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In vitro Antimicrobial Efficiency of Crude Extracts of Wild *Jatropha glauca* Plant Leaves Grown Naturally at Al-Baha Region, KSA

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Abstract The crude extracts from leaves of *Jatropha glauca* plant grown naturally at Al-Baha region obtained by extraction with ethanol and fractionated using three solvents: petroleum ether, ethyl acetate and methanol were investigated for their *in vitro* antimicrobial susceptibility. The antimicrobial activity were evaluated against two bacterial strains *S. aureus and E. coli*, and two common fungal strains *Candida albicans* and *Candida krusei* using disc diffusion assay. The crude ethanolic extract and its fractions methanolic and ethyl acetate showed an inhibitory effect against both bacterial and fungal microorganisms. The petroleum ether fraction had no antimicrobial effect. The ethanolic crude extract and methanolic fraction showed higher zone of inhibition, while the ethyl acetate fraction showed the least activity against both bacterial strains. The methanolic fraction was found to possess lowest MIC ≥250 μgml^{-1} against both tested bacterial strains. Similarly, the ethanolic crude extract and methanol fraction showed zone of inhibition against tested fungal strains with methanolic fraction showed the highest zone of inhibition. However, these observations indicated that the ethanolic crude extract and its methanolic and ethyl acetate fractions exhibited some antimicrobial potency that proves the leaves contain some gradients that have antibacterial and antifungal potential.

Keywords: *Jatropha glauca, crude extract, antimicrobial activity, MIC*

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1. Introduction

Natural products from medicinal plants have played very important role in health care and prevention of diseases and some research findings led up to design and production of natural plant-based pharmaceuticals [1]. Compared with synthetic compounds, natural products inherently consist of wide chemicals of structural diversity that are bioactive agents. This fact, encouraged researchers to search and discover many medicinal drugs based on natural products for treatment of various health problems [2,3,4,5]. In addition, the revitalization of interest in plant-derived drugs is mainly due to the invasive belief that 'herbal drugs' are safe and more reliable than the expensive synthetic drugs, many of which may have toxic and adverse side effects. Thus, there is a growing interest to explore the alternative drugs from different plant species that have biological activities and can be used as antibiotic resources [6,7]. The ancient civilizations of Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases [8]. Many plant materials have been used in traditional medicine long ago and still used and proved their efficiency [1]. Natural product medicines have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates [9].

Jatropha glauca is a medicinal plant belongs to genus *Jatropha* and family euphorbiaceae. It is a little-branched shrub growing up to 1 meter tall (Figure 1). It occurs in open bush land of semi-desert conditions, on lava and limestone, and present as natural vegetation in Al-Baha region and widely distributed in *Tihamah of As-Sarah* Mountains [10]. Locally it is known as "*Albukka*" and the name is due to the abundance of its juice that resembles white milk. *Jatropha gluaca* has potential moluscicidal activity with LD_{50} in the range of 10-100 ppm [11]. In Ethiopia traditional medicine the crushed plant with water is used for treatment of constipation and as ear drops for treatment of earache, and in Sudan the root and stem infusion mixture is used for treatment of epilepsy and rabies [12]. In Al-Baha region, Saudi Arabia, the extraction juice is used for treatment of camel and cattle scabies [10]. The juice of its stem causes excessive diarrhea that weakened the body [10]. Seeds of *J. gluaca* also showed toxicity in ruminants [13]. *J. multifida* roots extract showed antimicrobial activity, and the phytochemical constituents isolated from Jatropha species showed antimicrobial activity too [14,15,16]. Sanchez-Medina et al. (2001) reported antioxidant activity of crude extracts from some Jatropha species [17]. Chunkant et al and Oyi et al reported the antimicrobial activity of extract from *J.*

Curcas [18,19]. *J. curcas* also showed anti HIV activity [20]. *J. neopauciflora* bark reported as cytotoxic activity [21].

Figure 1. Jatropha glauca from Al-Baha region

Al Khaider (2016) carried out a comparative study of callus induction, oil constituent, phytochemical screening and genetic profiles of seeds extracts of *Jatropha glauca L.* and *Jatropha curcas L.* in Sudan [22].

The literature survey pointed out that *J. glauca* is multipurpose shrub with a variety of applications and enormous economic potentials for their seed oil, which can be converted into biodiesel an alternative to petro-diesel. *Jatropha* oil is an environmentally safe, cost-effective renewable source of nonconventional energy, and a promising substitute for diesel, kerosene and other fuels [23]. Most of the economically important *Jatropha* species viz, *J. glauca* and others are reported to have a cocktail of toxins including phorbol esters [24, 25].

