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Effect Of Hydroalcoholic Extract Of Tridax Procumbens On In Vitro Hemostasis And Anti Inflammatory Activity

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ABSTRACT

All across India, Tridax procumbens Linn is a common herbaceous weed. In traditional medicine, the extract of Tridax procumbens has been frequently used to reduce bleeding and increase its anti-inflammatory properties. The hydroalcoholic extract of Tridax procumbens is assessed for its potential function in hemostasis cascade and anti-inflammatory activities based on its traditional therapeutic value. The purpose of this study was to assess the hemostasis-related effects of Tridax procumbens hydro-alcoholic extract in order to support its procoagulant and anti-inflammatory properties. Using Lee White's approach, the hydro-alcoholic extract of Tridax procumbens was examined in vitro for hemostasis. Its in vitro anti-inflammatory effect was further examined using the protein denaturation and membrane stabilization techniques. The leaves' crude hydro-alcoholic extract showed potent anti-inflammatory and blood-coagulation properties. Tridax procumbens enhances anti-inflammatory action and shortens the time it takes for human blood to clot while also improving focus. Phenols and flavonoids present in the hydro alcoholic extract of Tridax procumbens exert their hemostatic and anti-inflammatory effects by scavenging free radicals, inhibiting platelet aggregation, reducing inflammation, protecting vascular integrity, and modulating inflammatory signaling pathways. The hydro-alcoholic extract clotted in the subject's blood sample in two to three minutes less time than usual. By using the membrane stabilization method, the hydro-alcoholic extract of Tridax procumbens showed a maximum percentage of stabilization of 74.39±3.25% at a concentration of 1000µg/ml. The highest inhibition of 63.39±1.38% was observed at a concentration of 1000µg/ml using the albumin denaturation method. Tridax procumbens hence possesses blood coagulant and anti-inflammatory qualities. The demand for natural sources of potent anti-inflammatory and blood coagulant drugs with minimal side effects to replace manufactured therapies is what spurs this research.

Keywords –

Tridax procumbens, phytochemical, hemostasis, anti-inflammatory, hydro-alcoholic extract, protein denaturation.

1. INTRODUCTION

Tridax procumbens Linn, sometimes referred to as Tridax Daisy or Coat Buttons, is a member of the Asteraceae family [1]. Hemostasis, anti-inflammatory qualities, and phytochemical elements are checked for in the plant [2, 3]. Leaf decoctions in water demonstrated strong blood coagulation activity; as a result, it has the potential to be a powerful hemostatic agent [4]. Since the ethanol extract shortens the clotting time in the blood samples used in all of the studies, the haemostatic property of the plant's leaves of the various solvent extracts was ascertained in vitro using Lee-White's method [5]. Procumbens tridax has strong anti-inflammatory qualities. The gain in weight indicates that the plant decoction's anti-inflammatory effect might be the result of corticotrophic effects [6].It was discovered that the plant's active portion, ethyl acetate fraction, contained moderately polar natural chemicals, such as flavonoids and alkaloids. These bioactive substances have been used to combat reactive oxidant species, which have been linked to the etiology of inflammation and associated diseases [7].

Blood coagulation, or hemostasis, is the procedure used to halt bleeding from injured blood vessels. This entails a series of actions involving several clotting components. To avoid excessive blood loss during tissue damage, the hemostatic system requires a precise balance between coagulation (fibrin creation) and fibrinolysis (fibrin dissolution). Any alteration to this equilibrium could result in bleeding or thrombosis [8]. Hemostasis, inflammation, proliferation, and extracellular matrix (ECM) remodeling are some of the overlapping phases that make up the intricately coordinated physiological process of wound healing, which restores tissue integrity [9, 10]. Additionally, hemostasis is essential for wound healing because it prevents excessive bleeding and starts the healing process by stimulating the blood coagulation cascade through fibrinogenolysis or the activation of coagulation factors like Factor X [11]. Numerous inactive precursors of enzymes generated from the liver and blood cells are involved in the successive chain of events that constitutes blood coagulation [12]. These enzymes are known as coagulation factors because they catalyze the proteolysis of the subsequent component in the cascade upon activation. Either intrinsic or extrinsic mechanisms can cause blood to coagulate, and both lead to a similar pathway that involves prothrombin activator or activated factor V complex [13]. Prothrombin (zymogen form) is changed into thrombin (active form), a serine type of protease, by the activated factor V complex. The soluble plasma fibrinogen is changed into a soft, insoluble fibrin clot by thrombin [14, 15]. After undergoing polymerization, the fibrin monomers fuse with platelet contractile protein to form a potent hemostatic plug that stops the bleeding. Later, a serine protease called plasmin cleaves the fibrin clot, speeding up the healing of wounds and reestablishing regular blood flow [16]. Proteases are therefore crucial in preventing excessive bleeding from recently cut and exposed wounds [17].

