

In vitro antimicrobial activity of crude extracts of Jatropha species

Rajesh S. Gaikwad¹, Rajendra B. Kakde², Anil U. Kulkarni³, Deepmala R. Gaikwad⁴ and V.H. Panchal⁵

^{1, 2,4}Swami Vivekanand, Arts, Commerce and Science College, Mantha, Jalna, (M.S.) India.
³Lal Bahadurshashtri Sr. College, Partur, Jalna, (M.S.) India.
⁵Nutan Mahavidyalaya, Selu, Jalna, (M.S.) India.

Abstract

Leaf extracts, stem extract, roots extract, latex and oil of *Jatropha curcas*, *J. glandulufera*, *J. integerrima* and *J. gossypofolia* were screened in order to study their effect on plant pathogenic fungi *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, and *Rhizoctonia solani* and plant pathogenic bacteria *Erwinia carotovora pv. Carotovora*, *Pseudomonas aeruginosa*, *Xanthomonas campestris pv. Citri* and *Xanthomonas campestris pv. mangiferaeindicae*. Degree of variation of antifungal and antibacterial activity of different parts of *Jatropha sp.* was observed.

Keywords: Jatropha curcas, J. glandulufera, J. integerrima and J. gossypofolia, plant pathogenic bacteria and plant pathogenic fungi.

INTRODUCTION

India possesses a variety of medicinal plants and it is one of the richest countries in the world in regard to genetic resources of medicinal plants. India exhibits a wide range in topography and climate, which bears varietal emporium of vegetation and floristic composition [1]. Historically plants have provided a good source of anti-infective agents with compounds which are highly effective instruments in the fight against microbial infections. Infectious diseases are the leading cause of death world-wide. Phytochemicals derived from plants have shown great promise in the treatment of obstinate infectious diseases. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Now a day's antibiotic resistance has become a global concern [2] as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [3]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind [4]. Therefore, researchers are increasingly turning their attention to folk medicine and looking for new leads day by day to develop better drugs against microbial infections [5]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [6]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections [7] in near future. Jatropha curcas. is a medicinal crop that belongs to the family Euphorbiaceae and has a long history of cultivation in tropical America, Africa, and Asia [8]. The seed kernels contain a high

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*Corresponding Author

Rajesh S. Gaikwad Swami Vivekanand, Arts, Commerce and Science College, Mantha, Jalna, (M.S.)

Swami Vivekanand, Arts, Commerce and Science College,Mantna, Jaina, (M.S.) India.

Email: drrsgaikwad@gmail.com

amount of oil (58-60%) [9] and serve as a potential source of biodiesel currently being used in India. The inhibitory activity of plant extracts is generally depends upon the concentration, type of parts used and microbes tested [10]. The accumulation and concentration of secondarymetabolites which are responsible for inhibitory activity is varied according the plant parts [11 and 12]. It may be a reason for the variation in the inhibitory activity of extracts of J. curcas. Extracts from various parts of Jatropha curcas, such as seeds and leaves, have shown molluscicidal, insecticidal, and fungicidal properties [13, 14, 15, 16 and 17]. Jatropha curcas seed extracts were found to inhibit the mycelial growth of Colletotrichum musae that causes anthracnose disease in bananas [18]. Its leaf extract was effective in controlling the fungal pathogen Sclerotium sp., which causes Azolla disease [19]. J. gossypifolia is used as a therapeutic agent in different ways. The leaf decoction of this is used for bathing wounds [20]. The leaf bath is used for sores, sprains, rash and bewitchment in Latin America and the Caribbean [21, 22].

Several studies have confirmed the antimicrobial efficacy of different *Jatropha* species; however, there is insufficient information regarding the antimicrobial activities of *J. curcas* Linn. Whatever limited information available on the medicinal properties of *J. curcas* is mostly on the leaf extracts of the plant. In this paper, the antimicrobial property of crude extracts of the stem bark extract, root extract, latex and oil of *Jatropha* sp. has been studied as part of the exploration for new and novel bio-active compounds.