There is a major problem because of the emergence of new resistance pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases, such as *Staphylococcus aureus* bacteria resistant to penicillin **[26]**. Despite much research and development, there is no approved vaccine for *S. aureus*. In view of this situation researchers are searching new antimicrobial substances that are natural plant-based economically feasible which are expected to be good sources of novel phytomedicine and an effective alternative antimicrobial, anticancer, anti-inflammatory, anti-diabetic and other chemotherapeutic agents [27,28].

To the best of our knowledge, there are no available reports on chemical composition and biological activities of the crude extracts from aerial parts of *J. glauca* growing naturally in Al-Baha region. Therefore, the present study was conducted to investigate the in vitro screening antimicrobial properties of ethanolic crude leaves extract of *J. glauca* plant grown in Al-Baha region and its fractionated solutions with methanol, petroleum ether and ethyl acetate. This is the first study reporting the activity of the crude extracts of *J. glauca* leaves and its fractions against bacterial and fungal strains.

2. Materials and Methods

2.1. Chemicals and Media

Methanol, ethyl acetate, petroleum ether, dimethyl sulfoxide (DMSO) and ethanol were of reagent grade and were purchased from Sigma-Aldrich and Merck chemical companies. The Muller Hinton Agar (MHA) and sabouraud dextrose agar media used for antibacterial and antifungal activity were purchased from Himedia. The standard drugs Ciprofloxacin and Fluconazole were purchased from local pharmacy.

2.2. Plant Material and Extraction

The plants material were collected from plants grown naturally in Al Baha region, south-west part of Saudi Arabia with an elevation of about 2155m above sea level. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, Dr Haider, Department of Biology, Albaha University, Al-Baha-Saudi Arabia. The aerial parts of *J. gluaca* plant were collected during the month of September 2017 from Al-Baha region. The leaves of the plant were separated and washed first with normal tap water and then with distilled water and finally with deionized water to remove any sediment particles or any other impurities that may attach to the surface of the leaves. The separated clean leaves were dried at normal air in the shade at room temperature conditions for nearly four days to avoid any loss of constituents. The dried leaves were kept in an oven for overnight at 35 °C until constant weight is reached. The dried leaves were then grinded in a grinder to get a homogeneous powder. The dried powdered leaves of plant (500 g) were extracted successively with 1 litre of ethanol (95% ethanol) by Soxhlet apparatus for 72 h at a temperature not exceeding the boiling point of the solvent. The ethanolic extract was evaporated using rotatory evaporator under reduced pressure to get crude ethanolic extract (47 g). The ethanolic crude extract was further

fractionated into three fractions utilizing petroleum ether, ethyl acetate and methanol solvents. These fractions were filtered using Whatman No. 1filter paper and then concentrated under reduced pressure at 40-45°C, using rotatory evaporator. The residues obtained were stored in freezer below -20°C until use.

2.3. Antimicrobial Activity

Two bacterial strains selected in this investigation, one is Gram-positive (*S. aureus*) and another Gram-negative (*E. Coli*) and two fungal strains *Candida albicans* the common pathogenic yeast and *Candida krusei* involved in chocolate production were used in this study. All the bacterial and fungal strains were provided by Department of Clinical Microbiology, Blood bank, Al Baha.

2.3.1. Disc-diffusion Assay

The dried ethanolic extract and the various fractions (petroleum ether, ethyl acetate and methanol) were dissolved in DMSO to a final concentration of 50 mg/mL and 25 mg/mL and were sterilized by filtration using 0.45 µm Millipore filters. Antimicrobial tests were carried out by disc-diffusion method [29] using 100 µL of suspension containing 108CFU/mL of bacteria, and 104 spore/mL of fungi. Muller Hinton Agar (MHA) used as growth medium for bacteria at 37°C, and sabourand dextrose agar (SDA) medium was used to culture fungal strains at 30°C. The discs (6mm in diameter) were impregnated with 10 µL of the extracts (500µg/disc) at the concentration of 50 mg/mL, (250µg/disc) at the concentration of 25 mg/mL and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ciprofloxacin (20 µg/disc) and Fluconazole (10 µg/disc) were used as positive reference standards for bacterial and fungal strains respectively to determine the sensitivity of one strain/isolate in each microbial species tested.

The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains and at 30°C for 72 h for fungal strains. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the test organisms. Each assay in this experiment was repeated twice and all data (Table 1 and Table 2) were expressed as arithmetic means of the duplicate measurements.

2.3.2. Microdilution Assay

The minimal inhibition concentration (MIC) of active fractions were performed using the successive microdilution test in 96 wells plate according to the methodology of Bicalho et al. (2003) [30]. The stock solution of the samples were prepared by dissolving 8mg of extract in 5mL of dimethylsulfoxide (DMSO), Ciprofloxacin and Fluconazole were used as positive control for bacterial and fungal strains respectively. Stock solution of positive controls was prepared in same concentration as that of plant extracts. The concentration of the plant extracts ranged from 500 µg/mL to 3.9 µg/mL and for the positive control from 100 µg/mL to 0.78 µg/mL. The incubation period was 24 h for bacteria and 72 h for fungi, MIC was determined when turbidity emerges in the wells. Four microbial strains were used to study the antimicrobial activity, two of which are bacteria *S. Aureus* (Gram-positive), *E. coli* (Gram-negative) and the other two are fungi *C. Albicans* and *C. Krusei*. All tests were performed in triplicate and all data (Table 3) were expressed as arithmetic means of the triplicate measurements. Extracts were considered active when there was inhibition at concentrations below or equal to 500 µg/ml. The substances were considered active when there was inhibition at concentrations below or equal to 100 µg/mL.

