



Antibacterial Activity of Jojoba Seed residue and Its Possible Active Compound

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Abstract

Jojoba (*Simmondsia chinensis*) (Link) C.K. is a shrub plant widely used in cosmetics, especially jojoba oil. The residue will remain when producing jojoba oil and become waste. This study aimed to determine the antibacterial activity of Jojoba seed residue (JSR) and its possible active antibacterial compounds. JSR was collected from Sudan and extracted by maceration with *n*-hexane, ethyl acetate, and 70% ethanol. The antibacterial activity was determined with the microdilution method against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). The 70% ethanol extract showed the value of MIC and MBC against *E. coli*, which was 7.8 mg/mL; meanwhile, against *S. aureus* was 3.9 mg/mL and 7.8 mg/mL, respectively. Fractionation of 70% ethanol extract using silica gel column chromatography with gradient elution produced ten fractions. Fraction 3 showed the MIC and MBC values in *E. coli* which were 3.1 mg/mL and 12 mg/mL, and in *S. aureus*, which were 3.1 mg/mL and 6.2 mg/mL. The fractionation continued to Fraction 3 using preparative thin layer chromatography to collect subfraction 3.2 at an *R_f* value of 0.76, actively based on contact autobiography against *E. coli* and *S. aureus*. Embelin was detected in Subfraction 3.2 using liquid chromatography-mass spectrometry (UHPLC-Q-Orbitrap HRMS) and suggested as the active antibacterial component in JSR.

1. Introduction

Jojoba (*Simmondsia chinensis* (Link) C.K.) is a shrub plant (20–50 cm in 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 [1]. On the other hand, jojoba leaves were reported as eco-friendly detoxicant [2]. Jojoba is famous for its seed oil (liquid wax ester), consisting mainly of straight-chain monoesters in the C₄₀–C₄₄ range. Jojoba oil is used in skincare products, especially as moisturizers, hair conditioners, lubricants [3], anti-herpes simplex 1 [4], and also as plasticizers [5]. After extracting the oil, jojoba seed residue (JSR) is still rich in

protein 29–30%, cyanogenic glycosides (cyanomethylene cyclohexyl glycosides), simmondsin, and its derivatives [6]. Therefore, toxicants such as simmondsin should be removed before using JSR as a livestock feed ingredient [7].

Some researchers tried to extract the polyphenols from the JSR using microwave-assisted extraction [8]. On the other hand, JSR was reported as a potent antioxidant [9, 10]. In this research, the antibacterial activity of JSR extracts will be determined. *S. aureus* and *E. coli* are some bacteria that can cause infectious diseases in humans. *S. aureus* is gram-positive bacteria that are the primary human pathogens. Infection with *S. aureus* can cause illness with characteristic signs, namely inflammation, ulcers, acne, and wound infection [11]. These problems

often interfere with the physical and psychological appearance. The organ that is often attacked by *S. aureus* is the injured skin and can spread to other people who are also injured. *E. coli* are pathogenic bacteria commonly found in the human intestine. The disease caused by *E. coli* infection in the intestine is diarrhea. Diarrhea caused by bacteria is still a health problem in developing and developed countries. Infection due to bacteria still dominates the potential for severe disease, sepsis, shock, septic, and multiorgan dysfunction [12].

The activity of plant extract is related to its chemical compounds. Therefore, it is essential to separate, fractionate, and characterize the active component using spectrometry analysis to identify the active component from the extract. So, this research aimed to determine the antibacterial activity of JSR extract and the possible active component from JSR.

2. Methodology

The jojoba seed residue (JSR) powder was collected from Sudan (University of Khartoum), and the taxonomist from University of Khartoum performed the determination. The voucher specimen was deposited in the Faculty of Agricultural Herbarium of University of Khartoum. The moisture and ash content of jojoba leaves residue powder was determined using the Association of Official Analytical Chemists (AOAC) method before the extraction [13]. The active extract was separated using column chromatography, and the active fraction was separated further using preparative thin-layer chromatography. The bioactivity of extract and fraction was determined as antibacterial activity against *S. aureus* and *E. coli* using thin layer chromatography bioautography and microdilution method to obtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The bioactive component was determined using Ultra High-Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-Q-Orbitrap HRMS).

