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Antioxidant Activity and Phytochemical Components of *Jatropha curcas* Linn. Root Extracts

Shahirah Atiqah Osman[1], Norhani Abdullah[1,2*] and Syahida Ahmad[1,3]

[1]Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[2]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.
[3]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

*Corresponding author:
Norhani Abdullah
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.
Phone: 0389471998
Fax: 03 8938 1612
Email: norhani@upm.edu.my / norhani.biotech@gmail.com

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ABSTRACT

Jatropha curcas Linn. is a multipurpose plant in the *Euphorbiaceae* family. Numerous reports have indicated the antioxidant properties of phenolics and flavonoids present in *J. curcas* root methanolic extract. In the present study, 80% methanolic extract of *J. curcas* root was prepared and used for extraction of bioactive compounds with five solvents (hexane, chloroform, ethyl acetate, n-butanol and water) by liquid-liquid fractionation. The fractions were evaluated for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities by using the 2-2'-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing/antioxidant potential (FRAP) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays. The phytochemical compounds present in each fraction were identified by liquid chromatography mass spectrometry (LCMS) analysis. The TPC of ethyl acetate and n-butanol fractions were 34.0 ± 0.02 µg GAE/g DW and 33.1± 0.01 µg GAE /g DW, respectively, while the TFC were 9.2 ± 0.04 µg CE/g DW and 10.1 ± 0.01 µg CE/g DW, respectively. The free radical scavenging activity varied with the solvents used. Ethyl acetate fraction showed the highest inhibition for DPPH (48.7%) and FRAP (79.6%) assays, while n-butanol fraction showed the highest ABTS radical scavenging activity (94.8%). The LCMS analysis showed the compounds present in the various fractions were phenolic and flavonoid derivatives such as coumaric acid, epigenin, quercetin, leuteolin and p-coumaroylquinic acid. The results showed that TPC, TFC and antioxidant activity for ethyl acetate and n-butanol fractions were higher compared to the other solvent fractions.

INTRODUCTION

Jatropha curcas Linn. is a multipurpose plant which belongs to *Euphorbiaceae* family. It is a plant species that is resistant to drought but can grow in low to high rainfall areas, such as Africa and South-east Asia including Malaysia [1]. Usually, this plant could be found in the farm as a commercial crop, or as boundaries or hedges for protection from grazing animals and to avoid erosion [1]. It has gained importance in Malaysia, as a source of seed oil for biofuel production.

The ethnomedical practice in West Africa showed the application of Jatropha leaves in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism [2]. The roots of *J. curcas* have been used as a decoction for bleeding gums, toothache, eczema, ringworm, and scabies and to cure dysentery [3]. The notable antioxidant, anticancer and anti-inflammatory activities of the extracts obtained from the root, latex and seed and the antimicrobial activity of root and stem have been reported [4,5].

Plant derived-bioactive compounds have received considerable attention due to their therapeutic potentials as antimicrobial, anti-inflammatory, anticancer and antioxidant activities. Numerous reports have indicated the antioxidant and anti-inflammatory properties of phenolics and flavonoids present in various plant extracts [4, 6].

It was reported [7] that various berries contained bioactive compounds such as phenolic acids, flavonoids, anthocyanins and flavonols, and tannins, either individually or combined, are responsible for various health benefits including prevention of inflammation disorders, cardiovascular diseases, or protective effects to lower the risk of various cancers.

A study reported by Oskoueian et al. [4] showed that methanolic extract of *J. curcas* root containingvarious phenolic and flavonoids compounds possessed high anti-antioxidant activity. However, the nature of solvents used for extraction would influence the bioactive compounds extracted and consequently affect the therapeutic activities. Therefore, this study was conducted to evaluate the phytochemical contents and antioxidant properties of various solvent fractions obtained from the methanolic extracts of *J. curcas* root.

