

Antimicrobial activity in leaf, seed extract and seed oil of *Jatropha curcas* L. plant

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Abstract: The study on antibacterial and antifungal efficacy of leaf and seed extract and seed oil of *Jatropha curcas* reveals that the inhibition zones ranged from 7 to 22 mm for antibacterial and from 10 to 20 mm for antifungal activity. Among all extracts tested, seed extract showed significant activity while leaf extract showed intermediate activity against gram-positive bacteria. In case of gram-negative bacteria, seed extract and seed oil has shown some moderate activity whereas, no activity was observed in leaf extract. In case of fungal cultures, among all test extracts only seed extract showed significant activity against *Mucor* and *Tilletia* fungus, while in case of *Rhizopus* no activity was observed against all test extracts. These results indicated the possibility of using *Jatropha* extract and seed oil for medicinal uses and natural food preservation.

Keywords: Antimicrobial activity, *Jatropha curcas* L., Crude leaf and seed extract, Seed oil, Agar disc diffusion assay

INTRODUCTION

In recent years there has been an increasing interest in the use of natural bioactive compounds, and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources. Infectious diseases caused by bacteria and fungus accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance to many antibiotics due to indiscriminate use of commercial antimicrobial drugs encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants (Karaman *et al.*, 2003).

Jatropha curcas (Euphorbiaceae family) is a multipurpose plant that has a long history of cultivation in tropical America, Africa, and Asia with many attributes, mainly as potential source of bio-fuel because its seed kernels contain a high amount of oil (58-60%) (Fairless, 2007). Besides oils, *Jatropha* seeds also contain high protein, anti-nutritional factors including trypsin inhibitor, lectin, saponin, phytic acid and toxic compounds called phorbol esters (Martinez-Herrera *et al.*, 2006). The natural compounds of *Jatropha* exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint heumatism (Aiyelaagbe *et al.*, 2007; Bhushan *et al.*, 2008). Furthermore, extracts from various parts of *Jatropha curcas*, such as seeds, seed oil, stem barks, roots and leaves have shown fungicidal (Nwosu and Okafor, 1995; Saetae and Worapot, 2010) and bactericidal properties (Igbinosa *et al.*, 2009).

The present study was aimed to evaluate the antibacterial and antifungal efficacy of crude leaf and seed extract and seed oil of *Jatropha curcas* on two gram-positive bacteria (*Bacillus subtilis* and *Bacillus thuringiensis*), four gram-negative bacteria (*Pseudomonas fluorescens*, *Escherichia coli*, *Agrobacterium tumefaciens* and *Acinetobacter junii*) and three fungal cultures (*Rhizopus oryzae*, *Mucor indicus* and *Tilletia indica*) causing infections/diseases.

MATERIALS AND METHODS

Bacterial cultures namely, *B. subtilis* (NCTC 10073), *B. thuringiensis* (MTCC 4714), *P. fluorescens* (NCTC 10038), *E. coli* (NCTC 9002), *A. tumefaciens* (MTCC 609) and *A. junii* (MTCC 1686) and fungal cultures namely, *Rhizopus oryzae* (MTCC 262), *M. indicus* (MTCC 3318) and *T. indica* were obtained from Department of Molecular Biology Genetic Engineering, G. B. Pant University of Agriculture & Technology, Pantnagar.

Jatropha curcas leaves were collected from DIBER, Haldwani, where the plant grows under natural conditions. The fresh leaves (5 gm) were ground in porcelain mortar and the leaf paste was squeezed through muslin cloth to give fresh crude leaf extract and 10 µl of the extract was applied on disc 1. The same extract was concentrated (four fold) in centrivap concentrator (Labconco, Biogentek, India) and 10 µl of this was applied on disc 2. The extract was also dried completely in centrivap concentrator and dissolved in 50 µl of Dimethyl Sulphoxide (DMSO) by keeping the tubes in the

