

Bioautography screening of *Jatropha gossypifolia* L. Leaves against the Gram positive organisms

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Abstract

Jatropha gossypifolia L., belonging to the family Euphorbiaceae is traditionally used in curing various ailments. It is commonly called as cotton leaf, physic nut and Belly ache. In Tamil commonly called as Kattamanakku. Humans are natural hosts for many bacterial species that colonize the skin as normal flora. *Staphylococcus aureus* and *Streptococcus epidermis* is infrequent resident flora, but they account for a wide variety of bacterial pyodermas. Predisposing factors to infection include minor trauma, pre-existing skin disease, poor hygiene, and rarely, impaired host immunity. The present study deals with the antibacterial activity and phytochemical screening of *Jatropha gossypifolia*. Sequential extraction was carried out using solvents such as chloroform, ethanol, and water from leaf of the plant were investigated for preliminary phytochemical and antibacterial property. Results of the study showed that all the extracts had good inhibitory activity against Gram-positive test organism. Although all four extracts showed promising antibacterial activity against test bacterial species, yet maximum activity was observed in ethanol extract. The minimum inhibitory concentration ranged in between 64 to 1024 µg/ml. Evaluation of phytochemicals such as alkaloids, flavonoids, saponin, tannin, glycosides, phenolic compound, proteins and amino acids revealed the presence of most of constituents in polar extracts of aqueous. All the findings exhibit that the leaf extracts have broad-spectrum activity and suggest its possible use in treatment of infectious diseases.

Keywords: *Jatropha gossypifolia* L., Pathogenic organisms, MIC, Phytochemical compound

1. Introduction

Jatropha gossypifolia L., belonging to the family Euphorbiaceae is traditionally used in curing various ailments. *J. gossypifolia* is commonly called "Cotton leaf, Physic nut, Wild physic nut, Belly ache" in English, "Ratan jyoti" in Hindi and "Kattamanakku" in Tamil. It is a perennial shrub reaching 3 m height with purplish stems. Leaves are palmate lobed, alternate leaf margins, petioles and stipules covered with glandular hairs and are featured with red to purple tinges. Inflorescence corymb. Flowers small, 5 unisexual, monoecious, deep maroon coloured with yellow centres. Bioautography is sensitive method for detection of antimicrobial compounds even in small amounts. Hence for detection of antimicrobial compounds, bioautography is suitable method in initial stages as compared to agar dilution methods^{3,10}.

Medicinal plants are useful to treat curing various disorders in humans from time immemorial and an integral part of the Indian traditional medicinal system, such as the Ayurveda and Siddha. It is estimated that about 80,000 species of plants are utilized by the different systems of Indian medicine. Modern day synthetic and chemical drugs often exhibit some negative effects or side effects but traditional herbals are safer and easy to access. Traditional healers and medicine man in India use a few medicinal plants for curing various diseases and they are cheap as compared to pharmaceutical drugs. Such plant based drugs are obtained from roots, stem, flower, bark or fruit of different medicinal parts at different stages of their growth and development. *Jatropha gossypifolia* is used to treat various kinds of human diseases. Root is used in diarrhoea and dysentery. Oil used as purgative and locally applies in skin disease and arthritis. Latex and leaf juice are used to treat ulcer, skin disease (leprosy) and gum infections. *J. gossypifolia* has great potential as a source of antimicrobial compounds against microorganisms. Thus *J. gossypifolia* can be used in the treatment of infectious diseases caused by pathogenic bacteria. *J. gossypifolia* is used as a therapeutic agent in different ways. The leaf decoction of this is used for bathing wounds. The present study deals with the "Bioautography screening of *Jatropha gossypifolia* L. Leaves against the Gram positive organisms".

2. Materials and Methods

2.1 Plant materials

The plant leaves was collected from areas of Viralmalai, Pudukkottai, Tamilnadu. The leaves were shade dried and pulverized to powder in a mechanical grinder.

2.2 Antibacterial Activity

Detection of antimicrobial activity of *Jatropha gossypifolia* by bioautography: Test microorganisms selected for antimicrobial activity: *Streptococcus epidermis*, *Staphylococcus aureus*.

2.3 Disc Diffusion Method¹

2.3.1 Principle

The disc-diffusion method provides a simple and reliable test in routine clinical bacteriology in order to find out the effect of a particular substance on a specific bacterium. This method consists of impregnating small circular discs of standard filter paper with given amount of a chosen concentration of substance. The discs are placed on plates of culture medium previously spread with a bacterial inoculum to be tested. After incubation the degree of sensitivity is determined by measuring the inhibition zone produced by the diffusion of the antibiotic substances from the discs into the surrounding medium.

