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Total Phenolic Contents and Free Radical Scavenging Activity of Different Parts of Jatropha Species

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Jatropha species is a worldwide important crop. Each part of this species is used in traditional medicine to treat the various diseases. In this research, antioxidant activity and total phenolic content of different parts of *Jatropha* species were investigated by using two extraction processes: ultrasound-assisted extraction and maceration. Based on the antioxidant activity and phenolic content, it can be summarized that the extracts obtained by ultrasound-assisted extraction process showed potent antioxidant activity and may be related to their phenolic content. The root extracts of *J. gossypiifolia* obtained by ultrasound-assisted extraction process showed the highest antioxidant activity and leaves of *J. curcas* showed highest phenolic contents as compared to maceration process. Among the different parts of these species, all the parts of *J. gossypiifolia* showed the higher activity as compared to *J. curcas*. Finally, *Jatropha* species' by-products with strong radical scavengers can be considered as potential sources of natural antioxidants.

Keywords: *J. gossypiifolia*, *J. curcas*, Euphorbiaceae, Antioxidant activity, Phenolic compounds.

INTRODUCTION

Oxidation reactions naturally occur in the human body due to continuous respiration and oxidative metabolism in human cells. Although oxygen is necessary for aerobic cells to generate energy, it can produce various ageing and diseases causing substances by incorporating reactive oxygen species (ROS). These species mainly occurs in free radical forms ($^{*}OH$, $^{1}O_2$, O_2^{*-}, $ONOO^{-}$) and non-free radical forms (R-OOH, NO and H_2O_2). These radicals can damage the macromolecules and imbalance the body that cause chronic degenerative diseases such as cancer, rheumatoid arthritis, atherosclerosis, emphysema, cirrhosis, diabetes and others [1,2]. The human body is vulnerable to these reactive species; natural antioxidants are the important compound for reducing the concentration of these species and preventing the above-mentioned chronic diseases. Antioxidants in the body are primarily derived from diet and can promote good health. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), 2-*tert*-butyl-4-methylphenol (TBMP) and gallic acid esters, *e.g.* propyl gallate (PG) are the most widely used antioxidants in food products but these synthetic products are restricted due to its carcinogenic and other toxic properties [3]. Therefore, the attention of natural antioxidants has been raised considerably in the study of certain fruits, vegetables and leaves with high antioxidant contents to boost their consumption. Among natural antioxidants, phenolic compounds are one of the abundant and extensively distributed antioxidants in the plants and over 8,000 phenolic compounds presently occur.

The genus *Jatropha* that belongs to the Euphorbiaceae family, which contains around 800 species and belongs to around 321 genera and it is also the largest genera of angiosperms in the world. These species are widely distributed in countries of tropical, subtropical and dry tropical weather and tropical semi-arid regions of Africa and Americas [4]. Its different parts, such as, leaves, stem barks, fruit and roots are used in traditional medicine to treat several diseases, namely, anti-hypertensive, anti-inflammatory, anti-ophidic, analgesic, antipyretic, anti-microbial, healing, haemostatic, anti-anemic and anti-diabetic applications [4-8]. Other uses are also related to this plant, such as biodiesel production, pesticide, insecticide, vermifuge, ornamentation, and even its use in religious rituals [9,10]. However, these are the rich sources of secondary metabolites such as terpenoids (diterpenes, triterpenes, sesquiterpenoids), flavonoids, lignans, neolignans, phenols, saponnins, steroids, coumarins and alkaloids [11-14].

Fruits of *Jatropha* are not only valuable from a biofuel point of view, but also they are source of phenolic compounds,

which have a potential positive effect on human health and regulate its product quality. Phenolic compounds have positive correlation with antioxidant activity. Most of the researchers investigated the antioxidant activity (DPPH, ABTH, FRAP, *etc*.), phenolic content, and others bioactivities of different parts of *Jatropha* [4-7,9,10]. On the contrary, few studies have focused on leaves [4,8,15-17], roots [17], fruits [18] and others parts [19-21] of *Jatropha*. However, some studies suggested that leaves exhibited higher phenolic content and antioxidant capacity as against to others parts due to phenolic compounds [15-20]. Concomitantly, roots of *J. gossypiifolia* displayed stronger antioxidant activity by both the mechanisms of single electron transfer and hydrogen atom transfer [22]. Moreover, there are also a few reports dealing with the extraction processes to maximize antioxidant activity. However, this preliminary screening could help to develop antioxidant formulations for food and health applications.

