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Anti-Inflammatory and Antiarthritic Activity Properties of *Euphorbia hirta* Methanol Extract

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Abstract: *Euphorbia hirta*, commonly known as asthma-plant, is recognized as a tropical weed likely originating from India. It is reported to possess numerous therapeutic properties, including but not limited to antiasthmatic, antidiabetic, anticancer, wound healing, gastrointestinal, diuretic, antiparasitic, immunological, antioxidant, antimicrobial, sedative, anxiolytic, antiepileptic, analgesic, antipyretic, antihistaminic, "Protective effects on the liver, galactagogue activity, inhibition of angiotensin-converting enzyme, and anti-thirst properties."This study focused on the methanolic extract of the whole plant to analyse the presence of various phytochemicals and assess its anti-inflammatory effects *via* protein denaturation assay, as well as its antiarthritic properties through hypotonicity-induced haemolysis and heat-induced haemolysis assays. The plant extract was tested across several concentrations ranging from 100 to 500mg/ml) in bioassays to validate its anti-inflammatory and antiarthritic capabilities. Diclofenac sodium (100 mg/ml) served as the reference standard drug in this study. Qualitative phytochemical screening of the methanolic extract of *Euphorbia hirta* revealed the presence of various bioactive constituents, including flavonoids, glycosides, phenolic compounds, tannins, alkaloids, terpenoids, and sterols. The antiarthritic activity, assessed through the inhibition of protein denaturation, showed that the methanol extract at 500 mg/ml achieved a 43.24% inhibition, which was lower than the standard drug diclofenac 64.86% inhibition. The IC_{50} value for the extract was determined to be 548.48 mg/ml. Furthermore, anti-inflammatory activity was evaluated by examining hypotonic and heat-induced haemolysis of human RBCs, where the methanol extract at 500 mg/ml demonstrated 43.01% and 51.14% protection, respectively. The EC_{50} values were calculated to be 568.2 mg/ml for hypotonic haemolysis and 440.87 mg/ml for heat-induced haemolysis. This study indicated that methanol extract *Euphorbia hirta* may serve as a therapeutic agent for management of inflammation.

Keywords: *Euphorbia hirta; Antiarthritic Activity; Anti-Inflammatory Activity; Phytochemical Analysis.*

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I. INTRODUCTION

In India, various parts of medicinal plants have been traditionally used to treat specific diseases and health conditions since ancient times. Indigenous systems of medicine, such as Ayurveda, Siddha, and Unani, have been practiced for centuries and continue to play an important role in traditional healthcare. Some drugs from Ayurveda approaches modern diseases, have already reached the market place [1-3]. Medicinal plants play a significant role in modern healthcare as important sources of raw materials for the production of various therapeutic drugs. Although synthetic medicines are effective in managing numerous diseases, their high cost and limited accessibility make them unaffordable for millions of people. It is estimated that around 70,000 plant species have been used for medicinal purposes [4]. Medicinal herbs serve as valuable sources of raw materials for the development and synthesis of conventional pharmaceutical drugs. Their therapeutic properties are attributed to the presence of diverse and complex bioactive compounds. India has documented more than 2,500 plant species with medicinal significance, while approximately 1,400 species are recognized in Sri Lanka and around 700 in Nepal [5]. *Euphorbia* is the largest genus within the family Euphorbiaceae, comprising approximately 1,600 species. Members of this genus are typically characterized by the

