

Article

Antioxidant Properties of *Jatropha curcas* L. Seed Shell and Kernel Extracts

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Abstract: The purpose of this study was to determine the antioxidant activity of the seed shells and kernels of *Jatropha curcas* L. The extracts obtained from five solutions (0%–95% ethanol) were tested and compared. Overall, the antioxidant capacity of seed shell extracts was higher than that of seed kernel extracts. The seed shell extract obtained using 95% ethanol exhibited the best antioxidant activity among the five solutions. The half-maximal inhibitory concentration (IC₅₀) of 1,1-diphenyl-2-picrylhydrazyl and free radical scavenging ability of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) were 13.63 ± 0.15 and 6.75 ± 0.51 µg/mL, respectively. The reduction ability and total phenol content were 95.14 ± 27.04 µg ascorbic acid equivalents/mg of extract and 536.33 ± 8.62 µg gallic acid equivalents/mg of extract, respectively. In in vitro cytotoxicity assays, solutions with less than 250 µg/mL of seed shell extract had no major cytotoxicity. The seed shell of *Jatropha curcas* L. can be used as an antioxidant material and has potential for biomedical applications.

Keywords: *Jatropha curcas* L., seed; shell; kernel; extract; antioxidant; cytotoxicity

1. Introduction

Plant-derived bioactive compounds have received considerable attention because of their therapeutic potential as anti-inflammatory, anticancer, and antioxidant substances [1]. *Jatropha curcas* L. is a tropical and subtropical plant that has multiple uses and considerable economic potential [2–4]. It belongs to the Euphorbiaceae family [5], whose aqueous and organic extracts have been traditionally used as laxatives, scavengers, emetics, and therapeutics [6]. Although *J. curcas* is toxic, some of its parts are suitable for therapeutic uses [7]. For example, its root and latex, which contain phenolic and flavonoid compounds, exhibit notable antioxidant, antihypertensive, antiplatelet, and anti-inflammatory activities [8]. The methanolic extract of leaves has anticrustacean and antiviral activities [9]. The stem and root barks have antibacterial properties and can be a source of antibacterial compounds [10]. The root contains curcusone A–E [11], which can be used for the treatment of many diseases [12]. Curcusone A and B, which are found in the stem, have anticancer activity. Moreover, curcusone B effectively suppresses metastatic processes [12]. The main source of toxicity in seeds is the kernel [13]. However, no information exists regarding the toxicity of the seed shell.

The seed extract of *J. curcas* exhibits significant antioxidant activity, and the hydroalcoholic extract of *J. curcas* exhibits potent activity for protecting against oxidant and free radical injuries [14]. The kernel of *J. curcas* has a high total phenolic content and exhibits strong antioxidant activity [15]. The extract of *J. curcas* seed shells has a high phenolic content and exhibits relatively strong bioactivity. *J. curcas* seed shells are a new natural antioxidant source and can be used in the industry [16]. The kernels of *J. curcas* contain nitrogen, phosphorous, and potassium and can be used as fertilizers [16,17].

To the best of our knowledge, the antioxidant capacity of *J. curcas* seed shells and kernels has rarely been studied. Water, methanol, and ethanol are suitable extraction solvents for polyphenolic compounds to optimize the antioxidant capacity of *J. curcas* seed shell and kernel extracts [18]. Studies have been conducted on different solvent extractions [16]; however, the differences in ethanol extraction at a series of concentration have been less studied. Therefore, this study investigated the in vitro antioxidant activity of the seed shell and kernel extracts of *J. curcas* by using five types of ethanol solutions with different polarities.

2. Materials and Methods

2.1. Reagents

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Alfa Aesar (Tewksbury, MA, USA). Ascorbic acid and trichloroacetic acid were obtained from Acros Organics (Fair Lawn, NJ, USA). Potassium persulfate and potassium ferricyanide were obtained from Showa Chemical (Tokyo, Japan). Phosphate buffer and sodium carbonate were purchased from Riedel-de Haën (Seelze, Germany). Folin-Ciocalteu phenol reagent and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Iron (III) chloride hexahydrate was purchased from J.T. Baker (Phillipsburg, NJ, USA), and Dulbecco's modified Eagle's medium (DMEM) was obtained from HyClone Lab (Logan, UT, USA). Dulbecco's phosphate-buffered saline (PBS) was provided by Medicago (Uppsala, Sweden), and MTT was procured from Gold Bio (St Louis, MO, USA).

