

Antioxidant activity of flavonoids from leaves of *Jatropha curcas*

Qiulan Huang, Yiran Guo, Rao Fu, Tong Peng, Yuting Zhang, Fang Chen*

Key Laboratory Bio-Resources and Eco-Environment, Ministry of Education, College of Life Science, Sichuan University, 610064 Chengdu, China

*Corresponding author, e-mail: Cfang@263.net

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ABSTRACT: The objective of this study was to characterize the antioxidant properties of ethanol extracted from leaves of *Jatropha curcas* and to determinate the compounds. The IC₅₀ values of DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) were found to be 7.47 and 6.6 µg/ml, respectively, for the extracts. The reducing power of the extracts increased from 0.09 to 0.16, respectively, for 1–32 µg/ml of standard reagent. The assignment of the flavone compounds using HPLC-DAD-MSⁿ was based on the evaluation of their UV-Vis absorption maxima (λ_{\max}) and mass spectral analyses. Tentatively, 4 compounds (orientin, vitexin, isovitexin, rhoifolin) were identified.

KEYWORDS: oil plant, in vitro antioxidant, HPLC-MS

INTRODUCTION

Jatropha curcas belongs to the Euphorbiaceae family and is an ideal biodiesel crop in most arid areas of Asia, particularly in southeast Asia^{1,2}, South America, and Africa, because of its high oil (43–61%) seed kernel^{3,4}. Traditionally, *Jatropha* seeds and other plant parts have been used to produce oil, soap, and medicinal compounds^{4–6}. *J. curcas* grows readily in poor and stony soil, resists drought and disease, and yields high quality biodiesel⁷. The plant is a perennial plant, native and is widespread throughout many tropical countries⁸.

All parts of *J. curcas* can be used in traditional medicine or as raw materials for pharmaceutical and cosmetic industries. It cures many diseases, such as arthritis, jaundice, dental complaints, tumours, allergies, burns, cut wound, leprosy scabies, and small-pox^{6,9}. *J. curcas* contains proteins, peptides, diterpenes and a variety phytochemicals exhibiting a broad spectrum of biological activities¹⁰. The extract of root and latex of *J. curcas* plant which contains phenolic, flavonoid, diterpene, and saponin compounds shows notable antioxidant, anticancer, and anti-inflammatory activities^{11,12}. The oil pulp can therefore be used as a protein source, and the root be used to produce acridine. Currently, there are many studies that examine the structure and function of natural source phenolic and flavonoid compounds, as well as their antioxidant, anticancer roles in noncommunicable diseases^{13,14}.

The research for antioxidants from natural sources aims to identify compounds that may help to prevent oxidative damage in the body and contribute to the prevention of noncommunicable diseases^{15,16}. The by-products of *J. curcas* such as leaves have not been comprehensively studied, therefore limited information is available on the feasible application of the meal as a potential source of bioactive compounds⁹. Hence the objective of the present study was to characterize this plant flavone compounds and its antioxidant activity. The information gathered would indicate that the leaves can be a potential source of bioactive compounds.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the study were of analytical or HPLC grade. Except for AlCl₃, TCA, ascorbic acid (AA), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt), potassium ferricyanide, apigenin, luteolin, DPPH (2,2-diphenyl-1-picrylhydrazyl), apigenin-7-O-neohesperidoside, all other chemicals used were of analytical grade from Chengdu Kelon Chemical Reagent Company.

Plant materials and extract preparation

J. curcas leaves were collected from Sichuan, China, and were dried naturally and crushed. According to the method established by our tests, the compounds were quantified under the optimal conditions (data

not shown). The ethanol extracts were concentrated until all the ethanol was removed. The solution was sequentially extracted by petroleum ether, ethyl acetate, and n-butyl alcohol. The ethyl acetate phase was the test sample and the other phases were kept to be analysed later.

Radical DPPH scavenging activity

The free radical activity with DPPH as a free radical was determined following a modified method^{17,18}. Different concentrations (1–32 µg/ml) of the sample and AA, as positive control, were prepared. A 100 µl aliquot of each sample/standard solution was mixed with 100 µl of DPPH solution. The mixture was incubated at RT for 30 min. The DPPH reacted with the tested extract and antioxidant to decrease the absorbance at 517 nm. The scavenging capability to the DPPH radical was calculated as $(1 - (A_i - A_j)/A_c)$, where A_i is the initial absorbance of the DPPH radical, A_j is the absorbance of the sample, and A_c is the absorbance of the DPPH without sample.