2.1. Equipment and Materials

JSR powder was collected from the University of Khartoum, while the test bacteria used were *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. The instrument used was Vanquish Flex UHPLC-Q-Orbitrap HRMS (Thermo Fisher Scientific, Waltham, MA, USA) with an accucore TM C18 column, 100 × 2.1 mm, 1.5 μm (Thermo Fisher Scientific, Waltham, MA, USA).

2.2. Extraction and Fractionation

The extraction method was maceration with *n*-hexane in a 4:1 for 24 hours 3 times. The resulting residue was extracted three times using ethyl acetate solvents for 24 hours. Furthermore, the resulting residue was extracted three times using 70% ethanol for 24 hours. 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 and used to separate 0.9 grams of extract. The column used was 1.5 cm

in diameter and 42 cm in height. A gradient elution system separated the most active extract. Eluate was accommodated every 5 minutes at a 1 mL/minute flow rate. The collection of reservoirs was tested by thin-layer chromatography (TLC) using Silica gel GF₂₅₆ as stationary phase, the chloroform: methanol (9:1; v/v) as mobile phase, and detected at UV wavelengths 254 and 366 nm. Spots with a retention factor value (*R_f*) and the same TLC pattern were combined as one fraction. The most active fraction was then separated further using preparative thin-layer chromatography (PTLC).

2.3. Determination of Antibacterial Activity

The Nutrient Broth (NB) and Trypticase Soy Broth (TSB) were used as a medium for *S. aureus* and *E. coli*, respectively. The 100 μL of extract/fraction with a concentration range of 0.98–125 mg/mL was inserted into every 96-well plate. The extract or fraction was diluted in DMSO 20%. Each well was added with 100 μL of sterile medium and 5 μL of bacterial inoculum (10⁷ coliform unit (CFU)/mL). Then the plates were incubated at 37°C for 24 hours. The lowest sample concentrations showing no bacterial growth were characterized by clear wells after incubation and selected as MIC. Next, a 10 μL medium that did not show bacterial growth was inoculated on 100 μL of a new medium and incubated for 18–22 hours at 37°C. The lowest concentration that did not show bacterial growth after the second inoculation was chosen as the MBC. The negative control was DMSO 20%, and the positive control was chloramphenicol [14].

2.4. Determination of Antibacterial with TLC-Bioautography Contact

The most active extract was applied as much as 10 μL with a concentration of 2% on the TLC plate G60 F254 using CAMAG Linomat 5. The plates were put into the chromatography vessel for 15 minutes with the selected mobile phase. After the eluent reached the propagation limit, the TLC plate was removed and air dried to remove the solvent; then, the TLC plate was attached to a suspension of bacteria (*S. aureus* and *E. coli*) growing on TSB and NA medium for 18–22 hours. The medium was incubated at 37°C for 24 hours. The medium was observed, and the appearance of a clear zone on the medium indicated antibacterial activity. The inhibition area on the chromatogram was marked and compared with the chromatogram detected previously with UV light at 254 nm to determine the *R_f* value, which had inhibitory activity.

2.5. LC-MS/MS Analysis

The most active fraction was analyzed using a Vanquish Flex UHPLC-Q-Orbitrap HRMS with a C18 column. The sample was dissolved in 5 mL of methanol, and 5 μL of the sample was injected into the column 2.1 mm × 50 m, a flow rate of 0.2 mL/minute. The column temperature was 40°C, and the final time was 25 minutes. Aqueous + 0.1% formic acid (A) and Acetonitrile + 0.1% formic acid (B) was used as mobile phase. A gradient elution system of 5–35% B (0–4 minutes), 35–65 % B (4–7 minutes), 65–80% B (7–15 minutes), 80–95% B (15–20 minutes), 95% B (20 minutes), 95–5% B (22–

22,010 minutes) was used. MS–MS condition with positive ion mode (ESI) and scan range (m/z) 80–1000.

