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RESEARCH ARTICLE

In vitro evaluation of antibacterial property of *Jatropha curcas* L. against different pathogens

■ **SEEMA DWIVEDI**

SUMMARY

The various plant parts (root, stem, leaf) of *Jatropha curcas* were dried and powdered , they were soaked in different solvents *i.e.* water, 70 per cent ethanol, 80 per cent methanol, 100 per cent acetone, 100 per cent hexane, 100 per cent petroleum ether, chloroform and 100 per cent ethylacetate so that secondary metabolites may get dissolved. Screening of these extracts for antibacterial property was performed by using antibacterial suspensibility assay by agar well diffusion method also called cup plate method to compare their effectiveness against various pathogens. In case of leaf extract maximum zone of inhibition was observed against *E.coli* (methanol extract) . The antibiogram analysis of root extract gave the maximum zone of inhibition for *E coli* (ethanol extract). The antibiogram analysis of stem extract gave the maximum zone of inhibition for *E.coli* (cold water extract).

Key Words : *Jatropha curcas*, Secondary metabolites, Antibacterial property, Antibiogram

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Jatropha curcas is a small tree belonging to the family of *Euphorbiaceae*. *Jatropha curcas* is found in central America (Janick and Robert, 2008), but now available at different parts of the tropics and sub-tropics in Africa/Asia (Gübitz *et al.*, 1999; Kumar and Sharma, 2008 and Martinez *et al.*, 2006). The genus name Jatropha derives from the Greek word jatr´os (doctor) and troph´e (food), which indicates its medicinal value (Kumar and Sharma, 2008). In our conventional medicines the *Jatropha curcas* used for the treatment of fever, mouth infections, jaundice and joint rheumatism (Irvine,1961 and Oliver-Bever, 1986). People belonging to rural communities ofIndia, used the juice from leaves *of Jatropha curcas* to cure diseases such as dysentery and colic (Upadhyay *et al*., 2007) and it have potential for wound healing (Balaji *et al*., 2009). This plant get more focused in the research studies due to its anti cancerous activities (Rathee *et al*., 2009 and Shetty *et al*., 2006). The latex of this plant use for healing the cuts and bleeding wounds which soon stops the bleeding due to its anticoagulant activity (Daziel, 1955; Watt *et al.*,1932 and Neuwinger, 1996). Earlier research tell us about the presence of antibacterial agents in different parts of *Jatropha curcas* (Oskoueian *et al.*, 2011; Namuli *et al.*,2011 and Garba and Okeniyi, 2012). The present work is focused to compare the antibacterial potential of leaves, root and stem various pathogens.

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MATERIAL AND METHODS

Fresh and healthy parts (leaf, root and stem) of plant *Jatropha curcas* were collected from Biotech park ,Jankipuram and VKC Railway crossing (Lucknow, U.P.) (Fig. A).

Biotech park | VKC Railway crossing | Stem, root and leaf

Fig. A : Sample collection

Plant leaves, stem and root were washed with the help of running tap water followed by sterilization by help of distill water and were packed for drying in sunlight for several days and then used as raw material for the extraction of anti-bacterial compounds.

Requirements :

Plant material (Dried and powdered root, stem, leaf and bark) (Fig. B), mortar-pestle, tray, distill water, 70 per cent ethanol, 80 per cent methanol, 100 per cent acetone, 100 per cent hexane, 100 per cent petroleum ether, chloroform and 100 per cent ethylacetate, waterbath, oven, beaker, whattman's filter paper, funnel, Petri plates, conical flask and eppendorf tubes.

Leaf powder | Stem powder | Root powder

Fig. B : Shows dried and powdered leaf, stem and root

Media used :

Nutrient broth (NB), Nutrient agar (NB), Luria-Bertani broth (LB), Potato dextrose agar (PDA).

Test micro-organism :

The bacterial strain used in the study were *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram negative) and *Pseudomonas aeruginosa* (Gram negative). Bacterial cultures were maintained on NA plates.

(A) (B) (C)

Fig. C : Shows quaderent streaking on NA plates of pathogen A- *Staphylococcus aureus*, B- *Escherichia coli* and C- *Pseudomonas aeruginosa*

Preparation of plant extract by solvent extraction method :

Various plant parts of *Jaropha curcas* was used (root, stem, leaf) in this study. The samples was extracted by hot water, normal water,70 per cent ethanol, 80 per cent metahnol, 100 per cent acetone, 100per cent hexane, 100 per cent petroleum ether, 100 per cent chloroform, 100 per cent ethylacetate (secondary metabolites).Four grams of leaf, bark root and stem was soaked in 40ml of above mentioned secondary metabolites and was kept in dark for four days so that the metabolites get dissolved properly. After 4 days the filtrate was collected in a pre weighted Petri plate and it was covered with silver foil having fine pores and then the plate was kept in oven at 50^0C (Chen *et al.*, 2007). The Petri plate was again weighed and the final amount of extract was calculated by subtracting the pre-weighted Petri plate from the final weight of plate. Extract obtained was dissolved in double volume of the DMSO, thus, giving the concentration of plant extract to be 50 µg/ml and was cryopreserved.