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production of white, milky latex that may exhibit varying degrees of toxicity. Latices of *E. ingens, E. mey, E. tirucalli*, and *E. triangularis* are possible sources of rubber [6]. This group of plants has been reported to possess phytochemical compounds which include: - flavonoids, triterpenoids, alkanes, amino acids, and alkaloids [7]. *E. ipecacuanha* is known as wild ipecac; *E. ant quorum* is known as *Tridhara; E. lathyrus* is known as caper spurge; and *E. thymifolia* is known as *Laghududhika*[8]. Several species of *Euphorbia* have been traditionally utilized for medicinal purposes. When the plant tissues are damaged, most *Euphorbia* species release milky latex that can be toxic to varying degrees and has historically been used as a component of arrow poisons. *E. hirta* possesses antibacterial, anthelmintic, antiasthmatic, sedative, antispasmodic, antifertility, antifungal, and antimalarial properties[9].It has many medicinal properties like anticancer, anxiolytic, analgesic, antimalarial, antidiarrheal, antioxidant, ant amoebic, anti-inflammatory [10, 11]. In the present investigation the methanolic extract of *Euphrobia hirta* whole plant was investigated for presence of different phytochemicals, anti-inflammatory activity by protein denaturation assay and antiarthritic activity by hypotonicity induced hemolysis assay and heat induced hemolysis assay in order to substantiate its anti-inflammatory property.

II. MATERIAL AND METHODS

➢ *Plant Material*

The whole plant of *Euphorbia hirta* was collected from Torvi village, Vijayapura. The collected plant material was thoroughly washed with tap water to remove impurities and subsequently shade-dried under suitable conditions for 15 days. After drying the plant material was powdered using mechanical blender and stored in polythene bags at room temperature [12, 13].

➢ *Phytochemical Extraction*

The powdered plant material was subjected to cold extraction using methanol as solvent system for 72 hours in dark with constant agitation. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in desiccators [14].

➢ *Qualitative Phytochemical Analysis*

The crude extracts of *Euphorbia hirta* obtained from the different organic solvents and water, where subjected to qualitative phytochemical analysis for different phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids, sterols using standard methods [15].

III. EVALUATION OF INVITRO ANTI-INFLAMMATORY ACTIVITY

➢ *Inhibition of Albumin Denaturation Assay*

The anti-inflammatory activity of *Euphorbia hirta* was studied by using inhibition of albumin denaturation technique which was studied according to followed with minor modifications [16].The reaction mixture contained the test extracts and a 1% aqueous solution of bovine serum albumin fraction. The pH of the mixture was adjusted appropriately using a small quantity of phosphate-buffered saline (PBS). The sample extract concentration in (100 mg/ml -500 mg/ml) were incubated at 37 ºC for 20 min and then heated to 51 ºC for 20 min, after cooling the samples the turbidity was measured at 660nm (Eppendorf Bio Spectrophotometer) The experiment was performed in triplicate. Diclofenac was used as a standard drug (100µg/ml). [17].

The percentage inhibition of protein denaturation was determined using the following formula:
Percentage inhibition = (Abs Control –Abs Sample)/Abs Control X 100

IV. EVALUATION OF INVITROANTIARTHRITIC ACTIVITY

➢ *Evaluation of Protection Against Heat Induced Haemolysis*

The reaction mixture, with a total volume of 2 mL, contained 1 mL of the test sample at different concentrations (100–500 mg/mL) and 0.5 mL of a 10% RBC suspension. For the control, saline was used in place of the test sample. Diclofenac (100 µg/mL) was employed as the standard drug. The reaction mixtures were incubated in a water bath at 56 °C for 30 minutes. Following incubation, the tubes were cooled under running tap water and centrifuged at 3000 rpm for 10 minutes. The absorbance of the resulting supernatants was then measured at 560 nm. The experiment was performed in triplicates for all the test samples [18].

The Percentage protection of Haemolyses was calculated as follows:

Percentage protection = [(Abs control –Abs sample) / Abs control]X 100

➢ *Evaluation of Protection Against Hypotonicity-Induced Haemolysis*

Different concentration of extract (100-500 mg/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of water and 0.5ml of HRBC suspension [19]. Diclofenac sodium (100 mg/mL) was employed as the reference standard. All assay mixtures were incubated at 37 °C for 30 minutes and subsequently centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560nm [20].