MATERIALS AND METHODS

Plant material

The plant *J. curcas* Linn. was identified by Mr. Shamsul Khamis of the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). Three plants about 4 years old were used as the source of roots. The plants were at the Faculty of Agriculture farm, Universiti Putra Malaysia with the GPS location of 3˚0'26.91"N latitude and 101˚42'13.24"E. Roots from the peripheral section of each plant were collected, washed to remove soils and cut into small pieces (3-5 mm thickness). Roots from each tree were weighed separately and stored at -20 °C for 24 hours. Then, they were freeze-dried using a freeze dryer (Labcanco, United State) for three to five days. The dried roots were ground to powder form by using a dry blender.

Methanolic extraction

The three root samples were separately used for preparation of the methanolic extracts. The methanol:water (80:20) mixture was used to soak 10mg of the three root samples in total volume of 200mL in 250mL flask. Individual root sample was placed in a shaker (Orbital Shaker SK300, JEIO Tech) at 150 rpm for 24 h. After 24 h, the sample was filtered using filter paper Whatman no.4, followed by Whatman no.1. The filtrate was then centrifuged at 3000 x g, 4°C for 10 minutes. The supernatant was stored at -20°C. Extraction steps were repeated daily for one week. All supernatants were evaporated by using a rotary evaporator (R-210 Buchi, Switzerland) until the volumes were about 100mL and the samples were freeze dried to a powder form. Dried extracts were labelled as crude extracts to be used for fractionation.

Liquid-liquid fractionation

In order to separate the compounds into polar, semi-polar and non-polar fractions, the crude extracts (10mg) obtained from the roots were dissolved in 200mL of distilled water and fractionated by using different solvents, hexane, chloroform, ethyl acetate and n-butanol. The 200-mL aqueous solution was mixed with 200mL hexane (1:1) and poured into a separating funnel. The solution was shaken slowly for 10 minutes. A double layer that formed by the two solvents separated the non-polar (hexane) and polar compounds (water). The hexane fraction was collected. This step was repeated thrice. The remaining water extract was then mixed with chloroform to separate semi-polar compounds from the polar compounds. The same procedure was repeated by using ethyl acetate, n-butanol and leaving the residual soluble aqueous fraction. All collected fractions were evaporated using a rotary evaporator to concentrate the solution to a small amount of liquid, which cannot be further evaporated. Then, the oily liquid was freeze-dried to a powder form. The crude extracts were then collected and weighed. All samples were stored at -20 °C for further analyses. The liquid-liquid fractionation process is as shown in **Fig. 1**.

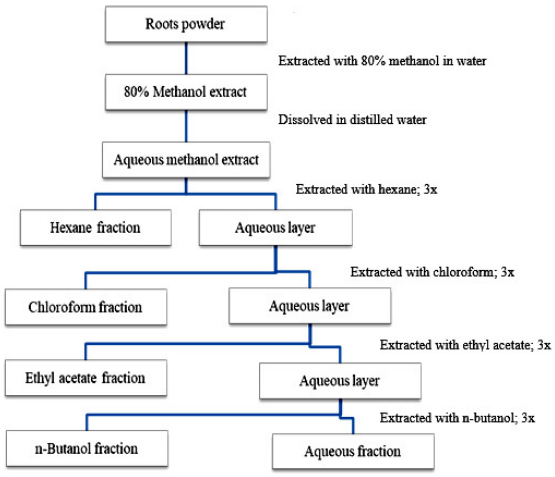

Fig. 1. Liquid-liquid fractionation process [13].

Total phenolic content

All fractions were analysed for total phenolic content (TPC) by using the Folin–Ciocalteu method [8] with some modifications. Briefly, 25µL of crude extract (1µg/mL) were prepared for each fraction. Then, 125µL of 10% Folin Ciocalteu were added and the mixtures were allowed to stand at room temperature for about four minutes. After that, the solution was mixed with 1000µL of 7.5% (w/v) of sodium carbonate (Na_2CO_3) and incubated in the dark at room temperature for 2 hours. The absorbance was measured at 765nm using a UV/Vis microplate reader (Spectramax plus 384; Molecular Devices Inc., USA). All assays were performed in triplicates. Gallic acid was used as the standard to form the calibration curve and the results were expressed as µg gallic acid equivalents/g dry weight (DW) of each fraction of the methanolic extract from *J. curcas* roots.