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. The aim of this study is to investigate the antimicrobial activity of *Jatropha sp.*

MATERIALS AND METHODS Antifungal activity

For evaluating antifungal activity of different extracts fungal broth assay [23] was used. Crude water extracts of root, stem and leaves of *Jatropha* sp. were prepared. 10gm of each sample was extracted with 100 ml of solvents. Allow the maturation of extracts for overnight. On the next day extracts were filtered then used as test

samples. Extracts were used in the concentration 20 %. Similarly, latex and oil of *Jatropha* sp. were used as test samples. Latex and oil were used in percentage 5 to 30 %. Test organisms used were *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Rhizoctonia solani* and *Trichoderma viride*. All test organisms were inoculated in the Glucose Nitrate Broth containing 0.1 ml test sample and incubated at a room temperature for 72 hrs. After incubation period fungal mass was filtered and wet weight was taken. Fungal mass was dried and dry weight was taken.

Antibacterial activity

For evaluating antibacterial activity of different samples extracts, agar well diffusion assay [24] was used. Crude water extracts of root, stem and leaves of Jatropha sp. were prepared. 10 gm of each sample was extracted with 100 ml of solvents. Allow the maturation of extracts for overnight. On the next day extracts were filtered then used as test samples. Similarly latex and oil of Jatropha sp. were used as test samples. Test micro-organisms used are plant pathogenic bacteria like Erwinia carotovora pv. Carotovora. Pseudomonas aeruginosa, Xanthomonas compestris pv. Citri, Xanthomonas compestris pv. Mangiferaeindicae and human pathogenic bacteria like Bacillus cereus, Bacillus megaterium, Escherishia coli, Proteus vulgaris, Staphylococcus aureus and Salmonella typhi. First all test organisms were inoculated in a 10 ml of nutrient broth and incubated for overnight at 37°C. On the next day the 2 ml aliquot of inoculum mixed with nutrient agar and poured in sterile petriplates. The medium was allowed to cool. After solidification wells of 6mm diameter were prepared with cork borer. 50 µl and 100 µl of each test samples were added in the well. All procedures were carried out in sterile conditions. Then plates were incubated at 37°C for 24 hrs. Water was used as negative control. Each sample was done in triplicate. Antibacterial activity was evaluated by guantifying zones of inhibition of bacterial growth after 24 hrs.

RESULTS AND DISCUSSION

Bioactivity of *Jatropha* sp. leaf extracts against plant pathogenic fungi

In order to understand the fungal properties of leaf extract of different species of *Jatropha*, the extracts of four species of *Jatropha* at 20% concentration were tested against growth and sporulation of six plant pathogenic fungi. The results are given in table 1.

All the four species of *Jatropha* showed inhibitory nature for mycelial growth of all the fungi tested, however *Jatropha curcas* exhibited maximum inhibitory action as compared to *J. glandulufera, J. integerrima, J. gossypofolia* (Fig. 1).

Bioactivity of *Jatropha* sp. stem extracts against plant pathogenic fungi

In order to study the fungal properties of stem extracts of different *Jatropha* sp., the extract of four species of *Jatropha* at 20% concentration were tested against plant pathogenic fungi. The results are summarized on table 2.

All four species of *Jatropha* showed inhibitory nature for mycelial growth of all the six fungi tested. However stem extract of *Jatropha glandulifera* against *Alternaria alternata* showed simulating nature. Stem extract of *Jatropha gossypifolia* against *Aspergillus*

flavus exhibited similar response as in control.

Bioactivity of *Jatropha* species root extracts against plant pathogenic fungi

Fresh root extract of four *Jatropha* sp. at 20% concentration were tested against six different plants to know their effect of pathogens nutrient media without root extracts used as control. The results are given in table 3.

All four species of *Jatropha* showed inhibitory growth of mycelia of all the six plant pathogenic fungi tested. Only *Jatropha* gossypifolia showed stimulatory effect on *Aspergillus niger*.

Bioactivity of *Jatropha curcas* latex against plant pathogenic fungi

In order to study bioefficacy of latex against plant pathogenic fungi. The latex of four species of *Jatropha* was freshly collected and at a particular concentration was tested against growth of six pathogenic fungi and results are summarized in table 4.

Latex of all the four species of *Jatropha* showed inhibitory action with more or less degree with different fungi. Latex of *Jatropha curcas* proved to be highly inhibitory for mycelial growth of all the fungi tested. It was interesting to note that *Aspergillus niger* was found to be stimulated for growth in presence of latex of *J. gossypifolia* while it was inhibited by other three species of *Jatropha*.