2.3.2 Preparation of Discs

Discs usually consisted of absorbent paper impregnated with the compound (plant extracts). It is most convenient to use whatman No.1 filter paper for preparing the discs. Dry discs of 6 mm diameter were prepared from whatman No.1 filter paper and sterilized in an autoclave. These dry discs were used for the assay.

2.3.3 Procedure

Circular discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These paper discs were impregnated with test compounds (Plant extract) in the respective solvents for overnight and placed on nutrient agar plates seeded with the test bacterium. The plates were incubated at 37°C for 24 hours. After 24 hours the zone of inhibition around each disc was measured and the diameter was recorded.

2.4 Phytochemical tests²

The analysis of phytochemicals from the solvent free extract of *Jatropha gossypifolia* was individually performed using different quantitative tests for alkaloids, flavonoids, saponins, tannins, glycosides, phenols, proteins and amino acids.

2.4.1 Extraction of phytochemicals

The individual phytochemical was extracted in the suitable solvent and stored in air-tight containers at 4°C for further use.

2.4.2 Test for alkaloids

To a little of extract a few drops of Mayer's test reagent was added. Formation of precipitate indicates presence of alkaloids.

2.4.3 Test for flavonoids

1 ml extract was taken and a few drops of very dilute solution of ferric chloride were added. The colour changed to pale green or red brown colour which indicates the presence of flavonoids.

2.4.4 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.

2.4.5 Test for tannins

To 5ml of extract solution, 1ml of lead acetate solution was added. Flocculent brown precipitate indicates the presence of tannins.

2.4.6 Test for glycosides

A small portion of extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's test

To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extracts produced pink to red colour, it indicates the presence of glycosides.

2.4.7 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.

2.4.8 Test for Amino acids and Proteins (1% Ninhydrin solution in acetone)

2ml 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.

3. Result

The results of antimicrobial activity test of different solvents extracts of *J. gossypifolia* are Shown in (Table-1) (Fig 1 & 2). The results suggest that the ethanol extract of *J. gossypifolia leaves* exhibited considerable antibacterial activity against both test organisms. All the tested phytochemicals were found to be present in dried plant material (leaves) as shown in (Table-2).

Table 1. Zone of Inhibition formed by ethanol, aqueous, chloroform extract of *Jatropha gossypifolia* leaves against gram positive bacterial strain

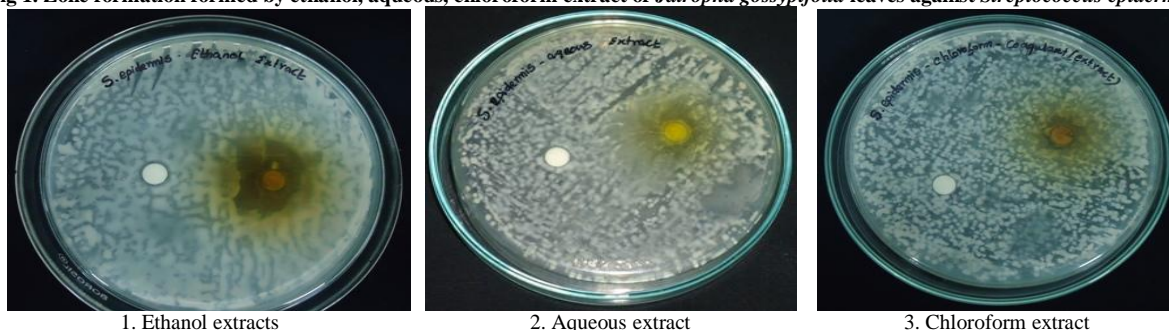
Sample	Bacterial strain	Standard Value	Zone Of Inhibition In mm					
			Ethanol Extract		Aqueous Extract		Chloroform Extract	
			Observed value	$X^2 = \sum [(O-E)^2]/E$	Observed value	$X^2 = \sum [(O-E)^2]/E$	Observed value	$X^2 = \sum [(O-E)^2]/E$
<i>Jatropha Gossypifolia Leaves</i>	<i>S. epidermis</i>	23	22 mm	0.0435	19mm	0.695	17mm	1.565
	<i>S. aureus</i>	23	21mm	0.173	18mm	1.086	16mm	2.13

Note: The chi-square values obtained was less than the calculated table value $\chi^2(0.05) = 3.841$ at 5% level of significance.

The chi- square value obtained has 3.1739 which was less than the calculated table value, $\chi^2(0.05) = 3.841$ at 5% level of significance. The above results lead to the conclusion that the data was consistent with the hypothesis and diameters of zone of inhibition obtained from observed data showed similarities with experimental data¹⁰.

