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Investigation of invitro anti-inflammatory activities of ethanolic Euphorbia hirta flower extract

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ABSTRACT: The present study investigated the in vitro anti-inflammatory activity of ethanolic Euphorbia hirta flower extract. Euphorbia hirta, a plant used in traditional medicine, was investigated for its antiinflammatory properties. The flower extract showed significant anti-inflammatory activity, reducing inflammation and inhibiting inflammatory responses. The study supports the traditional use of Euphorbia hirta in treating inflammatory disorders and suggests its potential as a natural remedy for inflammation.

KEYWORDS: Euphorbia hirta, anti-inflammatory activity, flower extract, traditional medicine, natural remedy. The study demonstrates the potential anti-inflammatory activity of ethanolic Euphorbia hirta flower extract, supporting its traditional use in the treatment of inflammatory disorders.

I. INTRODUCTION

PHARMACOLOGY

Pharmacology is the science of drugs and medications, including a substance's origin, composition, pharmacokinetics, Pharmacodynamics, therapeutic use, and toxicology. More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function .If substances have medicinal properties, they are considered pharmaceuticals.

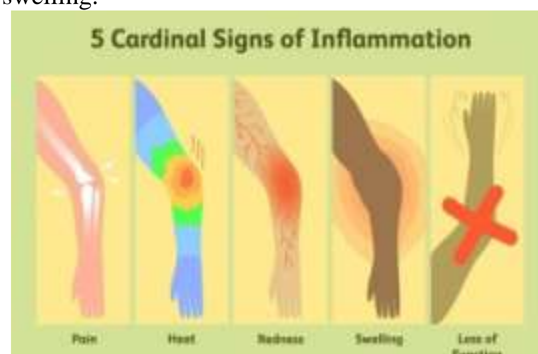
The field encompasses drug composition a properties, functions, sources, synthesis and drug design, molecular and cellular mechanisms, organ/systems mechanisms, signal transduction/cellular communication, molecular diagnostics, interactions, chemical biology, therapy, and medical applications and antipathogenic capabilities.

The two main areas of pharmacology are pharmacodynamics and pharmacokinetics. Pharmacodynamics studies the effects of a drug on biological systems, and pharmacokinetics studies the effects of biological systems on a drug.

INFLAMMATION

Inflammation is your body's response to an illness, injury or something that doesn't belong in your body (like germs and chemicals). Inflammation is a normal and important process that allows your body to heal. Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation, and acts by removing injurious stimuli and initiating the healing process.

When an invader (like a virus) tries to enter your body, or you get injured, your immune system sends out its first responders. These are inflammatory cells and cytokines (substances that stimulate more inflammatory cells). These cells begin an inflammatory response to trap germs or toxins and start healing injured tissue .Inflammation can cause heat, redness, pain, swelling.



Inflammation is therefore a defense mechanism that is vital to health. Usually, during

acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation.

However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases. At the tissue level, inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function, which result from local immune, vascular and inflammatory cell responses to infection or injury.

Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release.Various pathogenic factors, such as infection, tissue injury, or cardiac infarction, can induce inflammation by causing tissue damage.

In response to tissue injury, the body initiates a chemical signaling cascade that stimulates response aimed at healing affected tissues. These signals activate leukocyte chemotaxis from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses.

TYPES OF INFLAMMATION

There are two main types of inflammation acute and chronic. Acute inflammation is sudden and temporary, while chronic inflammation can go on for months or years.

ACUTE INFLAMMATION

This is your immune system’s response to a sudden injury or illness. Inflammatory cells travel to the site of injury (like a cut on your finger) or infection and start the healing process. Infections in different parts of your body can cause sudden, and usually short-lived, inflammation.

For example, bacterial infections like strep throat and viral infections like the flu can cause throat inflammation. Other bacterial and viral infections can cause inflammation of your small intestine (enteritis). Acute inflammation may last for a few hours to a few days, depending on your condition.

CHRONIC INFLAMMATION

This is when your body continues sending inflammatory cells even when there’s no danger. For example, in rheumatoid arthritis, inflammatory cells and substances attack joint tissues. This leads to inflammation that comes and goes and can cause severe damage to your joints.

With chronic inflammation, processes that normally protect your body end up hurting it. Chronic inflammation can last for months or years. You may have periods where it improves and other times when it gets worse.