Total flavonoid content

Total flavonoid content was measured by the aluminium chloride colorimetric assay [9] with some modifications. A 96 wells microplate was used. The reagents 5% (w/v) of sodium nitrate, ($NaNO_2$) 10% (w/v) of aluminium chloride (AlCl3) and 1M of sodium hydroxide (NaOH) were freshly prepared together with 10mg/mL of catechin which was used as the standard and then diluted to 0.5µg/µL with 100% DMSO. Then, 25µL of samples were pipetted into wells accordingly in triplicates and 50µL of distilled water were added, followed by 10 µL of 5% $NaNO_2$ and 15µL of 10µM $AlCl_3$. The solution was mixed well and incubated for 5 minutes before adding 50µL of 1M NaOH and incubated at room temperature for 25 minutes. The absorbance was measured by using a UV/Vis microplate reader (Spectramax plus 384; Molecular Devices Inc., USA) at 510nm. The results were expressed as µg catechin equivalents (CE)/g DW.

Liquid chromatography mass spectrometry (LCMS)

Liquid chromatography mass spectrometry analysis was conducted to determine the nature of compounds present in each fraction prepared. The LCMS analysis was performed by using the AB Sciex 3200QTrap LCMS/MS with Perkin Elmer FX 15 HPLC system, Advance Chemistry Solutions [10].

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The antioxidant activity of each sample was determined by the DPPH free radical scavenging activity [8]. A 0.1mM solution

of DPPH in DMSO was prepared. 25µL of samples (hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions) were pipetted into the 96 wells plate and serially diluted with DMSO to different concentrations (3.125, 6.25, 12.5, 25, 50, and 100µg/mL), followed by 175µL of DPPH solution in the dark room. The absorbance at 540nm was immediately determined by using a UV/Vis microplate reader (Spectramax plus 384; Molecular Devices Inc., USA). The assay was performed in triplicate. Gallic acid was used as the reference antioxidant. The DPPH radical scavenging activity was calculated by using the formula below:

$$\text{DPPH free radical scavenging activity (\%)} = [(A_{control} - A_1)/ A_{control}] \text{ X } 100$$

where $A_{control}$ was the absorbance of the control (DMSO + DPPH) and A_1 was the absorbance of the sample.

Ferric-reducing antioxidant power (FRAP) assay

The antioxidant activity of samples was determined by FRAP assay [11]. The ferric reducing properties of each fraction was determined at different concentrations through serial dilution of 25µL samples (3.125, 6.25, 12.5, 25, 50, and 100µg/mL) with DMSO in 96 wells plate. Each diluted sample was mixed with 25µL of phosphate buffer (0.2M, pH 6.6) and 25µL of potassium ferricyanide (1% w/v). The mixtures were incubated at 50°C for 20 minutes. After that, 25µL of 10% trichloroacetic acid (TCA) were added into the mixture to stop the reaction. Distilled water at 100µL were then added, followed by 16µL of 0.1% (w/v) ferric chloride ($FeCl_3$). The mixtures were incubated for 30 minutes in a dark condition at room temperature. The absorbance was measured at 593nm by using an UV/Vis microplate reader (Spectramax plus 384; Molecular Devices Inc., USA). The assay was performed in triplicate. Ascorbic acid was used as a reference antioxidant. Percentage of antioxidant activity of all samples in the FRAP assay was calculated by using the formula below:

$$\text{Antioxidant activity (\%)} = [(A_1 - A_{control})/ A_1] \text{ X } 100$$

where $A_{control}$ was the absorbance of the control (potassium phosphate buffer + FRAP reagent) while A_1 was the absorbance of the sample.

2,2′-Azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay

The antioxidant activity of samples was determined by ABTS assay [12]. The ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS•1) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for generation of radicals for 16 h before use. After that, the solution was diluted with distilled water to adjust the absorbance at 734nm to 0.7 ± 0.02. All samples with different concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) in 96 wells plate and added with 100 µL ABTS solution and mixed for 10 seconds. The absorbance was determined at 734nm using an UV/Vis microplate reader (Spectramax plus 384; Molecular Devices Inc., USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the antioxidant reference. The antioxidant activity of all samples was calculated by determining the decrease in absorbance at different concentrations by using the formula below:

Percentage of inhibition = [(Absorbance ABTS-Absorbance sample)/Absorbance ABTS] X100

All IC_{50} values for each solvent fraction were calculated based on 50% inhibition of radical scavenging activity for all three roots accordingly.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and treatments mean were compared to control by using Dunnett's Multiple Comparison Test at $p<0.05$. GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA) was used for all the statistical analyses as well as IC_{50} value calculation.

