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Antibacterial activity of *Jatropha curcas* extract against *Pseudomonas fluorescence* and *Xanthomonas auxinopodis* P.V. *citri*)

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Abstract
Plants are considered to be the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds. In the present investigation, the antibacterial activity of methanolic extracts of *Jatropha curcas* was investigated using agar well diffusion method against two bacteria entitled *Pseudomonas fluorescence* and *Xanthomonas auxinopodis* P. V. *citri*. The antibacterial activity was measured by diameter zone of inhibition and minimum inhibitory concentration (MIC). The extracts exhibited broad spectrum antibacterial activities against the microorganisms. The extracts of seed, seed coat, root, leaves and bark were used at different concentrations at 1%, 2.5%, 5%, 7.5%, 10% and 15%. Amongst all the crude methanolic root extract with 5% concentration found to be the best extractant. The root extract showed (15mm and 14.8mm) maximum zone of inhibition at 5% concentration against *Pseudomonas fluroscence* and *Xanthomonas auxinopodis P.V. citri* respectively. The result of this study indicates that this plant contains compounds with antibacterial activity which validates their use for treatment of various microbial infections in traditional medicine. The findings provide the basis for further study on the plants with aim of isolating and identifying the active substances.

Keywords: *Jatropha curcas*, *Pseudomonas fluroscence, Xanthomonas auxinopodis* P.V. *citri*, Methanol, Antibacterial activity

1. Introduction
Plants and herbs, since times immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from traditional/folklore practices have been traced to the occurrence of natural products with medicinal properties. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed and about 80% of the world's population relies on herbal medicines (UNESCO, 1996) [16].
Despite the availability of different approaches for the discovery of therapeuticals, natural products still remain as one of the best reservoirs of new structural types. Plants face many stresses in their life cycle and in the process produce secondary metabolites. These secondary metabolites are not important for the metabolic functions of the plant but help to face many stressful conditions like diseases, pests, etc. Some of these secondary metabolites have capacity to fight microorganisms and thus can be used for medicinal purposes. The active screening of natural products to yield synthetic pharmacologically active compounds is one of the most important aspects in drug development (Cragg *et al*., 1997) [5].
Aromatic and medicinal plants employed on a large scale in medicine against drug-resistant bacteria, considered as possible reason for unsuccessful treatment in infectious diseases. However, medicinal plants are the rich source of new medicines and are alternatives to the usual drugs Medicinal plants have been conventionally used as antimicrobial and anti-inflammatory agents (Tepe *et al*., 2004) [14].
Jatropha is originated from the hot region of Central and South America (Brazil) and is widely distributed in tropical and subtropical parts of the world belong to family *Euphorbiaceae* (Becker and Francis 2001) [2]. *Jatropha curcas* Linn that have been identified as a plant widely used in traditional medicine in various parts of Africa (Iwu, 1993, Burkill, 2009) [10]. *J. curcas* variously known as physic nut, purging nut or pig nut (Uche and Aprioku, 2008; Igbinosa *et al*., 2009) [15, 8] is used in folklore remedies for treatment of various ailments such as skin

infections, gonorrhea, jaundice and fever (Akinpelu *et al*., 2009) [1]. They also suggested that the antibacterial activity of the methanolic extract of the leaves of *J. curcas* against 13 bacterial species including *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The extract showed appreciable inhibitory activity against these organisms (Akinpelu *et al*., 2009) [1].

Jatropha curcas is a source of secondary metabolites of medicinal importance. The leaf, fruits, latex and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins that exhibit wide range of medicinal properties. The plant product exhibit antimicrobial activity. The seed of the plant are not only the source of biodiesel but also contains several metabolites of pharmaceutical importance. (Debnath and Bisen, 2008) [6]. The seeds are also used in the treatment of syphilis (Orwa *et al*, 2009) [11]. With a view, the present study has exploited the probability of having any antimicrobial activity present in *Jatropha curcas*. Such studies are important either for exploration of new biomolecules to be used by pharmaceutical and agrochemical industry directly or a lead molecule to synthesize more potent molecules.

Materials and methods

Plant extraction preparation

For extraction purpose, leaf, bark, seeds, seed coat and roots were used in the present investigation. The collected plant materials were watery washed, disinfected, rinsed with distilled water and dried completely in hot air oven at 40^0C. The dried plant materials were powdered using mixer grinder and used for further extraction using different solvents (methanol, aqueous methanol, acetone, ethyl acetate and hexane).