Percentage protection = [(Abs control –Abs sample) / Abs control] X 100

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V. RESULTS

➢ *Qualitative Phytochemical Analysis*

Table 1. Results of Phytochemical Analysis

Phytochemical Constituents	Observation
Alkaloids	++
Flavonoids	++
Glycosides	++
Phenolic compounds	++
Tannins	++
Terpenoids	+
Steroids	+

The qualitative phytochemical screening demonstrated that the sample contains a variety of bioactive secondary metabolites. Alkaloids, flavonoids, glycosides, phenols, and tannins were identified at moderate levels (++), whereas terpenoids and steroids were detected at comparatively lower levels (+). The occurrence of these phytochemical constituents indicates that the sample has the potential to exhibit a range of biological activities. These compounds are commonly associated with antioxidant, antimicrobial, anti-inflammatory, and other pharmacologically beneficial properties, highlighting the potential therapeutic significance of the sample.

VI. EVALUATION OF INVITRO ANTI-INFLAMMATORY ACTIVITY

➢ *Inhibition of Albumin Denaturation Assay*

This investigation showed that concentration of sample at 100 mg/ml showed 5.40%, at 200mg/ml showed 9.00%, at 300 mg/ml showed 30.63%, at 400 mg/ml showed 36.03%, and 500 mg/ml showed 43.24 % of inhibition. While standard drug showed 64.86%. In this study, 500mg/ml showed the highest percentage of inhibition. Whereas standard was highest percentage of inhibition as compare to concentration of *E. hirta*. IC_{50} value is 548.48 mg/ml.

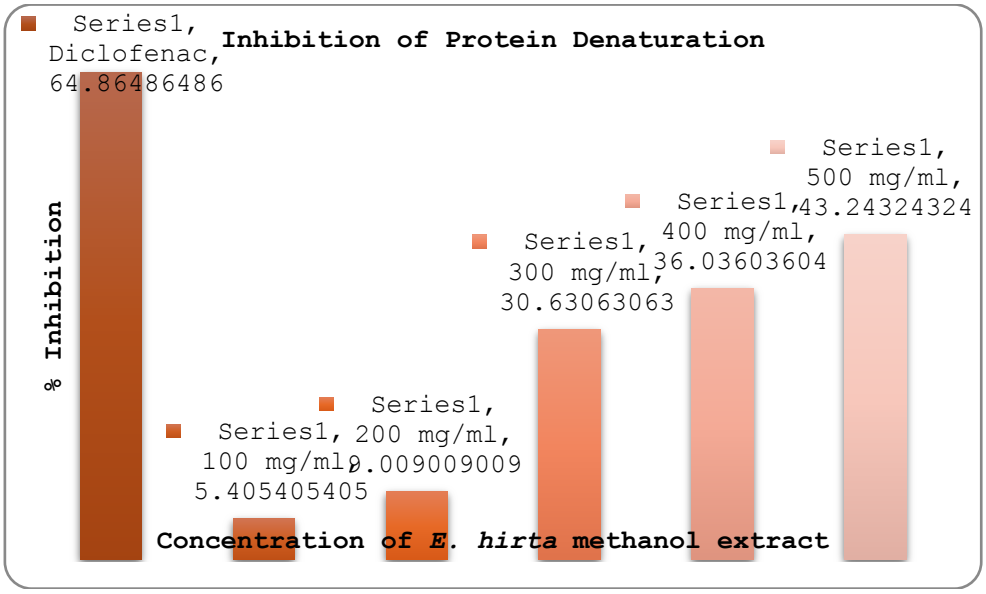

Fig 3. Graph Showing the Percentage Inhibition Effect of *E. hirta* Methanol Extract Against Protein Denaturation.

