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Research Article

A Comparative Study of Antibacterial Activity of Leaves and Latex of *Jatropha curcas* L.

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ABSTRACT

Different parts of *Jatropha curcas* L. has been traditionally used for medicinal purposes to cure various diseases. In the present study the comparison of antibacterial activity of latex, leaves and their various extracts (methanolic and ethanolic) has been investigated against *Escherichia coli* (gram negative species) and *Staphylococcus aureus* (gram positive species) by using disc diffusion method. Antibacterial activity of pure latex and its ethanolic extracts has been found only against *E. coli*. But the ethanolic extract of leaves of *Jatropha curcas* L. showed antibacterial activity against both the bacterial test species. Methanolic extract of latex as well as leaves also exhibit antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. The magnitude of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* found to be significantly higher in latex and its extracts as compare to leaves. Antibacterial activity of latex and its extracts found to be higher then the antibacterial activity of standard tetracycline as compared to leaves extracts.

Key Words: Antibacterial activity, *Jatropha curcas* L., Latex, *Escherichia coli*, *Staphylococcus aureus*.

INTRODUCTION

Jatropha curcas L.is commonly known as physic nut, purging nut or pig nut or jablota (Himachali) (Fig.1). It is a multipurpose shrub or small tree belonging to the family of Euphorbiaceae. *Jatropha curcas* L originated in central America[1], but now thrives in many parts of the tropics and sub-tropics in Africa/Asia[2,3,4].

These plants have been used since time immortal to treat various diseases. In our traditional medicines the *Jatropha curcas* used for the treatment of fever, mouth infections, jaundice, guinea worm sores and joint rheumatism[5,6]. Members of rural communities of Churu district in the Thar Desert, India, used the juice from leaves of *Jatropha curcas* to cure diseases such as dysentery and colic[7].This plant also get attention due to its anti cancerous activities[8]. The latex of this plant applied on the cuts and bleeding wound soon stops the bleeding due to procoagulant activity [9,10,11]. Previous studies reveals the presence of antibacterial agents in different parts of *Jatropha curcas* L.[12,13,14]. For developing herbal treatment from various parts of *Jatropha curcas* L. which part must be more effective against bacteria.The present study aimed to compare the antibacterial potential of leaves, latex and their various extracts (methanolic and ethanolic) against *Escherichia coli and Staphylococcus aureus*. These two bacterial species causes various diseases in human beings.

MATERIALS AND METHODS

Collection of Samples: The samples were collected from the wild patch of *Jatropha curcas* L. plants grown at the Solag village of Distt. Bilaspur (N : $31^0$21.356', E :$76^0$49.737', Height: 938m amsl) in the month of April**.**

Latex from the plants collected as liquid exudates from the cut stalk of leaves and young stem and stored in coloured sterile bottles. Fresh leaves has been collected from the same plants and stored at 4°C in aluminum foil **(Fig.2).**

Preparation of Plant Extract

Latex: Known quantity of fresh latex (1 ml) was mixed with varying amount of ethanol (1 ml,2 ml,3 ml,4 ml,5 ml,6 ml) for preparing ethanolic extract and methanol (1 ml,2 ml,3 ml,4 ml,5 ml,6 ml) for preparing methanolic extract. Then mixtures were placed in auto shaker with very low speed for overnight and then filtered through the Whattman's filter paper for use.

Leaves: Fresh leaves were collected from *Jatropha curcas* L. were dried at room temperature and then ground into fine powder by using the mortal pastel. Then the known concentration of powder of leaves (10 mg, 20 mg, 30 mg, 40 mg, 50 mg and 60 mg) were mixed with 10 ml of ethanol or methanol for preparing respective extracts and kept undisturbed for 24 hrs and filtered through the Whatman's filter paper for use.

Media Used: Nutrient broth, Nutrient agar, Eosin Methyl Blue (EMB), Alcohol were used from Hemidia Laboratories Bombay. All the solutions and media were prepared in distilled water.

Test Organisms Used: In the present study two bacterial strains *Escherichia coli* (Gram-negative bacterium) and *Staphylococcus aureus* (Gram-positive bacterium) were procured from Department of Microbiology, Abhilashi

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Fig.1 : Jatropha curcas L. plant in their natural habitat.

