

Evaluation of Preliminary Phytochemical Screening and Acute Dermal Toxicity studies of Ethanolic Extract of *Jatropha glandulifera* Roxb. leaves

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Abstract:

The Present research work was undertaken to investigate the phytochemical constituents and acute Dermal toxicity of the Ethanolic Leaf extract of *Jatropha glandulifera* (EJGL). Fresh Leaves were collected, shade-dried at room temperature, coarsely powdered, and extracted with ethanol using the Soxhlet extraction method. The extract was concentrated using an electrical water bath to obtain a semisolid crude extract with a percentage yield of 6.59%.W/W. Preliminary phytochemical screening was carried out using standard procedures described by Trease and Evans to identify the major bioactive constituents present in the extract. The Acute Dermal Toxicity of test extract was determined in Wistar Albino rats weighing between 150-200g and the Acute Dermal Toxicity were performed according to Organisation for Economic Co-operation and Development (OECD) guideline 402 to determine the safe dose of the plant extract in experimental animals. The Preliminary phytochemical evaluation of the *Jatropha glandulifera* ethanolic leaf extract revealed the presence of alkaloids, flavonoids, quinones, phytosterols, glycosides, carbohydrates, tannins, and phenolic compounds. In Acute Dermal Toxicity studies, EJGL did not cause any mortality of the animals upto dose of 1000mg/kg.

Key words: *Jatropha glandulifera* leaves, Phytochemical screening, Acute dermal toxicity studies.

INTRODUCTION:

Medicinal plants have served as an essential component of the Indian healthcare system for more than thousand years, contributing significantly to the prevention and management of various diseases. Their therapeutic potential is recognized across diverse healthcare practices, including traditional folk medicine, indigenous systems of medicine, and modern pharmaceutical research, where they provide a valuable source of bioactive compounds for the development of therapeutic agents.^[1] Almost 70% of modern medicines in India are derived from natural products. The herbal products are highly been accepted due to low cost, high availability and less side effects.^[2]



Figure 1: *Jatropha glandulifera* Roxb. leaves.

Jatropha glandulifera Roxb. is a medicinal plant belonging to the family Euphorbiaceae and is commonly known as **Kattamanaku** or **Vellai Athalai** in Tamil. The name *Jatropha* is derived from the Greek words *iatros* ("physician") and *trophe* ("nourishment"), reflecting its medicinal relevance.^[3] It is predominantly distributed across various parts of India, particularly in the southern states such as Tamil Nadu, Kerala, and Maharashtra. It has diverse therapeutic properties, *J. glandulifera* has long been recognized in traditional and folk medicine, where it is widely used by indigenous communities for the management of various ailments.^[4] In addition, several bioactive molecules such as jatrophin, jatropholone-A, Fraxetin, coumarino-lignan, 3,3-dimethylacrylshikonin, acetyl shikonin, scopoletin, and gallic acid have been identified, highlighting the plant's considerable pharmacological potential.^[2] *Jatropha glandulifera* Roxb. have been used in ethno-medicines for a long time and widely recognized in traditional medicine for its diverse therapeutic applications. Various parts of the plant are used in the treatment of skin infections, wound healing, snake bites, cuts, and ulcers to help arrest bleeding and promote healing.^[6] And also treat paralysis, and rheumatic disorders.^[7] It possesses anti-inflammatory, antimicrobial, antibacterial, analgesic, and antidiabetic properties and it is used in management of respiratory disorders such as asthma and bronchitis.^[5]

Although *Jatropha glandulifera* has been widely used in traditional medicine, its phytochemical composition and toxicological profile have not been thoroughly investigated. Therefore, the present study was designed to undergo phytochemical screening of EJGL and to evaluate its acute dermal toxicity in Albino rats to provide scientific evidence for its safe medicinal use.

MATERIALS AND METHODS

Plant materials:

Fresh leaves of *Jatropha glandulifera* were collected from in and around Tiruvannamalai district. The plant material was authenticated at the Siddha Central Research Institute (CCRS), Ministry of AYUSH, Government of India, Arumbakkam, Chennai, The plant Code is (J18032602G).

Preparation of Extract:

The collected leaves were cleaned and shade dried for 12-15 days at room temperature, until they were free from the moisture and then pulverized into coarse powder by the means of mechanical grinder and sieved using sieve No.10 and further used for extraction process.

The powdered plant material was first defatted with petroleum ether and the plant material was subjected to continuous hot extraction method with ethanol using Soxhlet apparatus. The obtained alcoholic filtrate was evaporated using electrical water bath to obtain semisolid crude extract. The percentage yield of extract was found to be 6.59% w/w. The extract was stored in the airtight container in refrigerator below 10°C. The

obtained ethanolic extract of *Jatropha glandulifera* leaves was subjected to preliminary phytochemical studies and Acute Dermal Toxicity studies.