RESULTS AND DISCUSSION

Total phenolic and flavonoid content

Ethyl acetate and n-butanol fractions showed the highest phenolic content of 34.0 ± 0.02 µg GAE/g DW and 33.1 ± 0.01 µg GAE/g DW, respectively (**Table 1**). Aqueous, chloroform and hexane fractions showed lower phenolic contents. The presence of phenolic compounds has been reported in *J. curcas* root, seed, leaf and stem [4]. As shown in Table 1, TFC of ethyl acetate (9.2 ± 0.04 µg CE/g DW), n-butanol (10.1 ± 0.01 µg CE/g DW) and aqueous (8.6 ± 0.02 µg CE /g DW) fractions were significantly higher ($p<0.05$) than hexane and chloroform fractions.

Table 1. Total phenolic and flavonoid contents of each solvent fraction extracted from 80% methanolic extract of *J. curcas* roots.

Fractions	Phenolic (µg GAE/g DW)	Flavonoid (µg CE /g DW)
Hexane	4.8 ± 0.01^{d}	5.3 ± 0.01^{c}
Chloroform	8.9 ± 0.01^{c}	6.3 ± 0.01^{c}
Ethyl Acetate	34.0 ± 0.02^{a}	9.2 ± 0.04^{a}
N-Butanol	33.1 ± 0.01^{a}	10.1 ± 0.01^{a}
Aqueous	26.6 ± 0.031^{b}	8.6 ± 0.02^{b}

Note: Each value is a mean ± SE of 3 root samples. Means with different superscripts within column indicate significant difference at $p<0.05$.
GAE: gallic acid equivalent
CE: cathecin equivalent

The choice of solvents is an important factor to determine the phytochemical compounds present in the plant extracts. Therefore, it is important to select the right solvent to maximize the amount of compounds extracted. A general principle is, non-polar solvents will extract out non-polar substances, and polar solvents will extract out polar materials [13]. Therefore, liquid-liquid fractionation of the 80% methanol extract was carried out based on polarity. All five solvents were chosen based on the increasing polarity. In order to eliminate the lipoidal material of the extracts, hexane and chloroform were first used to defatting the sample due their non-polar characteristic [13]. N-butanol, ethyl acetate, and water are polar solvents, but water and n-butanol are polar protic solvents and would extract negatively charged solutes, while ethyl acetate is a polar aprotic solvent that solvate positively charged species [13]. The higher concentrations of phenolics and flavonoids in the polar solvents indicated that *J. curcas* root contained higher levels of polar molecules than non-polar phenolic and flavonoids compounds.

Phenolic and flavonoid compounds are abundant in plants, and various biological activities have been attributed to these metabolites. They have been reported to have antioxidant, anti-inflammatory, antimicrobial, cytotoxic effects, and hypolipidemic activities. Most of these biological functions are related to their free radical scavenging activities [14]. The

activity of an antioxidant is determined by its reactivity as a hydrogen or electron-donating agent, the fate of the resulting antioxidant-derived radical, its reactivity with other antioxidants and the transition metal-chelating potential [15].

Among the natural antioxidants, phenolic compounds comprise the most powerful secondary metabolites that contribute to their multifunctional purpose in pharmaceutical, medicinal material, food industry and cosmetics [16]. Polyphenols possess ideal structural chemistry for free radical-scavenging activities, and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis [17]. Thus, phenolic compounds present in *J. curcas* root are expected to enhance the properties of natural antioxidants and exhibit several biological activities such as anti-inflammatory and antibacterial properties [4,5].