3. Results and Discussion

3.1. JSR and The Extraction Yield

The moisture and ash content of JSR in this study was $9.05 \pm 0.78\%$ and $2.31 \pm 0.04\%$ dried basis, respectively. Three types of solvents were used in this study to extract the active component of JSR, namely *n*-hexane, ethyl acetate, and 70% ethanol. The lowest yield was found in *n*-hexane extract (3.33%), which means that only a small amount of nonpolar compound is left in JSR. The highest yield was found in ethyl acetate extract, about 4.74%. The yield of this extract is almost the same as in our previous reports, which was about 3.63%; 4.40%, and 3.65% for *n*-hexane, ethyl acetate, and ethanol extract, respectively [9].

3.2. Antimicrobial Activity of Extract

The potency of the three JSR extracts as an antibacterial against *E. coli* and *S. aureus* is shown in Table 1. Each JSR extract could inhibit the growth of *S. aureus* and *E. coli* bacteria. The presence of clear wells indicated these results after being given bacterial inoculants in the concentration range of 0.98–125 mg/mL. When the extract suppresses the growth of *S. aureus* more than *E. coli*, lower MIC and MBC values were discovered. Because *S. aureus* is a Gram-positive bacteria with a peptidoglycan-based cell wall, it will be simpler for antibacterial substances to penetrate the cell. Lower MIC and MBC values were observed when the extract suppressed the growth of *S. aureus* more than *E. coli*. *S. aureus* is a Gram-positive bacteria with a peptidoglycan-based cell wall, allowing antibacterial substances to penetrate cells more easily.

In contrast, the cell wall of *E. coli*, a Gram-negative bacteria, is relatively more complex. The 70% ethanol extract had the lowest MIC and MBC values compared to other extracts. This result follows Abu Salem and Ibrahim [15], stating that 70% ethanol extract has good antibacterial activity. Chloramphenicol was used as a positive control because it has a broad spectrum with a mechanism inhibiting protein synthesis. Chloramphenicol showed better MIC and MBC values against *E. coli* and *S. aureus* than the three JSR extracts.

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

Sample name	Extraction yield (%)	<i>E. coli</i> antibacterial		<i>S. aureus</i> antibacterial	
		MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>n</i> -hexane extract	3.33	125	> 2000	> 2000	> 2000
Ethyl acetate extract	4.74	62.5	62.5	15.62	31.25
70% ethanol extract	3.56	7.8	15.6	3.9	7.8
Chloramphenicol*	-	0.98	1.98	0.98	0.98

* = Positive control

The MIC of 70% ethanol extract of JSR is higher than the citronella essential oil (MIC of 625.21 µg/mL and MBC

of 1250.28 µg/mL) [16]. The JSR extract is not as active as citronella essential oil. The JSR extract was much more potent when compared to the antibacterial activity of jojoba seed, which was no antibacterial activity against *S. aureus* and *E. coli* [17]. Since the 70% ethanol extract of JSR is the most potent compared to other extracts, the 70% ethanol extract was chosen for the following research step.

3.3. Fractionation of Active Extract and The Fractions Antimicrobial Activities

The 70% ethanol extract of JSR was separated by silica gel column chromatography using the step gradient method, followed by chloroform and methanol. Elution began with 100% chloroform eluent, then continued with a mixture of chloroform and methanol using the following ratios 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. The fractions were then grouped based on the same TLC pattern resulting in 10 fractions. Antibacterial activity of the 10 fractions was carried out again using the microdilution method and contact bioautography TLC against *S. aureus* and *E. coli*. Bioautography is a specific method for determining compounds with antibacterial activity by detecting TLC plates. Contact bioautography is an analytical technique for separating organic compounds by chromatography and is identified by studying their effects on microorganisms [18].