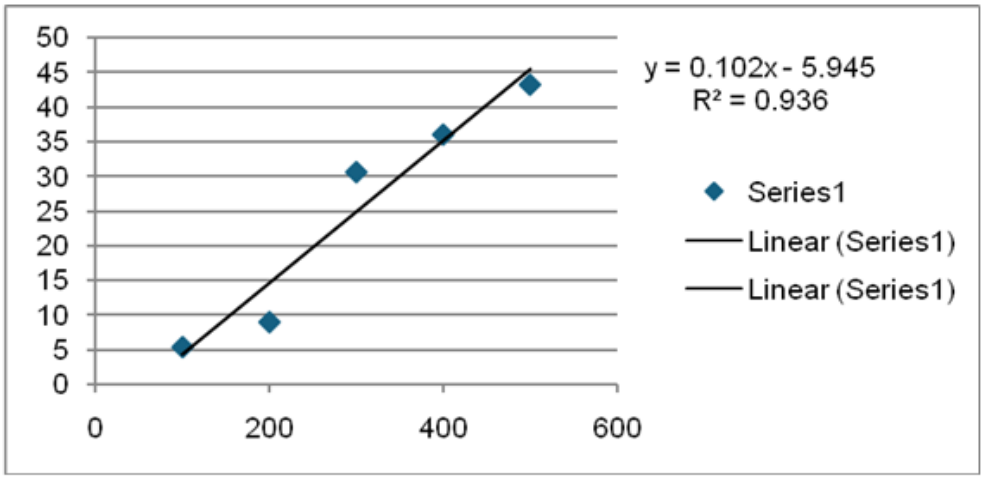

Fig 4. Regression Line Graph Showing the R-Square Value of 0.936 for Analysis of Inhibition of Protein Denaturation.

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VII. EVALUATION OF INVITROANTIARTHRITIC ACTIVITY

➢ *Evaluation of Protection Against Heat Induced Haemolysis*

This investigation showed that concentration of sample at 100 mg/ml showed 8.57%, at 200mg/ml showed24.76%,at 300 mg/ml showed 34.28%, at 400 mg/ml showed 43.80% and at 500 mg/ml showed57.14 % of protection. While standard drug showed 64.86%. This 500mg/ml showed the highest percentage of protection. Whereas standard was highest percentage of protection as compare to concentration of *E. hirta* IC_{50}value was 440.87 mg/ml.

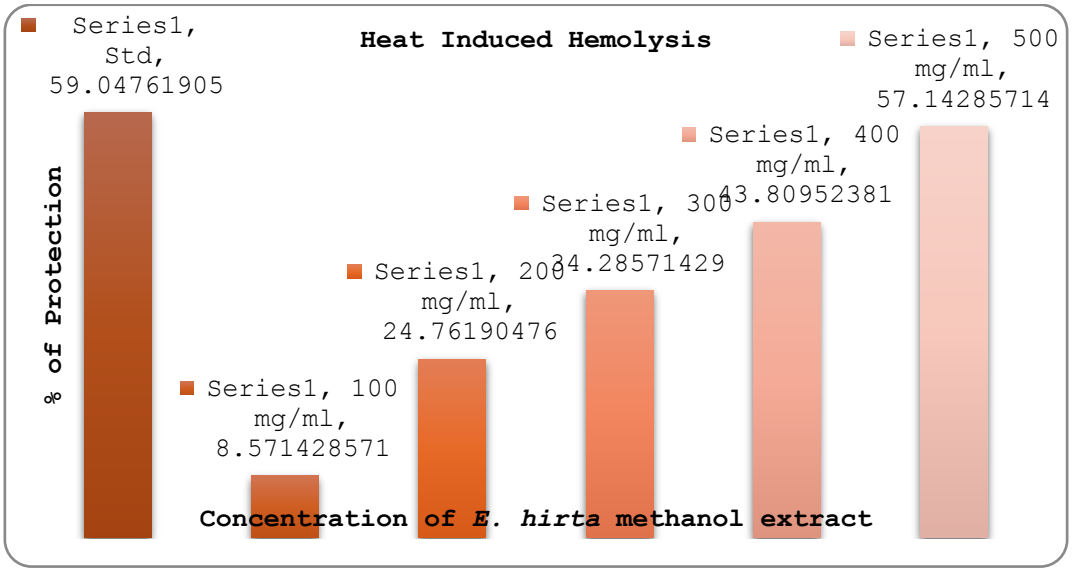

Fig 5. Graph Showing the Percentage Protection Effect of *E. hirta* Methanol Extract Against Heat Induced Haemolysis.