Antibacterial suspensibility assay :

Antibacterial activity was performed by the agar well diffusion assay (Esimone *et al.*,1998 and Adamu *et al*., 2013).) also called Kirby Bauer method. Autoclaved NA media was poured in the autoclaved Petri plates and after the solidification process 25µl of pathogen culture was spread on the respective plates which were earlier labeled as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Three to four wells of 8 mm diameter were bored on NA plate using a sterile cup-borer and 50µl of tetracycline, 50µl of crude plant (stem,leaf, root) extract (Different in different wells) and autoclaved DMSO were poured in the respective wells and the plates were incubated at 37^0C overnight. The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) at the end of the incubation period and compared to the standard antibiotic tetracycline. Tetracycline

(50µg/ml) was used as a standard antibiotic throughout the study. The concentration of the pathogens used was 5µl/ml.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized in Tables 1 to 14 and Fig 1 to 14.

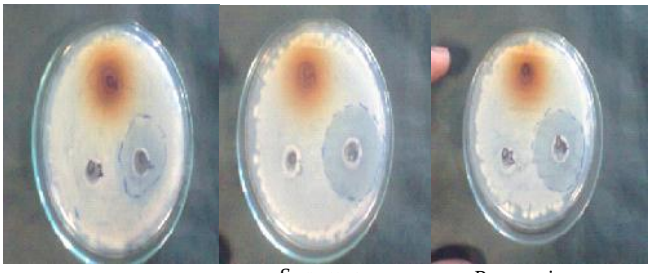
Antibiogram analysis of hot water extract of leaf against various pathogens :

No antibacterial property was found.

Table 1 : Antibiogram analysis of hot water extract of leaf against various pathogens

Sr.No.	Pathogens	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3
1.	*E. coli*	0.00 mm	18 mm	0 mm
2.	*P.aeruginosa*	0.00 mm	14 mm	0 mm
3.	*S.aureus*	0.00 mm	14.5mm	0 mm

1=Hot aq. Extract of leaf.;2=Tetracycline ;3=Autoclaved DMSO



E.coli *S. aureus* *P. aeruginosa*

Fig. 1: Antibiogram analysis of hot water extract of leaf against various pathogens

Antibiogram analysis of ethanol and methanol extract of leaf against various pathogens :

Antibacterial property was found only in case of ethanolic and methanolic extract against *E.coli* and *S. aureus* pathogens.

Table 2 : Antibiogram analysis of ethanol and methanol extract of leaf against various

Sr. No.	Pathogen	Zone of inhibiti on by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	15 mm	16 mm	28 mm	0 mm
2.	*P.aeruginosa*	0.0 mm	0.00 mm	22 mm	0 mm
3.	*S.aureus*	15 mm	16mm	28.5mm	0 mm

1=Ethanolic extract ; 2=methanolic extract ; 3=Tetracycline ; 4=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 2: Antibiogram analysis of water and methanol extract extract of leaf against various pathogens

Antibiogram analysis of hexane and petroleum ether extract of leaf against various pathogens :

No antibacterial property was found in this case.

Table 3 : Antibiogram analysis of hexane and petroleum ether extract of leaf against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	0 mm	0 mm	25.6 mm	0 mm
2.	*S.aureus*	0 mm	0 mm	24.4 mm	0 mm
3.	P.aeruginosa	0mm	0mm	27.8mm	0 mm

1=Hexane extract ; 2=Petroleum ether extract ; 3=Tetracycline ; 4=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 3: Antibiogram analysis of hexane and petroleum ether extract of leaf against various pathogens

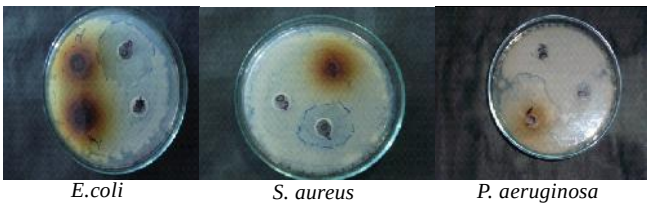
Antibiogram analysis of acetone and eyhyacetate extract of leaf against various pathogens:

Antibacterial activity was found against pathogens.

Table 4 : Antibiogram analysis of acetone and eyhyacetate extract of leaf against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	13mm	15 mm	29.5 mm	0 mm
2.	*S.aureus*	12.5 mm	11.5 mm	23.5 mm	0 mm
3.	P.aeruginosa	14mm	11mm	19mm	0mm

1=Acetone extract;2=ethyacetate ;3=Tetracycline ; 4=Autoclaved DMSO



E.coli *S. aureus* *P. aeruginosa*

Fig. 4: Antibiogram analysis of acetone and ethyl acetate extract of leaf against various pathogens

Antibiogram analysis of cold water extract of leaf against various pathogens :

No antibacterial property was found.