water bath at 37°C for one hour and cleared by centrifugation at 10,000 x g for 2 min and 10 µl of the supernatant was applied on disc 3. Clarified crude leaf extract (centrifuged at 10000 x g for 10 min) was concentrated four fold in centrivap concentrator and 10 µl of this was applied on disc 4. The remaining aliquot of this supernatant was dried completely in centrivap concentrator and dissolved in 50 µl of DMSO as described earlier and 10 µl of this was applied on disc 5. Mature seeds (5 g) of *Jatropha curcas* were also ground in porcelain mortar using 5 ml DMSO as described earlier to give fresh crude seed extract and 10 µl of the extract were applied on disc 6. The crude seed extract was concentrated (four fold) in centrivap concentrator and 10 µl of this was applied on disc 7. Dried seeds were powdered and successively extracted in a Soxhlet extractor for 8 h at 65°C with petroleum ether for the extraction of *Jatropha* seed oil according to the standard procedure of Soxhlet (1879) and 10 µl of fresh seed oil was applied on disc 8. Standard antibacterial (Kanamycin, 1000 µg/ml) and antifungal (Clotrimazole, 100 µg/ml) drugs were applied separately on disc 9 as positive control. The DMSO solvent was also applied on disc 10 as negative control.

In vitro antimicrobial studies were carried out on different bacterial and fungal cultures against test extracts (leaf, seed extract and seed oil) by agar disc diffusion assay method (Gupta *et al.*, 2009). A 100 µl aliquots of bacterial (about 10⁶ cells/ml) and fungal cultures (about 10⁸ cfu/ml) were evenly spreaded on to LA (Luria agar, Himedia) and PDA (Potato dextrose agar, Himedia) plates (90 mm

diameter) using a sterile glass rod spreader, respectively. The plates were left at room temperature for 15 min. to allow the agar surface to dry. The sterile disc (6 mm, Himedia) containing the standard drug and test compounds were placed on the inoculated plates. The LA and PDA plates were incubated at 37°C and 30°C for 24 to 48 h, respectively. Experiment was performed under strict aseptic conditions. Cleared zones were scored as antimicrobial activity of standard drug and test compounds against different tested microorganisms. The antibacterial and antifungal activity was evaluated by measuring the diameter of the inhibition zone. The experiments were performed in triplicate and the data were calculated as mean ± SD.

RESULTS AND DISCUSSION

In order to investigate the antibacterial and antifungal activity of different extracts (leaf, seed extracts and seed oil) of *Jatropha* plant, disc diffusion assay was performed. The presence of zones of clearance speculates that the extract present in the disc has antimicrobial activity. In our experiment among 8 extracts (Fresh crude leaf extract, concentrated crude leaf extract, dried crude leaf extract dissolved in DMSO, concentrated supernatant of fresh crude leaf extract, dried supernatant of fresh crude leaf extract dissolved in DMSO, fresh crude seed extract, concentrated crude seed extract and seed oil) tested, 2 extracts (Crude seed extract and concentrated crude seed extract) of *Jatropha* showed significant and 3 extracts (Dried crude leaf extract dissolved in DMSO, concentrated supernatant of crude leaf extract and dried supernatant

Table 1. Antimicrobial activity in leaf, seed extract and seed oil of *Jatropha curcas*.

Disc No.	Compound	Antibacterial activity (Diameter of inhibition zone in mm)					Antifungal activity (Diameter of inhibition zone in mm)			
		BT	BS	AGRO	ESH	PSE	ACI	RHI	MUC	TILL
1	Fresh crude leaf extract	-	-	-	-	-	-	-	-	-
2	Concentrated crude leaf extract	-	-	-	-	-	-	-	-	-
3	Dried crude leaf extract dissolved in DMSO	7	7	-	-	-	-	-	-	-
4	Concentrated supernatant of fresh crude leaf extract	7	7	-	-	-	-	-	-	-
5	Dried supernatant of fresh crude leaf extract dissolved in DMSO	8	8	-	-	-	-	-	-	-
6	Fresh crude seed extract	13	10	7	7	7	7	-	10	-
7	Concentrated crude seed extract	14	11	8	7	7	8	-	11	13
8	Seed oil	-	7	-	7	-	7	-	-	-
9	Standard drug	18	22	21	20	15	17	20	19	18
10	DMSO	-	-	-	-	-	-	-	-	-

