Evaluation of Phytochemical screening and in vitro Anti-inflammatory activity of Ethanolic extract of *Jatropha gossypifolia* Linn.

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**A B S T R A C T**

The present study was aimed to evaluate the phytochemical screening and in-vitro anti-inflammatory activity of ethanolic extract of *Jatropha gossypifolia* Linn. It reveals the presence of considerable amount of alkaloid, steroid, phenolic substances and vitamin C (Ascorbic acid), moderately saponins and carbohydrates, trace amount of glycoside and resins were explored from the phytochemical screening. The investigation is based on the need for newer anti-inflammatory agents from natural source with potent activity and lesser side effects as substitutes for chemical therapeutics. Realizing the fact this study was carried out to evaluate the in vitro anti-inflammatory activity of ethanolic extract of *J. gossypifolia*. Results of the study is obtained that the ethanolic extract of *J. gossypifolia* was exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane in concentration dependent manner It is due to the presence of active principles such as flavonoids and triterpenoids may responsible for this activity. Hence, *J. gossypifolia* can be used as a potent anti-inflammatory agent.

**Keywords:** *Jatropha gossypifolia* Linn, Anti-inflammatory activity, Phytochemical Screening, Ethanolic extract, Hypotonicity

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1. **Introduction**

Inflammation is a response of a tissue to injury, often injury caused by invading pathogens. It is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is...
one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.

**Pathophysiology of inflammation:**

The main cells involved in chronic infection are macrophages and lymphocytes. Because both these cells have single nuclei, they are known as mononuclear cells. With the aid of chemical mediators such as lymphokines, macrophages do an excellent job of engulfing and neutralizing or killing foreign antigens. Lymphocytes are the predominant cell in chronic inflammation. There are two types, labeled T and B cells. T-lymphocytes are produced in the thymus gland. They ensure cell based immunity from infection. B-lymphocytes originate in the bone marrow and ensure humoral (bodily fluid) immunity.

**Plant profile:**

Jatropha gossypifolia, commonly known as bellyache bush, black physicnut or cotton-leaf physicnut, is a species of flowering plant in the spurge, family- Euphorbiaceae.

**Scientific Classification:**

- **Kingdom:** Plantae
- **Sub kingdom:** Tracheobionta
- **Division:** Angiosperms
- **Genus:** jatropha
- **Class:** Eudicots
- **Sub class:** Rosidae
- **Order:** Malpighiales
- **Family:** Euphorbiaceae
- **Species:** J. gossypifolia

**Fig 1: Mechanism of Inflammation**

**Traditional Uses:**

- The leaves are used as traditional folk medicine to treat inflammatory and painful diseases.
- Leaf juice is used to heal wound as external application, bruises and also used for intermittent fevers, carbuncles, eczema, itches, sores on the tongues of babies, stomachache, and venereal disease.
- Whole plant used for antihypertensive, antimicrobial, anti-inflammatory analgesic, hemostatic, anticholinesterase, antioxidant, contraceptive, tocolytic, antineoplastic, local anaesthetic, neuropharmacological, antidiarrheal, immunomodulatory, hepatoprotective actions.

**2. Materials and Methods**

**Phytochemical Screening:**

The ethanolic extract of jatropha gossypifolia Linn was subjected to preliminary phytochemical screening for detection of various phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, cardiac glycosides, steroids, terpenoids,anthroquinones, phlobatannins, proteins and carbohydrates. Following standard procedures were used.

**Test for Carbohydrates:**

- **Molisch test:** To 2ml of test solution, add alcoholic α-naphthol and then add a few drops of conc. sulphuric acid through sides of the test tube. Formation of purple to violet coloured ring at the junction confirmed the presence of carbohydrates (reducing sugar).
- **Barfoed's test:** To 1 ml of test solution, add 1 ml of barfoed's reagent and heat on a water bath, the formation of cupric acid gives a green colour confirmed the presence of the monosaccharide (reducing sugar).
- **Fehlings test:** To 1 ml of test solution, add 1 ml of fehling's A and B and heat to water bath, the formation of brick red precipitate confirms the presence of the carbohydrate (reducing sugar).36
- **Benedicts test:** To 2 ml of test solution, add 1 ml of benedicts reagent and heat on a water bath, the formation of reddish brown precipitate confirms the presence of the carbohydrates (reducing sugar).

**Test for Glycosides:**

**Keller Killiani Test:**

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.
Baljets test: Treat 2ml of test solution with picric acid or sodium picrate. Formation of orange colour confirms the presence of cardiac glycosides.

Test for Saponins:
Foam Test: Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Steroids and Triterpenoids:
Liebermann Burchard test: Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red colour in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Salowsk test: Treat the extract with a few drops of conc. Sulphuric acid, the formation of red colour at lower layer indicates the presence of steroids or the formation of yellow colour at a lower layer indicates the presence of triterpenoids.

Test for Alkaloids:
Hager's Test: Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Dragondroffs reagent test: To 1ml of test solution, add dragondroffs reagent (potassium bismuth iodide). Formation of reddish brown precipitate confirms the presence of the alkaloids.