SYMPTOMS OF INFLAMMATION

- Discolored or flushed skin.
- Pain or tenderness that should be mild and only in the area of the injury.
- Swelling (for example, knee inflammation).
- Skin that feels hot to the touch.
- Inability to use that part of your body as you normally would (for example, reduced range of motion).
- Chest pain
- Mouth sores
- Abdominal pain

CAUSES OF INFLAMMATION

- Low levels of physical activity.
- Chronic stress.
- Having a BMI at or above 30 (obesity), especially when excess weight is deep within your belly (visceral fat).
- An imbalance of healthy and unhealthy microbes in your gut (dysbiosis).
- Regularly eating foods that cause inflammation, such as foods high in trans fat or salt.
- Disrupted sleep and circadian rhythm.
- Exposure to toxins, including air pollution, hazardous waste and industrial chemicals.
- Using tobacco products.
- Regularly drinking too much alcohol.

PLANT PROFILE

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Tamil Name: Amman Pacharisi
Kingdom: Plantae**Binomial name:** Euphorbia hirtaL.
Order: Malpighiales**Family:**Euphorbiaceae
Genus: Euphorbia**Species:** E. hirta

Synonyms

- Chamaesycegemella (Lag.) Small▪Chamaesycehirta (L.) Millsp.
- Chamaesycekarwinskyi (Boiss.) Millsp.
- ChamaesyceroseiMillsp.

Euphorbia hirta

Euphorbia hirta (sometimes called asthma-plant) is a pantropical weed, originating from the tropical regions of the Americas. It is a hairy herb that grows in open grasslands, roadsides and pathways.It is widely used in traditional herbal medicine across many cultures, particularly for asthma, skin ailments, and hypertension. It is also consumed in herbal tea form as folk medicine for fevers in the Philippines (where it is known as tawa-tawa), particularly for dengue fever and malaria.

DESCRIPTION

This erect or prostrate annual herb can grow up to 60 cm (24 in) long with a solid stem that is furnished with many yellow to reddish coarse hairs, and produces abundant white latex. There are stipules present. The leaves have an oblique base and are simple, elliptical to slightly rhombic, hairy (on both upper and lower surfaces but particularly on the veins on the lower leaf surface), with a finely dentate margin, the veins upper side being deep-set and conspicuous on the underside, and the leaf surface somewhat leathery.
Leaves occur in opposite pairs on the stem. The flowers are unisexual and found in axillary cymes at each leaf node, held as dense balls of flowers and fruit capsules usually close to the stem, the flower glands with tiny white/pinkish petal-like appendage.

The fruit is a capsule with three valves (creating 3 sides), uniformly appressed hairy, containing tiny (0.7–0.9 mm), oblong, and four-sided orange to pink or red seeds. It has a white or brown taproot.

MORPHOLOGY

The plant Dudhia/ Euphorbia hirta is a small annual herb, frequently seen occupying open waste spaces, roadsides, grasslands, pathways, rice field and as a weed of cultivation. The plant is a common herb, found in pan-tropic , partly sub tropic areas and worldwide including Australia, Western Australia, Northern Australia, Northern territory, Queensland, New south wales, Central America, Africa, Indo malaysia, Philippines, China and India. It is native to Central America. It is usually erect, grows up to a height of 40cm tall and it can also be seen lying down. The stem is slender, reddish in color, covered with yellowish bristly hairs especially in young parts.

Leaves – simple, arranged oppositely, distichous, leaf blades are lanceolate, unequal base, cuneate one side, round other side, acute apex, finally toothed margins, dark green above, pale beneath, purple bloth in middle, measures about 1-2.5 cm long. Flower unisexual, male flowers are sessile, linear bracteoles, fringed, single stamen, with absent perianth. Female flowers are short pedical, rimmed perianth, superior ovary; three celled, three styles, minute, covered with short hairs, twofid apex.

Inflorescence – cluster of flowers called cyathium at terminal or auxiliary. Several cyathia densely clustered into a cyme. Fruits – yellow, three lobed, three – seeded, keeled capsules, containing three brown, four- sided, angular, wrinkled seeds, base truncate, hairy, 1-2mm in diameter. Seeds- oblong, four – sided, slightly wrinkled, pinkish brown, caruncle absent.