3. Results and Discussion

In the present study, the disc diffusion observations reveal that the ethanolic crude extract, methanolic fraction and ethyl acetate fraction were active against the tested bacterial and fungal strains, whereas the petroleum ether fraction was inactive against all the tested strains. Zone of inhibition of the tested extracts were given in Table 1 and Table 2.

Table 1. Zone of inhibition (mm) of ethanolic crude extract and various fractions against bacterial strains

S.No.	Plant extract	Antibacterial activity (Zone of inhibition in mm)			
		S. aureus		*E. coli*	
		500 µg/disc	250 µg/disc	500 µg/disc	250 µg/disc
1	95% Ethanol	20	12	16	10
2	Petroleum ether	-	-	-	-
3	Ethyl acetate	15	8	12	8
4	Methanol	22	14	14	12
5	Ciprofloxacin (20µg/disc/10µg/disc)	24	14	22	15

Table 2. Zone of inhibition of ethanolic crude extract and various fractions against fungal strains

S.No.	Plant extract	Antifungal activity (Zone of inhibition in mm)			
		C. albicans		*C. krusei*	
		500 µg/disc	250 µg/disc	500 µg/disc	250 µg/disc
1	95% Ethanol	18	14	12	8
2	Petroleum ether	--	--	--	--
3	Ethyl acetate	12	8	10	--
4	Methanol	20	16	14	8
5	Fluconazole (10 µg/disc)	23		12	

Table 3. MIC assay of *J. glauca* leave extract and fractions against bacterial and fungal strains

S.No.	Plant extract	Minimum inhibitory concentration (MIC) μgml^{-1}			
		Bacterial Strains		*Fungal strains*	
		S. aureus	*E. coli*	*C. albicans*	*C.Krusei*
1	95% Ethanol	≥250	≥500	≥500	---
2	Ethyl acetate	≥500	≥500	---	--
3	Methanol	≥250	≥250	≥250	--
4	Ciprofloxacin	≥ 12.5	≥ 25.0	NT	NT
5	Fluconazole			≥ 6.5	----

** NT: not determined.

The ethanolic crude extract were active against both bacterial strains and showed 20, 12mm for *S. aureus* and 16, 10mm for *E. coli* zone of inhibition at 500 µg/disc and 250 µg/disc respectively. Similarly, the methanolic fraction was active against both the bacterial strains and showed 22, 14mm for *S. aureus* and 14, 12mm for *E. Coli* zone of inhibition at 500 µg/disc and 250 µg/disc respectively. The ethyl acetate fraction was less active and showed zone of inhibition in the range 8-15 mm. However, petroleum ether fraction of J.*glauca* had no activity against all the tested bacterial strains. The crude ethanolic extract, methanolic fraction and ethyl acetate fractions were also active against the tested fungal strains, where methanolic extract showed highest zone of inhibition against the tested fungal strains and the zone of inhibition was in the range 16-20 for *C. albicans* and 8-14mm for *C. krusei* at 500 µg/disc and 250 µg/disc respectively. The two most active extracts (ethanolic extract and methanolic fraction) were further evaluated for their minimum inhibitory concentration (MIC).The microdilution assay showed that that the ethanolic extract and methanolic extract were more active against bacterial strain *S. aureus* with MIC ≥250 µg/mL. The methanolic extract also exhibited same MIC against *E.Coli.* Microdilution assay was also performed against fungal strains, where methanolic extract was the only fraction, which showed MIC ≥250 µg/mL against *C. albicans.* While *C. Krusei* was resistant towards both extracts, with MIC ≥ 500 µg/mL (Table 3).

Literature also showed that polar solvent extracts (Methanol and chloroform) have antimicrobial active substances [31]. The results observed in this investigation demonstrate that ethanol and methanol extract of *J. glauca* may contain antimicrobial active substances with antibacterial and antifungal potential.

4. Conclusion

It may be concluded that *Jatropaglauca* leaves possess significant antimicrobial activity that comparable to standard drug ciprofloxacin and fluconazole. *Jatropa glauca* may be a potent medicinal plant for the treatment of some antibacterial and antifungal diseases. The phytochemical investigation is required to isolate the active compounds present in the ethanolic extract and the various fractions of *Jatropa glauca* leaves.

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