Cold extraction method

About 10 g of dried powder of seed, seed coat, root, leaves and bark were taken in separate 250 ml conical flasks with screw cap separately. Then 100 ml of each solvent (methanol, aqueous methanol, acetone, ethyl acetate, and hexane) was added to the flask. The flasks were kept at room temperature for seven days with 30 minute shaking after every 24 hr. The extracts were then filtered by using Whattman filter paper under vacuum and dried at room temperature in watch glass dish. The weight of extracts was calculated from the weight of glass dish prior to drying of extracts and weight of glass dish after drying of extracts (Harborne, 1973) [7].

Reflux extraction method

Soxhlet extraction was carried out with Universal Extraction System (Buchi). Ten grams dried powder (seed, seed coat, root, leaves and bark) was taken in glass thimble and extracted with solvents such as methanol, aqueous methanol, acetone, ethyl acetate and hexane. The procedure was carried out for 10 cycles for each extract and the temperature was adjusted just below the boiling point of the respective solvents. Most of the solvent from each extract was evaporated by using the same instrument. Further drying of the extract was carried out at room temperature. The weight of each extract was also noted as described earlier (Harborne, 1973) [7]. Amongst all the yield percentage of methanolic extract was found high compared to other solvents and therefore carried further for antibacterial activity.

Antibacterial activity of the plant extracts

Bacterial strains

The antibacterial potency for each plant part (seed, seed coat, root, leaves and bark) was evaluated using two bacterial strains *viz*. *Pseudomonas fluorescence* and *Xanthomonas auxinopodis* P. V. *citri*. The bacterial strains were obtained from the Department of Plant Pathology, Post Graduate Institute, Dr. P. D. K. V., Akola.

Inoculums preparation

The microorganisms were maintained by sub-culturing on nutrient agar medium and used to carry the antimicrobial activity. A 24 hr old culture of each indicator strain with the $O.D._{590}$ =$0.D._{650}$ was used for the preparation of bacterial suspension.

Preparation of sample for screening of antibacterial activity

The extracts and their fractions were dissolved in DMSO (Dimethyl sulfoxide) and methanol to prepare final concentration of 1%, 2.5%, 5%, 7.5%, 10%, 15%. DMSO was used as control. For antibacterial activity, 100 µL of each extract was used for the disc diffusion method, whereas, Ampicillin (10ug/ml) was used as standard. The assay was carried out in duplicates.

Screening of crude methanolic extracts from *J. curcas* for antibacterial activity

The antibacterial activity of the only crude methanolic extract was determined (because of high yield percent of extract) using agar well diffusion method described by Irobi et. al. (1994) [9] against two bacterial cultures viz., *Pseudomonas fluorescence* and *Xanthomonas auxinopodis* P. V. *citri*.

Agar Well Diffusion Method

Briefly, nutrient broth was used to culture bacteria. Fresh overnight cultures of inoculum (0.1 ml) of each culture, was spread on nutrient agar plate. The DMSO treated as test control, whereas, Ampicillin (10ug/ml) was used as positive control and the extract of different concentrations that is 1%, 2.5%, 5%, 7.5%, 10%, 15% were tested against test bacteria. 20µl of each extracts were added in wells made in agar plates. The plates were kept inverted in an incubator set at 28˚C. The zone of inhibition in mm was determined after 24 hrs. of incubation period. The microbes were plated in duplicates and average zone diameter was calculated. The plates were kept in the fridge at 4^0C for 2 hr. to permit plant extracts diffusion then incubated at 35^0C for 24 hr incubation period. The presence of inhibition zones were measured by Vernier caliper, recorded and considered as indication for antibacterial activity.

Determination of minimum inhibitory concentrations (MIC's) of the effective extract

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h of incubation. The most effective plant extracts (seed, seed coat, root, leaves and bark) with different concentrations exhibit a strong antibacterial activity to determine their MIC using disk diffusion method and evaluate their efficiency in controlling bacterial strains.

Result and Discussion

Extraction methods from *J. curcas*

The details of extraction, cold percolation and reflux extraction are given in Table 1. For cold percolation the dried tissue powder (seed, seed coat, root, leaves and bark) was extracted with both polar and non polar solvents such as

methanol, aqueous methanol (75%), acetone, ethyl acetate and hexane. Similarly reflux extraction was achieved using Universal extraction system.