Table 2. Phytochemical analysis of *Jatropha gossypifolia* aqueous extract

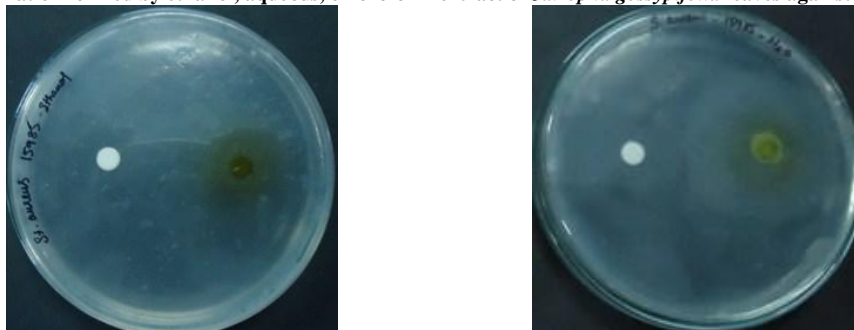
Phytochemical constituents of <i>Jatropha gossypifolia</i>		
S. No	Constituents	Aqueous extract
1.	Alkaloids	Positive
2.	Flavanoids	Positive
3.	Saponin	Positive
4.	Tannin	Positive
5.	Glycoside	Negative
6.	Phenolic compound	Negative
7.	Protein and amino acid	Negative

Fig 1. Zone formation formed by ethanol, aqueous, chloroform extract of *Jatropha gossypifolia* leaves against *Streptococcus epidermis*

1. Ethanol extracts

2. Aqueous extract

3. Chloroform extract

Fig 2. Zone formation formed by ethanol, aqueous, chloroform extract of *Jatropha gossypifolia* leaves against *Staphylococcus aureus*

1. Ethanol extracts

2. Aqueous extract

4. Discussion

In previous studies the bioactivity of leaf extract of different *Jatropha* species against plant pathogenic bacteria, the leaf extract of *J. curcas*, *J. gossypifolia*, *J. glandulifera* and *J. integerrima* at two different concentration i.e 50 HI and 100 HI were tested against the growth of four plant pathogenic bacteria namely *Erwinia carotovora* pv. *Carotovra*, *Pseudomonas aeruginosa*, *Xanthomonas campestris* pv. *Citri*, *Xanthomonas campestris* pv. *Mangiferaeindicae*¹⁵.

Qualitative phytochemical analysis of the crude powder of the leaves collected was determined. Foam test for saponins. Salkowski and Liebermann-Burchard test for terpenoids and triterpenoids. FeCl₃ test for tannins. Keller-Killiani test for cardiac glycosides. Fehling's test for reducing sugars, xanthoproteic test for proteins, iodine test for starch and ammonia test for detection of flavonoids were performed to identify the constituents present in the extracts of the leaves of the plant¹⁵.

In the earlier studies investigation the leaves of *J. gossypifolia* L. were screened for antibacterial activity by extracting them successively in various solvents such as Petroleum ether, Benzene, Chloroform, Acetone, Alcohol and Water. Crude powder of leaves was screened for the presence of chemically active compounds by standard methods and for antibacterial activity by zone of inhibition. The results revealed the presence of saponin, tannin, flavonoid, reducing sugars, cardiac glycosides, terpenoids, triterpenoids, steroids, xanthoprotein and starch. The medicinal values of this plant could be attributed to the presence of one or more of the detected metabolites¹⁵. Plant extracts are used to treat numerous human diseases¹⁰ and have prominent effect on the animal system, important therapeutic properties and antimicrobial activities against various pathogens^{7,5,8}.

The leaves of *J. gossypifolia* are used for intermittent fevers, carbuncles, eczema, itches, and sores on the tongues of babies, swollen mammae, stomach ache, and venereal disease and as blood purifier². The leaf decoction is used for bathing wounds^{7,11} (wound healing).

The present study deals with the Plants are of great importance to the health of individuals and communities from time immemorial. Plant kingdom provides a tremendous reservoir of various phytochemicals with potential therapeutic properties.

The present studies concluded that the leaves of *J. gossypifolia* contained a number of phytochemical compounds such as alkaloids, flavonoids, saponins, tannins, glycoside, phenolic compound, proteins and amino acids.

5. Conclusion

Staphylococci can cause many forms of infection. *S. aureus* causes superficial skin lesions (boils, styes) and localized abscesses in other sites. *S. aureus* causes deep-seated infections, such as osteomyelitis and endocarditis and more serious skin infections (furunculosis). *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds. The importance of plant is well known. Life and its growth cannot be imagined without plants. *S. epidermis* is commonly caused infection in adults and children. Throat infection, swollen glands, Impetigo, Cellulites, middle ear infection, sinusitis. They not only produce food for survival but also create healthy environment and eco-friendly Atmosphere to live. Results reveal leaves of *J. gossypifolia* have quite a number of chemical constituents, which may be responsible for many pharmacological activities. This work is required to investigate the extracts of leaves of *J. gossypifolia* for various pharmacological activities for the benefit of human beings.

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