

## BROAD-SPECTRUM ANTIMICROBIAL PROPERTIES OF MEDICINALLY IMPORTANT PLANT JATROPHA CURCAS L.

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### ABSTRACT

In the present study the effectiveness of *Jatropha curcas* on inactivation of some microorganisms i.e. *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were determined. The filter paper disc method was used for screening of crude ethanolic extract of leaves for antimicrobial activity. The paper discs saturated with extract were placed on the surface of the sterilized nutrient agar medium that had been inoculated with the test organisms by using a sterile swab. The diameters of microbial inhibition zones were measured after 24 hours of incubation at 37°C. According to the methodology used, it was possible to conclude that the ethanolic extract presented antimicrobial activity against *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. No antimicrobial activity was found against *Bacillus subtilis*. Ethanolic extract of *Jatropha* leaves presented the largest inhibition zones (i.e. 11mm.) against *E. coli*.

**Keywords:** *Jatropha curcas* L., Ethanolic extract, *E. coli*, *P. fluorescens*, *P. aeruginosa*, *S. aureus*, *B. subtilis*.

### INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. Recent estimations suggest that over 9,000 plants have been known for medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities<sup>1</sup>.

The search for plants with antimicrobial activity has gained increasing importance in recent years, due to a growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms or multi-resistant microbes. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new, antimicrobial compounds<sup>2-4</sup>. Plants are widely used for medicinal purpose in different countries and are a source of many potent and powerful drugs<sup>5</sup>.

The interest in the scientific investigation of *Jatropha curcas* L. is based on the claims of its effective use for the treatment of many diseases. The sap (latex) has antimicrobial properties against *Staphylococcus* and *Streptococcus* sp. and *E. coli*. Latex is used to dress sores, ulcers and inflamed tongues<sup>6</sup>. Latex from the stem is used to arrest bleeding of wounds. Seeds are used for dropsy, gout, paralysis and skin ailments<sup>7</sup>.

Therefore, this research regarding the antimicrobial activity of this plant is expected to enhance the use of *Jatropha* against diseases caused by the test pathogens. It is expected that screening of plant extract against wide variety of test organisms will be helpful in obtaining new antimicrobial substances.

### MATERIALS AND METHODS

#### Plant material

Plant material used for this study was collected from University Botanical Garden, Botany Department, University of Rajasthan, Jaipur, India. All the plants were identified at taxonomic section, Botany Department, University of Rajasthan, Jaipur, India.

#### Culture and Maintenance of Bacteria

For the present antimicrobial assay Gram-negative bacteria *Escherichia coli*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* were used as test organisms.

Pure cultures of above mentioned microorganisms were obtained from S.M.S. Medical College, Jaipur, Rajasthan, India. These microbes were grown in Nutrient Broth Medium, prepared by autoclaving Nutrient broth (Hi-Media) in distilled water at 15 psi for 25-30 min. and incubated at 37°C for 48 hours. Each bacterial culture was further maintained on the same medium after every 48 hours of transferring. A fresh suspension of test microorganisms in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay. Suspensions used in the experiments were adjusted to an O.D. of 0.07 (I 10%).

#### Preparation of plant extract

Fresh leaves of *Jatropha* were collected from a 15 year old shrub growing in University Botanical Garden, Jaipur, India and used for experimental work. Leaves were dried at room temperature and then ground into fine powder using a grinder. A sample (200 gm.) of powdered plant material was exhaustively extracted by soxhlet extraction method using 80% Ethanol. At the end of the extraction,



extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30°C and stored at 4°C until further use. The final crude extract was diluted with ethanol to a standard volume and tested separately against the test organisms.

#### Determination of Antimicrobial activity

The filter paper disc method<sup>8</sup> was used for screening of crude extract for antimicrobial activity. Standard size blank Whatman filter paper discs, 6.00 mm. in diameter, sterilized by dry heat at 140°C for 1 hour, were saturated with the extract (0.04 ml) and known quantity of standard reference antibiotics separately. Now the discs were air dried at room temperature to remove any residual solvent which might interfere with the determination. The discs were then placed on the surface of the sterilized nutrient agar medium that had been inoculated with the test organism by using a sterile swab and air dried to remove moisture.