2.2. Preparation of Extracts

J. curcas seeds (Figure 1) were collected from the location with GPS coordinates (24.446718, 120.792095), Miaoli, Taiwan. The collection and further experiments were conducted from December 2018 to January 2019. The seeds were placed in an oven and dried at 50 °C. The shell and kernel were then manually separated, further dried, and powdered using a pulverizer. Seed shell and kernel extracts were prepared in three replications by dissolving seed shell and kernel powder in 0%, 25%, 50%, 75%, and 95% ethanol at a ratio of 1:10 g/mL at a constant temperature of 30 °C for 1 h as original sample solutions. The solvent was removed using a vacuum rotary evaporator. The dried extracts were examined for determination of extraction yield and extract concentration in original sample solutions. All antioxidant experiments used the original sample solutions, but cytotoxicity test used the redissolved extract solutions with 2.5% DMSO.



Figure 1. Seed shell and kernel of *Jatropha curcas* L.

2.3. 1,1-diphenyl-2-picrylhydrazyl Radical Scavenging Assay

According to the procedure developed in previous studies [19–23], a total of 2 mL of the tested sample was mixed with 2 mL of 0.2 M DPPH solution and then left in the dark for 30 min. The absorbance of the resulting mixture was measured at 517 nm. A lower absorbance value resulted in a stronger ability of the sample to scavenge free radicals. Ascorbic acid was used as the standard, and a solution with a corresponding ethanol concentration was used as the control.

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{517} \text{ of sample}}{A_{517} \text{ of blank}}\right) \times 100\% \quad (1)$$

2.4. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) Radical Scavenging Assay

By the previous procedure [19,22], an ABTS solution was prepared by reacting equal proportions of 7 mM ABTS with 2.45 mM potassium persulfate at room temperature in the dark for 16 h. The background absorbance was diluted to 0.7 ± 0.02 by using 95% ethanol. A total of 1.5 mL of the tested sample was mixed with 1.5 mL of the diluted ABTS solution for 10 min in the dark. The absorbance of the resulting mixture was then measured at 734 nm. A lower absorbance value corresponded to a stronger ability to scavenge free radicals. Ascorbic acid was selected as the standard, and a corresponding ethanol solution was used as the control.

$$\text{ABTS radical scavenging activity (\%)} = \left(1 - \frac{A_{734} \text{ of sample}}{A_{734} \text{ of blank}}\right) \times 100\% \quad (2)$$

2.5. Reducing Power Assay

Based on the previous procedure [19,22], an aliquot of the sample (0.5 mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of 1% potassium ferricyanide at 45 °C. After 30 min of reaction, the mixture was rapidly cooled. Subsequently, 0.8 mL of trichloroacetic acid (1%) and 1.5 mL of iron (III) chloride hexahydrate (1%) were added. The absorbance of the resulting mixture was measured at 700 nm. A higher absorbance value corresponded to a stronger reducing power of the sample. Ascorbic acid was used as the standard in the reducing power assay. The reducing power of the tested sample is expressed in μg of ascorbic acid equivalents (AAEs)/mg of extract.

2.6. Determination of the Total Phenolic Content

A total of 0.4 mL of each extract was mixed with 2 mL of 10% Folin–Ciocalteu phenol reagent [20–24]. After 5 min, 1.6 mL of 7.5% sodium carbonate was added to the mixture. The mixture was then incubated for 30 min. The absorbance of the resulting mixture was measured at 760 nm. Gallic acid, which is a major component of hydrolyzable tannis, was used as the standard when determining the total phenol content. The total phenolic content of the tested sample is expressed as micrograms of gallic acid equivalents (GAEs) per milligram of extract.

2.7. In Vitro Cytotoxicity Test

The cytotoxicity of the extract was evaluated using a mouse fibroblast L929 cell line. The cell line was obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). The cells were cultured in Dulbecco's MEM with 10% horse serum at 37 °C in a humidified incubator with 5% CO₂. The seed shell extract samples were prepared by dissolving them in a 2.5% DMSO solution, adjusted to the final concentrations, and then sterilized with a sterile filter.

The cells were seeded in a 96-multiwell plate at a cell density of 1.0×10^4 cells/well in 100 μL of culture medium and maintained in a humidified incubator with 5% CO₂ at 37 °C for 24 h. The culture medium was then removed, and the cultured cells were treated with serial dilutions of the extract (17–1000 $\mu\text{g}/\text{mL}$). After an incubation time of 24 h, the culture medium was removed and 100 μL of

methylthiazol tetrazolium (MTT) solution (5 mg/mL in PBS) was added and incubated for 3 h. After incubation, the MTT solution was removed and 100 μ L of DMSO was added to each well and left in the dark for 15 min. The absorbance was then measured at 570 nm to calculate the percentage of surviving cells.

$$\text{Cell viability (\%)} = \frac{OD_{\text{Sample}} - OD_{\text{Blank}}}{OD_{\text{Control}} - OD_{\text{Blank}}} \times 100\% \quad (3)$$

2.8. Statistical Analysis

To ensure statistical representation, all of the experiments were collected at least in triplicate, and means between the treatment levels were compared by using the SAS software (version 9.4, SAS Institute, Cary, NC, USA). Statistical analyses based on the *t*-test and analysis of variance (ANOVA). If the treatment was significant ($p < 0.05$) in ANOVA, the treatment mean compared with the Tukey test.