Twenty distinct clotting factors interact intricately during the coagulation process in a cascade mechanism, whereby one factor triggers the activation of the subsequent in the sequence by proteolytic activations [18, 19]. The transformation of soluble fibrinogen into insoluble fibrin is the final step in the clotting cascade. The majority of clotting factors are proteins, and very few are not [20]. This fibrin clot seals the wound and stops the bleeding, working in tandem with the platelet plug.

The body's reaction to damage, infection, or injury is called inflammation, and it is characterized by heat, redness, discomfort, swelling, and altered physiological processes. Chemical mediators released by wounded tissue and migratory cells cause it to occur [21]. Increased vascular permeability, increased protein denaturation, and altered membranes are some of the many processes that make up inflammation, which is commonly linked to pain. Protein denaturation is the process by which proteins are subjected to external stressors or substances, such as heat, an organic solvent, a strong acid or base, or concentrated inorganic salt. As a result, proteins lose their secondary and tertiary structures. When denatured, the majority of biological proteins cease to function biologically. Inflammation is known to be caused by protein denaturation [22, 23]. There are two types of inflammation: acute and chronic. Increased vascular permeability, capillary infiltration, and leukocyte emigration are linked to acute inflammation. Fibroblast activation, proliferation (angiogenesis), fibrosis, and the infiltration of mononuclear immune cells, macrophages, monocytes, and neutrophils are all linked to chronic inflammation. Rheumatoid arthritis (RA) is a chronic, disabling autoimmune disease, and inflammation is a prevalent clinical symptom [24]. It is thought that medicinal plants are a significant source of novel chemicals with possible medical use. Therefore, it is reasonable to consider research into plants that have a folkloric use for their anti-inflammatory properties as a fruitful and rational research method in the hunt for novel anti-inflammatory medications. Progression of organ damage and potentially fatal hypersensitivity reactions are two possible negative effects of inflammation [25]. It has been claimed that NSAIDs prevent proteins from becoming denaturated, which can contribute to auto-immune disorders and act as antigens [26].

Vascular tissues intricate biochemical reaction to damaging stimuli is inflammation. Additionally, it is an attempt by the organism to protect itself by eliminating harmful stimuli and starting the healing process. The cells become activated and release inflammatory mediators when an inflammation starts. Histamine, serotonin, prostaglandins, slow-reacting substances of anaphylaxis (SRS-A), and some plasma enzyme systems, such as the kinin, coagulation, complement, and fibrinolytic systems, are some of these mediators. Together, these mediator molecules result in enhanced blood flow permeability and vasodilatation, fluid and protein exudation from the plasma membrane, and migration of leukocytes primarily neutrophils outside the blood vessels and into the wounded tissues. Acute and chronic inflammation are two different categories for inflammation.

2. MATERIALS AND METHODS:

2.1 PLANT MATERIAL:

Leaves of Tridax procumbens was collected from the premises of Late Bhagirathi Yashwantro Pathrikar College of D Pharmacy (D. Pharm, B. Pharm) Chatrapati Sambhajinagar, Maharashtra, India. The plant material was identified and authenticated by professor Dr. Arvind S. Dhabe, Head of department of botany, Dr. Babasaheb Ambekar Marathwada Univercity, Chatrapati Sambhajinagar, Maharashtra, India.

2.2 CHEMICALS:

Methanol, Water, Chloroform, H_2SO_4, Lead acetate, $FeCl_3$, NH_4OH, Benzene, HCL, Wagner’s reagent, Hager’s reagent, Ninhydrin reagent, NaOH, Iso saline, Alsever solution, Phosphate buffer, Diclofenac, Toluene, Ethyl acetate, Formic acid.

2.3 PREPARATION OF EXTRACT:

After being gathered, fresh, healthy leaves were left to dry in the shade for two to three weeks. The components that had been shade-dried were ground into a powder, weighed, and then separated into three sections. In a separating funnel, each component was macerated with 50% methanol and 50% water, 50% petroleum ether, or 50% water. For 72 hours, the combinations were agitated vigorously and periodically. The extracts were separated into individual beakers and heated to 45 ℃ in a water bath to concentrate them. At least three iterations of this procedure were done in order to produce colorless marc for each solvent. It was oven-dried at 45 ℃, and the crude extract powder was gathered and weighed. Following this, the crude extracts were utilized to investigate hemostasis, anti-inflammatory efficacy, and phytochemical screening.

2.4 PHYTOCHEMICAL SCREENING OF EXTRACT:

The crude hydro-methanolic extract was subjected to qualitative chemical tests to identify various classes of bioactive chemical constituents present in the plant using standard procedures [27, 28, 29, 30]. Tests were performed for Steroids, Tannins, Emodins, Alkaloids, Saponin, Amino acids, Phenol, Cardial glycosides and Flavonoids.

A. Steroid:

1ml extract was dissolve in 10 ml of chloroform and equal volume of concentrated H_2SO_4 acid was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicate the presence of steroids.