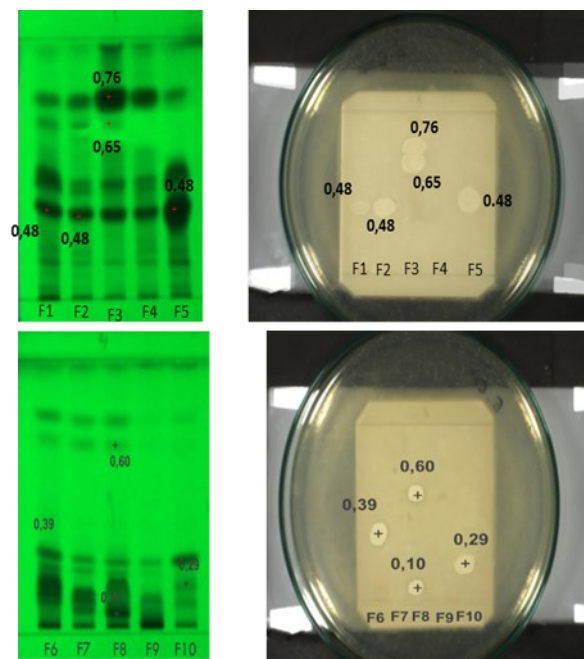


Figure 1. TLC contact bioautogram fractions (F1–F10) against *S. aureus* and with information on Rf values under 254 nm of UV light

The antibacterial activity of the 10 fractions (F1–F10) against *S. aureus* bacteria is shown in Figure 1. The results showed that Fractions 1, 2, and 5 have the same inhibition zone on the spot with an Rf value of 0.48. Fraction 3 has an inhibition zone at spot Rf 0.65 and 0.76, Fraction 6 at Rf 0.39, Fraction 8 at Rf 0.10 and 0.60, and Fraction 10 at Rf 0.29. Only Fractions 4, 7, and 9 showed no clear zones, meaning no activity as anti-*S. aureus*. Against *E. coli*,

Fraction 1 has an inhibition zone at the spot with an *R_f* value of 0.66, Fraction 3 at *R_f* value of 0.46 and 0.66, Fraction 4 at *R_f* value of 0.46 and 0.10, Fraction 5 at *R_f* value of 0.46, Fraction 6 and 7 at *R_f* value of 0.40, and Fraction 10 at *R_f* value of 0.16. Unlike *S. aureus*, Fractions 2, 7, and 8 showed no inhibition zone formed. A clear zone with the same spot indicates the possibility that the spot contains a similar compound.

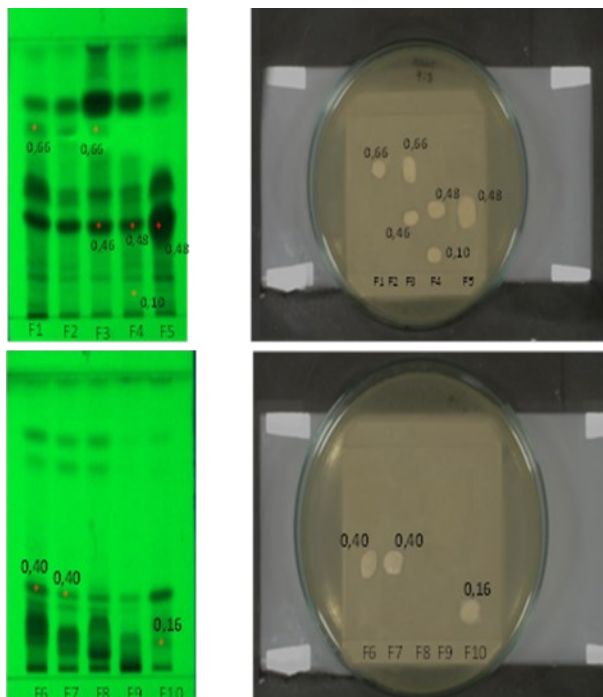


Figure 2. TLC contact bioautogram fractions (F1–F10) against *E. coli* and with information on the *R_f* value under 254 nm of UV light

Table 2. Antibacterial activity of the 70% ethanol extract fractions (F1–F10) of JSR

Fraction	<i>E. coli</i>		<i>S. aureus</i>	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
F1	12.5	25.0	12.5	12.5
F2	12.5	12.5	12.5	12.5
F3	3.12	12.5	3.12	6.25
F4	12.5	25.0	6.25	12.5
F5	6.25	12.5	6.25	12.5
F6	6.25	12.5	6.25	12.5
F7	50.0	50.0	12.5	25.0
F8	12.5	50.0	12.5	50.0
F9	25.0	12.5	25.0	12.5
F10	25.0	25.0	12.5	25.0
Chloramphenicol	0.78	1.56	0.78	1.56