ABTS radical-scavenging activity

Various concentrations (1–32 µg/ml) of samples and AA were prepared. ABTS was dissolved in distilled water to a final concentration of 7 mM and mixed with a potassium persulphate solution at a final concentration of 2.45 mM. The mixture was left to settle at room temperature for 12–16 h in the dark before use. For each experiment, a freshly prepared ABTS solution was diluted with 0.2 M phosphate buffer (PBS, pH 7.4) to adjust its absorbance to 100 ± 100 at 734 nm wavelength. Then, 100 µl of each sample/standard solution was mixed with 100 µl ABTS solution, and the absorbance were measured at 734 nm¹⁹. The scavenging capability of ABTS was calculated as before using A_i , A_j , and A_c for the initial absorbance of ABTS radical, absorbance of the samples, and absorbance of the ABTS without sample, respectively.

Reducing power

Reducing power activities of the extracts were determined following the method of Hu and Xu²⁰ with some modifications: 0.1 ml of each sample of the standard reagent (AA as positive control) at various concentrations (1–32 µg/ml) was mixed with 0.2 ml of Na_3PO_4 buffer (pH 6.6) and 0.1 ml potassium ferricyanide (100 mg/ml) solution. Then the mixture was incubated at 50 °C in a water bath for 1 h and mixed with 0.16 ml of TCA (100 mg/ml) and 0.24 ml of FeCl_3 solution (1 mg/ml). The absorbance was measured at 700 nm wavelength²¹.

The antioxidant activity of the main composition

HPLC-DAD analysis was performed on the Shimadzu series HPLC systems equipped with a G13798 degasser, a G1312A binary pump, a G1329A autosampler, and a G1315D diode array detector. HPLC separation was achieved by using a C18 column (250 × 4.6 mm, 5 µm, Agilent Technologies) at 25 °C. Elution was performed using a mobile phase A (water) and a mobile phase B (methanol), and the samples (10 µl) were eluted at a flow rate of 0.8 ml/min. The UV-Vis spectra were scanned from 190–700 nm on DAD with a detection wavelength of 268 nm. The solvent gradient in volumetric ratios varied from 40–70% B over 30 min.

LC-DAD-MS (ESI-) analyses

The mass spectra were obtained using an LTQ Orbitrap XL (Thermo, USA). The ESI conditions were as follows: methanolic extracts were used for the analysis of the HPLC-DAD conditions with a little modification. LC-ESI/MS/MS, a source voltage of +3.0 kV, an auxiliary gas flow rate 10.01, a vaporizer temperature of 43.5 °C, a capillary temperature 300 °C, a capillary voltage –34.99 V, and tube lens voltage –110 V.

RESULT AND DISCUSSION

Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl is a stable free radical, and it has a single electron and strong absorption at 517 nm. The synthetic nitrogen-centred DPPH free radical is not biologically relevant but widely used to determine antioxidant activity. In this study, the extracts and AA displayed radical scavenging activity (Fig. 1a). When mixed with an antioxidant, the antioxidant compound donates an electron to DPPH and the colour changes from purple to light yellow. The scavenging activity were ranged from 2% to 94% and –1% to 96% for the extracted compounds and AA, respectively, with their concentrations from 1–32 µg/ml. The concentrations at which 50% of the DPPH radicals were scavenged (IC_{50}), were 12.5 and 7.5 µg/ml for the extracts and AA, respectively.

ABTS is a well-known nitrogen-centred synthetic radical and is widely used to determine antioxidant activity. The ABTS radical is generated by oxidation of ABTS with potassium persulphate and when antioxidants are added to it, it is converted to a non-radical form. Different concentrations of extracts and AA, ranging from 1–32 µg/ml, were examined for scavenging activity. The scavenging activity (Fig. 1b) ranged from 20% to 99.7% for the extract and from

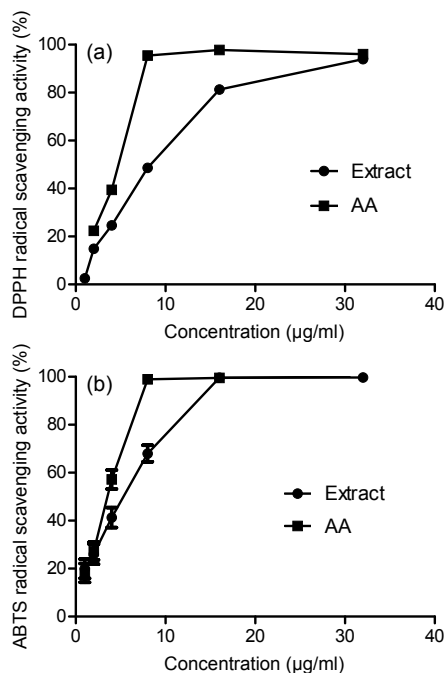


Fig. 1 Scavenging effects of *J. curcas* extract on (a) DPPH' radicals, and (b) ABTS' radicals.