B. Tannin:

(a) Lead Acetate Test: 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.

(b) Ferric Chloride Test: 4ml extract was treated with 4 ml $FeCl_3$ formation of green colour indicates that presence of condensed tannin.

C. Saponin:

5 ml extract was mixed with 20 ml of distilled water then agitated in the graduated cylinder for 15 min formation of foam indicates presence of saponin.

D. Emodin:

2ml of NH_4OH and 3ml of benzene was added to extract appearance of red colour indicates presence of emodin.

E. Alkaloids:

A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following tests:

(a) Wagner's test: Filtrate was treated with Wagner's reagent; formation of reddish brown precipitate indicates presence of alkaloids.

(b) Hager's test: Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow coloured precipitate.

F. Amino acid:

Ninhydrin test: To the 2 ml extract 2 ml of ninhydrin reagent was added and boil for few minutes, formation of blue colour indicates the presence of amino acid.

G. Phenol:

Ferric Chloride test: Test extract were treated with 4 drops of alcoholic $FeCl_3$ solution, formation of bluish black colour indicate the presence of phenol.

H. Cardial Glycosides:

Keller-Killani Test: Plant extract treated with 2 ml glacial acetic acid containing a drop of $FeCl_3$, formation of brown colour ring indicates the presence cardial glycosides.

I. Flavonoid:

(a) Alkaline reagent test: extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of flavonoid.

(b) NH_4OH test: 3 ml of extract were treated with 10% NH_4OH solution development of yellow fluorescence indicates presence of flavonoid.

2.5 THIN LAYER CHROMATOGRAPHY:

TLC technique was used to determine the presence of flavonoid in the extract. Toluene, ethyl acetate, formic acid at 5:4:0.2 proportions were used respectively for identification of flavonoids. The extract was run on silica gel G plate. Visualization was carried out in iodine chamber by dipping the plate in it, till the colour of spot appears. Rf value was calculated as migration distance of substance to the solvent front.

2.6 IR SPECTROSCOPY:

Weigh the hydro alcoholic extract of Tridax procumbens accurately. Dissolve the extract in a suitable solvent such as methanol. Prepare a uniform solution by through mixing or sonication. Place a sample amount of the prepared solution onto an IR-transparent substrate such as a potassium bromide pellet or a suitable IR cell. Set up the IR spectrometer according to the manufacturer's instruction. Ensure the instrument is calibrated properly. Select the appropriate wavelength range for analysis. Record the IR spectrum of the sample by scanning within the selected wavelength range. Save the spectrum for further analysis. Analysed the obtained IR spectrum for characteristics absorption bands corresponding to functional groups present in the hydro alcoholic extract of Tridax procumbens.

2.7 IN-VITRO HEMOSTASIS ACTIVITY:

The Lee-Whites method was utilized to evaluate the solvent extract's clotting time in vitro, which served as a guide for investigating the hemostasis activity of these leaves [31]. For the same, venous blood was drawn and the amount of time needed for clotting was recorded in a clean, dry test tube without the use of an anticoagulant (the normal clotting time is 5–12 min). The extract was primarily tested on subject blood samples to determine how it affected clotting time. Venous blood was drawn, and as soon as the blood reached the syringe, a stopwatch was set in motion. Three test tubes were filled, the first for normal clotting time and the other two with blood up to the 1 ml threshold. 0.5 ml of solvents were added to the second test tube in the same set to create the corresponding blanks, and 0.5 ml of hydro-

alcoholic extract was applied to the remaining test tubes. Next, a water bath set at 37 ^{0}C was used to hold all of these test tubes. After three minutes, each test tube was pulled out and slanted at a 45^0 angle to check for clotting. Returned to the water bath, the test tubes in which clotting had not begun were checked every 30 seconds to see whether clotting had taken place. When clotting was detected in a certain test tube, the watch was stopped right away, and the minutes were recorded. The clotting times of the remaining samples were also noted. Hydro-alcoholic extract reduced clotting time considerably than normal.

2.8 IN-VITRO ANTI-INFLAMMATPRY ACTIVITY:

2.8.1. Membrane Stabilization Method [32]:

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The 10% packed cells were washed with isosaline. Various concentrations of extracts were prepared (200,400,600, 800 and 1000µg/ml) using distilled water and 1 ml of plant extracts, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 370C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated by spectrophotometrically at 560 nm. Diclofenac (1 mg/ml) was used as reference standard drug and a control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula,

$$\% \text{ Protection} = \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

2.8.2. Albumin Denaturation Method [33]:

The reaction mixture (5ml) consist of 0.2 ml of egg albumin (from fresh hen's egg), 2.8ml phosphate buffered saline (pH: 6.4) and 2ml of varying concentration of plant extracts. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37±2 °C in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. After cooling, their absorbance was measured at 660nm by using vehicle as blank. Diclofenac at the final concentration of (1mg/ml) was used as reference drug and treated similarly for determination of absorbance. The Percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

3. RESULT:

3.1 PHYTOCHEMICAL ANALYSIS:

The leaves of Tridax procumbens in hydro-alcoholic extract was showed the presence of steroid, phenol, emodin, saponin, alkaloids, amino acids, and tannin. Whereas absence of flavonoid in NH_4OH test and cardial glycosides.