Table 1: Yield of seed, bark, seed coat, leaves and roots extract with various solvents and extraction methods.

		Yield of extract %	
SN	**Name of solvents**	**Cold extraction**	**Reflux extraction**
Seed			
1	Methanol	16.0	20.8
2	Aqueous methanol (75 %)	15.1	18.1
3	Acetone	13.5	13.5
4	Ethyl acetate	11.2	14.4
5	Hexane	15.4	18.0
Bark			
1	Methanol	8.9	11.1
2	Aqueous methanol (75 %)	8.4	13.4
3	Acetone	9.1	11.0
4	Ethyl acetate	6.1	6.5
5	Hexane	5.1	7.2
Seed Coat			
1	Methanol	2.4	3.8
2	Aqueous methanol (75 %)	3.0	3.4
3	Acetone	3.1	2.8
4	Ethyl acetate	2.5	3.0
5	Hexane	2.9	3.6
Leaves			
1	Methanol	8.7	11.9
2	Aqueous methanol (75 %)	8.1	10.5
3	Acetone	8.0	11.8
4	Ethyl acetate	5.5	7.9
5	Hexane	4.6	8.3
Roots			
1	Methanol	10.4	13.5
2	Aqueous methanol (75 %)	10.2	12.2
3	Acetone	7.8	10.6
4	Ethyl acetate	6.8	7.5
5	Hexane	7.1	6.8

It was observed that in general, the extraction yield with polar solvents such as methanol was more as compared to non polar solvents such as ethyl acetate and hexane. In case of seeds the hexane has extracted the oil as *Jatropha* seed is rich source of oil. This is also reflected in the results. In almost all the methods amongst all the solvents methanol has given better yields and has been chosen as best extractants and further used to screen antibacterial activity.

Amongst the methods of extraction, reflux extraction was found to be the best method in terms of extract yield. In many cases where the bioactive molecule does not degrade with temperature, warm extraction is a method of choice as it gives comparable yields in less time than cold percolation method. Because of the purity of solvents (due to reflux) and temperature, reflux extraction may have given higher yields compared to other methods. In almost all the cases reflux extraction with methanol has given better results as compared to other methods and solvents. Similar results were also reported by Shen and Shao in 2005. Amongst all the tissues, highest yield were obtained with seeds (20.8 %), while lowest yield was obtained with seed coat (2.4%). As reflux extraction of samples with methanol has given higher yields in most cases, crude methanolic reflux extracts was selected for further exploitation of antibacterial activity/screening.

Screening of crude methanolic extract of *J. curcas* against selected bacterial strains