Bioactivity of Jatropha curcas oil against plant pathogenic fungi

In order to study the antifungal activities of oil of four different *Jatropha* sp. oil at 5%, 10%, 15%, 20%, 25%, 30% concentration were tested against growth and sporulation of four plant pathogenic fungi. The results are summarized in table 5.

Oil inhibits the growth of plant pathogenic fungi. The results also highlighted that as increased the oil concentration the fungi inhibited their growth with respect to increased concentrations of *Jatropha* oil.

Bioactivity of *Jatropha* species leaf extracts against plant pathogenic bacteria

In order to study the bioactivity of leaf extract of different *Jatropha* species against plant pathogenic bacteria, the leaf extract of *J. curcas, J. gossypifolia, J. glandulifera* and *J. integerrima* at two different concentration i.e 50 µl and 100 µl were tested against the growth of four plant pathogenic bacteria namely *Erwinia carotovora pv. Carotovroa, Pseudomonas aeruginosa, Xanthomonas campestris pv. Citri, Xanthomoas campestris pv. Mangiferaeindicae.* The results are mentioned in table 6.

All the leaf extract of four *Jatropha* species at both concentration (50 μ I and 100 μ I) prove to be highly inhibitory for the growth of plant pathogenic bacteria tested along with control. *J. curcas* was found highly effective at 100 μ I concentration against *Erwinia carotovora, Pseudomonas aeruginosa* and *Xanthomonas campestris pv. citri*, while it is less effective against *Xanthomonas campestris pv.* The leaf extract of *J. gossypifolia* at both the concentrations against all four plant pathogenic bacteria showed inhibition than other species of *Jatropha* (Fig. 2).

Fungi used			Specie	s of Jatropha		Contro
_		J. curcas	J. gossypifolia	J. glandulifera	J. integerrima	(GN)
Alternaria alternata	Mycelial growth (mg)	0.153	0.163	0.146	0.154	0.198
	Sporulation	++	++	++	++	+++
Aspergillus flavus	Mycelial growth (mg)	0.115	0.179	0.132	0.148	0.223
	Sporulation	+	++	++	++	+++
Aspergillus niger	Mycelial growth (mg)	0.133	0.162	0.135	0.138	0.187
	Sporulation	++	++	++	++	++
Fusarium oxysporum	Mycelial growth (mg)	0.113	0.193	0.168	0.169	0.250
	Sporulation	+	+++	++	++	+++
Rhizoctonia solani	Mycelial growth (mg)	0.126	0.183	0.138	0.146	0.201
	Sporulation	+	++	++	++	+++
Trichoderma viride	Mycelial growth (mg)	0.108	0.159	0.132	0.124	0.183
	Sporulation	+	++	++	+	++

Table 1. Bioactivity of Jatropha species leaf extracts against plant pathogenic fungi

Sporulation : - = Absent, + = minimum, ++ = moderate, +++ = maximum

Fungi used			Species	of Jatropha		Control
_		J. curcas	J. gossypifolia	J. glandulifera	J. integerrima	(GN)
Alternaria alternata	Mycelial growth (mg) Sporulation	0.137 ++	0.163 ++	0.205	0.158 ++	0.183 ++
Aspergillus flavus	Mycelial growth (mg) Sporulation	0.154 ++	0.224	0.217	0.170 ++	0.223
Aspergillus niger	Mycelial growth (mg) Sporulation	0.148 ++	0.167 ++	0.166 ++	0.164 ++	0.187 ++
Fusarium oxysporum	Mycelial growth (mg) Sporulation	0.132 ++	0.183 ++	0.175 ++	0.209	0.250
Rhizoctonia solani	Mycelial growth (mg) Sporulation	0.121 +	0.181 ++	0.120 +	0.188 ++	0.201
Trichoderma viride	Mycelial growth (mg) Sporulation	0.148 ++	0.169 ++	0.124 +	0.103 +	0.198

Table 2. Bioactivity of Jatropha species stem extracts against plant pathogenic fungi

Sporulation : - = Absent, + = minimum, ++ = moderate, +++ = maximum

Table 3.Bioactivity of Jatropha species root extracts against plant pathogenic fungi