3. Results and Discussion

3.1. Extraction Yield

The extraction yield of *J. curcas* seed shells and kernels is dependent on the extraction solution and seed material. Table 1 presents the extraction yields of the seed shells and kernel for different ethanol solutions. The extraction yield of the shell was lower than that of the kernel. The extraction yield of the shell ranged from 3.00 ± 0.45 to 14.81 ± 0.92 , and the extraction yield of the kernel ranged from 8.19 ± 1.26 to 24.23 ± 0.78 . Similar results have been obtained for other plant species, such as pecan [25] and hazelnut [26].

Table 1. Extraction yield (%) of seed shells and kernels with different ethanol solutions.

| Part | Ethanol conc. (%) | | | | | |
|-------------------|--------------------------|-------------------------|-------------------------|-------------------------|------------------------|--|
| | 0 | 25 | 50 | 75 | 95 | |
| Seed shell | 12.58±0.25 ^{b*} | 14.81±0.92 ^a | 13.35±0.78 ^a | 8.35±0.29 ^c | 3.00±0.45 ^d | |
| Seed kernel | 24.23±0.78 ^a | 23.27±0.57 ^a | 20.45±0.26 ^b | 17.60±0.82 ^c | 8.19±1.26 ^d | |
| <i>p</i> value ** | <0.0001 | <0.0001 | 0.0001 | <0.0001 | <0.0001 | |

* Mean within a row followed by the same lower case letter are not significantly different at 5% level by Tukey test.

** The *p* value in comparing yield between seed shells and seed kernels using *t*-test.

Moreover, a higher extraction yield was achieved with a lower ethanol concentration as shown in Table 1, which is in agreement with the finding that the extraction efficiency is low and unfavorable in a mixture with no aqueous content [27]. However, in this study, no obvious difference was observed in the extraction yield among the solutions with 0%–25% ethanol in seed kernels and with 25%–50% in seed shells. Furthermore, the use of different solvents in the extraction resulted in different antioxidant activities among the extracts [28].

3.2. DPPH Radical Scavenging Assay

Oxidative stress due to the accumulation of free radicals may cause heart disease, cancer, diabetes, ischemia, and aging. The negative effect of free radicals on the body can be resisted by antioxidants [29]. Figure 2 displays the DPPH radical scavenging activity of seed shell and kernel extracts obtained from different extraction solutions in this study. The result indicates that the DPPH radical scavenging activity increased with the concentration of the extracts, irrespective of the extraction solution used. The seed shells extracted from concentrated ethanol solutions had high DPPH radical scavenging activity. The half-maximal inhibitory concentration (IC₅₀) values in the DPPH radical scavenging assay of the shell were 721.84 ± 8.04 , 101.66 ± 8.92 , 40.82 ± 1.65 , 28.09 ± 3.41 , and 13.63 ± 0.15 μ g/mL for solutions obtained using 0%, 25%, 50%, 75%, and 95% ethanol, respectively (Figure 2a). The corresponding IC₅₀ values for the kernel were 1195.60 ± 87.56 , 1409.19 ± 65.83 , 1239.22 ± 44.79 , 1698.33 ± 54.28 , and 1729.31 ± 118.46 μ g/mL, respectively (Figure 2b). The IC₅₀ of ascorbic acid in a water solution was

$8.35 \pm 1.12 \mu\text{g/mL}$. Therefore, the shell extract obtained using 95% ethanol had the best DPPH free radical scavenging activity, similar to that of ascorbic acid. This result is in agreement with a previous report [16]. However, the kernel extracts had poor DPPH free radical scavenging activity, considerably lower than that of ascorbic acid. According to the previous study [30], an extract of defatted *J. curcas* seeds also exhibited DPPH radical scavenging activity.