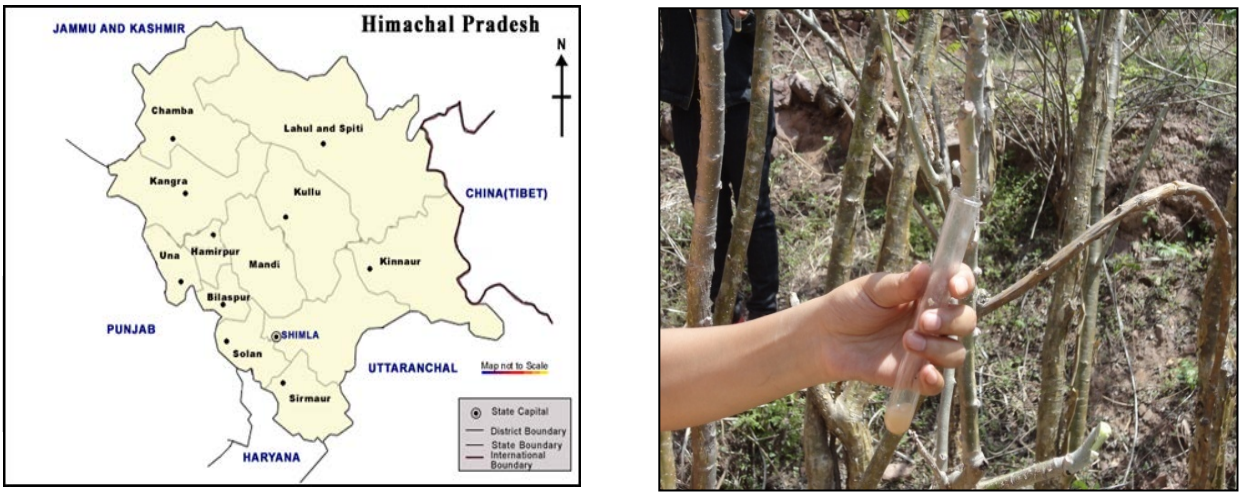

Fig.2: Place and Sample collections from Jatropha curcas L. plant.

Institute of Life Sciences, Tanda, Distt. Mandi, Himachal Pradesh.

Preparation of Suspension of Bacterial Culture**:** The tested organisms used in this study (*Escherichia coli, Staphylococcus aureus*) were firstly cultured in nutrient broth, incubated for 24 hrs in incubator shaker at 120 rpm at 37°C ± 1°C.

Determination of antibacterial activity

Paper Disc Method**:** In this Whatman's filter paper disc of 6mm in diameter were saturated with extract of known quantity. Sterile nutrient agar (for *Staphylococcus aureus*) and Eosin Methyl Blue (*Escherichia coli*) plates were inoculated with tested organism and allowed the plates to dry and then discs were placed. These plates were incubated at 37°C for 24 hrs in incubator, after which the zone of inhibition measured.

Determination of activity index

The activity index[15] of the crude plant extract was calculated as

$$\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition}}{\text{Zone of inhibition obtained for standard antibiotic drug}}$$

STATISTICAL ANALYSIS

All experiments were carried out in triplicate. Data analysis done by using MS office 2010. Data are presented as arithmetic means and the results obtained were analyzed in terms of standard deviation.

RESULTS AND DISCUSSION

Latex of *Jatropha curcas* L.: Pure latex showed inhibition zone of 7.6 mm against *Escherichia coli* and found to be ineffective against *Staphylococcus aureus*. Methanolic extract of this latex at concentrations 1ml: 2ml and 1ml: 3ml (Latex: methanol) has been found to exhibit maximum antibacterial activity with inhibition zone of 7.3 mm and 6.6 mm against *Escherichia coli* and concentrations 1ml:4ml and 1ml:5ml, 1ml: 6ml found to be ineffective as compared to tetracycline (zone of inhibition 7 mm), Where as against *Staphylococcus aureus*, maximum zone of inhibition 8.6 mm has been

Table 1: Antibacterial activity of latex of *Jatropha curcas* L.

