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Research Article

Antibacterial Evaluation of *Jatropha spinosa* Latex Extracts.

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ABSTRACT

This plant has been considered a medicinal plant in Yemen's traditional medicine, used as an antiseptic for wounds. This present study was carried out to evaluate the antibacterial properties of hexane, chloroform, butanol, and aqueous extracts of *Jatropha spinosa* latex. Agar disc diffusion tests were carried out to determine the antimicrobial effects of the *Jatropha spinosa* latex extracts against Gram-positive (*B. cereus*, *S. aureus*, and *S. pyogenes*), Gram-negative (*Klebsiella*, *E. coli*, and *P. aeruginosa*) bacterial strains. Among the various extracts, the hexane extract of *Jatropha spinosa* latex has the strongest antibacterial potency against *Staphylococcus aureus* (gram-positive), and the Butanol extract showed the highest effect on *S. aureus*, *S. pyogenes*, and *Klebsiella* at a higher concentration of 1mg/1ml with zones of inhibition 11.26mm, 11.53mm, and 11.6mm, respectively. Water extract showed the maximum (11.13± 1.02mm) inhibitory effect against *Klebsiella* at a higher concentration of 1mg/1ml, and no activity was observed at all concentrations against the Gram-positive *S. pyogenes* bacteria strain. The chloroform extract has potent antibacterial activity against the Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* bacterial strains at a concentration 1mg/1ml with zone inhibition of 10.53mm and 10.83mm. The positive control Beta-sitosterol 0.25% W/W ointment did not show inhibitory effect against four tested bacteria strains (*S. aureus*, *S. pyogenes*, *Klebsiella*, and *E. coli*) at all concentrations. It has exhibited lesser inhibitory potency when compared with the *Jatropha spinosa* latex extracts. The present study has revealed that the *J. spinosa* latex extracts are a potential antimicrobial

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agent having a broad-spectrum activity.

INTRODUCTION

Jatropha spinosa belongs to the family *Euphorbiaceae*, which comprises about 340 genera and more than 8000 species, mainly distributed in tropical regions across various habitats [1–4]. This family is considered one of the most important in Yemen, with over 106 species, including a high proportion of endemics [5,6]. *Euphorbiaceae* species have traditionally been used in Yemen for treating skin and infectious diseases [7,8], and in other regions of the world, they have also been applied in folk medicine for a wide range of disorders such as tumors, malaria, gastrointestinal and respiratory conditions, as well as renal, hepatic, rheumatic, and neurological ailments [9–10]. The genus *Jatropha* comprises about 175 species distributed in tropical regions worldwide [11]. The name *Jatropha* originates from the Greek words *Jatros* (physician) and *trophe* (food), reflecting its long-standing medicinal and nutritional value. The latex of several *Jatropha* species has been reported to possess pharmacological activities, including hemostatic, antibacterial, antifungal, antimalarial, antiparasitic, and insecticidal effects [12]. Traditionally, *Jatropha* trees have been utilized in Asia, Africa, and Latin America for the treatment of various ailments [13]. Their bioactivity is attributed to the presence of diverse phytochemicals such as terpenoids, tannins, and flavonoids, which contribute to their antimicrobial and therapeutic potential [14,15]. More broadly, medicinal plants have played a central role in human health care throughout history. They have been used for treating diseases, preventing epidemics, and preserving food [16]. Many modern drugs have been derived from these plants, and they continue to provide important leads for pharmacological development [17]. Previous research has shown that many medicinal plants

have pharmacological qualities such as antibacterial, anti-inflammatory, anti-diarrheal, anti-ulcer, antioxidant, gastroprotective, and anti-constipation activities [18–22]. Millions of infectious or toxic diseases are caused by bacteria [23, 24], and approximately 80% of the global population still relies on plant-based traditional medicine for primary healthcare needs [25]. Therefore, the present study was designed to investigate the antibacterial activity of *Jatropha spinosa* latex extracts as part of ongoing research on Yemeni medicinal plants. This work aims to validate the traditional use of the species in folk medicine, assess its potential as a source of new antibacterial agents against resistant wound pathogens, and compare its efficacy with standard pharmaceutical drugs. Ultimately, the study seeks to contribute to the development of pharmaceutical formulations containing bioactive compounds from *J. spinosa* that may provide effective and competitive alternatives in the drug market.

MATERIALS AND METHODS

Plant material

The materials were collected from the Al-Wagah region at Taiz governorate, Yemen, especially in the rainy season. The plant sap (latex) collection was gathered by the aerial part of the stem injury using a sterile surgical blade, which was collected into a small glass bottle of about 40ml and transferred to the university laboratory.



Figure 1. Extraction of *J. spinosa* latex.

Extraction

The 50ml of distilled water was added to the latex and put into a separating funnel, and 100ml of hexane solvent was added, and then shaken for 3 minutes, and the mixture was placed for 24 hours.

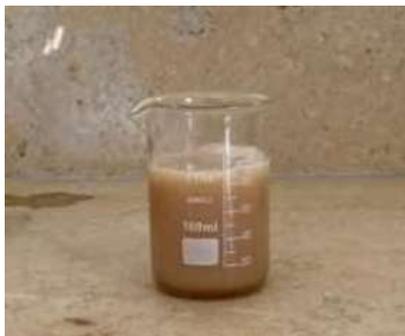


Figure 2. Distal water with latex.