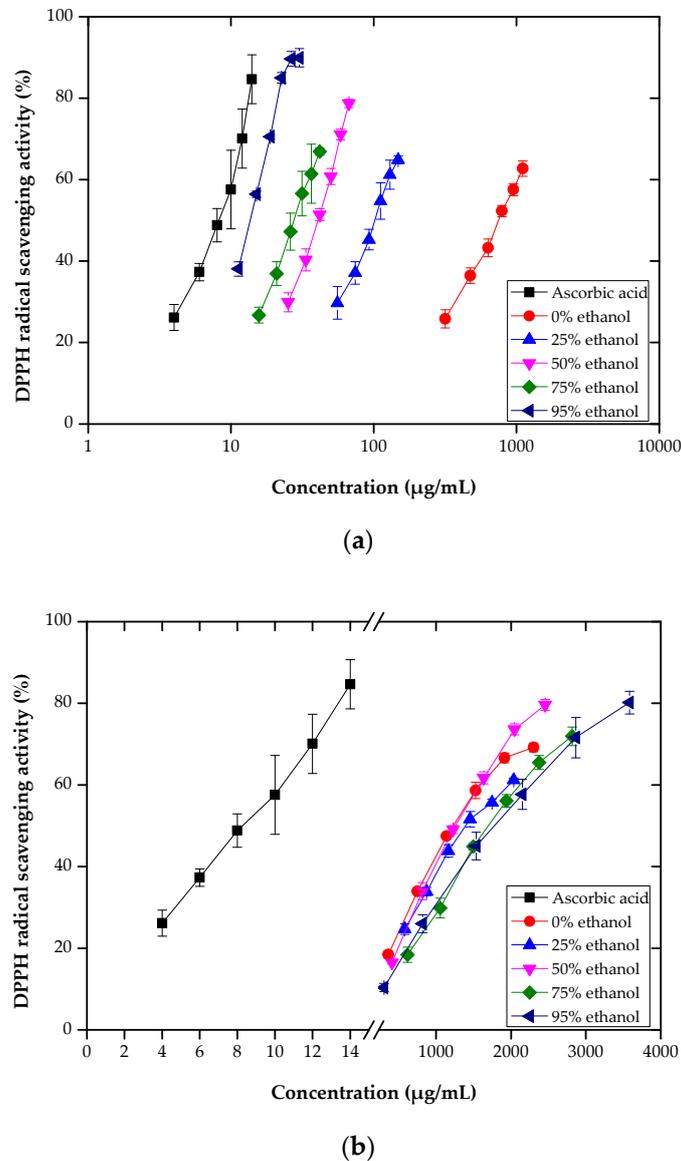
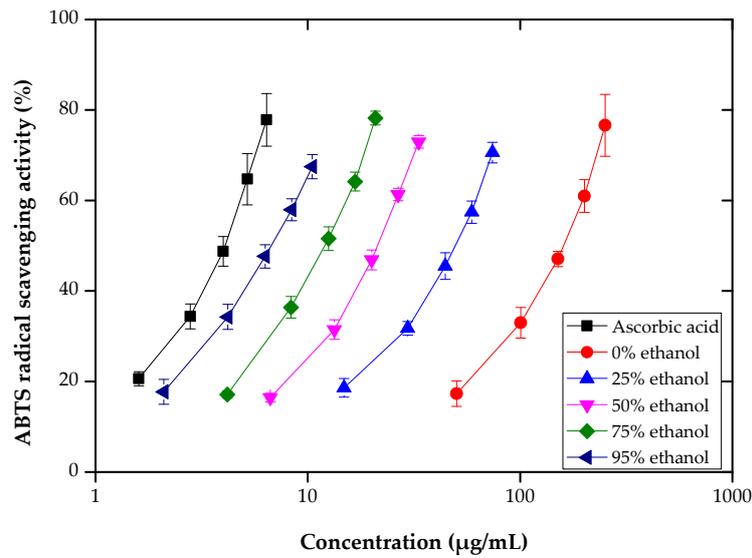


Figure 2. DPPH radical scavenging activities of the seed (a) shell and (b) kernel extracts from different ethanol solutions.

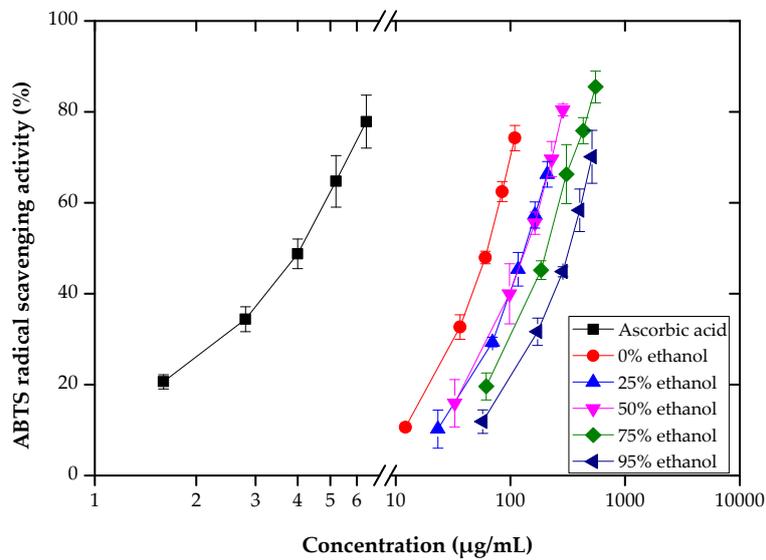
3.3. ABTS Radical Scavenging Assay

Figure 3 displays the ABTS radical scavenging results for the seed shell and kernel extracts obtained from different ethanol solutions. Similar to the trend displayed in Figure 2, a trend of increasing ABTS radical scavenging activity with increasing extract concentration was observed, regardless of the type of extraction solution used. The seed shell extracts obtained from concentrated ethanol solutions had high ABTS radical scavenging activity. The shell extract obtained using 95% ethanol had the best IC_{50} value in the ABTS free radical scavenging assay. The result that an increase of ethanol concentration had in a higher ABTS radical scavenging activity agrees with the report about *Salvia jurisicii* [31] and *Withania somnifera* [32]. The IC_{50} values in the ABTS assay were 161.00 ± 5.40 , 50.19 ± 2.96 , 21.47 ± 0.91 ,