II. MATERIAS AND METHODS

Processing of plant:

The collected plant has identified by Dr.J.Suresh Kumar, Assistant Professor, Dept of Botany, Government Arts College, Tiruvannamalai (District), Tamilnadu, India. The plant was washed with tap water 3 times.
The purified plant material was shade dried at room temperature to avoid chemical changes and frequently observed for any fungal contamination as the plant material rich in water content. When the plant material was dried material was dried entirely (Figure 1a), it has subjected to prepare coarse powder with help of pestle and mortar. (Figure 1b).
The coarse material powder is collected and used for extraction of the crude drug in solvent by soxhlet extraction method.

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Figure 1a (Dried flowers)
Figure 1b (Coarse powder)

Extraction by Soxhlet Apparatus:

Extraction by soxhlet apparatus the extraction procedure for the isolation of crude drug from plants has been practiced for a long time. The mode of extraction process on the presence of water content of the plant materials that have been extracted.

Usually the crude extract has taken from Soxhlet apparatus with the aqueous solvent. This apparatus mainly consists of three parts, a round bottom flask in which the solvent has taken, the main jar in which the material from which the compounds to been extracted has kept loaded, and a condenser in which condensation of vapours of solvents takes place.

Approximately 30 g of the powder of plant material from which the extract has to take into Soxhlet main jar. The solvent is poured in the round bottom flask and extract condensation under reduced pressure, and a controlled temperature of 60-80 ℃ has set to boil through the regulated heating mantle (figure 2). The vapour of the solvent pass-through drive tubes enters condenser through the main jar and gets condensed where there is a continuous flow of water in the condenser.

Figure2: Soxhlet Apparatus Extraction

PREPARATION OF EXTRACT

Shade-dried plant flower chopped into small pieces by using mortar and pestle, grinded into powdered form. The powdered plant material was subjected to sequential solvent extraction by soxhlet extraction method.

The extraction was done with ethanol solvent. The extracts were evaporated using rotary evaporator and the percentage yield was thus recorded.

Dried extracts were stored in airtight containers for further studies. Concentrated extracts were subjected to various chemical tests in order to detect the various phytoconstituents.

PHYTOCHEMICAL SCREENING

The concentrated extracts of selected plant were subjected to different chemical tests for the detection of different phytoconstituents using standard methods.

(i) Test for saponins

Crude extract when mixed with 5ml distilled water in a test tube then it was shaken briskly. The formation of stable foam which indicate the presence of saponins.

(ii) Test for flavonoids

Crude extract when mixed with 10ml distilled water; 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate solution then added 1ml concentrated sulphuric acid.
Indication of yellow color shows the presence of flavonoids.

(iii) Test for steroids

The crude extract of selected plant was dissolved in 0.5mL dichloromethane to prepare a dilute solution and then 0.5 mL of acetic anhydride was added followed by four drops of concentrated sulphuric acid. A blue-green colouration indicated the presence of steroids.

(iv) Test for tannins

Crude extract of plant was mixed with small amount of water and heated on water bath. The mixture was filtered and ferric chloride was added drop by drop to the filtrate. A dark green appear which indicates the presence of tannins.

(v) Test for Alkaloids

Crude extract was dissolved with 2ml of 1% HCl and heated gently. Wagner’s and Mayer’s reagents were added to the mixture. Turbidity of the resulting precipitate was taken as confirmation for the presence of alkaloids.

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(vi) Test for carbohydrate

Both Fehling A and Fehling B solution were mixed in equal volume. These reagents are added in crude extract and smoothly boiled. A brick red precipitate is appeared at the bottom of the test tube and indicates the presence of reducing sugar. Where,

Phyto-chemical constituent	Ethanolic extract	Methanolic extract
Alkaloid	+	+
Flavonoid	+	+
Terpenoid	+	+
Tannin	+	+
Saponins	-	-
Carbohydrate	+	-

Phyto-chemical constituent	Choloroform extract	Hexane extract
Alkaloid	+	-
Flavonoid	+	+
Terpenoid	+	-
Tannin	+	-
Saponins	-	-
Carbohydrates	+	-

+ = indicates presence of phytochemicals
- = indicates absence of phytochemicals

- The result of the preliminary phytochemical screening from flowers of Euphorbia hirta Showed the presence of alkaloid, flavanoid, tannin, terpenoid and carbohydrate in ethanol extract.