Table 5 : Antibiogram analysis of cold water extract of leaf against various pathogens

Sr.No.	Pathogens	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3
1.	*E. coli*	0 mm	29.5 mm	0 mm
2.	*S.aureus*	0mm	23 mm	0 mm
3.	*P.aeruginosa*	0mm	29mm	0mm

1=Cold water extract of leaf ;2=Tetracycline ;3=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 5: Antibiogram analysis of cold water extract of leaf against various pathogens

Antibiogram analysis of 70 per cent ethanol and methanol extract of stem against various pathogens:

No antibacterial property was found.

Table 6 : Antibiogram analysis of 70 per cent ethanol and methanol extract of stem against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	0 mm	0.00 mm	28 mm	0 mm
2.	*P.aerigenosa*	0 mm	0 mm	30 mm	0 mm
3.	*S.aureus*	0 mm	0 mm	23 mm	0 mm

1=Ethanol extract ; 2=methanol extract; 3=Tetracycline (50µg/ml); 4=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 6: Antibiogram analysis of 70 per cent ethanol and methanol extract of stem against various pathogens

Antibiogram analysis of petroleum and hexane of stem against various pathogens:

No antibacterial activity.

Table 7 : Antibiogram analysis of petroleum and hexane of stem against various pathogen

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	0 mm	0 mm	20 mm	0 mm
2.	*P.aeruginosa*	0 Mm	0 mm	22 mm	0 mm
3.	*S.aureus*	0 mm	0 mm	19mm	0 mm

1=Petroleum; 2=Hexane; 3=Tetracycline (50µg/ml); 4=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 7: Antibiogram analysis of petroleum and hexane of stem against various pathogen

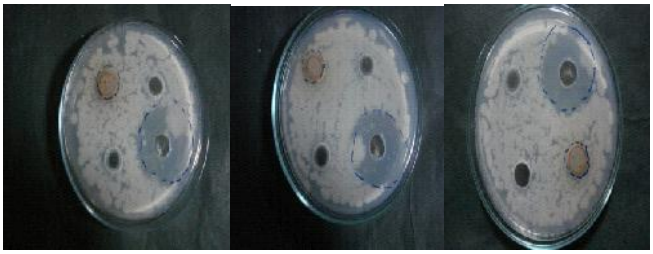
Antibiogram analysis of cold water extract of stem against various pathogens:

Antibacterial activity was found.

Table 8 : Antibiogram analysis of cold water extract of stem against various pathogens

Sr.No.	Pathogens	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3
1.	*E. coli*	11.5 mm	27 mm	0 mm
2.	*P.aeruginosa*	10.5 mm	26.5 mm	0 mm
3.	*S.aureus*	10.5 mm	26 mm	0 mm

1=cold water extract of stem ; 2=Tetracycline (50µg/ml); 3=Autoclaved DMSO



E.coli *S. aureus* *P. aeruginosa*

Fig. 8: Antibiogram analysis of cold water extract of stem against various pathogens

Antibiogram analysis of hot water extract of stem against various pathogens:

No antibacterial activity.

Table 9 : Antibiogram analysis of hot water extract of stem against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	0 mm	20. mm	0 mm	0 mm
2.	*P.aeruginosa*	0 mm	26 mm	0 mm	0 mm
3.	*S. aureus*	0 mm	28.9 mm	0 mm	0 mm

1=Hot water ; 2=Tetracycline (50µg/ml); 3=Autoclaved water; 4= Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

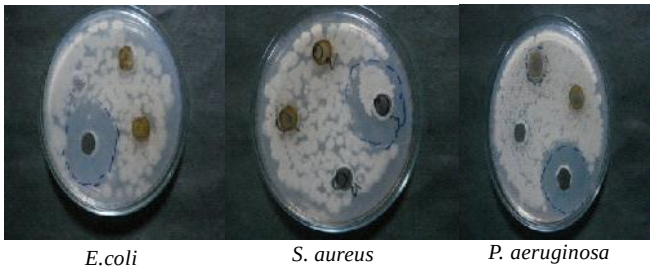
Fig. 9 : Antibiogram analysis of chloroform extract of stem against various pathogens

Antibiogram analysis of acetone and ethylacetate extract of stem against various pathogens:

No antibacterial activity was found.