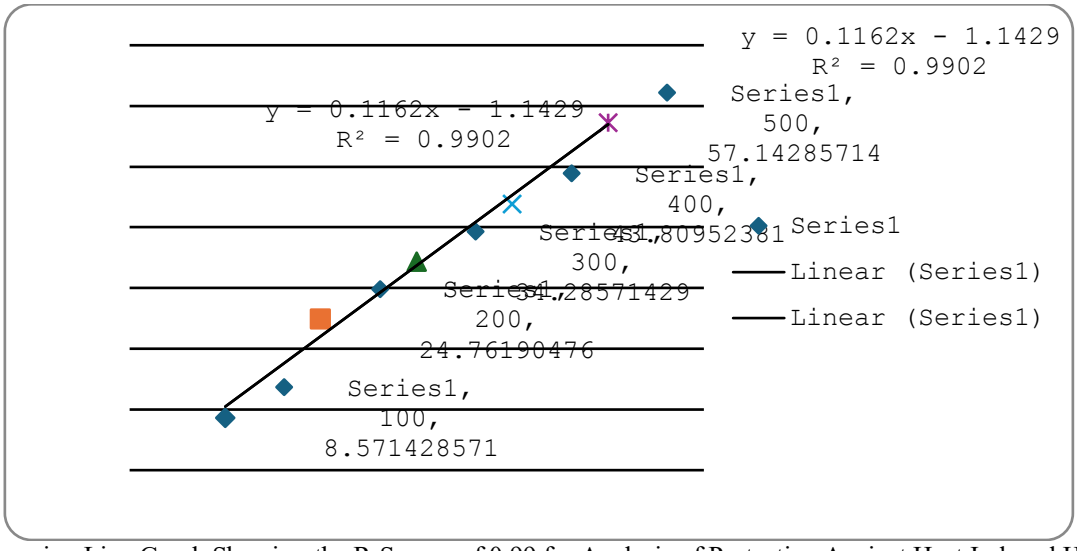

Fig 6. Regression Line Graph Showing the R-Square of 0.99 for Analysis of Protection Against Heat Induced Haemolysis

➢ *Evaluation of Protection Against Hypotonicity-Induced Haemolysis*

This study showed that concentration of sample is at 100 mg/ml showed 63.44%, at 200mg/ml showed 15.05%, at 300 mg/ml showed 27.95%, at 400 mg/ml showed 35.48%, and at 500 mg/ml showed 39.78% of protection. While standard drug showed 64.86%. In this 500mg/ml showed the highest percentage of protection. Whereas standard was highest percentage of protection was comparable to the concentration of *E. hirta*. IC_{50} value is 568.2 mg/ml.

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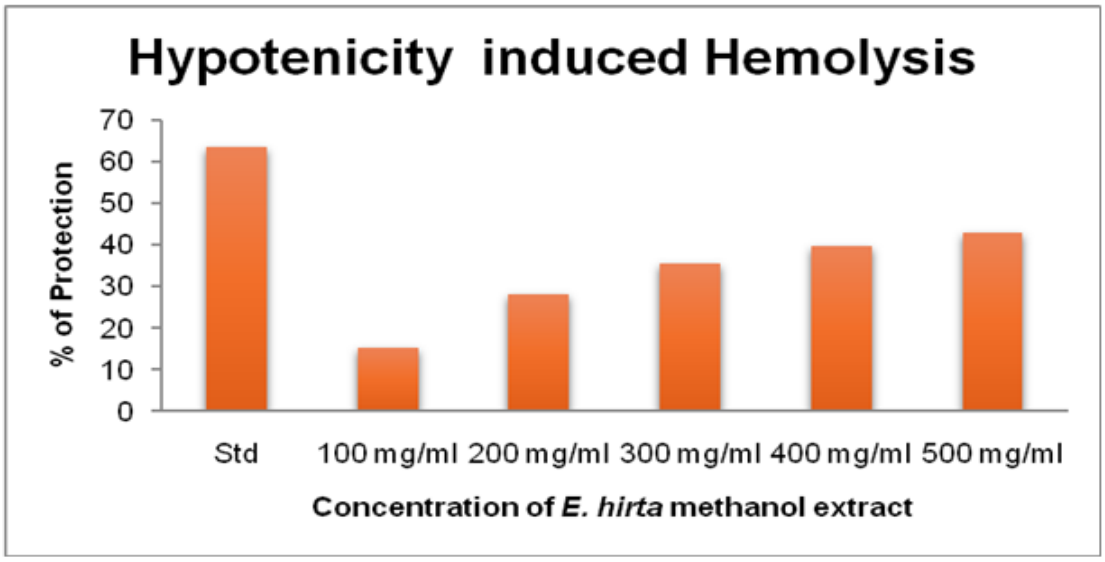

