IN VITRO EVALUATION OF THROMBOLYTIC ANDANTI-INFLAMMATORY ACTIVITIES OF THE WHOLE PLANT OFDRYNARIA QUERCIFOLIA (L.) (POLYPODIACEAE)

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Abstract:

Medicinal plants have long played a vital role in healthcare due to their rich content of bioactive compounds and therapeutic potential. Drynaria quercifolia, a medicinal fern belonging to the Polypodiaceae family, is widely used in traditional systems of medicine for treating inflammation, wounds, rheumatism, and circulatory disorders. The present study aims to evaluate the anti-inflammatory and thrombolytic activities of the whole plant extract of Drynaria quercifolia using in vitro experimental models and to scientifically validate its traditional uses. The plant extract contains important phytoconstituents such as flavonoids, phenolics, tannins, and saponins, which contribute to its pharmacological actions. Anti-inflammatory activity is assessed using the Human Red Blood Cell (HRBC) membrane stabilization method, while thrombolytic activity is evaluated using a clot lysis assay. These studies help determine the dose-dependent effectiveness of the plant extract. Inflammation and thrombosis are interrelated pathological processes involving cytokine release, oxidative stress, and fibrin clot formation. The phytochemicals in Drynaria quercifolia act by inhibiting inflammatory mediators, scavenging reactive oxygen species, stabilizing cellular membranes, and enhancing fibrinolysis. Compared to synthetic drugs, which often cause adverse effects such as gastrointestinal irritation and bleeding, plant-based therapies offer a safer and multi-targeted approach.

Keywords:

Drynaria quercifolia, Anti-inflammatory, Thrombolytic activity, Medicinal plants, Phytochemicals, HRBC method, Clot lysis assay

1. INTRODUCTION

Medicinal plants have been an integral part of human civilization for thousands of years and continue to play a vital role in modern healthcare. Traditional systems of medicine such as Ayurveda, Siddha, and Traditional Chinese Medicine rely heavily on plant-based formulations for the treatment of various diseases. These plants contain a wide range of bioactive compounds that exhibit pharmacological activities, making them valuable resources for drug discovery.

One such medicinal plant is *Drynaria quercifolia*, a tropical epiphytic fern widely distributed in India and other Asian countries. It has been traditionally used for treating inflammation, bone fractures, wounds, and circulatory disorders. The therapeutic potential of this plant is attributed to the presence of secondary metabolites such as flavonoids, phenolics, alkaloids, and saponins. These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and thrombolytic activities.

In recent years, there has been growing interest in exploring plant-based therapies due to the limitations of synthetic drugs. This article aims to provide a comprehensive overview of the anti-inflammatory and thrombolytic properties of *Drynaria quercifolia*, supported by scientific evidence.

INFLAMMATION: DEFINITION, TYPES, AND MEDIATORS

Inflammation is a complex physiological response to harmful stimuli, including pathogens, tissue injury, chemical irritants, or autoimmune processes. It serves as a protective mechanism designed to eliminate injurious stimuli and initiate tissue repair.

Types of Inflammation

Acute Inflammation: Rapid onset, short duration; characterized by redness, heat, swelling, pain, and loss of function. Mediated primarily by neutrophils, histamine, prostaglandins, and cytokines. Chronic Inflammation: Prolonged, persistent inflammation involving macrophages, lymphocytes, fibroblasts, often leading to tissue remodeling, fibrosis, or chronic disease states such as arthritis, atherosclerosis, and thrombosis.

Key Inflammatory Mediators

Cytokines: TNF-α, IL-1β, IL-6 regulate immune cell recruitment and inflammation amplification. (Barua *et al*., 2013)
Eicosanoids: Prostaglandins and leukotrienes derived from arachidonic acid via COX and LOX pathways.
Nitric Oxide (NO): Produced by inducible nitric oxide synthase (iNOS), contributing to vasodilation and oxidative stress.

Reactive Oxygen Species (ROS): Cause oxidative damage to tissues and amplify inflammatory signaling.

Drynaria quercifolia exerts anti-inflammatory effects by modulating these mediators. Flavonoids and phenolics in the plant inhibit COX and LOX enzymes, suppress pro-inflammatory cytokine release, and scavenge ROS, stabilizing cellular membranes and preventing tissue damage.

Molecular Basis of Inflammatory Response

At the molecular level, inflammation is regulated by intricate signaling pathways: NF-κB Pathway: Activated by cytokines and oxidative stress; drives transcription of inflammatory genes (COX-2, iNOS, TNF-α).MAPK Pathway: Mediates responses to extracellular stimuli, regulates cytokine production.