18% to 100% for AA. In addition, the IC_{50} values were 6.6 and 4.1 µg/ml for extracts and AA, respectively. Besides, the antioxidant activity in the ABTS or DPPH assay were also strongly correlated with the contents of phenolic and flavonoid compounds²¹.

Reducing force is an important indicator to show the ability of the antioxidant to provide electronic ability. The antioxidants give out electrons through their own reduction give and make free radicals into stable molecules, and then lose activity. Antioxidants reduces iron potassium cyanide, that reduces its ferric iron into bivalent iron ion. The reducing power is larger, the stronger the antioxidant effect is. The Fe-Fe reducing power of a compound is used to determine the electron donating capacity of antioxidants and is one of main characteristics of phenolic compounds²². The reducing power of the extract and AA increased from 0.09 to 0.16 and 0.11 to 1.00, respectively, at 1–32 µg/ml. The scavenging of the AA was significantly higher than that of the extract (Fig. 2).

The antioxidants clear the free radicals that are produced by the biological redox reactions. The radical DPPH scavenging activity, the ABTS radical-scavenging activity, and the reducing power in the experiment revealed that the flavone compounds from leaves of *J. curcas* had strong antioxidant activity, and the orientin played the main function (Fig. 3).

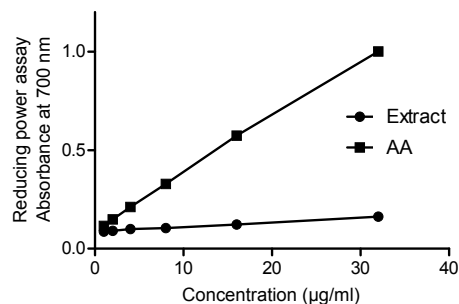


Fig. 2 Deoxidization capability of *J. curcas* extract.

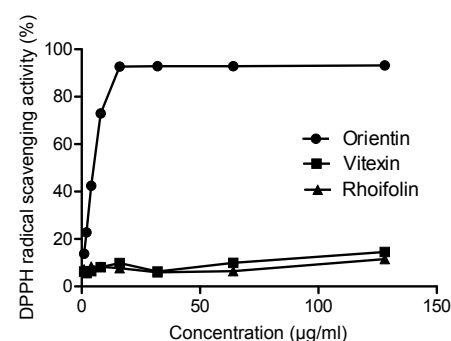


Fig. 3 Scavenging effects of flavonoids from leaves of *J. curcas* on DPPH' radicals.

Comparing to the other three flavonoids, the orientin had one more hydroxyl and contained pyrocatechol structure, so the orientin had a stronger ability to clearing free radicals²³.

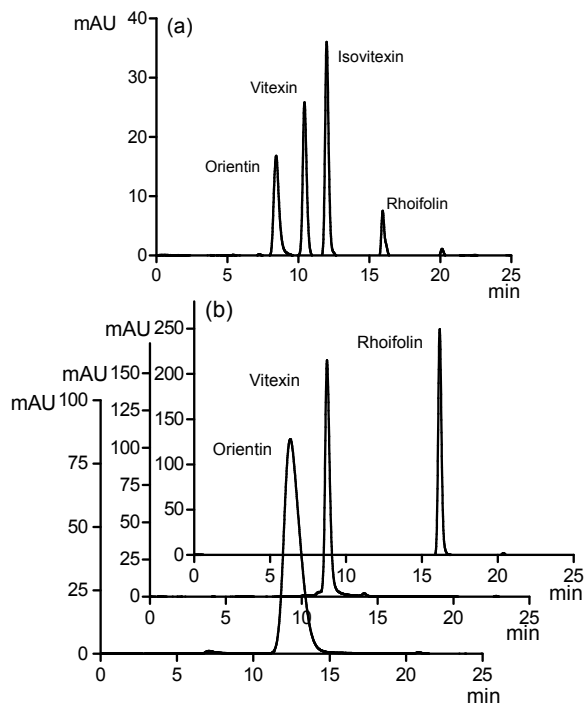
LC-DAD-MS (ESI-) analysis of the collected fractions

The ethanolic extract was further analysed by HPLC-DAD in order to characterize the flavone compounds. UV spectra of this peak showed λ_{max} at 268 (267) and 338 (350) nm, which are characteristic of apigenin (luteolin). LC-ESI/MS/MS indicates both methylated and sulphated forms of luteolin and apigenin are present as *c*-glycosides combined with up to one sugar residues, and apigenin is present as *O*-glycosides combined with up to two sugar residues. So it could choose standard substance according to these data. The data of the HPLC-DAD-ESI-MSⁿ is summarized in Table 1.

