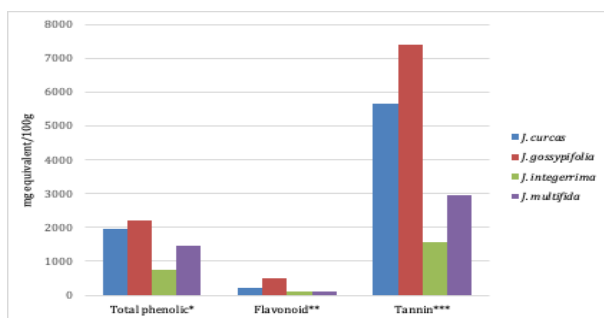


Figure 2: Bar chart representing the results of macronutrient analysis of the leaves of the selected *Jatropha* species (calculated on fresh weight basis)

Phenolic Contents

The phenolic contents determined in the examined leaves were also variable (Table 1, Figure 3). The highest concentrations of all quantified components viz., total phenolic compounds, flavonoids and tannins were observed in *Jatropha gossypifolia* followed by *J. curcas*, *J. multifida* and finally *J. integerrima* with flavonoid contents in the last two species almost similar.



*expressed as GAE; **expressed as QE; ***expressed as TAE

Figure 3: Bar chart representing the results of phenolic analysis of the leaves of the selected *Jatropha* species (calculated on dry weight basis)

Antioxidant Activity

The DPPH radical scavenging activity of the ethanol extracts of the leaves of the selected species was evaluated at different concentrations relative to the standard anti-oxidant, ascorbic acid. The IC₅₀s of the different samples, as determined from the curves represented in Figure 4, revealed that extracts of *Jatropha curcas*, *J. multifida* and *J. gossypifolia* exerted a relatively considerable to moderate anti-oxidant effect (IC₅₀s; 1.03, 1.26 and 1.70 mg/mL, respectively) as compared to ascorbic acid (IC₅₀, 23.53 µg/mL). On the other hand, that of *J. integerrima* showed the least radical scavenging activity with IC₅₀ >10 mg/mL. The sequence of the DPPH radical scavenging activities of the tested samples is obviously different from that of their phenolic contents; this could be referred to the synergistic effect of other non-phenolic anti-oxidant metabolites (Kolak *et al.*, 2009, El-Sayed *et al.*, 2008). Moreover, the influence of environmental factors on production of phenolic metabolites in the leaves of *Jatropha* species growing in different localities is evident and is consequently reflected on their respective anti-oxidant activity, as indicated by reported data (Jain *et al.*, 2013, Rampadarath *et al.*, 2014).

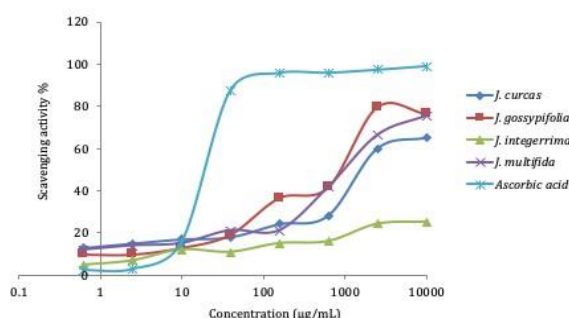


Figure 4: Line graph representing the radical scavenging activity of the ethanol extracts of the leaves of the selected *Jatropha* species

CONCLUSION

Results of proximate and macronutrient analyses could be considered as useful quality control criteria for comparative characterization of the selected *Jatropha* species cultivated in Egypt. The ethanol extracts of three of these namely; *Jatropha curcas*, *J. gossypifolia* and *J. multifida* exhibited appreciable anti-oxidant activities, partially correlated to their phenolic contents, suggesting their use as health beneficial natural anti-oxidants. Therefore, carefully selected cultural practices should be adopted for improving the production of these phytochemicals. Besides, intensive chemical and biological studies are necessary for evaluating the economic impact and possibility of implementation of these locally cultivated plants in the drug industry.

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