DRYNARIA QUERCIFOLIA PLANT

Pro-inflammatory Enzyme Activation: Cyclooxygenases (COX-1, COX-2) and lipoxygenases (LOX) catalyze synthesis of prostaglandins and leukotrienes. Phytochemicals in *Drynaria quercifolia* inhibit these pathways

Suppressing NF-κB activation, reducing transcription of inflammatory cytokines. Inhibiting COX/LOX enzymes, lowering prostaglandin and leukotriene synthesis.

Scavenging ROS, mitigating oxidative damage to cellular proteins, lipids, and DNA. These molecular interactions underpin the in-vitro and in-vivo anti-inflammatory efficacy observed in experimental studies of *Drynaria quercifolia*.

Mechanism of Fibrinolysis and Thrombolytic Therapy

Fibrinolysis is the enzymatic degradation of fibrin clots, primarily mediated by plasmin, which is activated from plasminogen. Standard thrombolytic drugs (e.g., streptokinase, tissue plasminogen activator) facilitate this conversion to dissolve clots. *Drynaria quercifolia* extracts contribute to fibrinolysis via: Saponins: Facilitate plasminogen activation and fibrin degradation.

DRYNARIA QUERCIFOLIA **RHIZOME**

Flavonoids/Phenolics: Reduce oxidative stress, destabilize fibrin cross-links and enhance clot lysis. (Patra *et al*., 2021; Senthilkumar & Venkatesalu, 2016).

The synergistic interaction between anti-inflammatory and thrombolytic constituents enhances therapeutic efficacy. By reducing endothelial inflammation and inhibiting platelet aggregation, anti-inflammatory compounds indirectly facilitate thrombolysis and improve vascular homeostasis.

MATERIALS AND METHODS

EXTRACTIVE VALUES OF *DRYNARIA QUERCIFOLIA* WHOLE PLANT USING DIFFERENT SOLVENTS

Chemicals / Solvents

- Petroleum ether
- Chloroform
- Ethyl acetate
- Ethanol
- Methanol

Plant Material

- Dried whole plant powder of *Drynaria quercifolia*
- Glassware & Apparatus
- Stoppered conical flasks
- Whatman No. 1 filter paper
- Evaporating dishes
- Hot air oven
- Water bath

MACERATION

DEFINITION

Maceration is defined as a process of extraction in which a coarsely powdered crude drug is allowed to remain in contact with a suitable menstruum in a closed container for a specified period, with periodic agitation, to dissolve the soluble constituents. The liquid is then separated by filtration and expression of the marc to obtain the extract.

PRINCIPLE

The extraction process in maceration is governed by diffusion, osmosis, and solubility principles. The solvent enters the plant cells by osmosis, softening and swelling the cellular structure. Soluble constituents dissolve in the solvent according to their chemical affinity and polarity.

A concentration gradient is established between the intracellular contents and the surrounding solvent. Dissolved constituents diffuse outward until equilibrium is attained. Agitation helps maintain the concentration gradient, thereby improving extraction efficiency. Thus, maceration relies on passive mass transfer rather than mechanical force or heat.