LCMS analysis

Hexane fraction

The LCMS chromatograms of the hexane fractions from *J. curcas* root showed three main compounds. These compounds were identified as 15,16-dihydroxy-9Z,12Z-octadecadienoic acid, benzenepropanoic acid conjugate and coumaric acid based metabolite (**Table 2**). Compounds that have been reported from *J. curcas* roots include gallic acid, quercetin, ellagic acid, coumaric acid, salicylic acid, and benzoic acid [18]. P-coumaric acid is known to have antioxidant activity. Sukhonthara et al. [19] had reported the presence of p-coumaric acid as the major phenolic compounds in commercially defatted rice bran extracts, which demonstrated high inhibitory effect on polyphenol oxidase.

Table 2. Compounds identified by LCMC analysis in different solvent fractions extracted from 80% methanolic extract of *J. curcas* roots.

Fractions	Compounds
Hexane	15,16-dihydroxy- 9Z,12Z octadecadienoic acid
	Benzenepropanoic acid conjugate
	Coumaric acid based compound
Chloroform	15,16-dihydroxy- 9Z, 12Z-octadecadienoic acid
	Coumaric acid conjugate
Ethyl Acetate	Epigenin based 433
	Epigenin based
	Kaempferol glucoside isomer
	Methyl 2-[cyclohex-2-en-1-yl(hydroxy)methyl]-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxoprolinate
n-Butanol	Coumaric acid
	Coumaric acid conjugate
	P-coumaroylquinic acid
Aqueous	Quercetin
	Quercetin conjugate
	P-Coumaroylquinic acid
	Leuteolin conjugate

Chloroform fraction

The results of LCMS analysis indicated 17 compounds were present in the chloroform fraction, but only two compounds were identified as 15,16-dihydroxy-9Z,12Z-octadecadienoic acid and coumaric acid conjugate. 15,16-dihydroxy-9Z,12Z-octadecadienoic acid has a molecular formula $C_{18}H_{32}O_4$ with average mass of 312.444 Da. This compound is a type of lipid that has been found in *Purmus persicae* Linn. which contributes to pharmacological action such as inhibiting platelet aggregation, anti-atherosclerosis, developing hemodynamic and also anti-inflammatory activities [20].

Ethyl acetate fraction

The LCMS analysis of ethyl acetate fraction showed 23 compounds, but only four could be identified. These compounds were apigenin based, kaempferol glucoside isomer, and methyl 2-[cyclohex-2-en-1-yl(hydroxy)methyl]-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxoprolinate. Apigenin, a naturally occurring plant flavone, abundantly present in common fruits and vegetables is recognized as a bioactive flavonoid shown to possess anti-inflammatory, antioxidant and anticancer properties [21]. Kaempferol is a type of phenolic compound that is present in black currant fruit and well known as a compound that contributes to antioxidant, anti-inflammatory, antihemostatic, vasomodulatory, anticancer, and also muscle relaxing effects [22]. This compound is also found in *Equisetum arvense* extracts that has the potential in pharmaceutical industry to treat inflammatory disorders [23].

n-Butanol fraction

The LCMS analysis of n-butanol fraction from *J. curcas* root methanolic extract showed 19 different compounds where three of them were identified as coumaric acid, coumaric acid conjugate, and p-coumaroylquinic acid. Coumaric acid is a bioactive compound that is present in medicinal plants that exhibits anti-inflammatory properties. Pragasam et al. [24] reported that coumaric acid was identified as an immunosuppressive agent to treat autoimmune inflammatory disorder such as rheumatoid arthritis. P-coumaroylquinic acid, a phenolic compound is a derivative of p-coumaric acid [25] which may be involved in antioxidant activity.

Aqueous fraction

Twenty-two different compounds were detected in the aqueous fraction, but only four were identified as quercetin, quercetin conjugate, p-coumaroylquinic acid and leuteolin conjugate. Quercetin, one of the most widely distributed flavonoids in the plant, is also present in both food sources and beverages. It possesses a broad range of pharmacological properties including anti-inflammatory, anti-proliferative effect in tumour cells and protective effects against oxidative stress. It also exerts a strong antioxidant, anti-inflammatory and anti-metastatic effects as well as antiviral and anti-estrogenic activities [26, 27, 28].