When comparing the activity of fractions against two bacteria, Fraction 3 shows better antibacterial activity. MIC and MBC values of all fractions were determined, and the results are summarized in Table 2. The results confirmed that Fraction 3 was the most active since this fraction had the lowest MIC and KBM values compared to

other fractions. The antibacterial activity of Fraction 3 is better than that of 70% ethanol extract (Table 1) but not as good as chloramphenicol as the positive control. Furthermore, Fraction 3 is more active against *S. aureus* compared against *E. coli*.

3.4. Further Fractionation and Characterization of Active Component

Fraction 3 separated further using preparative TLC using chloroform:methanol (9:1(v/v)) as mobile phase. Based on the spot activity with an *R_f* value of 0.76 in Figure 1, Sub-fraction 3.2 (*R_f* = 0.76), which had antibacterial activity and abundance, was identified using LC-MSMS. In addition, some compounds were identified based on the chromatogram peaks. The LC-MS-MS analysis was corrected with methanol blanks to show differences in the chromatogram peaks between the samples and the blanks. Chromatogram data analysis was obtained using Thermo Scientific Xcalibur software, and mass spectrum analysis was performed using Compound Discoverer 2.1.0.401 software. Four compounds were reported to have antibacterial activity (Table 3).

Table 3. Identified compounds that reported antibacterial activity in Subfraction 3.2

Retention time (min)	Molecular weight (m/z)	Compound name	Molecular formula	Activity reported
0.567	144.0896	2,6-diamino-4-hexenoic acid	C ₆ H ₁₂ N ₂ O ₂	Antibacterial against <i>Bacillus subtilis</i> [19]
10.832	294.1791	Embelin	C ₁₇ H ₂₆ O ₄	Antibacterial [20]
13.994	255.2558	Palmitamide	C ₁₆ H ₃₃ NO	Antibacterial [21]
24.684	104.0211	3-hydroxybutyric acid	C ₄ H ₈ O ₃	Antimicrobial against <i>S. aureus</i> , <i>Klebsiella pneumoniae</i> , and <i>Candida albicans</i> [22]

Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone, Figure 3) is a compound that has a quinone and a phenolic group on the same ring. Embelin also has a long alkyl chain (undecyl) as a substituent, which gives it a nonpolar phase solubility. This compound is reported to have antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Shigella flexneri*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Shigella boydii*, and *Proteus mirabilis* [20]. In addition, Embelin is also reported to exhibit anticancer, antioxidant, antimalarial, antiviral, antidiabetic, and anti-inflammatory activity [23]. Therefore, it could suggest that Embelin is the active antibacterial compound in JSR, even though other compounds are also prospective to be explored, such as 2,6-diamino-4-hexenoic acid, palmitamide, and 3-hydroxybutyric acid.

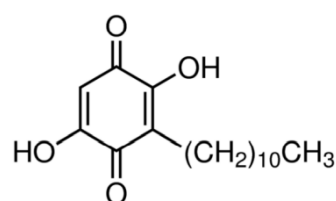


Figure 3. Structure of Embelin

4. Conclusion

The 70 % ethanol extract of JSR has an antibacterial activity with MIC and MBC values of respectively 7.81 mg/mL and 15.62 mg/mL against *E. coli*, 3.91 mg/mL and 7.81 mg/mL against *S. aureus*. The MIC and MBC values of Fraction 3 obtained in *E. coli* bacteria were 3.12 mg/mL and 12.5 mg/mL, while the *S. aureus* bacteria were 3.12 mg/mL and 25 mg/mL. The Embelin in Subfraction 3.2 could be the active component from the JSR 70 % ethanol extract. Another compound could be explored to obtain more potent antibacterial compounds from JSR. Therefore, further purification of Fraction 3 is required to ascertain the presence of active components such as 2,6-diamino-4-hexenoic acid, palmitamide, and 3-hydroxybutyric acid.

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