INVITRO METHODS OF ANTI INFLAMMATORY ACTIVITY

1)HRBC membrane stabilization assay:

Preparation of RBCs suspension:

3ml of fresh whole human blood were drawn from healthy nonsmoker's volunteers, who did not take any alcoholic drinks, and did not use any chemical medicine for one week at least.

Blood samples were pipette into heparin tubes and centrifuged for 10 minutes at a speed of 3000 rpm, then red blood cells were resuspended using a volume of normal saline solution equal to that of the supernatant and reconstituted as a 40% suspension with 10 mM sodium phosphate buffer (PH 7.4).The buffer solution consists of NaH_2PO_4 (0.2g), Na_2HPO_4 (1.15g) and NaCl (9g) in 1 L of distilled water.

Examining the ability of plant extract to stabilize red blood cells membrane:

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The above mentioned phosphate buffer was used to prepare series of dilutions of each plant extract; 5, 10, 15, 20, 25 µg per ml for Euphorbia hirta. A group of centrifuge tube were prepared in such a way that each tube contained 5m of extract, 4.85 ml of isotonic buffer solution and 0.15ml of RBCs suspension 40%.One of the group was incubated 20mins in a water bath at 54^0 C. The other group was placed in a refrigerator.Afterwards, the tubes were centrifuged for 7mins at 3500 rpm and the absorbance of the supernatant was measured at 560nm.
The ability of the plant extract to stabilize the membrane was calculated in percent by the formula (1);

Protection %= 1-(A2-A1÷A3-A1) × 100 Where:
A1=Absorbance of unheated test sample.
A2=Absorbance of heated test sample.
A3=Absorbance of heated control sample.
Saline solutions were used as a negative control and diclofenac sodium were used as a standard drug, respectively.

2) Evaluation of extract effect on protein denaturation assay:

The following reaction mixture was prepared; 0.2 ml of hen egg albumin, 2.8 ml of isotonic phosphate buffer (10 mM sodium phosphate buffer, pH 7.4) and 2 ml of extract solution at concentrations (5, 10, 15, 20, 25 µg ml) for Euphorbia hirta. The mixture was incubated for 20 minutes at 37º C and then for 5 minutes at 70º C. After cooling, the absorbance was measured at 660nm.

To calculate the ability of the extract to protect protein from denaturation in percent, formula (2) was used below:

Protection %= [(Absorbance of sample ÷ Absorbance of control) -1] × 100
Sodium diclofenac was used as a positive control, while distilled water used as a negative control.

III. RESULTS

1) Anti-inflammatory activity by HRBC membrane stabilization method

The flower extract of Euphorbia hirta different concentration showed significant stabilization towards HRBC membranes. The percentage protection of ethanol extract showed significant antiinflammatory activity in a concentration dependent manner. The results are tabulated in Table 1.

Table 1: In vitro anti-inflammatory activity of Euphorbia hirta flower by HRBC membrane stabilization
Table 1a: In vitro anti-inflammatory activity of Euphorbia hirta flower by HRBC membrane stabilization method

Concentrationµg/ml	Absorbance of the unheated sample (A1)	Absorbance of the heated sample (A2)
5µg/ml	0.015	0.013
10µg/ml	0.025	0.015
15µg/ml	0.033	0.022
20µg/ml	0.041	0.026
25µg/ml	0.053	0.034

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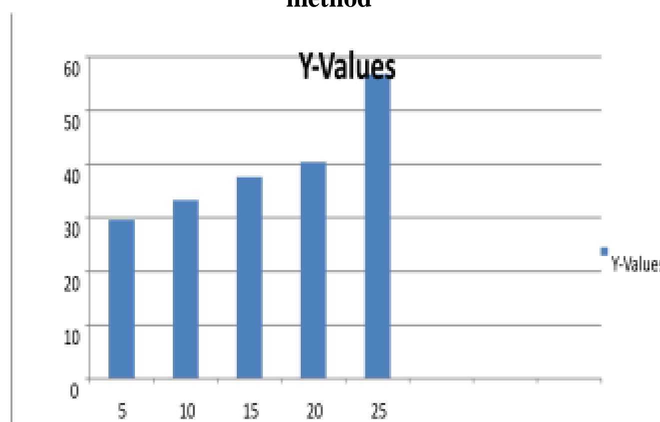
Table 1b: In vitro anti-inflammatory activity of Euphorbia hirta flower by HRBC membrane stabilization method