Antioxidant activity

DPPH radical scavenging activity

Different fractions of 80% methanolic extract of *J.curcas* roots showed variable DPPH scavenging activities as shown in **Fig. 2**. The fractions showed radical scavenging ability in a dose dependent manner. At the highest concentration of 100 µg/mL, both ethyl acetate and aqueous fractions reached almost similar percentage of DPPH inhibition at 48.7% and 47.4%, respectively. n-butanol fraction showed slightly lower (43.7%), while chloroform (26.8%) and hexane (24.8%) were the fractions with the lowest inhibition activity. Gallic acid as the reference phenolic compound, showed the highest value at 91.7%. The high free radical scavenging activity of crude extract of *J. curcas* root and stem had also been reported [18]. The antioxidant activity of phenolic compounds is due to their redox properties which make them act as hydrogen donors, reducing agents and hydroxyl radical quenchers [18].

In the present study, the IC_{50} value of gallic acid in the DPPH assay was 26.8 µg/mL. Ethyl acetate fraction possessed the lowest IC_{50} value of 85.4 µg/mL, followed by aqueous fraction (133.6 µg/mL). The IC_{50} value for hexane fraction was too high due to the low scavenging activity.

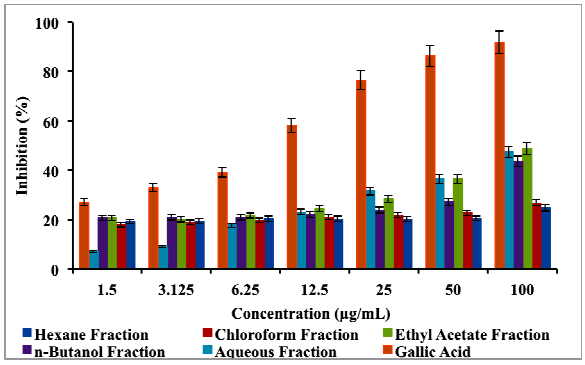

Fig. 2. DPPH free radical scavenging activity of different fractions prepared from 80% methanolic extract of *J. curcas* root at different concentrations. Each value represents mean+SD (n=3). Vertical bar represents standard deviation.

Ferric-Reducing Antioxidant Power (FRAP) assay

Different fractions of *J.curcas* roots showed dissimilar percentages of reducing power activity. Ascorbic acid was used as the reference antioxidant. The reducing power in FRAP bioassay for ascorbic acid was 94.7% at the concentration of 100 µg/mL. According to the results in **Fig. 3**, among the fractions, ethyl acetate fraction exhibited the highest reducing power with a value of 79.6% at the concentration of 100 µg/mL, while n-butanol had slightly lower activity at 73.4%. Both fractions contained high levels of phenolic compounds that are congruent to the reducing power property, which reduced Fe^{3+} to Fe^{2+} in the presence of tripyridyl-triazine (TPTZ). The aqueous fractions showed lower reducing power activity at 52.8%, while hexane (41.2%) and chloroform (39.2%) fractions demonstrated the lowest activity.

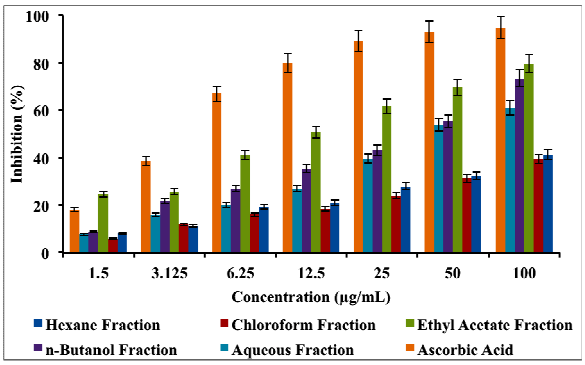

Fig 3. FRAP reducing power of different fractions prepared from 80% methanolic extract of *J. curcas* root at different concentrations. Each value represents mean+SD (n=3). Vertical bar represents standard deviation.