Mayers reagent test: To 1ml of test solution, add mayers reagent (potassium mercuric iodide solution). Formation of a cream colour precipitate confirms the presence of alkaloids.

Test for Flavonoids:
Schinods test: To the 2ml of test solution, add few magnesium turnings and add conc. HCl drops wise pink, crimson red, green to blue colour will appear after a few minutes if flavonoids were present.

Alkaline reagent Test: Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Zinc hydrochloride test: To the test solution add a mixture of Zinc dust and Conc. Hydrochloric acid. It gives red colour after a few minutes if flavanoids were present.

Test for Tannins:
Gelatin Test: Test solution when treated with gelatine solution would give white precipitate indicating the presence of tannins.

Phenzone test: Take 5ml of aqueous extract of drug. Add 0.5grams of sodium acid phosphate. Warm it and cool. Filter solution. To the filtrate, add 2% solution of Phenzone. Tannins will be precipitated. Precipitates will be bulky and colored.

Gold beater's skin test: Gold beater’s skin is a membrane prepared from intestine of Ox and it behaves similarly to un tanned skin. Soak a small piece of Gold beater’s skin in 2% hydrochloric acid. Rinse it with distilled water. Place it in solution to be tested for 5 minutes. Wash in water and transfer to 1% solution of ferrous sulphate. Black or brown colour of skin indicates presence of tannins. It is a quantitative test and only positive for true tannins.

Test for Proteins and Amino acids:
Biurettte Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour.

Ninhydrin Test: Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple colour suggesting the presence of free amino acids.

Millons test: To 1ml of test solution, add 2ml of millions reagent and heat on a water bath, the formation of a white precipitate confirms the presence of the proteins.

Invitro Pharmacological studies
Drugs and chemicals: Diclofenac was procured from Sigma Chemical Co.Hyd, dextrose, sodium citrate, citric acid, sodium chloride, sodium hydroxide, dihydrogen phosphate and Ethanol from chemical laboratory in Nellore, were used in the present study.

Instruments used: UV spectrophotometer, Digital balance and Weighing balance.

Evaluation of In-vitro Anti Inflammatory Activity: HRBC Membrane Stabilization Method:
Principle: The principle involved in stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline (0.36%), 0.5 ml HRBC suspension (10% v/v) with 0.5 ml of each plant extracts (leaf, stem and callus) of various concentrations (100, 250, 500, 1000 g/ml), standard drug diclofenac sodium (100, 250, 500, 1000 g/ml) and control distilled water instead of hypo saline to produce 10% hemolysis were incubated at 37°C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage hemolysis produced in the presence of distilled water was taken as 100%.

Procedure: The human red blood cell membrane stabilization method (HRBC) has been used as a method to study the invitro anti-inflammatory activity. Blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment.

- The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water).
- The blood was centrifuged at 3000rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2).
- The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.
- The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2ml hypo saline [0.36%], 0.5ml HRBC suspension [10% v/v] with 0.5ml of plant extracts and standard drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000 g/ml).
and control (distilled water instead of hypo saline to produce 100% hemolysis) were incubated at 37°C for 30min and centrifuged respectively.

- The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.
- The percentage of hemolysis of HRBC membrane can be calculated as follows:

\[
\text{% Hemolysis} = \left( \frac{\text{Optical density of Test sample}}{\text{Optical density of control}} \right) \times 100
\]

- The percentage of HRBC membrane stabilization can be calculated as follows: \( \text{% Protection}=100-\frac{\text{Optical density of test sample}}{\text{Optical density of control}} \times 100 \).

3. Results and Discussion

Phytochemical Screening: Preliminary phytochemical screening was studied in broad sense to explore its chemical nature, it reveals the presence of considerable amount of alkaloid, steroid, phenolic substances and vitamin C (Ascorbic acid), moderately saponins and carbohydrates, trace amount of glycoside and resins were explored from the phytochemical screening.

HRBC Membrane Stabilization Method:

In-vitro anti-inflammatory activity of J. gossypifolia were concentration dependent, the maximum protection of 90.59% was seen at the concentration of 1000 μg/ml. All results were compared with standard diclofenac sodium which showed 92.65% protection at the concentration of 1000 μg/ml.

<table>
<thead>
<tr>
<th>Conc. (μg/ml)</th>
<th>% Hemolysis of J. gossypifolia</th>
<th>% Stabilisation of J. gossypifolia</th>
<th>% Hemolysis of diclofenac sodium</th>
<th>% stabilisation of diclofenac sodium</th>
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<tbody>
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4. Conclusion

The Ethanolic extract of J-gossypifolia possess significant anti-inflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions. In conclusion, the present study has shown that the plant parts of J.gossypifolia have membrane stabilization effect by inhibition of hypo tonicity induced lysis of erythrocyte membrane. Hence, it implies the anti-inflammatory and analgesic properties mediated by prostaglandin synthesis inhibition. Membrane stabilization may contribute to the anti-inflammatory effect. Further research on isolation of the active principle for anti-inflammatory activity has to be worked out in future studies.

5. References