Fungi used			Species	of Jatropha		Contro
_		J. curcas	J. gossypifolia	J. glandulifera	J. integerrima	(GN)
Alternaria alternata	Mycelial growth (mg)	0.105	0.117	0.128	0.166	0.181
	Sporulation	+	+	+	++	++
Aspergillus flavus	Mycelial growth (mg)	0.138	0.129	0.142	0.133	0.173
	Sporulation	++	+	++	++	++
Aspergillus niger	Mycelial growth (mg)	0.138	0.174	0.157	0.145	0.165
	Sporulation	++	++	++	++	++
Fusarium oxysporum	Mycelial growth (mg)	0.154	0.147	0.178	0.148	0.191
	Sporulation	++	++	++	++	+++
Rhizoctonia solani	Mycelial growth (mg)	0.172	0.124	0.133	0.122	0.187
	Sporulation	++	+	+	+	++
Trichoderma viride	Mycelial growth (mg)	0.119	0.126	0.139	0.127	0.184
	Sporulation	+	+	++	+	++

Sporulation : - = Absent, + = minimum, ++ = moderate, +++ = maximum

Table 4. Bioactivity of Jatropha curcas latex against plant pathogenic fungi

Fungi used				Jatrop	ha curcas			Control
		5%	10%	15%	20%	25%	30%	(GN)
Alternaria alternata	Mycelial growth (mg)	0.158	0.143	0.115	0.99	0.83	0.72	0.192
	Sporulation	++	++	+	+	+	+	++
Aspergillus niger	Mycelial growth (mg) Sporulation	0.179 ++	0.165 ++	0.148 ++	0.121 +	0.98 +	0.81 +	0.212
Rhizoctonia solani	Mycelial growth (mg)	0.162	0.152	0.143	0.119	0.109	0.97	0.199
	Sporulation	++	++	++	+	+	+	+++
Trichoderma viride	Mycelial growth (mg)	0.170	0.154	0.139	0.116	0.95	0.89	0.218
	Sporulation	++	++	++	++	+	+	+++

Sporulation : - = Absent, + = minimum, ++ = moderate, +++ = maximum

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Fungi used				Jatroph	a curcas			Control
		5%	10%	15%	20%	25%	30%	(GN)
Alternaria alternata	Mycelial growth (mg) Sporulation	0.162 ++	0.151 ++	0.139 ++	0.119 +	0.104 ++	0.92 +	0.182
Aspergillus niger	Mycelial growth (mg) Sporulation	0.189 +++	0.176 ++	0.159 ++	0.144 ++	0.124 +	0.112	0.200
Rhizoctonia solani	Mycelial growth (mg) Sporulation	0.146 ++	0.134 ++	0.123	0.110 +	0.90 +	0.83 +	0.198 +++
Trichoderma viride	Mycelial growth (mg) Sporulation	0.162 ++	0.147 ++	0.136 ++	0.128 +	0.111 ++	0.95 ++	0.196 +++

Sporulation : - = Absent, + = minimum, ++ = moderate, +++ = maximum

Table 6. Bioactivity of Jatropha species leaf extracts against plant pathogenic bacteria

	Leaf extract of Jatropha species								
Plant pathogenic bacteria	J. cu	rcas	J. gossy	vifolia	J. gland	ulifera	J. integ	gerrima	Control
i ant pathogenic bacteria	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	(GN)
			Zor	ne of inhi	bition in	mm			
Erwinia carotovora pv. carotovora	11	14	09	10	00	00	00	00	00
Pseudomonas aeruginosa	09	16	11	14	12	16	11	14	00
Xanthomonas campestris pv. citri	11	13	10	12	00	00	00	10	00
Xanthomonas campestris pv. mangiferaeindicae	00	00	11	15	10	13	00	13	00

Table 7. Bioactivity of Jatropha species stem extracts against plant pathogenic bacteria

	Stem extracts of Jatropha species								
Plant pathogenic bacteria	J. cu	rcas	J. gossy	pifolia	J. gland	ulifera	J. integ	errima	Control
I mar pathogenie Sucteria	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	(GN)
			Zo	ne of inh	ibition in	mm			
Erwinia carotovora pv. carotovora	00	00	09	11	00	00	00	00	00
Pseudomonas aeruginosa	13	14	14	18	09	11	10	13	00
Xanthomonas campestris pv. citri	00	00	00	09	00	00	00	00	00
Xanthomonas campestris pv. mangiferaeindicae	11	15	11	14	00	09	09	11	00