	S.No.	Concentration (latex:methanol) Or (latex:ethanol) (ml/ml)	Test Bacterial spp. *Escherichia coli* Zone of Inhibition (mm) Mean (± SD)	Activity Index	*Staphylococcus aureus* Zone of inhibition (mm) Mean (± SD)	Activity Index
Tetracycline			7.0(± 0.0)	-	5.0(± 0.0)	-
Pure latex			7.6 (± 0.9)	1.08	NIZ**	0
	1.	1:1	NIZ	0	6.3 (± 2.6)	1.26
	2.	1:2	7.3(± 2.4)	1.04	NIZ	0
Methanolic	3.	1:3	6.6(± 1.4)	0.94	NIZ	0
Extract	4.	1:4	NIZ	0	NIZ	0
of Latex	5.	1:5	NIZ	0	8.6 (± 0.9)	1.72
	6.	1:6	NIZ	0	8.6(± 2.0)	1.72
	1.	1:1	NIZ	0	NIZ	0
	2.	1:2	7.6 (± 0.4)	1.08	NIZ	0
Ethanolic	3.	1:3	7.3 (± 0.9)	1.04	NIZ	0
Extract	4.	1:4	NIZ	0	NIZ	0
of Latex	5.	1:5	NIZ	0	NIZ	0
	6.	1:6	NIZ	0	NIZ	0

** Data are the arithmetic means ± S.D. n=3.*

*** No Inhibition Zone*

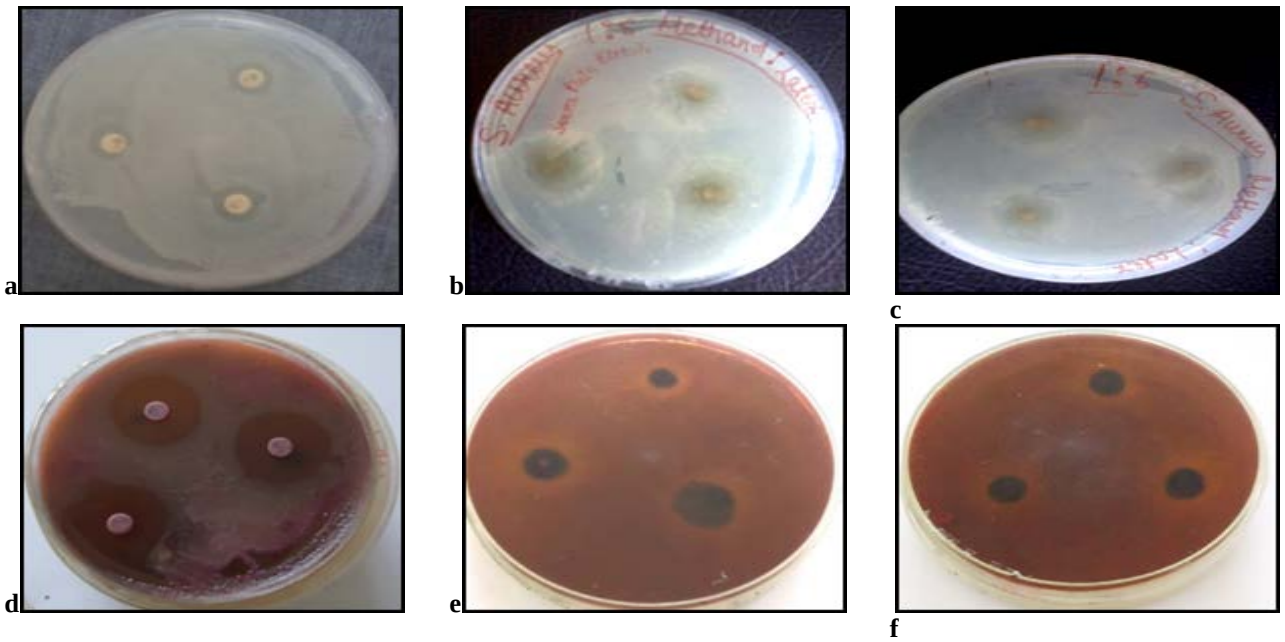

Fig. 3: Zone of inhibition against Staphylococcus aureus (a.- c.) ***a.*** *Tetracycline* ***b.*** *Latex Methanolic extract(1:5)* ***c.*** *Latex Methanolic extract(1:6) and Escherichia coli (d. – f.)* ***d.*** *Tetracycline* ***e.*** *Latex Methanolic extract (1:2)* ***f.*** *Latex Methanolic extract (1:3).*

observed with methanolic extract of latex at concentrations 1ml: 5ml and 1ml:6ml respectively and minimum zone of inhibition observed with methanolic latex extract of 1ml:1ml *i.e.* 3mm as compared to tetracycline (zone of inhibition 5 mm).

Ethanolic extract prepared in the ratio of 1ml: 2ml and 1ml: 3ml had showed maximum zone of inhibition 7.5mm and minimum 7.3 mm against *Escherichia coli* as compared to tetracycline (zone of inhibition 7 mm). Where as other concentrations 1ml:1ml, 1ml:4ml, 1ml:5ml, 1ml:6ml found to be ineffective, Ethanolic extract showed no antibacterial activity against *Staphylococcus aureus.* [Table 1][Fig.3].