Therefore, the aim of this work was to determine the antioxidant activity and total phenolic content of different parts of *Jatropha* species and to compare these activities by using two widely used extraction processes: ultrasonication and maceration. This is the first report to compare the different parts of *Jatropha* and their activity. The cluster and correlation analysis also performed for these cultivars.

EXPERIMENTAL

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich, USA. Folin-Ciocalteu reagent was purchased from Merck, Germany. Potassium persulfate, 99.9 % pure ethanol, monohydrate gallic acid and anhydrous sodium carbonate were purchased from Friendemann Schmidt Chemicals, Australia. All the chemicals used were analytical grade. 18 mΩ deionized water was used to prepare the standard materials and extraction.

Sample preparations: The different parts of *Jatropha* species were collected from Living lab energy and Future crops laboratories at Kuala Pilah under the FKAB, UKM in December 2015. Each part was washed with deionized water, given an airing at room temperature and then dried at 35-40 °C with the help of Septree Food Dehydrator, China. Finally, all the leaves were powdered using a special grinder (XY-2200B, Shenzhen Yason General Machinery Co., Ltd, Guangdong, China) and stored in an airtight container.

Extraction procedures of antioxidants

Ultrasound-assisted extraction: Ultrasonication extraction was fulfilled in a Thermoline ultrasonic bath (220 V and 40 kHz) at 35 °C. 250 mg of dried and ground powdered sample was transferred in a capped long test-tube (50 mL) and 15 mL of 70 % of the ethanolic solution was poured in the sample. Then the mixture was immersed in the ultrasonic bath and fixed well in the same position during sonication (60 min). After the extraction, the suspension sample was centrifuged at 4000 rpm for 15 min. Then the supernatant liquid was filtered and the extract thus obtained used directly for the determination of required properties.

Maceration extraction: The same amount of dried and powdered sample (250 mg) was kept in a capped long test-tube and extracted by same volume and percentages of aqueous ethanol. The samples were extracted at room temperature for 1 h by an orbital shaker at 200 rpm. After shaking, all the suspension samples were placed in a centrifuge machine at 4000 rpm for 15 min and subsequently the liquid was filtered and abovementioned extracts were stored at 4 °C for the analysis of biological activity within 2 days.

Total antioxidant capacity

DPPH free radical scavenging assay: The DPPH activity of different parts of *Jatropha* species were measured by using some reported method with some modifications [23]. In brief, 0.1 mM of fresh DPPH was prepared with the 70 % of aqueous ethanol. 100 µL of the standard Trolox solution (positive control) or appropriate dilutions of different extract were mixed with 2.9 mL of 0.1 mM DPPH solution. Then the control, standard and sample absorbance were measured at 520 nm after 30 min incubation at room temperature. Trolox equivalent antioxidant capacity (TEAC) was calculated by preparing a Trolox curve (the standard curve equation: $y = -0.0007x + 0.9396$, $R^2 = 0.9998$) from 31.25 µg/mL to 1.5 mg/mL of standard Trolox solution and the results were expressed as mg Trolox equivalent (TE)/g dry leaves (DL).The DPPH scavenging activity was expressed as a percentage of inhibition. The scavenging capacity or inhibition of DPPH (%) was calculated by using the equation below:

$$\text{Antioxidant capacity (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

where $A_{control}$ is the absorbance of radical solution with 70 % of aqueous ethanol; A_{sample} is the absorbance of radical solution mixed with sample extract or standard. Each sample and standard were measured in three replications. The absorbance was measured with 756 PC UV-visible spectrophotometer (Shanghai Yuefeng Instruments & Meters Co., Ltd.).