Table 1: Phytochemical Analysis of hydroalcoholic extract of Tridax procumbens.

Sr. No.	Phytochemicals	Result
1.	Steroids	+
2.	Tannin (a) Lead acetate test (b) Ferric chloride test	 + +
3.	Saponin	
4.	Emodin	+
5.	Alkaloids (a) Wagner's test (b) Hager's test	 + +
6.	Amino acid Ninhydrin test	 +
7.	Phenol	+
8.	Flavonoid (a) Alkaline reagent test (b) NH_4OH test	 + -
9.	Cardial glycoside	-

(+) indicates the presence of phytochemicals and (-) indicates absence of phytochemicals.

Fig 1. Preliminary study

3.2 THIN LAYER CHROMATOGRAPHY:

The hydro alcoholic extract of dried leaves of Tridax procumbens was subjected to thin layer chromatography for confirmation of the presence of quercetin flavonoid. The dark brownish yellow colour spot appeared under iodine chamber. It show Rf value 0.7, indicating presence of flavonoid in extraxt.

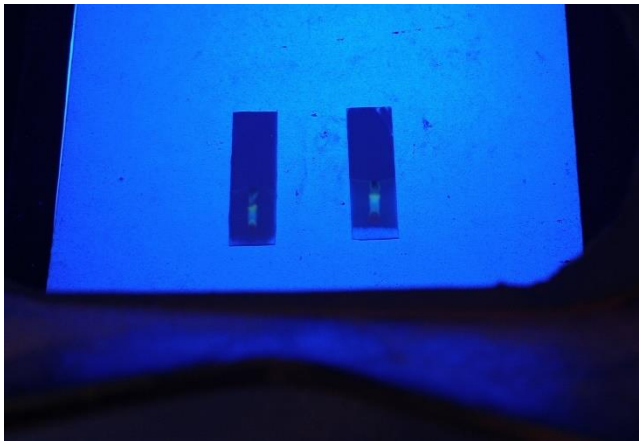
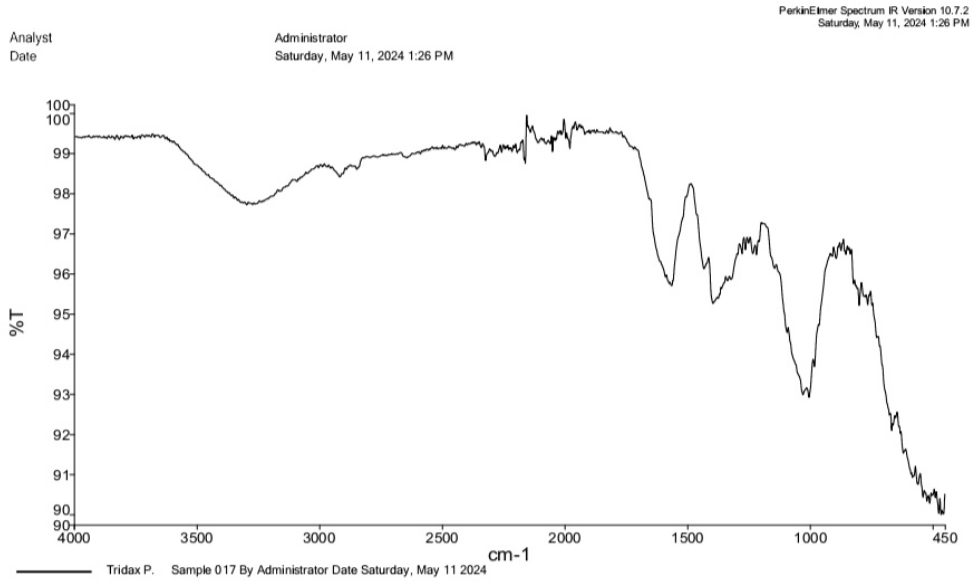


Fig 2. Thin layer chromatography

3.3 IR SPECTROSCOPY:

The hydro alcoholic extract of dried leaves of Tridax procumbens was subjected to IR spectroscopy for confirmation of presence of alcohol, phenol, and flavonoids. The –OH stretch around 3500-3100 cm^{-1} indicate the presence of phenolic compounds. Phenolic compounds are known to exhibit anticoagulant and procoagulant properties. They can influence blood clotting factors and platelet aggregation, contributing to hemostasis. Peak in the region of 1600-1650 cm^{-1} suggest the presence of conjugated C=C bonds. Compounds containing conjugated double bonds, such as flavonoids and polyphenols, often possess anti-inflammatory properties. They can inhibit inflammatory mediators, such as cytokines and prostaglandins, and modulate inflammatory pathways. The C–O stretch around 1300-1000 cm^{-1} indicate the presence of alcohol, ethers and phenols which is also responsible for hemostasis and anti-inflammatory activity.



