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## Phytochemical analysis and Preliminary screening of antimicrobial activity of *Jatropha curcas* L

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### Abstract

In the present study, the antimicrobial activity of *Jatropha curcas* L. (Euphorbiaceae) leaf extract was evaluated against one gram-positive species *Staphylococcus aureus*, and four gram-negative species *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* sp. The solvents used for extraction of plant leaves were Acetone, Chloroform, Ethanol and Distilled water. The *in vitro* evaluation of antimicrobial activity was carried out by agar well diffusion method. The alcoholic extract of leaves of *Jatropha curcas* L. showed maximum antimicrobial activity against all test organisms except *Proteus* sp., while aqueous extract of plant leaves was found to be ineffective against all test pathogens. The acetone and chloroform extract showed variable activity. *Proteus* sp. was found to be insensitive to all of the extracts. Ethanol extract of plant leaves was found to be equally potent against *Staphylococcus aureus* compared to standard antibiotics such as streptomycin. The study scientifically proves the importance of plant products in development of a potent antibacterial agent.

**Keywords:** Antibacterial activity, *Jatropha curcas* L., Leaf extract, Agar well diffusion method

### Introduction

Plants are considered as one of the most important source of medicine. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. Plant extracts were used for the treatment of various diseases and this forms the basis for all traditional systems of medicine (Kalimuthu *et al.*, 2010) [16]. The use of available medicinal plants for the treatment and control of diseases in a locality play significant roles in medical health care implementation in the developing countries (Ekundayo *et al.*, 2011) [7]. A large number of antimicrobial agents are currently available for the treatment and control of infections and diseases. However, the use of such medicines as therapeutic agents is limited due to various challenges such as drug solubility, stability, adsorption and toxicity. In addition, some of these drugs are expensive and generally not readily available to citizens of developing countries, especially those living in the rural areas (Sule *et al.*, 2011) [24].

Antimicrobial agents of plant origin have been successfully used in the treatment of various infectious diseases. There is need to search alternative therapeutic agents that could help reduce the challenges being posed by super bugs. Medicinal plants play important role in pharmacological research and drug development. The clinical, pharmaceutical and economic value of medicinal plants is still growing in various countries (Jayasuriya, 2013) [14]. Because of the wide-spread belief that herbal medicines are safer than synthetic drugs, demand of medicinal plants has increased many folds in the national and international markets (Singh & Krishnan Marg, 2011) [23].

*Jatropha curcas* L. is a member of the family Euphorbiaceae. It is a drought resistant small tree or large shrub and it is widely distributed all over the world. It is used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Igbinsosa *et al.*, 2009) [11]. It has been documented to have medicinal uses for human and veterinary purposes (Irvine, 1961) [12]. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Irvine, 1961; Oliver-Bever, 1986; Igbinsosa *et al.*, 2009) [12, 21, 11]. Fagbenro-Beyioku (1998) [9] investigated and reported the anti-parasitic activity of the sap and crushed leaves of *J. curcas*.

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The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity (Matsuse *et al.*, 1999; Igbinsosa *et al.*, 2009) [19, 11].

Antibiotic resistance in medically important bacteria is the major problem faced by the modern world. The indiscriminate use of commercial antimicrobial drugs has resulted in multiple drug resistance. Antibiotics may also cause adverse effects on the host including allergies, hypersensitivity and immune-suppression. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously minimizing many of the side effects that are often associated with synthetic antimicrobials (Cunha, 2001) [5]. Though antimicrobial properties of medicinal plants have been investigated by a number of researchers worldwide, very little information is available on such activities of medicinal plants and only a small number of plants have been systematically investigated for their antimicrobial activities.

In present study, the antibacterial property of crude extracts of the *Jatropha curcas* L leaves has been evaluated against some selected microorganisms which are known to cause diseases in human beings.