$ABTS^+$ free radical scavenging assay: The ABTS radical scavenging assay was calculated based on [24] with some modifications. First the radical solution was prepared by mixing both the stock solutions such as 7 mM aqueous solution of ABTS and 2.45 mM potassium persulfate ($K_2S_2O_8$) solution at a ratio of 1:1 [25]. The mixture was kept for 12-16 h in the dark conditions and room temperatures. Then the fresh working solution was prepared for each bioassay by diluting 1 mL ABTS radical solution with required amount of ethanol to obtain the absorbance of 0.700 ± 0.02 units at 745 nm. Afterwards, 100 µL of different extracts or different standard Trolox solution was added to 3.9 mL of an $ABTS^+$ solution. The absorbance was measured immediately at 745 nm after 6 min incubation at room temperature. 70 % of aqueous ethanol and Trolox were used as blank and positive control respectively. Trolox will be taken as positive control. TEAC was calculated by prepare a Trolox curve for ABTS assay (the standard curve equation: $y = -0.0006x + 0.5869$, $R^2 = 0.9998$) from 31.25 µg/mL to 1.0 mg/mL of standard Trolox solution and the results were designated as mg TE/g DL. The percentages of inhibition of ABTS was calculated by the eqn. 1. The equipment used was described before.

Determination of total phenolic content: The phenolic content of different parts of *Jatropha* species was analyzed by using Folin-Ciocalteu (FC) reagent with a little modification [26]. FC reagent was used as oxidizing agent. Firstly, 100 µL of the standard gallic acid or leaves extract were mixed with 3.25 mL of 12 times pre-diluted of Folin-Ciocalteu reagent. The samples and standards were properly mixed and allowed to stand for 7 min;then added 750 µL of 20 % Na_2CO_3 in the main solution containing test-tube and allowed to 2 h for incubation at room temperature and dark conditions. Finally, the absorbance was recorded at 760 nm based on colorimetric redox reaction from a standard curve ($y = 0.0027x + 0.0235$, $R^2 = 0.9998$) and using standard gallic acid solution of 31.25 µg/mL to 1.0 mg/mL. The results were presented as mg gallic acid equivalent (GAE)/g DL. Each standard and extract was measured in three times. The equipment used was as for previous assays.

Statistical analysis: To study the variance of antioxidant activity and phenolic content of different parts of *Jatropha* species, data were processed by one-way analysis of variance (ANOVA) using STATGRAPHICS Centurion XVII (Version 17.2.00, StatPoints Technologies Inc. 1982-2016).Correlation, regression and cluster analysis were also carried out in STATGRAPHICS Centurion XVII. Statistical significant differences were determined by Tukey honest significant difference (HSD) *post hoc* test. F values for which $p < 0.05$ were considered statistically significant. Pearson Product-Moment correlation matrix and regression analysis were used in order to evaluate the connection between DPPH, ABTS and total phenolic content in the extraction processes. The data of TEAC and GAE curve was done in Microsoft Excel 10 (Microsoft Inc., Redmond, WA, USA). Three replicates of each sample were used for statistical analysis. All data presented are expressed as means ± SD.

RESULTS AND DISCUSSION

Antioxidant activity: Antioxidant activity of plant materials can be analyzed by using various reported mechanisms. Generally, two modes of actions such as single electron transfer and hydrogen atom transfer have been used [27,28]. Therefore, in this research, the antioxidant activities of leaves of different cultivars were analyzed by two most common *in vitro* assays namely DPPH and ABTS and their results were expressed by different ways such as percentage of inhibition (%) and Trolox equivalent antioxidant capacity (mg TE/g DP). The data are shown in Table-1.

The DPPH assay is a broadly used and reliable antioxidant determination method as against to other assays [29]. In this process, DPPH solution reduced to non-radical DPPH-H in presence of hydrogen-donating antioxidant. The antioxidant compound containing crude extract reduced the stable purple colour to yellow-coloured diphenylpicryl hydrazine. The DPPH antioxidant activities of the studied *Jatropha* species are presented in Fig. 1. The percentages of inhibition and Trolox equivalent antioxidant capacity of different parts of *Jatropha* species were analyzed in DPPH assay and ranged from 24.33 ± 1.32 to 89.68 ± 1.26 % and 20.42 ± 1.05 to 72.34 ± 1.00 mg TE/g DP, respectively, for maceration as well as 33.61 ± 1.24 to 91.61 ± 1.37 % and 32.11 ± 0.91 to 74.42 ± 1.00 mg TE/g DP, respectively, for ultrasonication extraction (Fig. 1). For ultrasound-assisted extraction, all the parts of *Jatropha* species showed higher percentages of inhibition and Trolox equivalent antioxidant capacity than the maceration extraction and the results are shown in Table-1. These results agreed with the studies elsewhere, as in fact solvent system, extraction process and drying temperature could effect on the recovery of phenolic compounds and the antioxidant activity [4]. The highest antioxidant activity was detected in the roots of *J. gossypiifolia* and in the leaves of *J. curcas* and in the barks of *J. curcas* as a lowest antioxidant activity. From the ANOVA, there was no significant statistical difference between roots of *J. gossypiifolia* and leaves of *J. curcas* (Table-1). However, further studies should be performed to study the effect of growth location and cultivation practices, harvesting conditions and seasonality, since they could affect the antioxidant activity [30]. Moreover, non-antioxidant compounds may also interference the antioxidant activity [31].