Fig 7. Graph Showing the Percentage Protection Effect of *E. hirta* Methanol Extract Against Hypotonicity Induced Haemolysis.

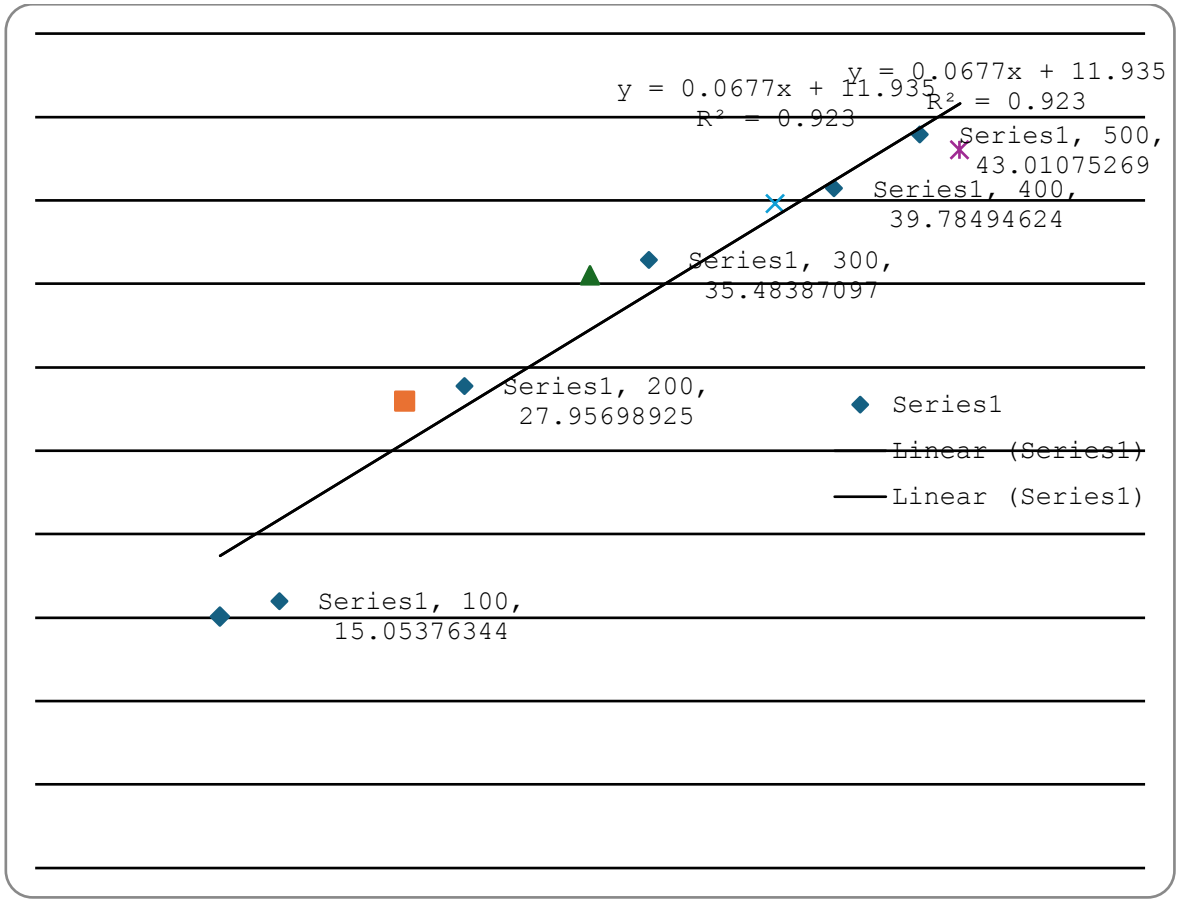

Fig 8. Regression Line Graph Showing the R-Square Value of More than 0.9 for Analysis of Protection Against Hypotonicity Induced Haemolysis.