## Materials and methods

### Bacterial Culture Collection

Five test pathogens *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* sp. used in this study were collected from stock culture collections of Department of Microbiology, D. A. B. Naik Arts and Science College, Chikhali, (Shirala) Shivaji University, Kolhapur, Maharashtra. Identity of the organisms was confirmed by morphological characteristics and conventional biochemical tests (Harley and Prescott, 2002) [10]. Pure cultures were preserved at 4°C on nutrient agar and MacConkeys agar slants.

### Collection of plant materials

The plant used for the experiment was *Jatropha curcas* L. The plant materials were collected from the fields of Shirala region.

### Preparation of crude extracts of leaves

Collected plant leaves were washed thoroughly, shade dried and then ground into fine powdered by using mortar and pestle.

#### a) Preparation of organic extracts

2 gm of powdered plant material was dissolved in 50 ml of organic solvents like Acetone, Chloroform or Ethanol. The mixture was placed on a shaker over night in order to allow extraction. The extraction was done at room temperature. The resulting mixture was then centrifuged and filtered through Whatman No.1 filter paper and evaporated at 50°C to near dryness and stored at 4°C in airtight tubes. These crude solvent extracts were diluted with 10% dimethyl sulphoxide (DMSO- which is to be used as negative control) before experiments.

#### b) Preparation of aqueous extract

2 grams of powdered plant material was weighed and suspended in 50 ml of sterile distilled water. The mixture

was boiled on slow heat for 30 min. The extract was then centrifuged and filtered through Whatman No.1 filter paper. The filtrate was collected. This procedure was repeated twice. The filtrate was concentrated at 80°C to make the final volume one-fourth of the original volume with the help of water bath and stored at 4°C in airtight tubes.

### Determination of antibacterial activity

Antimicrobial activity of different plant extracts was determined by agar well diffusion method. 0.1 ml of freshly grown culture of test organisms (10<sup>6</sup>cfu/ml) was spread on the surface of sterile nutrient agar plates. Wells of 6 mm diameter were made in agar plate with the help of sterile cork-borer. 200 µl of different plant extracts and same volume of positive and negative controls were filled in the wells with the help of pipette. Sterile distilled water or 10% DMSO was used as negative control and streptomycin was used as positive control for the test organisms. Plates were then kept in a refrigerator at 4 °C for some time till the extract diffuses in the surrounding agar medium with the lid closed and incubated at 37°C for 24 hr. The plates were observed for zone of inhibition. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition against the tested bacteria. Each assay in this experiment was replicated three times (Joshi *et al.*, 2011, Shinde *et al.*, 2015) [15, 25].

### Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, steroids, saponins, tannin, glycosides, phenols, amino acids and proteins by the following procedure.

#### 1. Test for Alkaloid

The alcoholic extract of plant was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation (Evans, 2002) [6].

#### 2. Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavonoid (Siddiqui and Ali, 1997) [22].

#### 3. Test for Steroid

10 mg of extract was dissolved in chloroform. Few drops of acetic anhydride were added followed by 1 ml of concentrated sulphuric acid. Blue colour in chloroform layer which changes to green shows the presence of steroids (Siddiqui and Ali, 1997) [22].

#### 4. Test for saponins

One ml extract and one ml alcohol diluted with 20 ml distilled water and shaken well for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

### 5. Test for Tannin

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour shows presence of gallic tannins while green black colour shows presence of catecholic tannins (Iyengar, 1995) [13].

### 6. Test for Glycosides

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer (Siddiqui and Ali, 1997) [22].

### 7. Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

### 8. Test for Amino acids and Proteins (1% Ninhydrin solution in acetone)

2 ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

### Result and discussion

In this study, some microorganisms which are known to cause diseases in human beings such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp.*, and *Proteus sp.* were selected and antibacterial activity of leaf extracts of *Jatropha curcas L.* was evaluated against them.