BT = *Bacillus thuringiensis*, BS = *Bacillus subtilis*, AGRO = *Agrobacterium tumefaciens*, ESH = *E. coli*, PSE = *Pseudomonas fluorescens*, ACI = *Acinetobacter junii*, RHI = *Rhizopus oryzae*, MUC = *Mucor indicus* and TILL = *Tilletia indica*.

of crude leaf extract dissolved in DMSO) showed intermediate activity against both gram-positive bacteria. In case of all gram-negative bacteria, Fresh crude seed extract, concentrated crude seed extract and seed oil has shown some moderate much activity whereas, no activity was observed in any leaf extracts (Table 1). Higher resistance of gram-negative bacteria to external agents has been earlier reported and it is attributed to the presence of lipopolysaccharides in their outer membrane, which make them inherently resistance to antibiotic, detergent and hydrophilic dyes (Nikaido and Varra, 1985). Similar trend for inhibition of bacterial growth has been observed in earlier studies with other plant extract (Negi and Jayaprakasha, 2003). The reason for higher sensitivity of the gram-positive bacteria than negative bacteria could be ascribed to the differences between their cell wall compositions. The gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier (Scherrer and Gerhardt, 1971). In case of fungal cultures, among all test extracts only crude seed extract (Disc 6) and concentrated crude seed extract (Disc 7) showed significant activity against *Mucor* and *Tilletia* fungus, while in case of *Rhizopus* no activity was observed against all test extracts. Concentrated crude seed extract (Disc 7) of *Jatropha* exhibited highest antifungal and antibacterial activity as compared to other extract tested. The inhibition zones ranged from 7 to 22 mm for antibacterial and from 10 to 20 for antifungal activity, respectively (Table 1). According to Bauer *et al.* (1996) the microbicidal activity is classified into resistant if the zone of inhibition in millimeter is less than 7. If it is 7-9 mm intermediate, if the inhibition is 10 or more it is sensitive. Hence in this study leaf, seed extract and seed oil of *Jatropha* gives sensitive microbicidal activity.

Jatropha curcas contains higher steroids, terpenoids (mainly, phorbol ester), flavonoids and alkaloids (mainly, jatrophine) compounds in the leaf and seed in comparison to other species of *Jatropha* (Krishnan and Paramathma, 2009). It is speculated that higher antibacterial and antifungal activities of different extract of *Jatropha* may be due to presence of these phenolic compounds. The *Jatropha* leaf extract was effective in controlling the fungal pathogens *Sclerotium* sp. and *Colletotrichum musae* which, causes azolla and anthracnose diseases, respectively (Thangavelu *et al.*, 2004). Furthermore, the latex of *Jatropha* contains an alkaloid known as 'Jatrophine' which is used as a hemostatic and wound dressing (Esimone *et al.*, 2009) and is said to be efficacious in treating scabies, wasp and bee stings, treat bleeding gums, toothache, anti-inflammation, eczema and ringworm (de-Feo, 1989; Van-den *et al.*, 1995). In addition, antibacterial activity of extracts of *Jatropha* seed oil, root and stem bark was also reported by many workers (Solsoloy and Solsoloy, 1997; Aiyelaagbe, 2007; Igbinsosa

et al., 2009). Recently, the ethanolic extract of *J. curcas* seed cake showed antifungal activities against important fungal phytopathogens (Saetae and Worapot, 2010). The microbicidal activity of *Jatropha* extracts is probably due to their ability to form complex with extra cellular and soluble proteins and bacterial cell walls by nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial actions may be related to their ability to inactive microbial adhesins, enzymes, cell envelope transport proteins, etc. There is also evidence for direct inactivation of microorganisms (Scalbert, 1991). Therefore, the present observations suggest that *Jatropha curcas* is a potential source of bioactive antimicrobial agents.

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