Table 10 : Antibiogram analysis of acetone and ethylacetate extract of stem against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	0 mm	0 mm	27.5 mm	0 mm
2.	*P.aeruginosa*	0 mm	0 mm	25 mm	0 mm
3.	*S.aureus*	0mm	0 mm	26 mm	0 mm



E.coli *S. aureus* *P. aeruginosa*

Fig. 10: Antibiogram analysis of acetone and ethyl acetate extract of stem against various pathogens

Antibiogram analysis of hot water extract of root against various pathogens :

Antibacterial activity was found in case of hot water extract against *E.coli* and *P.aeruginosa.*

Table 11 : Antibiogram analysis of hot water extract of root against various pathogens

Sr.No.	Pathogens	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3
1.	*E. coli*	1 mm	20.3 mm	0 mm
2.	*P.aeruginosa*	0.2 mm	26 mm	0 mm
3.	*S.aureus*	0mm	23mm	0 mm

1=Hot water extract of root ; 2=Tetracycline (50µg/ml); 3=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 11: Antibiogram analysis of hot water extract of root against various pathogens

Antibiogram analysis of methanol and 70 per cent ethanol extract of root against various pathogens:

Antibacterial activity was found.

Table 12 : Antibiogram analysis of methanol and 70 per cent ethanol extract of root against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	22 mm	17 mm	20 mm	0 mm
2.	*P.aeruginosa*	18 mm	14.5 mm	18.6 mm	0 mm
3.	*S.aureus*	16.5 mm	14.5 mm	19 mm	0 mm

1=70% Ethanol extract ; 2=Methanol extract (500µg/ml); 3=Tetracycline (50µg/ml); 4=Autoclaved DMSO

E.coli *S. aureus* *P. aeruginosa*

Fig. 12: Antibiogram analysis of methanol and 70 per cent ethanol extract of root against various pathogens

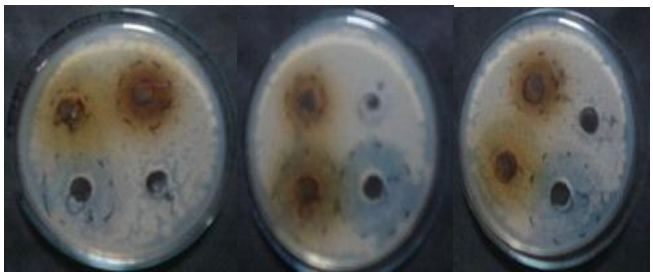
Antibiogram analysis of acetone and ethyl acetate extract of root against various pathogens:

Antibacterial activity was found.

Table 13 : Antibiogram analysis of acetone and ethyl acetate extract of root against various pathogens

Sr.No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	14 mm	17 mm	22 mm	0 mm
2.	*P.aeruginosa*	17 mm	15.5 mm	18.9 mm	0 mm
3.	*S.aureus*	15.5 mm	15.5 mm	21 mm	0 mm

1=Ethyl acetate; 2=Acetone; 3=Tetracycline (50µg/ml); 4=Autoclaved DMSO



E.coli *S. aureus* *P. aeruginosa*

Fig. 13: Antibiogram analysis of acetone and ethyl acetate extract of root against various pathogens

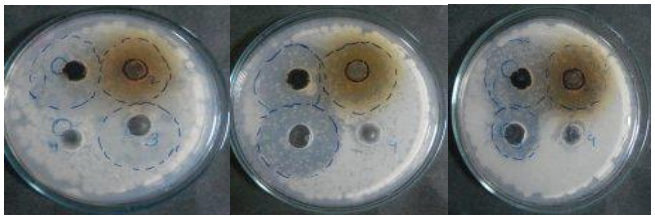
Antibiogram analysis of hexane and petroleum ether extract of root against various pathogens :

Antibacterial activity was found.

Table 14 : Antibiogram analysis of hexane and petroleum ether extract of root against various pathogens

Sr. No.	Pathogen	Zone of inhibition by 1	Zone of inhibition by 2	Zone of inhibition by 3	Zone of inhibition by 4
1.	*E. coli*	14 mm	17 mm	20 mm	0 mm
2.	*P.aeruginosa*	19 mm	14.5 mm	22 mm	0 mm
3.	*S.aureus*	20 mm	20 mm	18 mm	0 mm

1=Hexane extract (500µg/ml); 2=Petroleum ether extract (270µg/ml); 3=Tetracycline (50µg/ml); 4=Autoclaved DMSO



E.coli *S. aureus* *P. aeruginosa*

Fig. 14: Antibiogram analysis of hexane and petroleum ether extract of root against various pathogens

Conclusion :

Data obtained demonstrates the antibacterial activity of the plant depending upon the test organism tested for susceptibility assay. In the case of plant leaf methanolic extract gave the maximum zone of inhibition against *E.coli*, for aq. extract gave the maximum zone of inhibition against *E.coli* and for root maximum zone of inhibition was observed against *E.coli* for ethanolic extract. The active extract could be carried out for future pharmacological evalution by several methods such as NMR, MS, TLC, HPLC etc.

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