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Antioxidant Activity of Jojoba (*Simmondsia chinensis*) Seed Residue Extract

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Abstract. Jojoba (*Simmondsia chinensis* (Link)) is a shrub plant that widely used as cosmetic ingredients especially jojoba oil. When making jojoba oil, the residue still reminds and become waste. This study aimed to determine the antioxidant activity of jojoba seed residue (JSR). Jojoba seed residue was collected from Sudan. The JSR extracted by maceration with *n*-hexane, ethyl acetate, and 70% ethanol solvent. The antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The result showed that 70% ethanol extract of JSR had the highest antioxidant activity with 50% Inhibition Concentration (IC_{50}) value of 219.42 mg/L. The 70% ethanol extract was fractionated by using silica gel column chromatography with gradient elution produced 10 fractions. All fractions showed antioxidant activity (IC_{50} 106-447 mg/L). Fraction 3 had the highest antioxidant activity with IC_{50} value of 106.26 mg/L. Therefore, JSR has a great potential for use as a raw material in cosmetic and pharmaceutical products industry, or as a source of bioactive compounds.

Key words: *Simmondsia chinensis*; Seed residue; Antioxidant; DPPH; Bioautography

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INTRODUCTION

Jojoba (*Simmondsia chinensis* (Link)) is a shrub plant (20-50 cm height) that can grow to a height of 3 meters in dry areas. This plant is found in California (United States) and Mexico. Native Americans use this plant as a traditional medicine for infectious diseases, cancer, colds, dysuria, obesity, postpartum, sore throat, and sores. Jojoba is famous for its seed oil (liquid wax ester), which consists mainly of straight-chain monoesters in the C40-C44 range. This plant is widely used as a cosmetic ingredient because it has high antioxidant activity (Manoharan et al., 2016). Jojoba oil is used in skincare products, especially as moisturizers, hair conditioners, and lubricants (Mageed et al., 2014a). The jojoba seed residue (JSR) is rich in protein by 29–30% in addition to a group of cyanogenic glycosides (cyanomethylenecyclohexyl glycosides), simmondsin, and its derivatives (Elliger et al., 1974). To utilize it as livestock feed ingredient, the toxicant was removed (Abbott et al., 1999). Moreover, JSR can produce suitable activated carbons which are used in many applications, such as water and wastewater treatment, chemical and petroleum industries, and in mining (Tawalbeh et al., 2005).

Antioxidants are substances that can capture free radical molecules that inhibit oxidative reactions in the body. Basically, the human body produces free radicals / reactive oxygen and natural antioxidants in a balanced amount. However, several factors can cause excessive amounts of free radicals in the body. The present of free radicals will cause oxidative stress resulting degenerative diseases such as diabetes mellitus, heart diseases, cancer, cancer, etc. Antioxidants obtained from food/beverages can balance the number of free radicals that inhibit oxidative reactions in the body (Werdhasari, 2014).

Previously, many researches showed that jojoba seeds have activity as antifeedant, insecticidal, antifungal (Abbassy et al., 2007), cyclooxygenase inhibitors (Mageed et al., 2016), anti-inflammatory (Ranzato et al., 2011), lipoxygenase inhibitors (Abdul-Hafeez et al., 2014), antioxidants (Manoharan et al., 2016), and protective effect against hyperglycemia-induced oxidative stress (Belhadj et al., 2018). The jojoba leaves has also antioxidant activity, lipoxygenase inhibitors (Mageed et al., 2014a), and anticancer (Al-Qizwini et al., 2014). In this study, we utilized jojoba seed residues (JSR) which have been obtained from the fix oils extraction process. JSR was an attraction for this research due to the lack of literature on their antioxidant activity. The information from this research results will give information to the society that JSR is still have additional value as antioxidant raw materials.

METHODS

Preparation and Extraction

Jojoba seeds were obtained from Arkawit, Red Sea State, Sudan. Identification of plant material was performed by the taxonomist from Department of Botany, University of Khartoum. Seed oil was extracted by *n*-hexane solvent at University of Khartoum, Faculty of Agriculture, Department of Horticulture, Sudan. Jojoba seed residue powder was used for antioxidant assay and further fractionation. The moisture and ash content of JSR powder was determined using AOAC method (2005) prior to extraction process. The extraction used on this study was maceration with *n*-hexane in a ratio of 4: 1 for 24 hours (3 times). The resulting residue was extracted with ethyl acetate solvents for 24 hours (3 times). Furthermore, the resulting residue was extracted with 70% ethanol for 24 hours (3 times). The macerate was separated from the solvent by filtration. All macerates were concentrated with a rotary evaporator at 50 °C.