Figure 3. Hexane extraction.

Then, the hexane layer (lower layer) was separated from the other layers of the mixture. The 100ml of hexane solvent was again added to the mixture in a separating funnel and shaken for 3 minutes. After 24 hours, the hexane layer was separated from the other layers of the mixture. The 100ml of chloroform solvent was added to the mixture and shaken for 3 minutes, and the chloroform layer was separated after 24 hours. We added 75ml of butanol solvent, and the butanol layer was separated after 24 hours. This process was repeated three times to obtain a large amount of the extracts.



Figure 4. Chloroform extraction.



Figure 5. Butanol extraction.

All extracts were concentrated used rotary evaporator for antibacterial activity tests.



Figure 6. Extracts of *J. spinosa*

Disc diffusion method

Antibacterial activity of the tested plant extracts was carried out by the disc diffusion method [26]. First, the extracts of the plant tested were dissolved in DMSO at a concentration of 1mg/ml, 1mg/2ml, 1mg/3ml, and 1mg/4ml. Then, 100 μ L of bacterial inoculums containing 10^8 CFU/ml were spread over plates containing Mueller Hinton agar, and discs (5 mm in diameter) impregnated with different concentrations of the plant extract

solution as follows, were placed on the surface of the media. Also, we used reference four control discs containing DMSO and Meburn® (0.25 % disc) with different concentrations dissolved in DMSO: 1mg/ml, 2mg/ml, 3mg/ml, and 4mg/ml. The plates were incubated for 24 h at 37 °C. The diameters of the inhibition zones were measured, and antibacterial activity was assessed based on the inhibition zone diameters in mm [27]. This was applied three times for G+ve bacteria *S. aureus*, *S. pyogenes*, and *B. cereus*, and for G-ve bacteria *Klebsiella*, *E. coli*, and *P. aeruginosa*.

RESULTS AND DISCUSSION

The antimicrobial activities of *Jatropha spinosa* latex (hexane, chloroform, butanol, and water) extracts against the microorganisms examined in the present study, and their potency, were tested by the presence or absence of inhibition zones and zone diameter, and the results are given in Tables 1-4.

The results of *Jatropha spinosa* latex extracts showed increased activity with increasing concentration against all strains of Gram-positive and Gram-negative bacteria. The data indicated that *S. aureus* was the most sensitive strain tested with the hexane extract of *Jatropha spinosa* latex, which produced the largest inhibition zone, 14.3 mm at 1mg/1ml Table 1.

Table 1. Zone inhibition of the Hexane extract

Microorganism	Zone of Inhibitor (mm)			
	Hexane Extracts			
	1mg\1ml	1mg\2ml	1mg\3ml	1mg\4ml
<i>S. aureus</i>	14.3±1.52	8.6±0.57	5.56±0.51	-
<i>S. pyogene</i>	10.66±1.52	10.40±0.60	-	-
<i>Klebsiella</i>	11.46±0.50	7.46±0.5	5.33±0.57	-
<i>P. aeruginosa</i>	8.76±0.20	6.1±0.15	-	-
<i>E. coli</i>	10.50±0.50	7.2±1.05	-	-
<i>B. cereus</i>	7.83±0.15	5.7±0.64	-	-

The highest activity of *Jatropha spinosa* water extract was observed against *Klebsiella*, with an inhibition zone of 11.13 mm at 1mg/1ml Table 2.

Table 2. Zone inhibition of the Water extract.

Microorganism	Zone of Inhibitor (mm)			
	Water Extracts			
	1mg\1ml	1mg\2ml	1mg\3ml	1mg\4ml
<i>S. aureus</i>	9.5±0.5	5.7±0.4	-	-
<i>S. pyogene</i>	-	-	-	-
<i>Klebsiella</i>	11.13±1.02	9.0±0.20	6.03±0.57	-
<i>P. aeruginosa</i>	9.6±0.52	7.4±0.5	6.1±0.5	-
<i>E. coli</i>	9.13±0.15	5.1± 0.17	-	-
<i>B. cereus</i>	9.73±0.30	5.4±0.51	-	-

Jatropha spinosa chloroform latex extract also displayed a variable degree of inhibition against

the investigated bacterial strains. The maximum activity of chloroform extract was observed



against *S. aureus* with an inhibition zone of 10.53 mm at 1mg/1ml Table 3.

Table 3. Zone inhibition of the Chloroform extract.

Microorganism	Zone of Inhibitor (mm)			
	Chloroform Extracts			
	1mg\1ml	1mg\2ml	1mg\3ml	1mg\4ml
<i>S. aureus</i>	10.53±0.50	6.9±1.01	-	-
<i>S. pyogene</i>	8.56±0.51	5.33±0.57	-	-
<i>Klebsiella</i>	9.66±2.30	6.66±0.05	-	-
<i>P. aeruginosa</i>	10.83±0.76	8±1.0	6.0±0.11	-
<i>E. coli</i>	8.26±1.10	-	-	-
<i>B. cereus</i>	9.5±0.76	6.3±0.57	-	-

Also, the *Klebsiella*, found to be the most sensitive bacteria among the tested bacteria with butanol extract of *Jatropha spinosa* latex, which

demonstrated an inhibition zone of 11.6 mm at 1mg/1ml, Table 4.