12.17 ± 0.67, and 6.75 ± 0.51 µg/mL for shell extracts obtained using 0%, 25%, 50%, 75%, and 95% ethanol solutions, respectively (Figure 3a). The corresponding IC₅₀ values for the extracted kernels were 62.61 ± 2.71, 134.80 ± 9.04, 142.11 ± 16.83, 211.72 ± 23.64, and 329.71 ± 48.14 µg/mL, respectively (Figure 3b). Therefore, the shell extracts had in a higher ABTS radical scavenging activity than kernel extracts, corresponding to the report about *Corylus jacquemontii* [33]. The IC₅₀ of ascorbic acid in water solution was 4.08 ± 0.25 µg/mL. The results of the ABTS and DPPH free radical scavenging assays indicated that the extracted *J. curcas* shells had strong free radical scavenging activity and could be used as effective free radical scavengers.



(a)



(b)

Figure 3. ABTS radical scavenging activities of the seed (a) shell and (b) kernel extracts from different ethanol solutions.

3.4. Reducing Power Assay

The reducing power of a compound generally depends on the presence of molecules with antioxidative potential resulting from donating a hydrogen atom to break the free radical chain [34]. The results of a reducing power assay (Figure 4) indicated that the shell extract obtained using 95% ethanol had the highest reducing capacity. In agreement with the results of a previous study about *Corylus avellana* L. [26], the antioxidant activity decreased with the water content in the extraction solution; however, the extraction yield increased. As the water content in the extraction solution decreased, the antioxidant capacity of the shell extract increased considerably from 18.81 ± 4.50 (0% ethanol) to 695.14 ± 27.04 (95% ethanol) $\mu\text{g AAEs/mg}$ of extract. However, no difference was observed in the reducing power of the kernel extracts obtained using ethanol solutions of different concentrations.

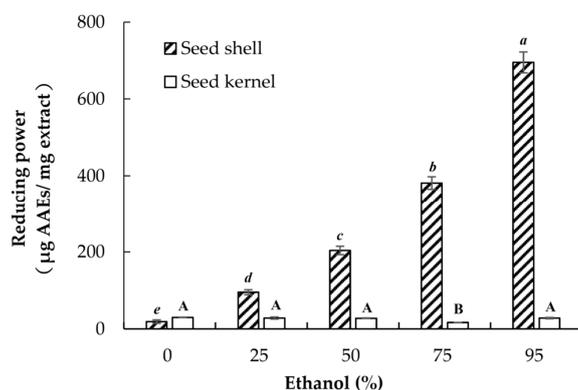


Figure 4. Reducing power of the seed shell and kernel extracts obtained using ethanol solutions of different concentrations. Mean within the seed shell (in small letter) and within the seed kernel (in capital letter) followed by the same letter(s) are not significantly different at the 5% level by Tukey test.

3.5. Determination of the Total Phenolic Content

The seed shells of *J. curcas* are thin and hard and have a relatively high phenolic content, which may be related to the function of seed protection [16]. Figure 5 indicates that the total phenolic content of the shell extract increased with the ethanol concentration, which is similar to the result in Figure 4. The phenomenon that an increase of ethanol concentration resulted in a higher extraction of phenolics agree with reports about black currant [35] and *Limnophila aromatic* [28]. The shell extract obtained using 95% ethanol had the highest total phenolic content of 536.33 ± 8.62 $\mu\text{g GAEs/mg}$ of extract. Moreover, a few major differences were observed in the total phenolic content of the kernel extracts.

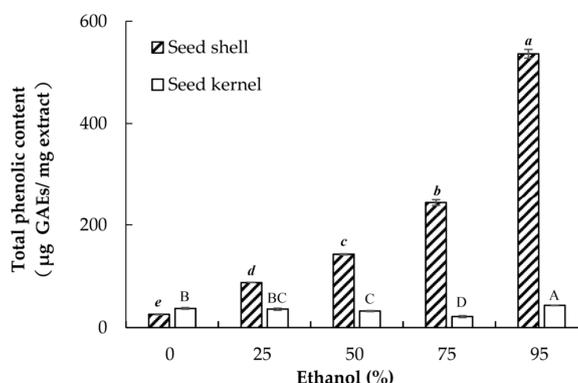


Figure 5. Total phenolic contents of the seed shell and kernel extracts obtained using ethanol solutions of different concentrations. Mean within the seed shell (in small letter) and within the seed kernel (in capital letter) followed by the same letter(s) are not significantly different at 5% level by Tukey test.