Fractionation was carried out by packing 18 grams of silica gel into the chromatography column that was used to separate 0.9 grams of extract. The column used was 1.5 cm in diameter and 42 cm in height. The most active extract was separated by a gradient elution system. Eluate was accommodated every 5 minutes at a flow rate of 1 mL/minute. Every eluate were tested by thin layer chromatography (TLC) using the best eluent and were detected at UV wavelengths of 254 and 366 nm. Stains with the same retention factor (Rf) value and pattern were combined as 1 fraction. Elution was stopped when no more stains were seen in the TLC test. The fractions were concentrated, then weighed respectively.

Determination of Antioxidant Activity

A total of 100µL DPPH 125 µmol/L was inserted into the 96 well plates in which there were 100 µL extract or fraction samples. The solution was incubated for 30 minutes at room temperature. The absorbance of the solution was measured at a wavelength of 517 nm using a multi-well plate reader. The positive control used was ascorbic acid. The inhibition was determined by measuring the absorbance variation in sample compared to control. Based on the relationship between inhibition on concentration, IC_{50} was determined (Tillah et al., 2017).

Determination of Antioxidant Activity with TLC-Bioautography

The antioxidant activity with bioautography was determined by the method of Ariansyah et al. (2019) with modification on eluent. TLC plate used was KLT $G_{60}F_{254}$ and eluted with chloroform:methanol (9:1). Separation on TLC plates were then observed under UV lights at wavelengths of 254 nm and 366 nm. The plate was then sprayed with 2.54 mM DPPH in methanol. The active sample was indicated by the presence of a yellow spot on purple plate.

RESULTS AND DISCUSSION

Jojoba plant used in this study is as shown in Figure 1a and the seed used is shown in Figure 1b. The moisture content of JSR in this study (9.05 ± 0.78%) was used to correct the yield value obtained so that the yield could be determined based on the dry weight. The measurement of ash content aimed to determine the amount of mineral content on JSR used in this study. The ash content of JSR was 2.31 ± 0.04% dried basis.

Figure 1. (a) Jojoba plant and (b) Jojoba seed

Table 1. Extraction yield and antioxidant activity of different JSR extracts.

Sample Name	Extraction yield (%)	IC_{50} value against DPPH (mg/L)
n-hexane extract	3.63	616.48
Ethyl acetate extract	4.40	478.72
Ethanol 70% extract	3.65	219.42
Ascorbic acid*	-	10.01

Note: *= Positive control

Table 1 shows that the highest yield of JSR extract was obtained by using ethyl acetate solvent which was 4.40% while the lowest yield was found in *n*-hexane solvent which was 3.63%. The use of differ-

ent solvent types with different polarities could influence the yield produced. The difference in solvent-based on its dielectric constant is expressed as a resisting repulsion between two electrically charged particles in a molecule. The higher dielectric constant indicates the more polar solvent. Dielectric constant in *n*-hexane, ethyl acetate, and ethanol 70% are 0, 0.45, and 0.68 respectively (Sudarmadji, 2010). The high yield value of JSR extract with ethyl acetate solvent showed that ethyl acetate solvents were able to extract more compounds than other solvents because the acquisition of compounds was based on the similarity of the polarity of the solvent. According to Mageed et al. (2014b) jojoba leaves contain secondary metabolites such as alkaloids, steroids, saponins, tannins, flavonoids, and phenols. Other polyphenols compounds are also reported by Belhadj et al, (2018) which contained different type of polyphenols such as hydroxybenzoic acid, flavonoids, hydroxycinnamicacids, and small amount of anthocyanins.

The measurement results of antioxidant activity were determined by IC_{50} values (Table 2). IC_{50} value is the concentration of test compounds needed to inhibit DPPH radicals by 50%. IC_{50} value is obtained from percentage of inhibitory activity (y) with sample concentration (x). Ascorbic acid was used as a positive control because it has good antioxidant properties. This antioxidant property is caused by the presence of hydroxyl groups from C2 and C3 atoms that donate H^+ ions together with their electrons to various oxidant compounds such as free radicals with oxygen or nitrogen, peroxide, and superoxide groups (Amić et al, 2007). Ascorbic acid had a smaller IC_{50} value compared to each extract. The results obtained showed IC_{50} values from the smallest to the largest were ascorbic acid, 70% of ethanol extract, ethyl acetate extract, and *n*-hexane extract with a mean value of 10 mg/L, 219.42 mg/L, 478.72 mg/L, and 616.48 mg/L respectively. This result has a different value compared to the IC_{50} value that has been reported by Kara (2017), who reported that the ethanol extract of jojoba seed had an IC_{50} value of 121 mg/L. This difference may be due to the different ethanol solvent polarity or plant materials (not seed residue) used.