Table 4. Zone inhibition of the Butanol extract.

Microorganism	Zone of Inhibitor (mm)			
	Butanol Extracts			
	1mg\1ml	1mg\2ml	1mg\3ml	1mg\4ml
<i>S. aureus</i>	11.26±0.64	6.9±0.26	-	-
<i>S. pyogene</i>	11.53±0.5	8.23±0.20	-	-
<i>Klebsiella</i>	11.6±1.52	8.0±0.20	6.8±1.05	-
<i>P. aeruginosa</i>	9.86±0.15	8.1±0.57	5.9±0.17	-
<i>E. coli</i>	10.86±0.20	7.50±0.45	5.1±0.17	-
<i>B. cereus</i>	10.03±0.05	7.56±0.51	6.4±0.52	-

Based on these results, the hexane extract exhibited stronger and broader antimicrobial activity than the chloroform, butanol, and water extracts. Yemeni people have a tradition of using several plant species for the treatment of infectious diseases and various ailments, and they used *J. spinosa* as an antiseptic and wound healing [28]. Methanolic stem extract and the fresh stem juice of *J. spinosa* demonstrated activity against *E. coli*, *S. aureus*, and *P. aeruginosa* [29], and as a bio-guided study for drug discovery, our fractionation of the crude extract into different extracts by several solvents may lead to determining the extract that has the strongest active compounds against bacterial strains. The phytochemical evaluation of the aerial part of *J. spinosa* showed

the presence of secondary plant metabolites, including flavonoids, carbohydrates, tannins, sterols, and triterpenes [30]. Whereas several studies have confirmed that some *Jatropha* genus plants contain many compounds that have a wound healing effect. The phytochemical investigation of *Jatropha curcas*, including Beta-sitosterol as an identified compound, which is an ingredient in wound healing ointment [31]. Several studies have been reported on the antibacterial activity of the plant extracts, which may be due to the presence of the natural compounds [32-36].

Significantly, in this study, it was found that (hexane, chloroform, butanol, and water) extracts of *J. spinosa* latex exhibited more antibacterial activity on tested bacteria compared to the Beta-

sitosterol 0.25% W/W ointment, which is used as an ointment drug for wound healing. In contrast, the Beta-sitosterol 0.25% W/W ointment showed no antimicrobial activity against the bacterial strains *S. aureus*, *S. pyogenes*, *Klebsiella*, and *E. coli* at all concentrations Table 5.

Table 5. Zone inhibition of Beta-sitosterol 0.25 % W/W.

Microorganism	Zone of Inhibitor (mm)			
	Beta – sitosterol 0.25% W/W			
	1mg\1ml	1mg\2ml	1mg\3ml	1mg\4ml
<i>S. aureus</i>	-	-	-	-
<i>S. pyogenes</i>	-	-	-	-
<i>Klebsiella</i>	-	-	-	-
<i>P. aeruginosa</i>	5.9±0.11	7.6±0.35	9.96±0.57	12.96±1.00
<i>E. coli</i>	-	-	-	-
<i>B. cereus</i>	7.13±0.23	8.76±0.68	10.56±0.51	11.96±1.0

It has been reported that the *Jatropha curcas* contains flavonoids which play a role not only inhibit bacterial growth but also inhibit the formation of prostaglandins and stimulate blood cells to accelerate the granulation of tissues in the body [37]. Saponins can increase the optimal supply of oxygen and nutrients and increase the new blood vessel formation needed in wound healing components (angiogenesis) [38]. The flavonoids and saponins contained in the *Jatropha curcas* sap stimulate the formation of new epithelial cells and support the epithelialization process, so that a reduction in wound size has a positive correlation with the re-epithelialization process. The faster the re-epithelialization process, the smaller the wound size will be, thus shortening the wound healing process [39]. The present study revealed the potential antimicrobial activity of the *J. spinosa* latex extracts, which could be attributed to the presence of secondary metabolite products that may be similar or close to *Jatropha curcas* latex constituents. To the best of our knowledge, this is the first antibacterial study report on the different extracts of the latex from *Jatropha spinosa*. More studies should be carried out to determine the active ingredients of the latex extracts to discover new compounds that have an antibacterial effect against resistant bacteria. Also,

to make an ointment formulation from the latex of *Jatropha spinosa*.

CONCLUSION

This study aimed to find effective plants to treat infections caused by Gram-positive bacteria *S. aureus*, *B. cereus*, *S. pyogenes*, and Gram-negative bacteria *E. coli*, *Klebsiella*, and *P. aeruginosa*. Based on the results of the study, the *Jatropha spinosa* latex extracts had antibacterial activity. The findings of the study support the view that certain medicinal plants are promising sources of potential antibacterial compounds and may be effective as a preventive pathogenesis of some diseases.

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