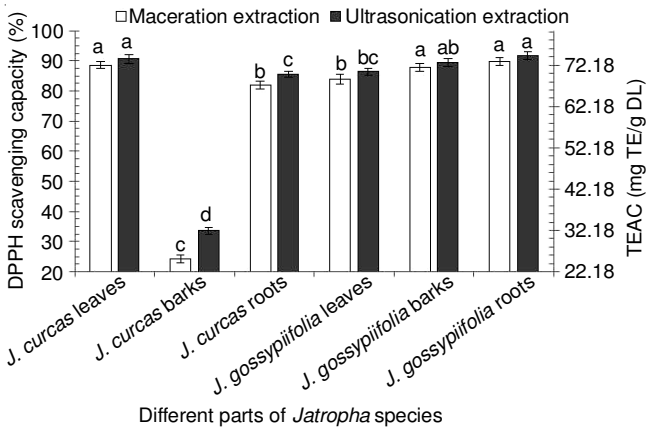

Fig. 1. The percentages of inhibitions and TEAC of different parts of *Jatropha* species in DPPH method

TABLE-1
DPPH AND ABTS RADICAL SCAVENGING ACTIVITY OF DIFFERENT PARTS OF *Jatropha* SPECIES IN DIFFERENT EXTRACTION PROCESS

Different parts of *Jatropha* species	DPPH radical scavenging capacity				ABTS radical scavenging capacity			
	Maceration		Ultrasonication		Maceration		Ultrasonication	
	Inhibition (%)	TEAC (mg TE/g DP)	Inhibition (%)	TEAC (mg TE/g DP)	Inhibition (%)	TEAC (mg TE/g DP)	Inhibition (%)	TEAC (mg TE/g DP)
J. curcas leaves	88.56±1.09a	71.45±0.87a	90.61±1.47a	73.69±1.08a	98.31±0.93a	57.76±0.51a	99.16±0.73a	58.22±0.40a
J. curcas bark	24.33±1.32c	20.42±1.05c	33.61±1.24d	32.11±0.91d	30.38±0.90c	20.19±0.50c	42.13±0.90b	26.69±0.50b
J. curcas root	81.94±1.34b	66.18±1.07b	85.53±1.05c	69.98±0.77c	95.60±0.91b	56.26±0.50b	98.31±0.93a	57.76±0.51a
J. gossypiifolia leaves	83.93±1.51b	67.77±1.20b	86.35±1.15bc	70.58±0.84bc	97.41±0.82ab	57.26±0.45ab	98.61±0.73a	57.92±0.40a
J. gossypiifolia barks	87.82±1.31a	70.86±1.04a	89.39±1.19ab	72.80±0.87ab	98.07±0.73a	57.62±0.40a	98.92±0.83a	58.09±0.46a
J. gossypiifolia roots	89.68±1.26a	72.34±1.00a	91.61±1.37a	74.42±1.00a	98.43±0.91a	57.82±0.50a	99.34±0.68a	58.32±0.38a

Present work showed the favourable results with previous studies on the leaves of *Jatropha* [8,15-20] and showed equivalent or higher antioxidant activity. As commented before, not only the species but also the extraction protocols used might affect the antioxidant activities. Alternatively, antioxidant activities of our studied *J. curcas* were lower than the methanolic extract (91.5 %) reported by of Igbinosa *et al.* [19] and root extract of *J. gossypiifolia* was higher than the reported data [8,15,19]. These results suggests that the plant extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove abnormal electron which is responsible for radical's reactivity.