3.6. In Vitro Cytotoxicity Test

The MTT assay can indicate whether a product can be used in biomedical supplies [36]. The *J. curcas* seed shell extract obtained using 95% ethanol was subjected to an in vitro cytotoxicity test with an MTT assay to calculate the 24-h L929 cell survival percentages in serially diluted extracts and an extract-free sample (Figure 6). The results indicated no major cytotoxicity, with a cell viability of $\geq 80\%$ [37] when the concentration of DMSO was less than 2.5%, which is in agreement with no morphological alterations or lysis [38]. Therefore, the seed shell extract was dissolved in a 2.5% DMSO solution for the cytotoxicity test in this study.

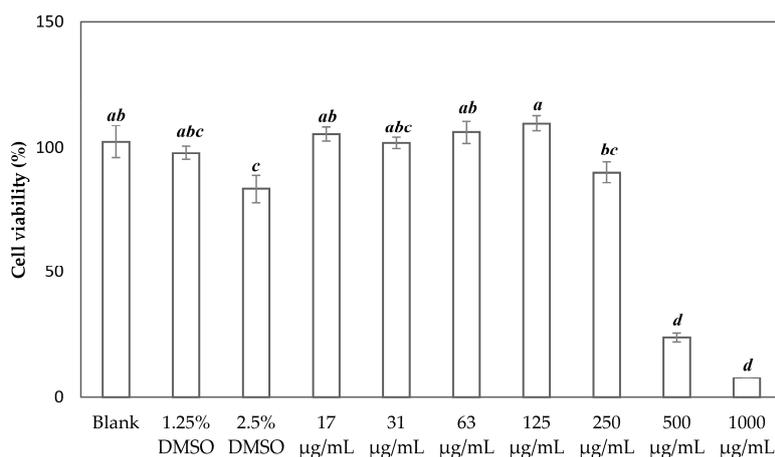


Figure 6. L929 cell viability after 24-h treatment with shell extracts of different concentrations. Mean followed by the same letter(s) are not significantly different at the 5% level by Tukey test.

The results revealed that no major cytotoxicity was observed when the seed shell extract had a concentration of less than 250 $\mu\text{g/mL}$. Seed shell extracts with concentrations of less than 250 $\mu\text{g/mL}$ did not alter the viability of fibroblasts. However, at concentrations greater than 500 $\mu\text{g/mL}$, the extract became potentially toxic and the cell viability decreased.

4. Conclusions

The extraction yield and antioxidant capacity of extracts depend on the plant part from which the extract is derived and the polarity of the extraction solution. In the case of *J. curcas* seeds, the extract yield of the seed shell was lower than that of the seed kernel. However, the seed shell extract had higher antioxidant capacity, including DPPH and ABTS radical scavenging activities, reducing power, and total phenolic content, than the seed kernel extract. The DPPH and ABTS radical scavenging activities of the seed shell extract obtained using 95% ethanol solution were close to those of ascorbic acid. The in vitro toxicity was conducted to determine the concentration of the seed shell extract that should be used. Further research and development of *J. curcas* seeds can considerably benefit domains such as health care and cosmetics.