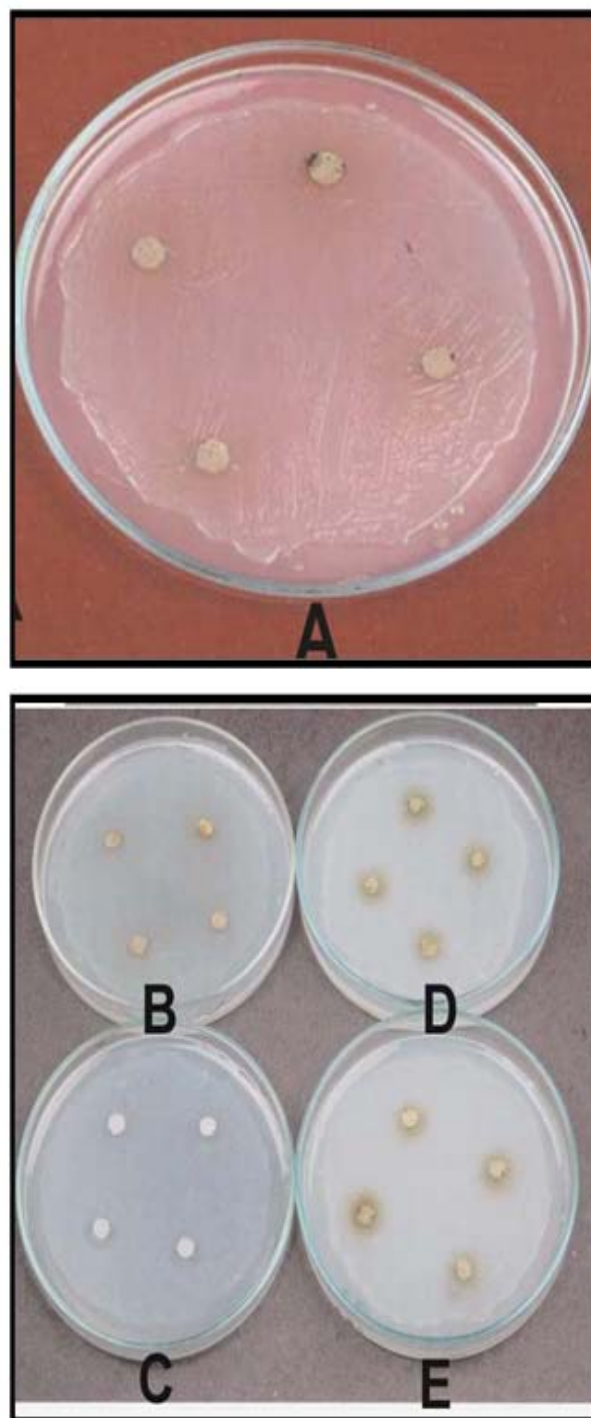
The thickness of the agar medium was kept equal in all Petri plates and the standard discs of Streptomycin were used, separately for all microbes tested, in the petriplates as control. Before incubations, petri plates were placed for 1 hour in a cold room (5°C) to allow diffusion of the compounds from the disc into agar plate. These were incubated at 37°C for 20 to 24 hours, after which the zones of inhibition of desired growth could be easily measured. The zone of inhibition was considered as an indicator for the antimicrobial activity. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones. All the experiments were in five replicates and the values were then computed.

Disc method for the comparative screening studies was selected for its reproducibility and precision.

#### RESULTS

Table-1 indicates the results of the antimicrobial activities of the crude ethanolic extract of leaves of *Jatropha curcas* L with respect to the test organisms. The extract showed significant activity against four test organisms. The data indicated that gram-negative *Escherichia coli* was the most sensitive strain of those tested with the ethanolic extract of *Jatropha* leaves, with the strongest inhibition zone of 11 mm and the activity index of 0.687. The ethanolic extract also exhibited high antimicrobial activity against *Staphylococcus aureus* (IZ-10 mm and AI-0.666). However, the ethanolic extract was found ineffective against *Bacillus subtilis*, where it showed no antimicrobial activity. However, the extract also showed moderate amount of antimicrobial activity against *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, respectively. The inhibition zone observed for *Pseudomonas fluorescens* was 7mm. (AI-0.466) and for *Pseudomonas aeruginosa* was found to be 6 mm (AI-0.545). (Figure 1).