**Table 1:** Antibacterial property of crude leaf extracts of *Jatropha curcas L.*

Name of the Extract	Diameter of zone of inhibition in mm of test pathogens against different extracts				
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus sp.</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
Acetone extract	18	08	-	-	-
Chloroform extract	-	07	-	11	-
Ethanol extract	15	12	-	08	21
Aqueous extract	-	-	-	-	-
Positive control (Streptomycin)	22	18	10	15	22
Negative control	-	-	-	-	-

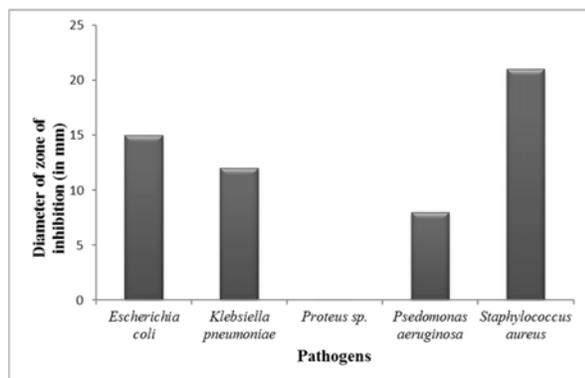
(Note- Figures indicate mean diameter of inhibition zone in mm) (- : not detected)

Ethanol extracts of plant leaves showed inhibitory activity against all test organisms except *Proteus sp.* Maximum zone of inhibition (21 mm) was obtained with ethanol extract of plant leaves against *Staphylococcus aureus*. Acetone and chloroform extract of showed variable antibacterial activity against test organisms, while aqueous extract of plant leaves did not show inhibitory activity.

*Proteus sp.* was found to be insensitive to all of the extracts. Ethanol extract of plant leaves was found to be equally potent against *Staphylococcus aureus* compared to standard antibiotics such as streptomycin.

For the comparison, positive and negative controls were used. Negative controls did not show inhibitory action on any of the test organisms, while positive control significantly inhibited growth of all test organisms.

Antibacterial activity of ethanol extract of plant leaves against test pathogens is shown graphically in figure 1.



**Fig 1:** Antibacterial property of crude leaf extracts of *Jatropha curcas L.*

In many parts of the world, plant extracts and their products are used as the active principles in herbal remedies and locally in the treatment of infectious diseases. The non activity of the aqueous extract against most bacterial strains investigated in the present study is in agreement with the previous works which show that aqueous extracts of *J. curcas* generally showed little or no antibacterial activities (Koduru et. al, 2006, Ashafa et. al, 2008) [17, 2].

**Table 2:** Phytochemical analysis of ethanol extract of leaves of *Jatropha curcas L.*

Sr. No.	Name of the constituent	Analysis
1.	Alkaloids	Positive
2.	Flavanoids	Positive
3.	Steroids	Positive
4.	Saponin	Positive
5.	Tannin	Positive
6.	Glycoside	Negative
7.	Phenolic compounds	Positive
8.	Protein and amino acid	Negative

The optimal effectiveness of a medicinal plant may not be due to the one main active constituent, but may be due to the combined action of different compounds originally in the plant (Bhandarkar et al., 2003) [3]. Though the results of this study agree with results of other workers, diameter of zone of inhibition formed varies from other study results. Probably the sources of microorganisms used may be the reason for this difference. Moreover, the effectiveness of plant extract against a particular pathogen is affected by various intrinsic and extrinsic factors. The antibacterial properties of medicinal plants may be due to presence of different bioactive antimicrobial compounds. Phytochemical constituents such as alkaloids, flavonoids, steroids,

terpenoids and several other compounds are secondary metabolites of plants. These compounds play important role in defence mechanism of plants against many microorganisms, insects and other herbivores (Arulmozhi *et al.*, 2007)<sup>[1]</sup>.

### Conclusions

This study supports the traditional use of *Jatropha curcas* L. for the treatment of various infectious diseases in different regions of the world, and may serve as a good source of novel bioactive compounds. It is quit safer to use as an herbal medicine as compare to chemically synthesized drug. But further studies must be carried out to enhance activity of plant extracts. The study scientifically proves the importance of plant products in development of a potent antibacterial agent.

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