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References

1. Osman, S.A.; Abdullah, N.N.; Ahmad, S. Antioxidant activity and phytochemical components of *Jatropha curcas* Linn. root extract. *J. Biochem. Microbiol. Biotechnol.* **2017**, *5*, 2–7.
2. Boudjeko, T.; Ngomoyogoli, J.E.K.; Woguia, A.L.; Yanou, N.N. Partial characterization, antioxidative properties and hypolipidemic effects of oilseed cake of *Allanblackia floribunda* and *Jatropha curcas*. *BMC Complement. Altern. Med.* **2013**, *13*, 352. [[CrossRef](#)]
3. Augustus, G.D.P.S.; Jayabalan, M.; Seiler, G.J. Evaluation and bioinduction of energy components of *Jatropha curcas*. *Biomass Bioenergy* **2002**, *23*, 161–164. [[CrossRef](#)]
4. Openshaw, K. A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biomass Bioenergy* **2000**, *19*, 1–15. [[CrossRef](#)]
5. Rampadarath, S.; Puchooa, D.; Ranghoo-Sanmukhiya, V.M. Antimicrobial, phytochemical and larvicidal properties of *Jatropha multifida* Linn. *Asian Pac. J. Trop. Med.* **2014**, *7*, S380–S383. [[CrossRef](#)]
6. Ling, T.; Hadi, V.; Guiguemde, A.; Landfear, S.M.; Rivas, F. *Jatropha* Natural Products as Potential Therapeutic Leads. In *The Formation, Structure and Activity of Phytochemicals*; Springer: New York, NY, USA; Cham, Switzerland, 2015; pp. 77–98.
7. Ohtani, M.; Nakano, Y.; Sano, R.; Kurata, T.; Demura, T. Toxic Substances in *Jatropha* Seeds: Biosynthesis of the Most Problematic Compounds, Phorbol Esters. In *The Jatropha Genome*; Springer: Cham, Switzerland, 2017; pp. 97–111.
8. Oskoueian, E.; Abdullah, N.; Ahmad, S.; Saad, W.Z.; Omar, A.R.; Ho, Y.W. Bioactive compounds and biological activities of *Jatropha curcas* L. kernel meal extract. *Int. J. Mol. Sci.* **2011**, *12*, 5955–5970. [[CrossRef](#)]
9. Sundari, J.; Selvaraj, R.; Prasad, N.R.; Elumalai, R. *Jatropha curcas* leaf and bark fractions protect against ultraviolet radiation-B induced DNA damage in human peripheral blood lymphocytes. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 875–882. [[CrossRef](#)]
10. Namuli, A.; Abdullah, N.; Sieo, C.C.; Zuhainis, S.W.; Oskoueian, E. Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn. extracts. *J. Med. Plants Res.* **2011**, *5*, 3982–3990.
11. Insanu, M.; Dimaki, C.; Wilkins, R.; Brooker, J.; Van der Linde, P.; Kayser, O. Rational use of *Jatropha curcas* L. in food and medicine: From toxicity problems to safe applications. *Phytochem. Rev.* **2013**, *12*, 107–119. [[CrossRef](#)]
12. Aiyelaagbe, O.O.; Hamid, A.A.; Fattorusso, E.; Tagliatalata-Scafati, O.; Schröder, H.C.; Müller, W.E. Cytotoxic activity of crude extracts as well as of pure components from *Jatropha* species, plants used extensively in African traditional medicine. *Evid. Based Complement. Altern. Med.* **2011**, *2011*, 134954. [[CrossRef](#)]
13. Devappa, R.K.; Makkar, H.P.; Becker, K. Localisation of antinutrients and qualitative identification of toxic components in *Jatropha curcas* seed. *J. Sci. Food Agric.* **2012**, *92*, 1519–1525. [[CrossRef](#)]
14. Verma, S.; Gupta, A.; Kushwaha, P.; Khare, V.; Srivastava, S.; Rawat, A.K.S. Phytochemical evaluation and antioxidant study of *Jatropha curcas* seeds. *Pharmacogn. J.* **2012**, *4*, 50–54. [[CrossRef](#)]
15. Nithiyantham, S.; Siddhuraju, P.; Francis, G. A promising approach to enhance the total phenolic content and antioxidant activity of raw and processed *Jatropha curcas* L. kernel meal extracts. *Ind. Crops Prod.* **2013**, *43*, 261–269. [[CrossRef](#)]
16. Fu, R.; Zhang, Y.; Guo, Y.; Liu, F.; Chen, F. Determination of phenolic contents and antioxidant activities of extracts of *Jatropha curcas* L. seed shell, a by-product, a new source of natural antioxidant. *Ind. Crops Prod.* **2014**, *58*, 265–270. [[CrossRef](#)]
17. Islam, A.K.M.A.; Yaakob, Z.; Anuar, N. *Jatropha*: A multipurpose plant with considerable potential for the tropics. *Sci. Res. Essays* **2011**, *6*, 2597–2605.
18. Pinelo, M.; Manzocco, L.; Nunez, M.J.; Nicoli, M.C. Solvent effect on quercetin antioxidant capacity. *Food Chem.* **2004**, *88*, 201–207. [[CrossRef](#)]
19. Tsai, C.C.; Chan, C.F.; Huang, W.Y.; Lin, J.S.; Chan, P.; Liu, H.Y.; Lin, Y.S. Applications of *Lactobacillus rhamnosus* spent culture supernatant in cosmetic antioxidation, whitening and moisture retention applications. *Molecules* **2013**, *18*, 14161–14171. [[CrossRef](#)]
20. Huang, W.Y.; Lee, P.C.; Hsu, J.C.; Lin, Y.R.; Chen, H.J.; Lin, Y.S. Effects of water quality on dissolution of yerba mate extract powders. *Sci. World J.* **2014**, *2014*, 768742. [[CrossRef](#)]
21. Chan, C.F.; Wu, C.T.; Huang, W.Y.; Lin, W.S.; Wu, H.W.; Huang, T.K.; Chang, M.Y.; Lin, Y.S. Antioxidation and melanogenesis inhibition of various *Dendrobium tosaense* extracts. *Molecules* **2018**, *23*, 1810. [[CrossRef](#)]