The smaller IC_{50} value indicates the higher antioxidant activity of the material. This is influenced by the polarity of the active compound distributed in the solvent used in the extraction process (Maser, 2014). Therefore, the use of solvents in the extraction process will affect the IC_{50} value. The high antioxidant activity of 70% ethanol extract compared to the other extracts was due to the more antioxidant compounds extracted. Based on the test results of the antioxidant activity of each extract, the 70% ethanol extract was chosen as the most active extract. This is because 70% ethanol extract had a lowest IC_{50} value than other extracts. This results give opportunity to agribusiness to ise the JSR as source of antioxidant source which can be used as raw material in cosmetic and pharmaceutical products.

The elution process of 70% ethanol extract of JSR was tested using the step gradient method, which was preceded by semi-polar chloroform, followed by the most polar methanol. Based on the nature of the solvent used, the semi-polar component will elute first while the polar component will be retained by silica gel. The use of the solvent selection method was done so that all components in the sample can be carried out from the column starting from the nonpolar to the polar. Elution began with chloroform 100% eluent then continued with a mixture of chloroform: methanol using the following ratio 9: 1, 8: 2, 7: 3, 6: 4, 5: 5, 4: 6, 3: 7, 2: 8, and 1: 9, ending with 100% methanol until all the bands come out of the column. Fractionation results can be seen in Figure 2.

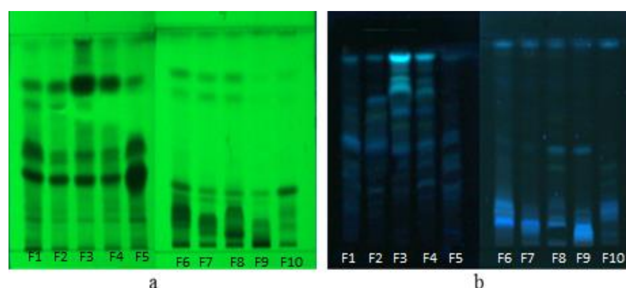

Figure 2. Chromatogram of Fraction 1-10. Mobile phase chloroform: methanol 9:1 (v/v). Documentation at: UV 254 nm (a) and UV 366 nm (b) lights.

Separation using column chromatography was tested by accommodating eluate in a vial every 5 minutes and obtaining 144 vials. All the vials were tested for their separation patterns with the best eluent, which was chloroform: methanol (9: 1) v/v. Elution was stopped when there were no more stains on TLC. Eluate which has the same TLC separation pattern was combined into one fraction. The fractions that had been combined were re-tested for separation in TLC to obtain 10 fractions. Vials that produced the same chromatogram pattern observed in UV light 254 nm and 366 nm were combined into 1 fraction.

Antioxidant Activity and TLC Bioautography

The antioxidant activity in the 10 fractions was tested again with the DPPH method with the results as shown in Figure 3. This method is often used to test antioxidant activity because of several advantages, i.e. direct, simple inhibition measurements, and fast analysis. TLC bioautography for antioxidants has the principle that antioxidants that react with DPPH will donate hydrogen atoms so that DPPH is reduced to DiphenylPicryl Hydrazine (DPPH-H)

which is stable and color decay occurs from purple to yellow (Molyneux, 2004). The color change shows the activity of free radical reduction which can be measured at a wavelength of 517 nm.

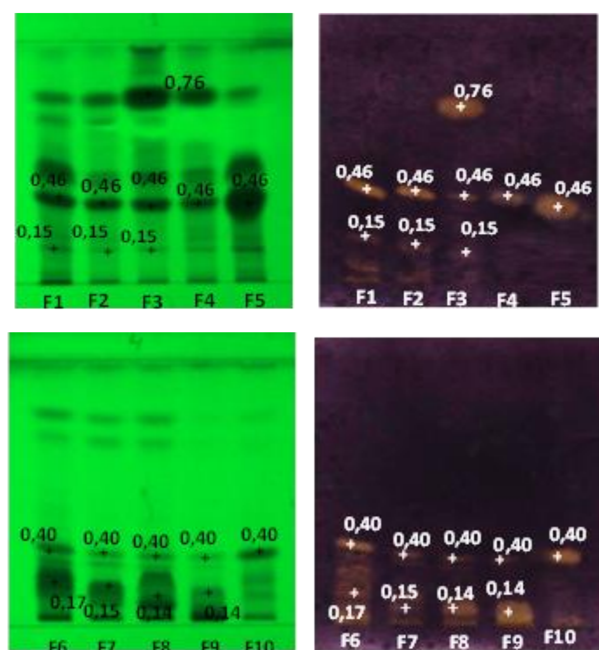

Figure 3. TLC-bioautogram of fractions 1-10 of 70% ethanol extract of JSR with Rf values under UV 254nm light. (a) TLC under UV 254 nm before spraying DPPH reagent; (b) TLC after spraying DPPH reagent without UV light