The ABTS free radical scavenging method is another important outstanding assay for analysing the antioxidant activity. In ABTS assay, the percentages of inhibition of different parts of *Jatropha* species were analyzed and ranged from 30.38 ± 0.90 to 98.73 ± 0.83 % and 42.13 ± 0.90 to 99.34 ± 0.68 % respectively, for maceration and ultrasonication extraction (Fig. 2). Whereas the Trolox equivalent antioxidant capacity (TEAC) were varied from 26.69 ± 0.50 to 58.32 ± 0.38 mg TE/g DP and 26.69 ± 0.50 to 58.32 ± 0.38 mg TE/g DP, respectively. The Trolox equivalent antioxidant activity was detected in the roots of *J. gossypiifolia* as the highest and in the barks of *J. curcas* as the lowest (Table-1). This study showed favourable results with previous studies and presented equivalent or higher. The percentages of inhibition of our studied species leaves and roots were higher than the Igbinosa and co-worker [19] reported methanol extract (89.0 %); ethanol extract (87.78 %) and aqueous extract (86.8 %).

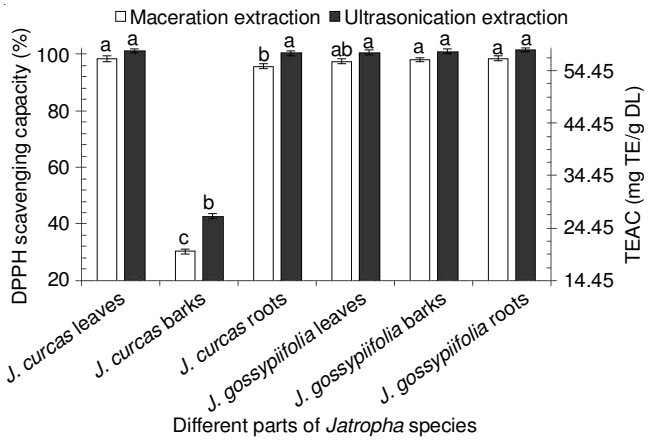

Fig. 2. The percentages of inhibitions and TEAC of different parts of *Jatropha* speciesin ABTS method

Total phenolic content: The polyphenolic content of different parts of *Jatropha* species obtained by ultrasound and maceration extraction process are shown in Table-2 and Fig. 3. In the extraction of phenolic compounds, the UAE allowed to higher phenolic contents ranged from 2.67 ± 0.35 to 44.06 ± 1.04 mg GAE/g DP as against to the total phenolic contents extracted by maceration extraction and varied from 3.21 ± 0.27 to 45.49 ± 1.16 mg GAE/g DP. Therefore, at the studied environments, higher phenolic content observed for all the parts in case of ultrasound extraction. The highest values of total phenolic contents were observed in the leaves of *J. curcas* as well as the lowest in the barks of *J. curcas* (Table-2). Actually,

TABLE-2
TOTAL PHENOLIC CONTENT OF DIFFERENT PARTS OF *Jatropha* SPECIES IN DIFFERENT EXTRACTION PROCESS

Jatropha species	Total phenolic content (mg GAE/g DP)	
	Maceration	Ultrasonication
J. curcas Leaves	44.06 ± 1.04a	45.49 ± 1.16a
J. curcas bark	2.67 ± 0.35e	3.21 ± 0.27e
J. curcas root	22.47 ± 0.54c	24.61 ± 0.86c
J. gossypiifolia leaves	26.20 ± 0.44b	26.41 ± 0.55bc
J. gossypiifolia barks	15.94 ± 0.45d	19.03 ± 0.53d
J. gossypiifolia roots	27.74 ± 0.42b	28.42 ± 1.19b