ABTS radical cation decolorization assay

As shown in **Fig. 4**, n-butanol, aqueous and ethyl acetate fractions exhibited high scavenging abilities compared to the other fractions. At concentration of 100 µg/mL, n-butanol, aqueous and ethyl acetate fractions demonstrated almost similar activities at 94.8%, 93.9%, and 90.8%, respectively. This is congruent with the high phenolic and flavonoid compounds present in these fractions. Both chloroform and hexane fractions had lower activity at 64.4% and 34.1%, respectively.

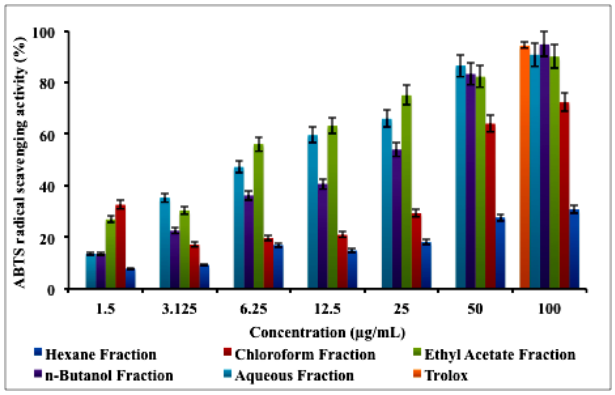

Fig 4. ABTS radical scavenging activity of different fractions prepared from 80% methanolic extract of *J. curcas* root at different concentrations. Each value represents mean+SD (n=3). Vertical bar represents standard deviation.

The relative antioxidant ability to scavenge the radical $ABTS^{+}$ was compared to that of standard Trolox. At the highest concentration of Trolox tested, the percentage of scavenging activity was 94.3%, comparable to the activities demonstrated by n-butanol, aqueous and ethyl acetate fractions. The ABTS radical cation scavenging activity reflects hydrogen-donating ability of the metabolites. Hagerman et al. [29] reported that the high molecular weight phenolics (tannins) had higher ability to quench free radicals ($ABTS^{\bullet}+$). The tannins were 15–30 times more effective at quenching peroxyl radicals than simple phenolics or Trolox. Hence, the results of the present study indicate the presence of high molecular weight phenolics in the active fractions.

CONCLUSION

Ethyl acetate and n-butanol fractions contained higher levels of total phenolic and flavonoid compounds compared to the other solvent fractions. The free radical scavenging activity varied with the fractions evaluated. Ethyl acetate fraction demonstrated the highest inhibition for DPPH and FRAP assays, while n-butanol fraction exhibited the highest ABTS radical scavenging activity. The LCMS analysis revealed the compounds present in the various fractions were phenolic and flavonoid derivatives such as coumaric acid, epigenin, quercetin, leuteolin and p-coumaroylquinic acid.

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REFERENCES

1. Islam AKMA, Yaakob Z, Anuar N. Jatropha: A multipurpose plant with considerable potential for the tropics. Sci Res Essays. 2011; 6(13):2597-05.
2. Sharma SKr, Singh H. A Review on pharmacological significance of genus *Jatropha* (Euphorbiaceae). Chin J Integr Med. 2012; 18(11):868-80.
3. Aiyelaagbe OO, Hamid AA, Fattorusso E, Taglialatela SO. Cytotoxic activity of crude extracts as well as of pure components from Jatropha species, plants used extensively in African traditional medicine. Evid Based Complement Alternat Med. 2011; 2011:1–7.
4. Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, et al., Antioxidant, anti-inflammatory and anticancer