Preliminary Phytochemical screening:

The preliminary Phytochemical test was carried out to detect the presence of phytochemicals in EJGL by following the standard procedures.

1. Detection of Alkaloids

a) Mayer's test:

The extract was treated with few drops of Mayer's reagent [Potassium mercuric iodide solution]. Formation of cream coloured precipitate indicates the presence of alkaloid.

b) Dragendorff's test:

The extract was treated with few drops of Dragendorff's reagent [Potassium bismuth iodide solution]. Formation of reddish-brown precipitate indicates the presence of alkaloids.

c) Wager's test:

The extract was treated with few drops of Wager's reagent [Solution of iodine in potassium iodide]. Formation of brown precipitate indicates the presence of alkaloids.

d) Hager's test:

The extract was treated with few drops of Hager's reagent [Saturated solution of Picric acid]. Formation of yellow colour precipitate indicates the presence of alkaloids.

2. Test for carbohydrates

a) Molisch's test:

The extract was treated with few drops of alcoholic alpha naphthol, then added few drops of concentrated sulfuric acid slowly through the sides of the test tube. Formation of purple to violet colour ring at the junction of test tube indicates the presence of carbohydrates.

b) Barfoed's test:

The extract was treated with few drops of barfoed's reagent and heated for 2 minutes. Formation of red precipitate indicates the presence of carbohydrates.

c) Seliwanoff's test:

The extract was treated with 3ml of Seliwanoff's reagent and heated on water bath for 1minute. Formation of rose red colour (ketoses) indicates the presence of carbohydrates.

3. Detection of reducing sugars

a) Benedict's test:

The extract was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath. Formation of reddish-brown precipitate indicates the presence of reducing sugar.

b) Fehling's test:

The extract was treated with Fehling's solution A and B, heated for few minutes. Formation of brick red precipitate indicates the presence of reducing sugar.

4. Detection of glycosides

a) Borntrager test:

The extract was treated with 5-10ml of dilute HCl, boiled on water bath for 10mins and filtered. The filtrate was extracted with benzene or CCl₄ and added equal amount of ammonia solution to filtrate and shaken. Formation of pink or red colour in ammoniacal layer due to presence of anthraquinone moiety indicates the presence of glycosides.

b) Modified Borntrager test:

The extract was treated with 5ml of dilute HCl followed by 5ml ferric chloride. Boiled for 10mins and filtered. The filtrate was extracted with benzene or CCl₄ and added equal amount of ammonia solution to filtrate and shaken. Formation of pink or red colour in ammoniacal layer due to presence of anthraquinone moiety indicates the presence of glycosides.

5. Detection of proteins and amino acids

a) Biuret test:

The extract was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Formation of violet colour indicates the presence of proteins and amino acids.

b) Ninhydrin test:

The extract was boiled with 0.2% solution of Ninhydrin (Indane 1,2,3 trione hydrate). Formation of violet colour indicates the presence of proteins and amino acids.

6. Detection of Flavonoids

a) Alkaline reagent Test:

The extract was treated with 10 ml of ammonium hydroxide. Formation of yellow fluorescence indicates the presence of flavonoids.

b) Ferric chloride test:

The extract was treated with few drops of 10% ferric chloride solution. Formation of Green precipitate indicates the presence of flavonoids.

c) Lead acetate test:

1 ml of the extract filtrate was taken in a test tube. 3–5 drops of 0.1% lead acetate solution were added, the formation of a gelatinous precipitate indicated the presence of tannins.

7. Detection of phenolic compounds

a) Iodine test:

The extract was treated with few drops of diluted iodine solution. Formation of transient red colour indicates the presence of phenolic compounds.

b) Ferric chloride test:

The extract was treated with 1ml of water and boiled for few minutes then it is filtered. The filtrate was treated with 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenolic compound.

8. Detection of Tannins

a) Gelatine test:

The extract was treated with 2ml of 1% gelatine and 10% of sodium chloride. Formation of white precipitate indicates the presence of Tannins

b) 10% sodium hydroxide test:

The plant extract was treated with 4 ml of 10% of sodium hydroxide solution and shaken well. Formation of emulsion indicates the presence of tannins.