22. Wu, C.T.; Agrawa, D.C.; Huang, W.Y.; Hsu, H.C.; Yang, S.J.; Huang, S.L.; Lin, Y.S. Functionality analysis of spent coffee ground extracts obtained by the hydrothermal method. *J. Chem.* **2019**, *2019*, 4671438. [[CrossRef](#)]
23. Chang, M.Y.; Lin, Y.Y.; Chang, Y.C.; Huang, W.Y.; Lin, W.S.; Chen, C.Y.; Huang, S.L.; Lin, Y.S. Effects of infusion and storage on antioxidant activity and total phenolic content of black tea. *Appl. Sci.* **2020**, *10*, 2685. [[CrossRef](#)]
24. Huang, W.Y.; Lin, Y.R.; Ho, R.F.; Liu, H.Y.; Lin, Y.S. Effects of water solutions on extracting green tea leaves. *Sci. World J.* **2013**, *2013*, 368350. [[CrossRef](#)]
25. De la Rosa, L.A.; Alvarez-Parrilla, E.; Shahidi, F. Phenolic compounds and antioxidant activity of kernels and shells of Mexican pecan (*Carya illinoensis*). *J. Agric. Food Chem.* **2010**, *59*, 152–162. [[CrossRef](#)]
26. Contini, M.; Baccelloni, S.; Massantini, R.; Anelli, G. Extraction of natural antioxidants from hazelnut (*Corylus avellana* L.) shell and skin wastes by long maceration at room temperature. *Food Chem.* **2008**, *110*, 659–669. [[CrossRef](#)]
27. Musa, K.H.; Abdullah, A.; Jusoh, K.; Subramaniam, V. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): Effect of extraction techniques and solvents. *Food Anal. Methods* **2011**, *4*, 100–107. [[CrossRef](#)]
28. Do, Q.D.; Angkawijaya, A.E.; Tran-Nguyen, P.L.; Huynh, L.H.; Soetaredjo, F.E.; Ismadji, S.; Ju, Y.H. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J. Food Drug Anal.* **2014**, *22*, 296–302. [[CrossRef](#)]
29. Rofida, S. Antioxidant activity of *Jatropha curcas* and *Jatropha gossypifolia* by DPPH method. *Farmasains* **2015**, *2*, 281–284.
30. Haq, M.N.U.; Wazir, S.M.; Ullah, F.; Khan, R.A.; Shah, M.S.; Khatak, A. Phytochemical and biological evaluation of defatted seeds of *Jatropha curcas*. *Sains Malays.* **2016**, *45*, 1435–1442.
31. Alimpić, A.Z.; Duletić-Laušević, S.N.; Matevski, V.S.; Marin, P.D. Antioxidant activity of *Salvia jurisicii* Košanin ethanol extracts. *Bot. Serbica* **2015**, *39*, 53–58.
32. Dhanani, T.; Shah, S.; Gajbhiye, N.A.; Kumar, S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arab. J. Chem.* **2017**, *10*, S1193–S1199. [[CrossRef](#)]
33. Kumar, A.; Kumar, P.; Koundal, R.; Agnihotri, V.K. Antioxidant properties and UPLC-MS/MS profiling of phenolics in jacquemont's hazelnut kernels (*Corylus jacquemontii*) and its byproducts from western Himalaya. *J. Food Sci. Technol.* **2016**, *53*, 3522–3531. [[CrossRef](#)]
34. Ghali, W.; Vaudry, D.; Jouenne, T.; Marzouki, M.N. Assessment of cyto-protective, antiproliferative and antioxidant potential of a medicinal plant *Jatropha podagrica*. *Ind. Crops Prod.* **2013**, *44*, 111–118. [[CrossRef](#)]
35. Nour, V.; Stampar, F.; Veberic, R.; Jakopic, J. Anthocyanins profile, total phenolics and antioxidant activity of black currant ethanolic extracts as influenced by genotype and ethanol concentration. *Food Chem.* **2013**, *141*, 961–966. [[CrossRef](#)]
36. Othman, A.R.; Abdullah, N.; Ahmad, S.; Ismail, I.S.; Zakaria, M.P. Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root. *BMC Complement. Altern. Med.* **2015**, *15*, 11. [[CrossRef](#)]
37. Marchesan, S.; Qu, Y.; Waddington, L.J.; Easton, C.D.; Glattauer, V.; Lithgow, T.J.; McLean, K.M.; Forsythe, J.S.; Hartley, P.G. Self-assembly of ciprofloxacin and a tripeptide into an antimicrobial nanostructured hydrogel. *Biomaterials* **2013**, *34*, 3678–3687. [[CrossRef](#)]
38. De Oliveira, R.B.; Vaz, A.; Alves, R.O.; Liarte, D.B.; Donnici, C.L.; Romanha, A.J.; Zani, C.L. Arylfurans as potential *Trypanosoma cruzi* trypanothione reductase inhibitors. *Memórias Do Inst. Oswaldo Cruz* **2006**, *101*, 169–173. [[CrossRef](#)]