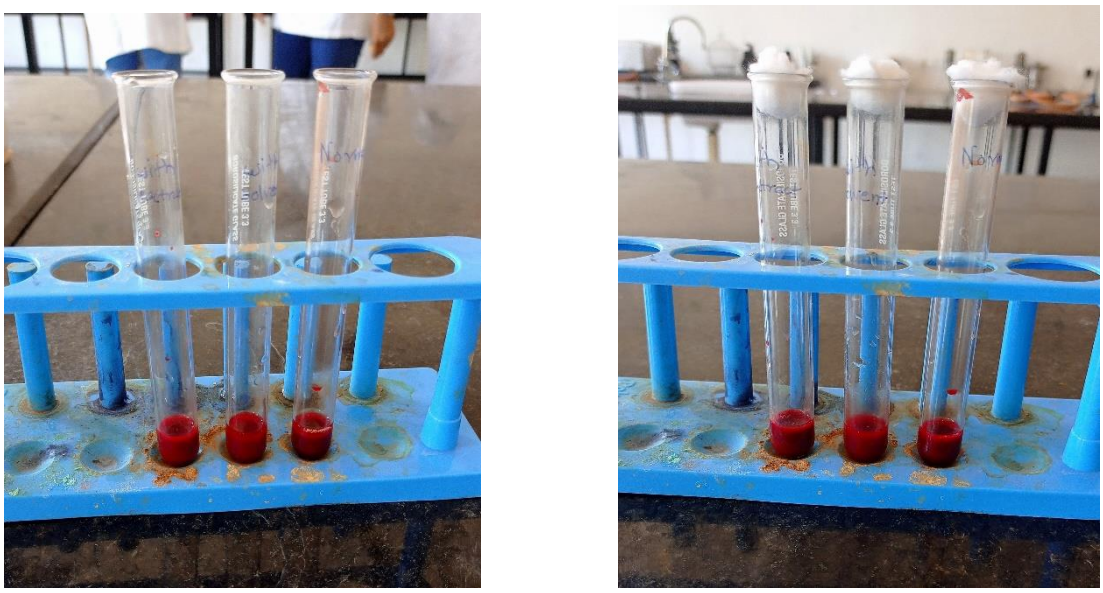
Spectrum 1: IR Spectrum

3.4 HEMOSTASIS ACTIVITY:

When the effects of solvents and the plant's hydro-alcoholic extract were investigated, it was discovered that blood containing solvents had clotting times longer than normal. Testing the Tridax procumbens hydro-alcoholic extract revealed a significant reduction in clotting time compared to normal. In the subject's blood sample, the hydro-alcoholic extract's clotting time was 2-3 minutes shorter than the normal clotting time.

Table 2: Analysis of Hemostasis activity

Sr. No.	**Sample Type**	**Clotting Time**
1.	Blood	6.4 min
2.	Blood + Solvents	6.7 min
3.	Blood + Hydro-alcoholic extract	3.9 min



A. Before coagulation B. After coagulation

Fig 3. In vitro Blood coagulation by Lee White's Method

3.5 ANTI-INFLAMMATORY ACTIVITY:

3.5.1. HRBC Membrane Stabilization Method:

Hemoglobin absorbance was measured in our study using the HRBC membrane stabilization technique. Because there is less membrane stability during RBC membrane lyses, hemoglobin is liberated. The erythrocyte membrane was lysed by the plant extracts under hypotonic conditions, demonstrating their ability to stabilize membranes. Table 3 and Figure 4 illustrate how Tridax procumbens hydroalcoholic extracts affect the stability of the RBC membrane. At a concentration of 1000µg/ml, the hydro alcoholic extract of Tridax procumbens demonstrated a maximum percentage of stabilization of 74.39±3.25%. At

200µg/ml, the minimal protection (24.53±2.45%) was noted. It exhibits strong anti-inflammatory properties on par with the reference standard medication, diclofenac sodium.

Table 3: In vitro anti-inflammatory activity of hydroalcoholic extract of Tridax procumbens by membrane stabilization method

Name of Sample	200µg	400µg	600µg	800µg	1000µg
Hydro-alcoholic Extract	24.53±2.45	36.33±2.56	49.15±3.40	61.53±4.36	74.39±3.25
Diclophenac Sodium	39.14±2.21	48.32±2.39	58.45±3.39	69.26±4.17	72.12±2.04