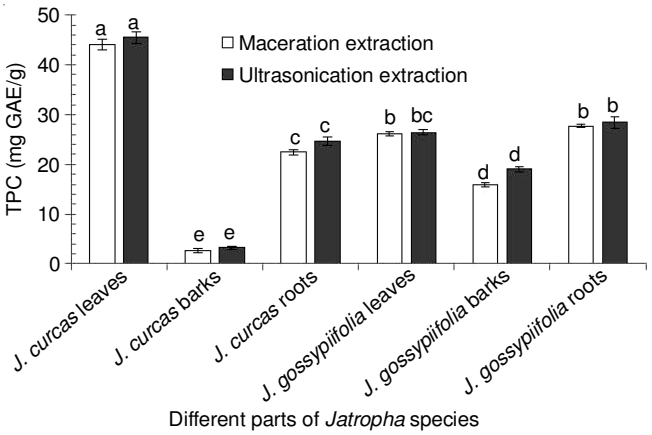

Fig. 3. Total phenolic contents of different parts of *Jatropha* species

ultrasound provokes a formation of tiny bubbles exposed to fast adiabatic expansions and compressions, which rises the temperatures and pressures within the system [32]. Thus, the ultrasound irradiation process could contribute to the higher yields of phenolic content. However, sonication process swells dried plants by adsorbing higher amount extraction solvent [33] resulting enlargement of the pores of cell walls that permit greater diffusivity across the cell walls. Finally, the process breakdown the cell walls, which enable to wash out the cell content and allow higher efficiency in phenolic release [34,35].

Cluster analysis: The hierarchical cluster analysis is useful in solving classification problems. In present study, the objective of using cluster analysis was to reveal clusters based on the antioxidant activity and total phenolic content. Higher total phenolic contents and antioxidant capacity (DPPH and ABTS) of *Jatropha* species are placed in same cluster, their linkage distance is higher than the lower antioxidant active and total phenolic contents cultivars, which are placed in different cluster. In hierarchical cluster analysis, the dendrogram was created using Ward's method and Euclidean distances was measured the similarity between samples, considering all the cultivars experimental properties and shown in Fig. 4(a-b). In case of antioxidant activity, two main clusters can be distinguished at euclidean distances of about 5.0 (Fig. 4a).

The first cluster includes five parts of *Jatropha* species, while second cluster contains residual parts, which indicated the least antioxidant activity (barks of *J. curcas*). The roots of *J. gossypiifolia* showed the highest antioxidant activity. From Fig. 4b, two main clusters also can be determined. The left cluster of the dendrogram includes 5 parts, whereas right cluster contains the remaining 1 part. The highest total phenolic content was detected in the leaves of *J. curcas*, which was

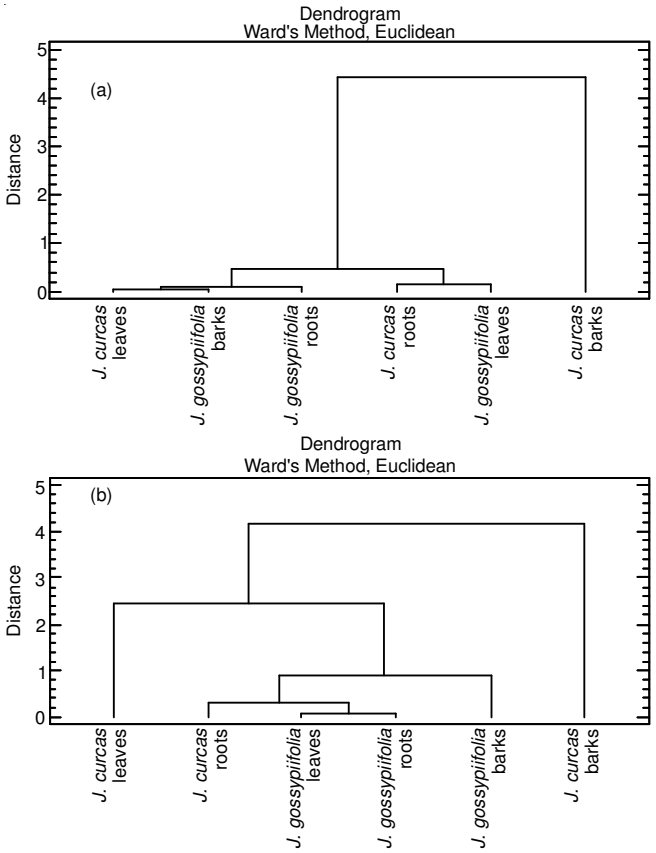

Fig. 4. Dendrogram of different parts of *Jatropha* specieson the basis of (a) antioxidant activity by and (b) total phenolic content

placed in left cluster. While right cluster contained only one part, which was barks of *J. curcas* and this part showed lowest phenolic content (Fig. 4b), as observed in antioxidant activity measured by both the scavenging method.