9. Detection of saponins

a) Foam test:

The extract was treated with 10 ml of water and boiled for few minutes then it is filtered. The filtrate shaken well and noted for the stable foam

10. Detection of Phytosterols

a) Salkowski Test:

The extract was treated with 2ml of chloroform, then added few drops of concentrated sulphuric acid, shaken and allowed to stand for some time. Formation of yellow colour ring at the junction which turns red after 2mins indicates the presence of phytosterols

b) Libermann Burchard Test:

The extract was treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of concentrated sulphuric acid. Change of colour from red to bluish green ring at the junction indicates the presence of phytosterols.

11. Detection of Quinones

a) Alcoholic KOH test:

To 1ml of plant extract, added few ml of alcoholic potassium hydroxide. Formation of red to blue colour indicates the presence of quinones.

b) Concentrated HCl test:

The extract was treated with few ml of concentrated HCl. Formation of green colour indicates the presence of quinones.

C) Concentrated Sulfuric acid:

The extract was treated with few ml of concentrated sulfuric acid. Formation of red colour indicates the presence of quinones.

12. Detection of fixed oils and fats**a) Stain test:**

Little quantity of plant extract was pressed in between two filter paper. Appearance of oil stain indicates the presence of fixed oils and fats.

Experimental design:

Female Wistar Albino rats were used in the experiments. The animals were procured from the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram Milk Colony, Chennai. After randomization into various groups and before start of the experiment, the animals were housed in polypropylene cages and maintained under standard environment conditions such as temperature ($26 \pm 2^\circ\text{C}$), relative humidity (45 – 55%) and 12 hr. dark/light cycle. The animals were fed with standard pellet diet and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethical committee (IAEC).

Skin preparation for Dermal Toxicity studies:

The Rat were anaesthetized by using General anaesthesia ketamine (50 mg/kg) and Xylazine (5mg/kg) carefully fur was removed from the dorsal trunk. Based on OECD guidelines 402, not less than 10% of the body surface area should be clear for the application of the test substance.

ACUTE DERMAL TOXICITY:

Acute dermal toxicity studies are performed to assess the safety profile of a test substance following a single topical application to the skin. This study is particularly important in evaluating plant extracts and other pharmaceutical materials intended for dermal use, as they provide preliminary toxicity data required for safe formulation development. The studies are conducted in accordance with OECD Guideline 402, ensuring standardized and internationally accepted testing procedures. Healthy young adult female rats (6-8 weeks) were acclimatized to the laboratory condition for 2 weeks. Before the test of 24 hr, the fur was removed from the dorsal area of the trunk of the test animals by electrical hair clippers. About 10% of the body surface area was cleared for the application of the test substance. Three group of female Wistar Albino rats (n=3) in each group was used for the study. For each dose, initial one animal was taken and based on the Morbidity and Mortality the next stage of usage of the other two animals were decided. 200mg/kg was taken as the starting dose of the topical administration and it was applied on the pre-determined area and it was held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. Following 200 mg/kg various dose levels, such as 1000 mg/kg, and 2000 mg/kg body weight, were used to evaluate safety, with animals observed over a total period of 14 days. These doses were applied locally only once at the first day of the study. Rats were monitored for the duration of 24 hrs, with special attention given to the first 6 hrs and once daily further for a period of 14 days. Based on the initial observation for toxicity and mortality in one animal for each dose decides the usage of the remaining two animals. During the study, animals are weighed and carefully observed for mortality, behavioural changes such as salivation, tremors, convulsions, diarrhoea, lethargy, sleep, and coma, as well as alterations in physical appearance, signs of pain or injury, and indications of illness. Additional observations include changes in fur, eyes, and mucous membranes, along with assessments of respiratory, circulatory, autonomic, and central nervous system functions and overall behavioural patterns. In addition, the treatment site may be observed at 24, 48 and 72 hours after removal of test sample using the Draize criteria.^[8]

Table 1: Draize dermal irritation scoring system (DDISS)

Erythema and Eschar formation	Value	Edema formation	Value
No erythema	0	No erythema	0
Very slight erythema (barely perceptible)	1	Very slight erythema (barely perceptible)	1
Well-defined erythema	2	Well-defined erythema	2
Moderate to severe erythema	3	Moderate to severe erythema	3
Severe erythema (beet- redness) to slight, eschar formation (injuries in depth)	4	Severe erythema (beet- redness) to slight, eschar formation (injuries in depth)	4

RESULTS:

Preliminary phytochemical screening:

The Preliminary phytochemical screening of ethanolic extract of leaves of *Jatropha glandulifera* revealed the presence of different kind of phytochemical components that are summarized in table No. 2

Table 2: Preliminary phytochemical screening of Ethanolic extract of *Jatropha glandulifera* Leaves