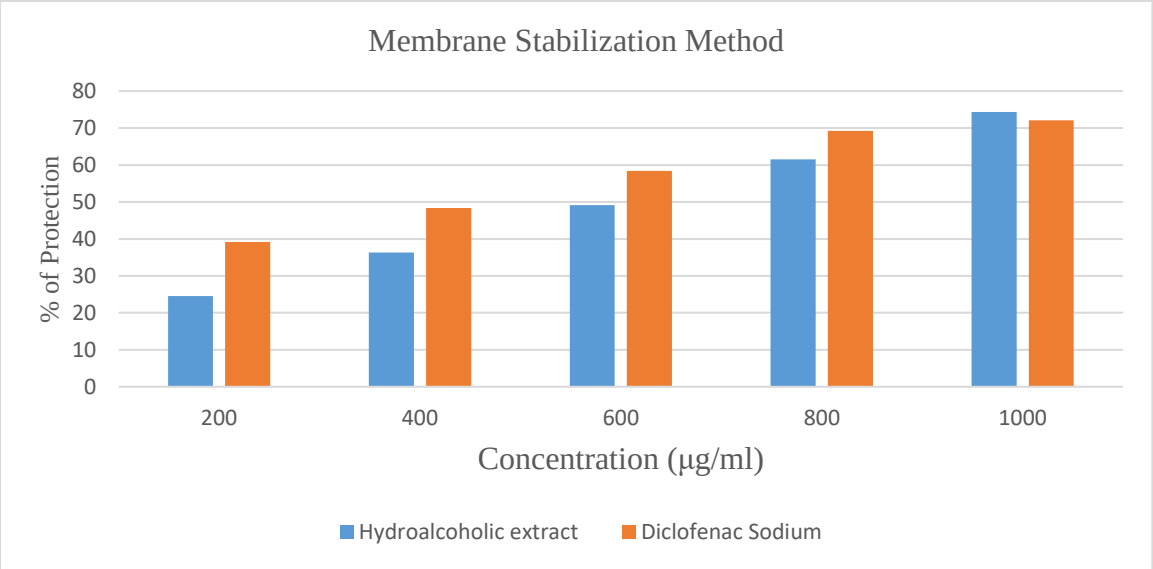
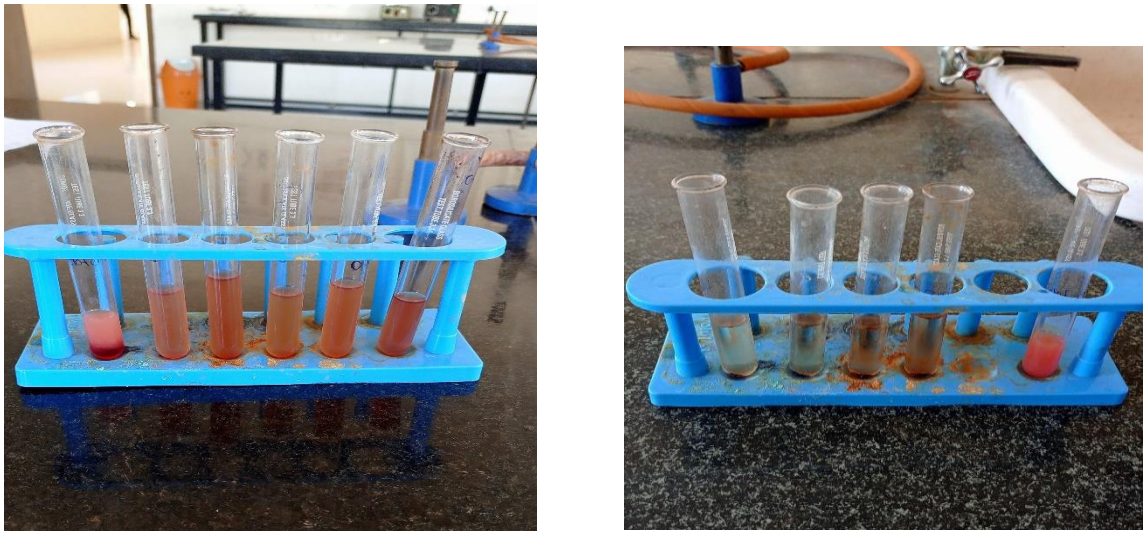


Fig 4: In vitro anti-inflammatory activity of Tridax Procumbens by Membrane stabilization method



A. HRBC Before centrifugation B. HRBC After centrifugation

Fig 5. HRBC centrifugation for in vitro anti-inflammatory activity

3.5.2. Albumin Denaturation Method:

Loss of biological characteristics of protein molecules is known as denaturation. In diseases like rheumatoid arthritis, diabetes, cancer, etc., inflammation is brought on by the denaturation of proteins. Therefore, avoiding protein denaturation may also aid in avoiding inflammatory diseases. Table 4 and Figure 6 of the current study demonstrate the hydroalcoholic extract of Tridax procumbens' in vitro anti-inflammatory efficacy by preventing protein denaturation. The concentration of 1000µg/ml showed the maximum inhibition of 63.39±1.38%. The concentration of 200µg/ml demonstrated the minimum inhibition of 30.15±1.12%.