Correlation analysis: The correlation analysis between ABTS, DPPH and total phenolic contents of *Jatropha* species were displayed in Fig. 5. A highly positive relationship ($p < 0.05$) was found between maceration DPPH assay and ultrasonic DPPH ($r = 0.998$), maceration ABTS ($r = 0.995$) and the ultrasonic ABTS ($r = 0.994$) on the other hand. In the same way, another significant correlation were observed between the maceration DPPH and maceration total phenolic content and the ultrasonic total phenolic content, but with poor r-value ($r = 0.753$ and 0.779, respectively). This significant correlation suggests that 75 and 80 % of the DPPH scavenging capacity of *Jatropha* species outcomes were due to the involvement of phenolic compounds. The remaining percentage may also come from others antioxidant compounds such as volatile oils, amino acids, vitamins and others, which are not limited to phenolics [36]. Moreover, a significant correlation between antioxidant activity and total phenolic contents proved that phenolic compounds may also contribute to the antioxidant activity of different parts of *Jatropha* species. Several previous studies have already been reported the significant positive relationship between polyphenols and antioxidant activity [37-39]. Therefore, the present study showed that roots of *J. gossypiifolia* and leaves of *J. curcas* are by-products with strong radical scavengers and can be considered as good sources of natural antioxidants for food applications, among others.

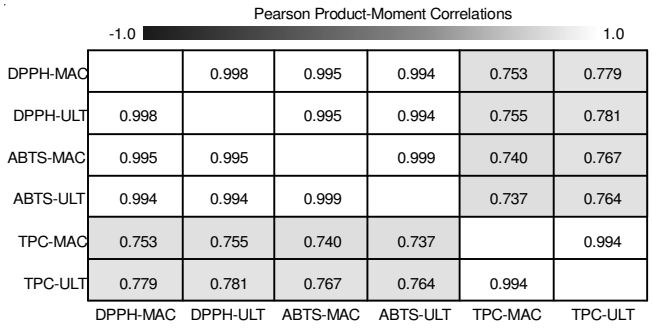

Fig. 5. Pearson product-moment correlation matrix of the studied parameters ($P < 0.05$)

Conclusion

Ultrasound-assisted extraction is one of the new, simple and economical extraction process. This was used to extract antioxidants from different parts of *Jatropha* species and compared with maceration extraction method. Using DPPH assay, the percentages of inhibition and Trolox equivalent antioxidant capacity were found to vary from 33.61 ± 1.24 to 91.61 ± 1.37 % and 32.11 ± 0.91 to 74.42 ± 1.00 mg TE/g dry parts (DP), respectively, for extracts obtained by ultrasound assisted extraction. In the case of maceration extraction, these values ranged from 24.33 ± 1.32 to 89.68 ± 1.26 % and 20.42 ± 1.05 to 72.34 ± 1.00 mg TE/g DP. While for ABTS assay, the parameters were found to vary from 42.13 ± 0.90 to 99.34 ± 0.68 % and 26.69 ± 0.50 to 58.32 ± 0.38 mg TE/g DP for ultrasonication, as well as from 30.38 ± 0.90 to 98.73 ± 0.83 % and 20.19 ± 0.50 to 57.99 ± 0.46 mg TE/g DP for maceration extraction. The total phenolic contents varied between 2.67 ± 0.35 to 44.06 ± 1.04 mg GAE/g DP and 3.21 ± 0.27 to 45.49 ± 1.16 mg GAE/g DP, respectively for the latter extraction processes. Therefore, roots of *J. gossypiifolia* and leaves of *J. curcas* could be a potential source of antioxidants from natural origin and could have greater significance as therapeutic agent in preventing or slowing oxidative stress and chronic related disorders, as well as for food applications (antioxidant additives). Therefore, the highest activity presented parts are desired to implicate *in vitro* and *in vivo* studies for considerating their mode of action as antioxidant. Also, these parts can be a potent candidates for further phytochemical and pharmacological studies to isolate and identify the secondary metabolites correlated to this antiradical activity or others bioactivity.

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