VIII. DISCUSSION

Phytoconstituents identified in the study include alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids, steroids, and carbohydrates. The methanol extract of the *E. hirta* fruit possesses a higher presence of these compounds compared to other aqueous and alcoholic solvents [22]. Specifically, glycosides and terpenoids were detected in the Chloroform-based extract [23]. While alkaloids and phenols were present in the Aqueous extract [24]. These findings align with previous phytochemical analyses of *E. hirta*, which indicated that methanol yielded a greater variety of phytoconstituents, including steroids, flavonoids, terpenoids, and alkaloids, when compared to other solvent extracts. The methanol extract was notably positive for alkaloids, flavonoids, tannins, and glycosides at levels

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comparable to those found in other solvent extracts. Based on the results obtained, the methanol extract of *E. hirta* demonstrates a rich composition of phytoconstituents. Notably, it is positive for the presence of phenols, which are recognized for their capability to impede lipid oxidation in oils and fatty acids, thus potentially lowering the risk of cardiovascular diseases [25]. Furthermore, phenols have been shown to disrupt the propagation of cancer cells at various stages [26], which may contribute to reduced cancer occurrence in individuals. Additionally, the presence of alkaloids in the extract is essential for protecting the plant against various pathogens, including fungi, yeasts, bacteria, and viruses [27, 28]. Tannins present in the extract are acknowledged for their antioxidant, antibacterial, and anti-inflammatory properties. Therefore, the presence of tannins in *E. hirta* yields for their astringents properties and also in treating wounds. As per the earlier reports, terpenoids do possess antibacterial and antineoplastic properties which explains the antibacterial activity of the *E. hirta*, *Pseudomonas aeruginosa, Escherichia coli*, *Bacillus subtilis* whilst *Candida albicans* and *Aspergillus niger* [29]. Apart from this activity, terpenoids were also studied for their anti-hepatoxic properties thus reducing the risk of liver damage. The presence of these bioactive phytoconstituents in the *E. hirta plant* explains its use as traditional medicine in treating various diseases, thus making the fruit of the plant is considered a valuable source of various bioactive compounds and has been reported to possess a wide range of medicinal properties.

Protein denaturation refers to the process whereby proteins lose both their tertiary and secondary structures due to external stresses or chemical agents, including strong acids or bases, concentrated inorganic salts, organic solvents, or heat [30]. When proteins undergo denaturation, they generally lose their biological functionality. This phenomenon is notably associated with inflammation. In an investigation aimed at understanding the mechanism behind anti-inflammatory activity, the efficacy of plant extracts in inhibiting protein denaturation was examined. The study found that the plant extract effectively inhibited heat-induced denaturation of albumin, achieving a maximum inhibition rate of 71% at a concentration of 500 mg/ml.

The extract demonstrated effectiveness in inhibiting heat-induced haemolysis across various concentrations. Notably, EHME at concentrations of 400 and 500 mg/ml significantly protected erythrocyte membranes from heat-induced lysis. Additionally, aspirin at a concentration of 100 µg/ml provided significant protection against the damaging effects of heat solution.

The results demonstrated that EHME at concentrations between 200-500 mg/ml significantly protects erythrocyte membranes from lysis induced by hypotonic solutions. Diclofenac sodium at 100 mg/ml also provided considerable protection against the damaging effects of hypotonic solutions. Notably, EHME at 500 mg/ml exhibited the highest level of protection, while Diclofenac sodium (100 mg/ml) showed inhibition of RBC haemolysis compared to the control.

IX. CONCLUSIONS

In the present study, results indicate that the methanol extracts of *Euphorbia hirta* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. This study gives on idea that the compound of the plant *E. hirta* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

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