Table 4: In vitro anti-inflammatory activity of hydro-alcoholic extract of Tridax procumbens by Albumin denaturation Method

Name of Sample	200µg	400µg	600µg	800µg	1000µg
Hydro-alcoholic extract	30.15±1.12	36.40±1.21	41.83±1.19	52.43±1.83	63.39±1.38
Diclofenac Sodium	31.53±2.13	35.94±2.43	40.25±2.34	54.67±2.19	66.59±2.52

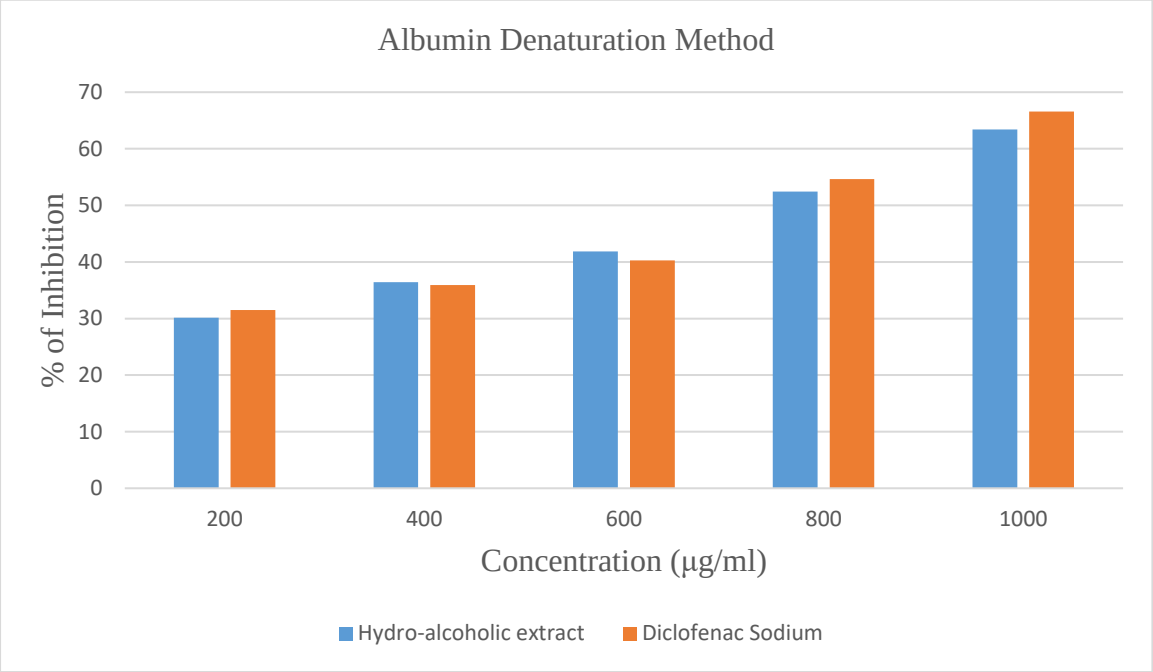


Fig 6: In vitro anti-inflammatory activity of Tridax procumbens by Albumin Denaturation Method

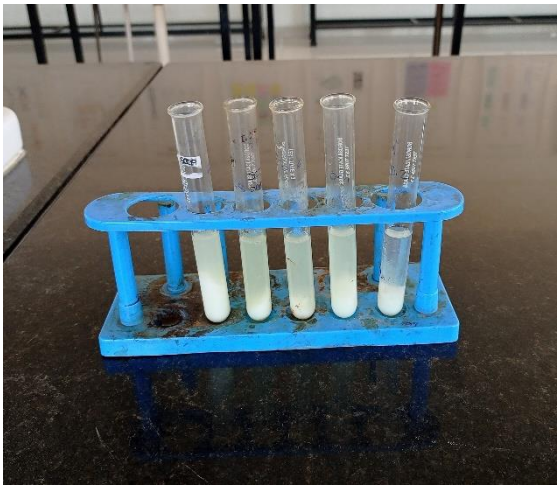


Fig 7. Albumin denaturation method

4. DISCUSSION:

Plant extracts are used in herbal therapy to treat a variety of illnesses. Depending on the local flora, medicinal plants can be found in a wide variety of local variants [34, 35]. Many contemporary medications were derived from plant sources at first, are now manufactured synthetically, and have ancestral roots in other plant materials [36, 37]. The initial screening tests for phytochemicals could be helpful in identifying the bioactive phytocompounds that could result in the creation of new medications. Additionally, these assays aid in the quantitative assessment and qualitative differentiation of phytochemical substances that are pharmacologically active [38]. The presence of several phytoconstituents, including steroids, tannins, emodins, alkaloids, amino acids, flavonoids, phenol, and saponin, was discovered by phytochemical studies on Tridax procumbens. Antioxidant, anti-microbial, wound healing, anti-malarial, anti-cancer, blood coagulation, repellency, anti-inflammatory, hepato-protective, immune-modulatory, anti-diabetic, anti-lithiatic, anti-obesity, antihyperglycemic, analgesic, hemostatic, and hypotensive qualities are just a few of the health benefits that these phytochemicals have to offer.