Concentration µg/ml	Absorbance of heated control sample (A3)	Protection %
5µg/ml	0.022	29.57%
10µg/ml	0.056	33.35%
15µg/ml	0.063	37.6%
20µg/ml	0.079	40.4%
25µg/ml	0.087	56.8%

Graph 1: Invitro anti-inflammatory activity of Euphorbia hirta flower by HRBC membrane stabilization method



X-CONCENTRATION (µg/ml)
Y-PROTECTION %

Protein denaturation method

The denaturation of protein is a well-documented cause of inflammation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. It was effective heat induced albumin denaturation at different concentration as shown in Table 2.

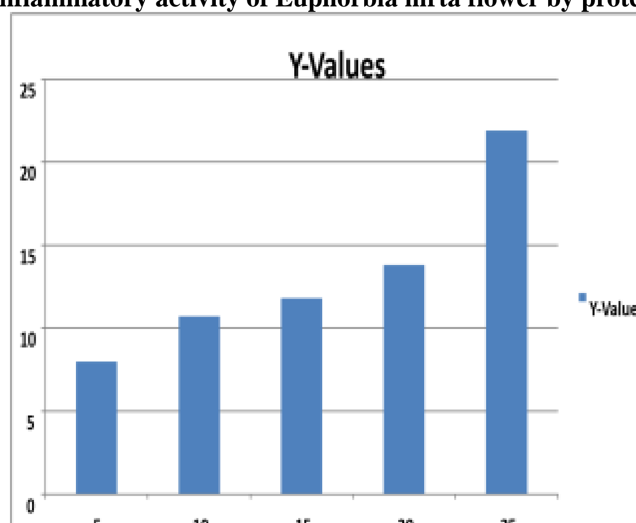
Table 2a: In vitro anti-inflammatory activity of Euphorbia hirta flower by protein denaturation method

Concentration µg/ml	Absorbance of sample	Absorbance of control
5µg/ml	0.49	0.45
10µg/ml	0.62	0.56
15µg/ml	0.66	0.59
20µg/ml	0.74	0.65
25µg/ml	0.89	0.73

Table 2b: In vitro anti-inflammatory activity of Euphorbia hirta flower by protein denaturation method

Concentration µg/ml	Protection %
5µg/ml	8%
10µg/ml	10.7%
15µg/ml	11.8%
20µg/ml	13.8%
25µg/ml	21.9%

Graph 2: Invitro anti-inflammatory activity of Euphorbia hirta flower by protein denaturation method



X-CONCENTRATION (µg/ml)
Y- PROTECTION %

IV. DISCUSSION

The Euphorbia hirta of the family Euphorbiaceae is found in the roadsides and pathways of Tiruvannamalai, Tamilnadu. The plant is used in folk medicine and also believed to have active components that help to treat and manage various diseases. Chemical evaluation of the ethanolic flower extract of Euphorbia hirta showed the presence of carbohydrates, proteins, amino acids, sterols, triterpenoids, cardiac glycosides, alkaloids, phenolic compounds, flavonoids. The phytochemicals like sterols and triterpenes have already been proved for preventing inflammatory process. Anti Inflammatory activity of its various extracts were performed to explore its bioefficiency.

The study was took HRBC membrane stabilization method for screening of activity. The results reveal the ethanolic extract of Euphorbia hirta showed significant activity (Membrane protection). The anti inflammatory activity and formation of collagen as well as prevention of release of inflammatory mediators is due to steroids and tannins content present in medicinal plants. Denaturation of protein is of the causes for the production of auto antigens in certain rheumatic diseases.

The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic, and disulphide bonding. The present study reveals that Euphorbia hirta flower might be capable of controlling the production of auto

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antigens due to denaturation of proteins and stabilize the lysosomal membrane. The invitro method showed significant anti inflammatory property of different concentrations tested.

V. CONCLUSION

In the present study, results indicate that the ethanolic Euphorbia flower extract possess antiinflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids and phenols. On the basis of the results,

we may concluded that the HRBC membrane stabilization and protein denaturation assay substantiated the anti-inflammatory potential of the extract, supporting conventional uses for pain and inflammation management. Overall this study provides valuable knowledge on the antiinflammatory activity.

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