activities of methanolic extracts from *Jatropha curcas* L. J Med Plants Res. 2011; 5:49–57.
5. Namuli A, Abdullah N, Sieo CC, Zuhainis SW, Oskoueian E. Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn. extracts. J Med Plants Res. 2011; 5:3982–90.
6. de Velde FV, Grace MH, Esposito D, Pirovani ME, Lila MA. Quantitative comparison of phytochemical profile, antioxidant, and anti-inflammatory properties of blackberry fruits adapted to Argentina. J Food Compost Anal. 2016; 47: 82-91.
7. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Socho J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015; 16(10): 24673-706.
8. Li HB, Cheng KW, Wong CC, Fan KW, Chen F and Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007; 102:771-6.
9. Ira S, Manisha M, Singh GP, Anirudha R. Quantitative estimation of phenolic and flavonoid content and antioxidant activity of various extracts of different parts of *Plumbago zeylanica Linn*. Int J Drug Dev Res. 2014; 6 (2):136 -40.
10. Advanced Chemistry Solutions *(The Scientific Consultation Company),* associated with GHOS Sdn Bhd, ACD/Labs, Inc. Kuala Lumpur, Malaysia.
11. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem.1995; 43:27–37.
12. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med. 1999; 26:1231–7.
13. Otsuka H. 2006. Purification by solvent extraction using partition coefficient. In: Sarker S.D., Latif Z., Gray A.I. (eds), Natural Products Isolation. Methods in Biotechnology, Humana Press. 20:269-73.
14. Sumanont Y, Murakami Y, Tohda M, Vajragupta O, Matsumoto K, Watanabe H. Evaluation of the nitric oxide radical scavenging activity of manganese complexes of curcumin and its derivative. Biol Pharm Bull. 2004; 27(2):170-3.
15. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci. 1997; 2:152-9.
16. Nasapon P, Ampai P, Maitree S, Chaiyavat C, Pimporn L. Phenolic content and in vitro inhibitory effects on oxidation and protein glycation of some Thai medicinal plant. Pak J Pharm Sci. 2010: 23 (4):403–8.
17. Rice-Evans C, Miller NJ, Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med. 1996; 20:933-56.
18. El Diwani G, El Rafie Sh, Hawash S. Antioxidant activity of extracts obtained from residues of nodes leaves stem and root of Egyptian *Jatropha curcas*. Afr. J. Pharm. Pharmacol. 2009; 3(11):521-30.
19. Sukhonthara S, Kaewka K, Theerakulkait C. Inhibitory effect of rice bran extracts and its phenolic compounds on polyphenol oxidase activity and browning in potato and apple puree. Food Chem. 2016; 19:922-7.
20. Yang NY, Liu L, Tao WW, Duan JO, Liu XH, Huang SP. Antithrombotic lipids from Semen Persicae. Nat Prod Res. 2011; 25:1650-6.
21. Shukla S, Gupta S. Apigenin: A promising molecule for cancer prevention. Pharm Res. 2010; 27(6):962–78.
22. Vagiri M, Ekholm A, Andersson S C, Johansson E, Rumpunen K. An optimized method for analysis of phenolic compounds in buds, leaves, and fruits of black currant (*Ribes nigrum* L.). J Agric Food Chem. 2012; 60(42):10501–10.
23. Gründemann C, Lengen K, Sauer B, Garcia-Kaufer M, Zehl M, Huber R. *Equisetum arvense* (common horsetail) modulates the function of inflammatory immunocompetent cells. BMC Complement Alternat Med. 2014; 14:283.
24. Pragasam SJ, Vijayalakshmi Venkatesan V, Rasool MK. Immunomodulatory and anti-inflammatory effect of p-coumaric acid, a common dietary polyphenol on experimental inflammation in rats. Inflammation. 2013; 36:169–76.
25. Usenik V, Fabcic J, Stampar F. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). Food Chem. 2008; 107: 185– 92.
26. Chuammitri P, Srikok S, Saipinta D, Boonyayatra S. The effects of quercetin on microRNA and inflammatory gene expression in lipopolysaccharide-stimulated bovine neutrophils. Vet World. 2017; 10(4):403–10.
27. Cao HH, Cheng CY, Su T, Fu XQ, Guo H, Li T, Tse AKW, Kwan HY, Yu H, Yu ZL. Quercetin inhibits HGF/c-Met signaling and HGF-stimulated melanoma cell migration and invasion. Mol. Cancer. 2015; 14(1):103.
28. Ma JQ, Li Z, Xie WR, Liu CM, Liu SS. Quercetin protects mouse liver against CCl 4-induced inflammation by the TLRr2/4 and MAPK/NF-κB pathway. Int. Immunopharmacol. 2015; 28(1):531–9.
29. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J Agric Food Chem. 1998; 46:1887–92.