The hemostasis process, which sequentially involves three processes: vasoconstriction, platelet plug development, and clot formation, was taken into consideration in order to establish a relationship between clotting time and hemostatic activity. In the last phase, coagulation takes place in the blood that has exited the blood artery (intrinsic clotting) and in the blood that has been occluded by vasospasm (extrinsic clotting); plugs are created as a result of intravascular and extravascular clots, respectively. Determining the clotting time is a standard laboratory test that is performed in cases of coagulation factor deficiency, such as factor VIII deficiency, which results in hemophilia. Thus, an increase in the normal clotting time indicates these coagulation deficiencies. Given that the hydro-alcoholic extract of Tridax procumbens leaves consistently shortens the clotting time in blood sample of subject, it can be hypothesized that the leaves have hemostatic activity that influences hemostasis [39].

The hydro-alcoholic extract of Tridax procumbens leaves demonstrated a stabilizing effect on membranes by preventing erythrocyte membrane lysis generated by hypotonicity. Since the erythrocyte membrane and lysosomal membrane are similar, the extract's stabilization of the former suggests that the latter may also be accomplished [40]. By blocking the release of lysomal components of active neutrophils, such as bactericidal enzymes and proteases, which further exacerbate tissue inflammation and damage upon extracellular release, stabilization of the lysosomal membrane plays a crucial role in reducing the inflammatory response [41]. Although the precise method by which the extract stabilizes the membrane is still unknown, hypotonicity-induced hemolysis may result from cell shrinkage brought on by the osmotic

loss of intracellular fluid and electrolyte. The processes that could promote or improve the outflow of these intracellular components could be inhibited by the extract [42, 43]. Both tannins and flavanoids have been shown to have anti-inflammatory properties. It is well known that flavonoids, like quercetin, can effectively reduce acute inflammation. A number of enzymes, including phospholipase A2, protein tyrosine kinases, protein kinase C, and phosphordiesterases, are effectively inhibited by certain flavonoids. The presence of flavanoids, tannins, and other precursors, either alone or in combination, may account for the extract's or fractions anti-inflammatory properties [44]. The hydro-alcoholic extract of Tridax procumbens may have anti-inflammatory properties, according to an in vitro study.

Inflammation is known to be caused by protein denaturation. Salicylic acid, phenylbutazone, and other anti-inflammatory medications have demonstrated dose-dependent potential to cause denaturation of proteins when heated [45]. The term "denaturation" refers broadly to the transformation of proteins from a soluble to an insoluble form caused by a wide range of chemical and physical agents, such as alcohol, acetone, acids, alkalies, dyes, heavy metal salts, heat, light, and pressure [46, 47]. Heat denaturation is viewed by Chick and Martin as a protein-water interaction that almost certainly involves hydrolysis [48]. According to some research, one of the causes of rheumatoid arthritis is protein denaturation [49, 50]. This is because some rheumatic disorders result in the formation of auto-antigens. It could lead to protein denaturation in vitro. Changes in hydrogen, hydrophobic, disulfide, and electrostatic forces are all part of the denaturation mechanism. A number of novel anti-inflammatory medications have demonstrated a dose-dependent capacity to prevent the denaturation of proteins caused by heat [51].

In summary, phenols and flavonoids present in the hydro alcoholic extract of Tridax procumbens exert their hemostatic and anti-inflammatory effects by scavenging free radicals, inhibiting platelet aggregation, reducing inflammation, protecting vascular integrity, and modulating inflammatory signaling pathways. These activities collectively contribute to the potential therapeutic benefits of Tridax procumbens extract in conditions involving impaired hemostasis and inflammation.

5. CONCLUSION:

According to the findings of the current investigation, hydro-alcoholic extracts of Tridax procumbens contained a variety of beneficial phytochemical components. Using Lee White's method, the hydro-alcoholic extract of Tridax procumbens was tested for its in vitro hemostasis activity as well as its anti-inflammatory and protein denaturation properties against human red blood cell membrane and egg albumin, respectively. With increasing concentration, the hydro-alcoholic extract of Tridax procumbens demonstrated noticeably greater hemostasis and anti-inflammatory action. The anti-inflammatory and hemostasis properties may be attributed to the active components of phytocompounds like flavonoids, tri-terpenoids, and related polyphenols. Phenols and flavonoids present in the hydro alcoholic extract of Tridax procumbens exert their hemostatic and anti-inflammatory effects by scavenging free radicals, inhibiting platelet aggregation, reducing inflammation, protecting vascular integrity, and modulating inflammatory signaling pathways. As a result, Tridax procumbens has anti-inflammatory and blood coagulant properties. The research is motivated by the need for natural sources of powerful blood coagulant and anti-inflammatory medicines with low side effects to substitute for synthetic therapeutics.

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