Figure 1: Antibacterial activity of *J. curcas* leaves



- A- *Bacillus subtilis*
- B- *Pseudomonas fluorescens*
- C- *Pseudomonas aeruginosa*
- D- *Staphylococcus aureus*
- E- *Escherichia coli*

**Table 1:** Antimicrobial Activity of Ethanolic extract of the leaves of *Jatropha curcas* L.

Test Microorganisms	Inhibition area of standard-streptomycin (10 µg)	Inhibition Zone (IZ) in mm.	Activity Index (AI)
<i>Escherichia coli</i>	16.0 mm.	11.0 mm.	0.687
<i>Staphylococcus aureus</i>	15.0 mm.	10.0 mm.	0.666
<i>Pseudomonas fluorescens</i>	15.0 mm.	7.0 mm.	0.466
<i>Pseudomonas aeruginosa</i>	11.0 mm.	6.0 mm.	0.545
<i>Bacillus subtilis</i>	19.0 mm.	—	0

IZ = Inhibition Zone in mm.

AI = Activity Index =  $\frac{\text{Inhibition area of the sample}}{\text{Inhibition area of the Standard}}$

## DISCUSSION

Many plants or plant parts have been studied for their antimicrobial activity<sup>9,10</sup>. In the present investigation ethanolic extract of the leaves of *Jatropha curcas* was screened for its antimicrobial activity. The antimicrobial activity of *Jatropha curcas* plant has been studied earlier by many workers<sup>11</sup>. Among the five microbes tested (i.e. Gram-negative bacteria *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*), ethanolic extract of the plant showed antimicrobial activity against four microorganisms (i.e. against *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The maximum antimicrobial activity was observed against *Escherichia coli* (11.0 mm.). *Staphylococcus aureus* was also inhibited by the ethanolic extract with the inhibition zone of 10.0mm.. Similarly, *Pseudomonas fluorescens* (I.Z.7.0 mm.) and *Pseudomonas aeruginosa* (I.Z.6.0 mm.) were also inhibited by leaves extract.

The ethanol extracts of *Jatropha curcas* plant has been reported to inhibit the growth of *Bacillus subtilis* (NCTC 8236) and *Candida albicans* (ATCC 10231)<sup>12</sup>; root extract was found to be active against *Staphylococcus aureus* and *Escherichia coli*<sup>13</sup>. So the present findings regarding the antimicrobial activity of the ethanolic extracts of the leaves of this plant is in agreement with the previous workers.

Besides the microbes tested, some other microbes (i.e. *Microsporium gypseum*<sup>14</sup>) are also sensitive to ethanolic and water extracts of *Jatropha*. The results obtained indicated the existence of antimicrobial compounds in the crude ethanolic extracts of *Jatropha curcas* and this shows that the ethanolic extract of *Jatropha* has a broad spectrum of microbial inhibition.

The presently tested microbes were also found to be inhibited by the extracts of other plants like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were inhibited by aqueous and organic solvents (acetone and ethanol) of *Tamarindus indica* Linn<sup>15</sup>. However; *Bacillus subtilis*, *Staphylococcus*

*aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were inhibited by *Stevia Rebaudiana* Bertoni leaves extract<sup>16</sup> and *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus* and *Staphylococcus aureus* were inhibited by the methanolic extract of leaves of *Salicornia brachiata*<sup>17</sup>, respectively.

The alcoholic extracts of many plants were found to be active against some microbes such as methanolic extract of *Palustriella commutate* showed antimicrobial activity against *Micrococcus luteus* (NRRL-B 1018), *Bacillus cereus* (NRRL B-3711), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (clinical isolate)<sup>18</sup>; extract of *Mitracarpus scaber* leaves against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Sarcina lutea*, *Candida albicans*<sup>19</sup>, *Proteus vulgaris*, *Serratia marcescens*<sup>20</sup>, *Fusarium oxysporum*, *Pythium aphanidermatum*, *Lasiodiplodia theobromae*, *Curvularia lunata*, *Fusarium semitectum*, *Colletotrichum capsici*, and *Colletotrichum gloeosporioides*<sup>21</sup>, respectively.

However, besides alcoholic extract, other extracts have also shown antimicrobial activity, such as the organic {dichloromethane : methanol (1:1)} and aqueous extracts obtained from *Guatteria riparia* and *Gnetum leyboldii* showed antimicrobial activity against *Staphylococcus aureus*<sup>22</sup>; chloroform and hot water extracts obtained from *Boswellia ameero*, *Buxus hildebrandtii* and *Commiphora parvifolia* inhibited the growth of *Staphylococcus epidermidis*, whereas both extracts of *Withania adunensis* also inhibited the growth of *Staphylococcus aureus* (ATCC 6538); *Bacillus subtilis* (ATCC 6051) and *Micrococcus flavus*<sup>23</sup>, respectively.

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