			Root e	xtracts of	Jatropha	species			
Plant pathogenic bacteria	J. cu	rcas	J. gossy	vifolia	J. gland	lulifera	J. integ	gerrima	Control
i mit pathogenie Saeteria	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	(GN)
			Zo	ne of inhi	bition in	mm			_
Erwinia carotovora pv. carotovora	11	14	10	12	11	13	10	13	00
Pseudomonas aeruginosa	13	16	11	12	13	14	09	11	00
Xanthomonas campestris pv. citri	09	11	09	11	00	10	09	10	00
Xanthomonas campestris pv. mangiferaeindicae	09	13	09	10	00	09	00	09	00

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Table 9. Bio	activity of Jati	ropha spec	cies latex a	gainst plan	t pathoger	nic bacteria			
	Latex from								
Plant pathogenic bacteria	J. cu	rcas	J. gossy	vifolia	J. gland	lulifera	J. integ	gerrima	Control
I mai painogenie succerm	50 µl	100 µl	50 µl	100 µl	50 µl	100 µ1	50 µl	100 µl	- (GN)
			Zo	ne of inhi	bition in	mm			
Erwinia carotovora pv. carotovora	00	00	00	00	00	09	00	10	00
Pseudomonas aeruginosa	09	14	09	11	10	12	11	14	00
Xanthomonas campestris pv. citri	00	00	00	00	00	00	00	00	00
Xanthomonas campestris pv. mangiferaeindicae	00	13	00	10	00	12	00	00	00

Table 10. Bioactivity of Jatropha species oil against plant pathogenic bacteria

	Oil from								
Plant pathogenic bacteria	J. curcas		J. gossy	vifolia	J. glandulifera		J. integerrima		Control
F B	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl	(GN)
Zone of inhibition in mm									
Erwinia carotovora pv. carotovora	10	13	09	12	10	12	09	12	00
Pseudomonas aeruginosa	12	16	12	14	11	14	09	10	00
Xanthomonas campestris pv. citri	09	10	09	12	09	09	09	00	00
Xanthomonas campestris pv. mangiferaeindicae	09	10	00	00	10	12	00	00	00

Bioactivity of *Jatropha* sp. stem extracts against plant pathogenic bacteria

In order to understand the effect of stem extract of all four *Jatropha* sp. against plant pathogenic bacteria, four different bacteria were tested at two different concentrations. The results are summarized in table 7.

Stem extract of all four *Jatropha* species at both the concentrations proved satisfactory inhibition of the growth of plant pathogenic bacteria tested along with the control. *J. gossypifolia* was found highly effective at 100 μ l concentrations against all four

bacteria which are *Erwinia carotovora pv. carotovora, Pseudomonas aeruginosa, Xanthomonas campestris pv* citri, *Xanthomonas campestris pv*. *Mangiferaeindicae.* On the other hand, the stem extracts of *J. glandulifera* was completely non-inhibitory at 50 μ l concentration against *Erwinia carotovora pv. carotovora, Xanthomonas campestris* pv. citri, *Xanthomonas campestris* pv. *mangiferaeindicae* but it was inhibitory against *Pseudomonas aeruginosa* at same concentration.

Bioacitivity of *Jatropha* sp. root extracts plant pathogenic bacteria

In order to know the bioactivity of root extract against plant pathogenic bacteria root extract of all four *Jatropha* species were tested. The root extract at two different concentrations i.e 50 μ l and 100 μ l were used against the four plant pathogenic bacteria namely *Erwinia carotovora pv carotovora, Pseudomonas aeruginosa, Xanthomonas campestris pv. Citri, Xanthomonas campestris pv. Mangiferaeindicae.* The results are given in table 8.

The root extract of all four *Jatropha* sp. at both the concentrations showed highly inhibitory response against tested plant pathogenic bacteria. *J. curcas* was found highly effective against all four bacteria at both the concentrations similarly the root extract of *J. curcas* as compared to other three species of *Jatropha* proved more inhibitory. *J. glandulifera* at 50 µl concentration against *Xanthomonas campestris pv. citrri, Xanthomonas pv. mangiferaeindicae* were completely non-inhibitory.