Qualitative tests on each fraction were tested out using TLC and continued with bioautography using DPPH reagent to determine compounds that play a role in antioxidant activity. The presence of these compounds was indicated by yellow against the purple background. Figure 3 shows all fractions having antioxidant activity. This can be seen in the presence of yellow color which was formed and compounds that are antioxidant, scattered in each fraction. These results indicated that the compounds (fractions 1 to fraction 5) have spots with the same Rf which is 0.46, Fraction 1 to fraction 3 had a spot with the same Rf of 0.15, fraction 6 to fraction 10 had a spot with Rf of 0.40, fraction 6 to fraction 9 had a spot with the same Rf (0.14-0.17), and fraction 3 shows yellow color on the spot with Rf of 0.76, which was different from the other fractions.

Based on the TLC chromatogram on UV 366 nm (Figure 2b), all spots had blue color. These spots was with black color on UV 254 nm. This indicated that the compounds on the spot could be flavone, flavanone, flavonols, and isoflavone. As reported previously, jojoba has a lot of flavonoids and 10 of them (quercetin and isorhamnetin derivatives) had reported by Abdel-Mageed et al. (2014). To find the real active compounds, further isolation process is needed.

The determination of IC_{50} value was done by making a series of concentrations of 10 isolated fractions with standard ascorbic acid as a positive antioxidant control. According to Molyneux (2004) classification, antioxidant compounds are very strong if the IC_{50} value is less than 50 mg/L, strong if the IC_{50} value is 50-100 mg/L, moderate if the IC_{50} value is 100-150 mg/L, weak if the value IC_{50} is between 150-200 mg/L, and is very weak if the IC_{50} value is more than 200 mg/L. In our study, the IC_{50} values obtained were very diverse. Table 2 shows that fraction 3 had the strongest antioxidant activity because it had the lowest IC_{50} value of 106.26 mg/L, and vice versa, the fraction 7 had the weakest antioxidant activity because it had the highest IC_{50} value of 446.83 mg/L. Positive control of ascorbic acid had IC_{50} value of 10 mg/L. According to Mageed et al. (2014a) the antioxidant activity of jojoba plant is caused by the presence of bioactive compounds which is flavonoids. Flavonoid compounds are secondary metabolites that are spread in plants and include phenolic compounds that tend to be easily soluble in polar solvents. The antioxidant effects of flavonoids are caused by free radical capture through a hydrogen proton donor from the flavonoid hydroxyl group (Amić et al, 2007). The antioxidant activity in flavonoids is mainly influenced by the substitution of hydroxy groups in the ortho and para position to OH and OR groups. The antioxidant activity of flavonoids is very dependent on the substituents in ring B which will increase their activity by the presence of ortho hydroxylation and catechol groups on the B ring (Amić et al, 2007). Quercetin has the greatest activity because O-hydroxy is present in ring B (Banjarnahor & Artanti 2014). The spot of active fractions produced need to be analyzed further by using NMR, FTIR, and MS to more accurately elucidate the molecular formula.

Table 2. Antioxidant activity of different fractions (1-10) of 70% ethanol extract of JSR

Sample Name	IC_{50} value against DPPH (mg/L)
Fraction 1	245.84
Fraction 2	245.66
Fraction 3	106.26
Fraction 4	157.74
Fraction 5	145.14
Fraction 6	259.41
Fraction 7	446.83
Fraction 8	241.53
Fraction 9	213.57
Fraction 10	249.93
Ascorbic Acid*	10.00

Note: *= Positive control

This research results give information that JSR could be utilized as cosmetics and pharmaceutical raw materials since it has good antioxidant activity. JSR is also could be a bioactive compounds source, even though the bioactive compound in the JSR still needs to be research further. This results also give added value to jojoba seed especially the JSR as agricultural waste.

CONCLUSION

Jojoba seed residue had antioxidant activity, especially the 70% ethanol extract with IC_{50} values of 219.42 mg/L. The results of column fractionation showed that Fraction 3 had the lowest IC_{50} value of 106.26 mg/L. Therefore JSR has a great potential to be used as a raw material in cosmetic and pharmaceutical products, or as a source of bioactive compounds which may enhance the jojoba seed profitability in agribusinesses.

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