The standard substances were given out at the same time with the extract by the same HPLC-DAD conditions (Fig. 4). Peak 2 and peak 3 are the isomeride from the data of the HPLC-DAD-ESI-MSⁿ (Fig. 5). All data could help to identify the four compounds which were orientin (peak 1), vitexin (peak 2), isovitexin (peak 3), and rhoifolin (peak 4).

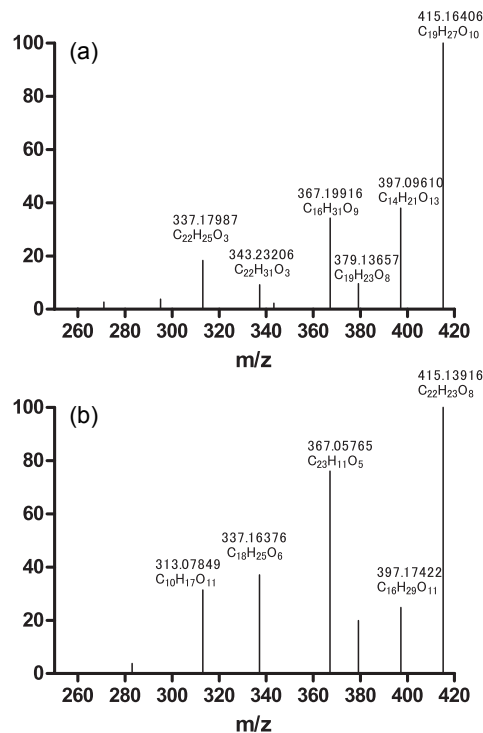
Table 1 Characterization of the individual compounds in extracts using HPLC-DAD-ESI-MS^a.

Peak	t_R (min)	λ_{max} (nm) ^a	$[M-H]^+$ m/z	Formula	HPLC-ESI-MS; expt, m/z (% base peak)
1	6.52	267, 350	449	C ₂₁ H ₂₁ O ₁₁	MS ² [449]: 431 (100), 413 (31), 383 (49), 329 (24)
2	7.83	268, 338	433	C ₂₁ H ₂₁ O ₁₀	MS ² [433]: 415 (100), 397 (35), 367 (32), 313 (17)
3	8.91	268, 338	433	C ₂₁ H ₂₁ O ₁₀	MS ² [433]: 415 (100), 397 (23), 367 (73), 337 (34)
4	12.01	268, 338	579	C ₂₇ H ₃₁ O ₁₄	

^a HPLC-DAD UV**Fig. 4** HPLC chromatogram of flavonoids from (a) leaves of *J. curcas* and (b) standard substances, at a detection wavelength of 336 nm.

The characterization of flavone compounds is important, as these compounds are associated with a range of different health properties. HPLC with diode array and mass spectrometric detection has proven to be very useful for the characterization of flavone compounds. In the present study, 4 compounds were characterized in *J. curcas* leaves, on basis of their UV spectral data and mass spectrometric analysis.

According to a previous report, *J. curcas* is not only a kind of very good oil plant, and the whole plant can be used in medicine for its subduing swelling eliminating stasis, antiviral, anticancer. The most promising phytochemicals are polyphenol and flavonoid compounds for their antioxidant properties. The vitexin, isovitexin, orientin, and rhoifolin are efficient antioxidants. For example, the antioxidant activity of rhoifolin was measured using the oxygen

**Fig. 5** MS² spectra of (a) isovitexin and (b) vitexin.

radical absorbance capacity which is an acknowledged method for antioxidant assessment^{23–26}. The anti-cancer and antiviral effect of *J. curcas* may then be related to vitexin, isovitexin, orientin and rhoifolin.

In conclusion, in the absence of phytochemical and pharmacological information it is worth to elucidate the chemical and biological properties of leaves of *J. curcas*. The high content of antioxidants, compared with other species, depicts *J. curcas* as a medical plant that should be further investigated, and the evaluation of the antioxidant capacity using cell-free and cell-based assays highlights this study over others. The *J. curcas* was taken as energy plant, and the role of leaves in medicine will be beneficial to increase the added value. This study of leaves of *J. curcas* in oxidation and chemical composition laid an important foundation on the development and utilization in medicine.

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