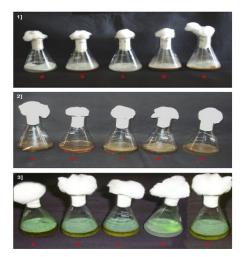


Fig 1. Bioactivity of Jatropha sp. leaf extracts against plant pathogenic fungi

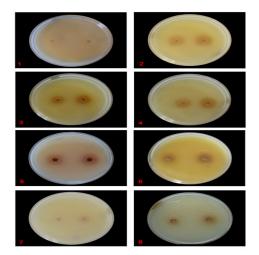


Fig 2. Bioactivity of *Jatropha* sp. leaf extracts against plant pathogenic bacteria

Bioactivity of *Jatropha* sp. latex against plant pathogenic bacteria

In order to study the bioactivity of latex of different *Jatropha* species against plant pathogenic bacteria, the latex of *J. curcas, J.*

gossypifolia, J. glandulifera, J. integerrima at two different concentrations were tested. The experiment was carried out against four different plant pathogenic bacteria namely *Erwinia carotovora pv*, *carotovora, Pseudomonas aeruginosa, Xanthomonas compestris* pv. *citri, Xanthomonas campestris* pv. *mangiferaeindicae* along with nutrient medium without latex served as control. The results are summarized in table 9.

All four *Jatropha* sp. at both the concentrations showed inhibitory action against plant pathogenic bacteria. All four species of *Jatropha* were found highly effective against *Pseudomonas aeruginosa*. While they were moderate effective against *Xanthomonas campestris pv citri*. Similarly latex of *J. glandulifera* showed satisfactory inhibitory at 100 μ I concentration than other three species of *Jatropha*.

Bioacitivity of Jatropha sp. oil against plant pathogenic bacteria

In order to know the effect of oil of *Jatropha* sp. against plant pathogenic bacteria, two different concentrations i.e 50 and 100μ I of oil were used. The activity was tested against the growth of four plant pathogenic bacteria namely *Erwinia carotovora pv carotovora*, *Pseudomonas aeruginosa, Xanthomonas campestris pv citri, Xanthomonas campestris pv Mangiferaeindicae.* The results are summarized in table 10.

Oil from *J. curcas, J. gossypifolia, J glandulifera, J. integerrima* at both the concentrations showed highly inhibitory for the growth of plant pathogenic bacteria tested. *J. curcas* was found highly effective against all four bacteria similarly the oil of *J. curcas* as compared to the three species of *Jatropha* at both the concentrations proved more inhibitory. It was interesting to note that all four species of *Jatropha* at both the concentrations showed inhibitory effect against *Ersinia carotovora* pv *carotovora, Pseudomonas aeruginosa.*

The antifungal activities of some plants extracts in controlling different pathogens have been reported by several workers [25, 26 and 27]. The inhibition is due to the fungitoxic activities of the plant extracts which agreed with the report of other workers [28]. Antimicrobial activity of stem bark extracts from *Jatropha curcas* was also carried out against different bacteria and fungi [29]. *E. coli, P. aeruginosa, S. aureus, B. cereus, B. megaterium* and *B. megaterium* were inhibited in crude extract of *Jatropha curcas*. On the contrary of that, *Salmonella typhi* was not inhibited by any of the crude extracts [30]. Castor oil seed crude extract lowers mycelia growth of *F. verticilliodes* significantly at (*P*≤0.05) compared to other treatments. Similarly, castor seed oil (crude extracts) had the lowest mycelial growth on *A. flavus* in-vitro [31].

The extract of *Jatropha curcas* seed would serve as a natural phytochemicals against bacterial and fungal phytopathogens for agricultural applications at a low cost and safe practice. *Jatropha curcas* seed, a by - product generated in large quantities by the biodiesel fuel industry, could thus be utilized as a source of the antibacterial and antifungal agents.

Results of the present study support the folkloric usage of this studied plant and suggested that its extract posses compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is thus also recommended that further studies under field condition should be done on these plants extracts to determine if their effect is fungicidal or fungistatic.

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