S.No	Name of the test to detect the Phytoconstituents	Presence (+) or Absence (-) of the Phytoconstituents
1.	Detection of Alkaloids	
	a) Dragendroff's test	(+)
	b) Hager's test	(+)
	c) Wagner's test	(+)
	d) Mayer's test	(+)
2.	Detection of Carbohydrates	
	a) Barfoed's test	(+)
	b) Molisch's test	(+)
	c) Selliwanoff's test	(+)
3.	Detection of reducing sugars	
	a) Benedict's test	(+)
	b) Fehling's test	(+)
4.	Detection of Glycosides	
	a) Borntrager's test	(+)
	b) Modified Borntrager's test	(+)
5.	Detection of Proteins and Amino acids	
	a) Biuret test	(+)
	b) Ninhydrin test	(+)
6.	Detection of Flavonoids	
	a) Alkaline reagent test	(+)
	b) Ferric chloride test	(+)
	c) Lead acetate test	(+)
7.	Detection of Phenolic compounds	
	a) Iodine test	(+)
	b) Ferric chloride test	(+)
8.	Detection of tannins	
	a) Gelatin test	(+)
	b) NaOH test	(+)
9.	Detection of saponins	
	a) Foam test	(-)
10.	Detection of Phytosterols	

	a) Salkowski's test	(+)
	b) Libermann Burchard test	(+)
11.	Detection of quinones	
	a) Alcoholic KOH test	(+)
	b) Con.Hcl test	(+)
	c) Con Sulfuric acid	(+)
12.	Detection of fixed oils and Fats	
	a) Stain test	(+)

Determination of acute dermal toxicity :

In Acute dermal toxicity evaluation of the ethanolic extract of *Jatropha glandulifera* leaves in the rats revealed that the initial test dose treated with one animal for 200 mg/kg did not show any morbidity or mortality observed for 14 days. Based on these observations, two additional animals were treated at the same dose level. No signs of toxicity or mortality were observed at 200 mg/Kg. Based on the above observation the next dose 1000 mg/kg was tested using single animal, no toxicity was observed up to 14th day. The next two animal tested with the same dose 1000mg/Kg also did not produce any toxicity. The next selected dose was 2000mg/kg, the initial test dose in the first animal did not produce any toxicity or mortality, but the next two animal with the same dose showed mild erythema on the 2nd day of test dose application and on the 12th both the animal underwent mortality. Based on the Draize dermal irritating score system dose of 200 and 1000mg/kg of EJGL did not produce any erythema or oedema in the animals, the value is scored as 0 respectively. In the High Dose of EJGL 2000mg/kg – slight erythema with no oedema was noted and the value was scored as 1. So, the 1000 mg/kg was considered as the safe dose for dermal usage.

DISCUSSION:

The phytochemical screening carried out in the present study revealed that the extract of *Jatropha glandulifera* contains a diverse range of bioactive phytoconstituents, it shows the presence of secondary metabolites such as alkaloids, flavonoids, tannins, phytosterols, glycosides, phenolic compounds, carbohydrates, quinones, proteins, and terpenoids. The occurrence of these phytochemicals may account for the wide range of biological and pharmacological activities attributed to *Jatropha glandulifera* in traditional medicine. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. The presence of alkaloids in the extract which can exhibit a variety of pharmacological activities, including antimicrobial, analgesic, antioxidant, anti-inflammatory, and neuroprotective effects.^[9] The detection of flavonoids and phenolic compounds in the extract highlights its possible antioxidant potential. Flavonoids also regulate inflammatory responses by inhibiting oxidative enzymes and reducing the production of pro-inflammatory mediators.^[10] Quinones exhibit antimicrobial, antioxidant, and wound-healing properties.^[11] Therefore, the detection of these phytochemical constituents in *Jatropha glandulifera* may contribute to its reported medicinal applications. These findings support the plant's potential as a source of bioactive compounds with therapeutic value. Based on Acute dermal toxicity studies 1000mg/kg is considered as safe dose for dermal usage to carry out the pharmacological evaluation regarding dermal activity.

CONCLUSION:

The outcome of the present study shows that the ethanolic extract of *Jatropha glandulifera* contains a variety of phytochemical constituents, including alkaloids, carbohydrates, reducing sugars, glycosides, flavonoids, proteins and amino acids, phenolic compounds, tannins, phytosterols, quinones, fixed oils, and fats. The acute dermal toxicity study showed that the EJGL did not produce any mortality or adverse effects in experimental animals up to the dose of 1000 mg/kg. These results confirm the safety of the extract and it is safe at the tested dose up to 1000mg/Kg and has the potential for further pharmacological evaluation.

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