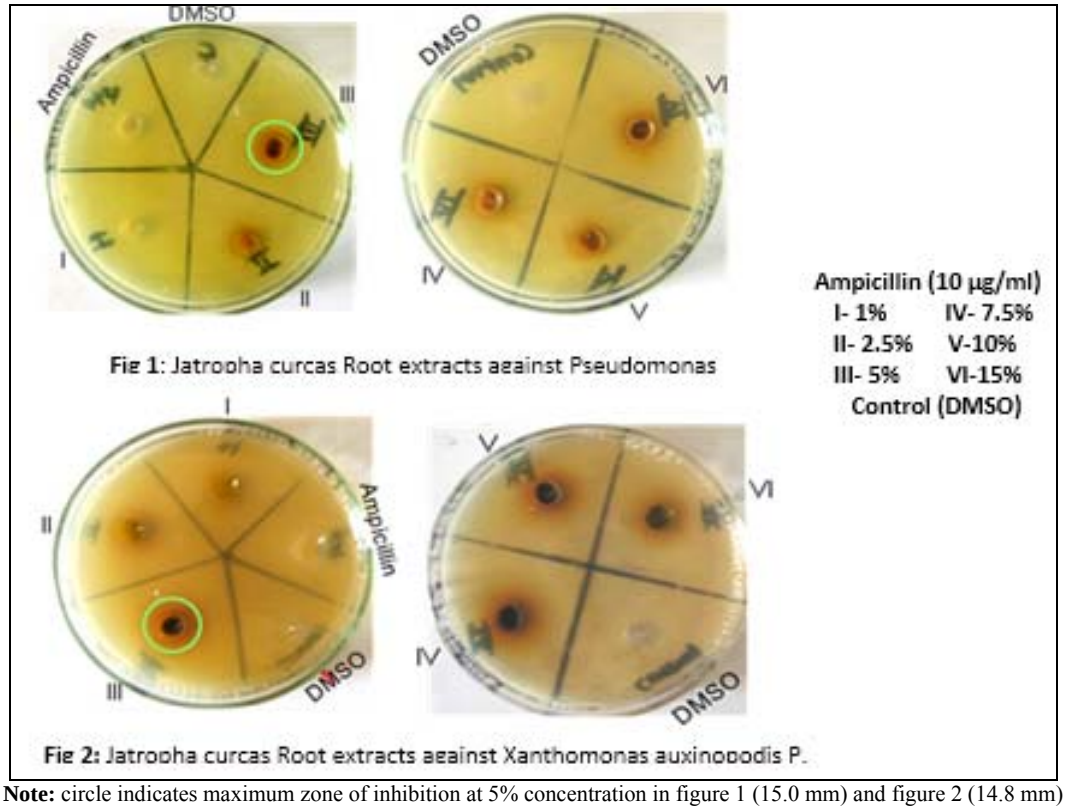
The crude methanolic extract of seed, seed coat, root, leaves and bark of *Jatropha curcas* were evaluated at different concentration (1, 2.5, 5, 7.5, 10, 15 %) against *Xanthomonas auxinopodis P. V.citri* and *Pseudomonas fluorescence* by agar well diffusion assay. Ampicillin (10 □g/ml) was used as standard while (DMSO) was used as a control. Amongst all extracts, root extract showed some antibacterial activity against both *X. auxinopodis* P. V. *citri* and *P. fluorescence* at 5% concentration. The root extracts showed (15mm and 14.8mm) maximum zone of inhibition at 5% concentration against *P. fluroscence* and *X. auxinopodis* respectively. The details are given in Table 2 and figure 1 and 2 showed zone of inhibition.

Table 2: Screening of antibacterial activity of crude methanolic root extract at different concentrations.

SN	Microorganisms	Treatment	Zone of Inhibition (mm)
1	*Pseudomonas fluroscence*	Control (DMSO)	0
		Standard (Ampicillin 10 µg/ml)	15.2
		1%	6.2
		2.5%	9.6
		5%	15.0
		7.5%	14.8
		10%	13.8

		15%	14.4
2	*Xanthomonas auxinopodis*	Control (DMSO)	0
		Standard (Ampicillin 10 µg/ml)	15.2
		1%	6.5
		2.5%	10.7
		5%	14.8
		7.5%	14.1
		10%	14.5
		15%	14.6

* Mean value of two replicates



Note: circle indicates maximum zone of inhibition at 5% concentration in figure 1 (15.0 mm) and figure 2 (14.8 mm)

For screening antibacterial activity only two organisms were selected as prokaryotic system representative. There are reports of antimicrobial activity of Jatropha against human pathogens including bacteria and fungus. Vishwanathan *et al*. (2004) [17] reported antimicrobial activity of *Jatropha maheshwari* stem solvent extract against 12 human pathogens. There are recent reports of antimicrobial activity of *J. curcas* stem extract. Igbinosa *et al*. (2009) [8] reported antibacterial activity of aqueous, methanolic and ethanolic stem extract of *J. curcas* comparable to that of standard streptomycin with minimum inhibitory concentration (MIC) less than 0.5mg/ml. Saossong and Ruangviriyachai in 2016 [12] found the potency of methanolic extract of *J. curcas* fruits against *P. putida*, *P. syringae* pv. *sesami*, *X. campestris*, *X. campestris* pv. *glycines*, *X. campestris* pv. *vesicatoria* and *R. solanacearum.* Although reports suggested the potential antibacterial activity in other extracts, our observations concluded that most of the extracts except root were having negligible or weak antibacterial activity.

Conclusion

Jatropha curcas is a medicinal plant, with its roots having very good potency against bacteria. This study has given a direction to search new molecules with antibacterial activity from *Jatropha curcas*. Further purification of extracts, characterization of active biomolecules and confirmation of bioactivity against a wide range of plant pathogens will be helpful to identify new source of antimicrobial activity which can be exploited for product development.

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