Leaves of *Jatropha curcas* L.: Methanolic leaf extract of *Jatropha curcas* L. has been showed maximum antibacterial activity with inhibition zone 6.6 mm at concentration 50mg/10ml and minimum antibacterial activity with inhibition zone 3.6 mm at concentration 60mg/ml against *Escherichia coli,* other concentrations of methanolic leaf extracts *i.e.* 10mg/10ml, 20mg/10ml, 40mg/10ml showed equal effect with zone of inhibition 4.0 mm against *Escherichia coli*. Where as extract concentration 30mg/10ml showed maximum antibacterial activity with inhibition zone 6.3 mm and minimum antibacterial activity with inhibition zone 3.0 mm at concentration 20mg/10ml against *Staphylococcus aureus.*

On the other hand ethanolic leaf extract of *Jatropha curcas* L. showed maximum antibacterial activity with

Table 2: Antibacterial activity of leaves of *Jatropha curcas* L.

	S.No.	Concentration of Leaf : ethanol or methanol (mg /10 ml)	Test Bacterial spp. *Escherichia coli* Zone of Inhibition (mm) Mean (± SD)	Activity Index	*Staphylococcus aureus* Zone of inhibition (mm) Mean (± SD)	Activity Index
Tetracycline			7.0 (± 0.0)	-	5.0 (± 0.0)	-
	1.	10	4.0 (±1.4)	0.57	4.0 (± 0.0)	0.80
Methanol	2.	20	4.0(± 0.8)	0.57	3.0 (± 0.8)	0.60
Leaf	3.	30	5.3 (± 1.2)	0.76	6.3 (± 2.0)	1.26
Extract	4.	40	4.0(± 1.6)	0.57	4.0(± 0.8)	0.80
	5.	50	6.6 (± 0.9)	0.94	3.3 (± 0.9)	0.47
	6.	60	3.6(± 1.2)	0.51	4.3(± 1.2)	0.61
	1.	10	6.3 (± 2.6)	0.90	3.0(± 0.8)	0.60
Ethanol	2.	20	3.3(± 1.2)	0.47	7.0 (± 1.4)	1.40
Leaf	3.	30	2.0 (± 0.8)	0.29	4.3 (± 0.4)	0.61
Extract	4.	40	5.3 (± 2.0)	0.76	5.3 (± 0.4)	1.06
	5.	50	4.0(± 1.4)	0.57	5.3 (± 0.4)	1.06

** Data are the arithmetic means ± S.D. n=3*

inhibition zone of 6.3 mm at concentration 10mg/10ml and minimum antibacterial activity with inhibition zone 2.0 mm has been observed at concentration 30mg/10ml against *Escherichia coli* and extract of 40mg/10ml showed 5.3 mm of zone of inhibition and 50mg/10ml showed 4.0 mm zone of inhibition against *Escherichia coli*, whereas against *Staphylococcus aureus* extract showed maximum antibacterial activity with inhibition zone 7.0 mm at concentration 20mg/10ml and minimum antibacterial activity with inhibition zone 3.0 mm at concentration 10mg/10ml. Extract concentrations 40mg/10ml and 50mg/10ml exhibited zone of inhibition 5.3mm against *Staphylococcus aureus* [Table 2].

Results reveals that latex of *Jatropha curcas* L. and its extracts showed antibacterial activity against both types of bacteria (gram positive & gram negative) indicates its broad spectrum antibacterial activity. Such observations also reported previously[16,17,18]. Antibacterial activity of Latex due to the presence secondary metabolites [19,20,21,22] . Methanolic extracts of latex of *Jatropha curcas* L. found to be more effective then the ethanolic extract. Methanolic and ethanolic extracts of leaves of *Jatropha curcas* L. showed antibacterial activity against *Escherichia coli* (gram negative species) and *Staphylococcus aureus* (gram positive species). Such results were also observed by other workers in their work of antimicrobial activity with *J. curcas* leaves extracts [23,24,25].

Out of latex and leaves of *J. curcas*, the magnitude of antibacterial activity against both the test pathogenic bacteria found to be significantly higher in latex as compared to leaves as clear from the activity index, indicating that the latex of *J. curcas* is more effective against bacteria then its leaves.

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