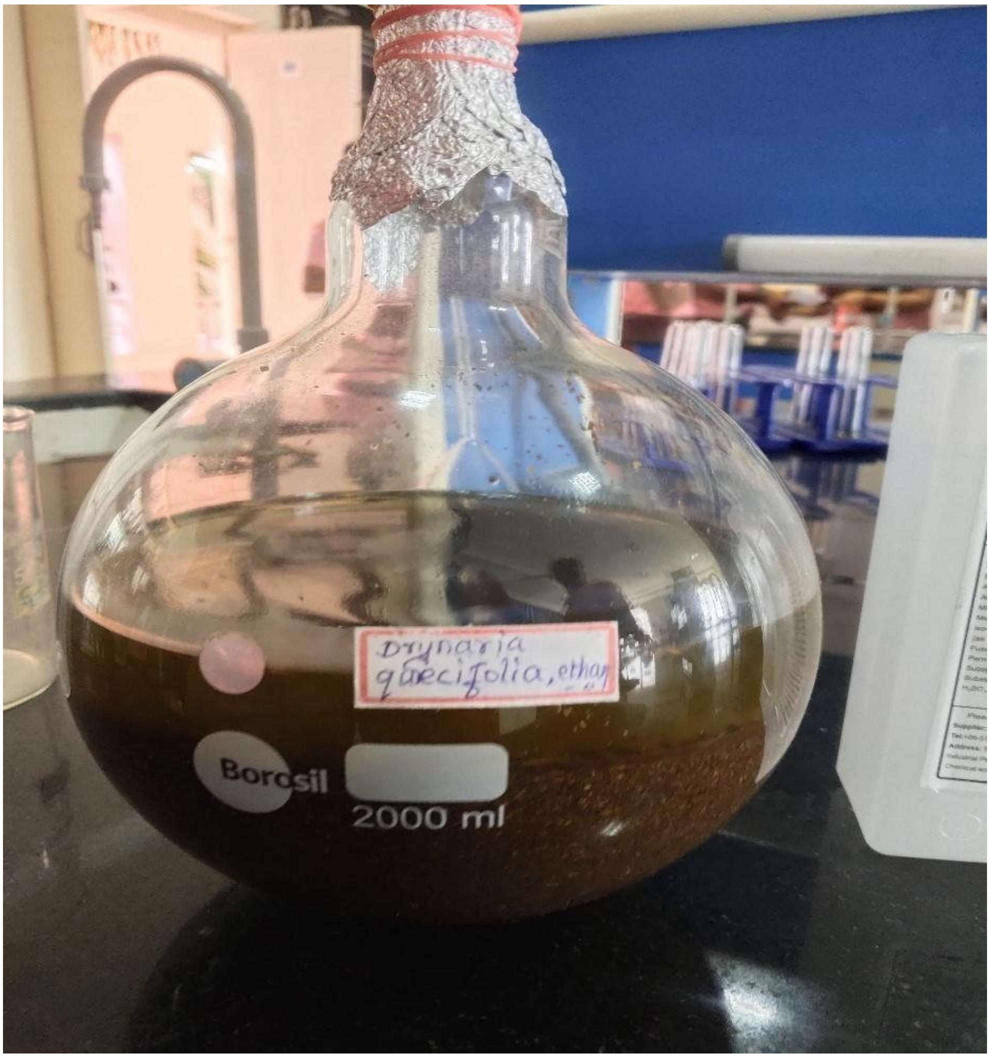
METHODOLOGY/PROCEDURE

Step 1: Preparation of Crude Drug The crude drug is thoroughly cleaned, dried, and coarsely powdered. Coarse particles ensure effective solvent penetration while preventing formation of impermeable masses.

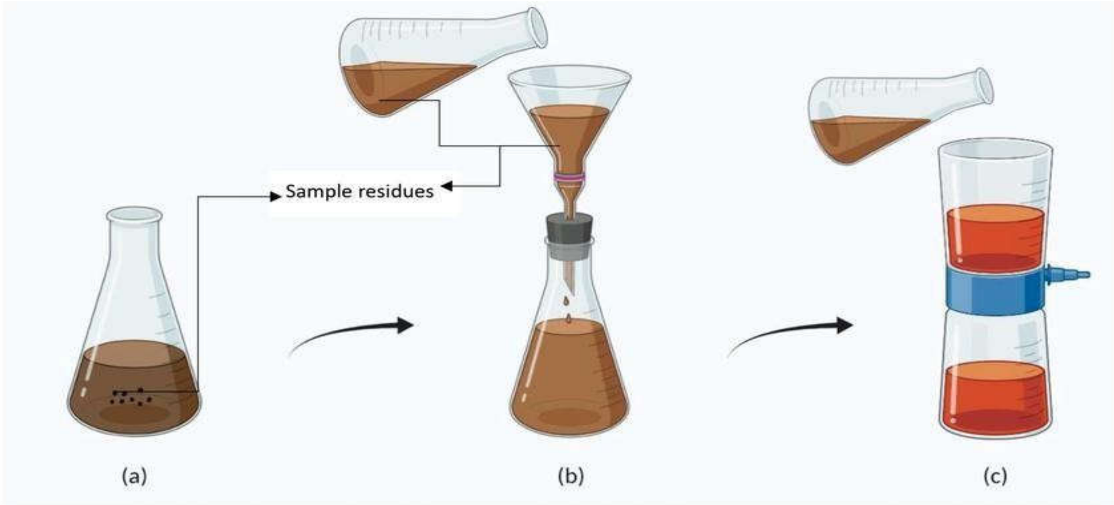
Step 2: Selection of Menstruum The choice of solvent is crucial and depends on: Nature of phytoconstituents Polarity and solubility Stability of constituents Commonly used menstruum's include water, ethanol, methanol, glycerin, and hydro-alcoholic mixture.

Step 3: Soaking (Maceration) The powdered drug is transferred to a maceration vessel, and sufficient solvent is added to completely cover the drug. The vessel is closed to prevent evaporation and contamination.

Step 4: Maceration Period The mixture is allowed to stand for 3–7 days, during which occasional shaking or stirring is carried out to enhance diffusion and prevent sedimentation.
Step 5: Separation of Extract After the completion of maceration, the liquid portion is separated by filtration. Step 6: Expression of Marc The residual plant mass (marc) is pressed mechanically to recover the absorbed solvent, ensuring maximum yield.
Step 7: Clarification and Preservation The combined filtrate is allowed to stand for clarification and may be preserved using suitable preservatives, especially in aqueous extracts.



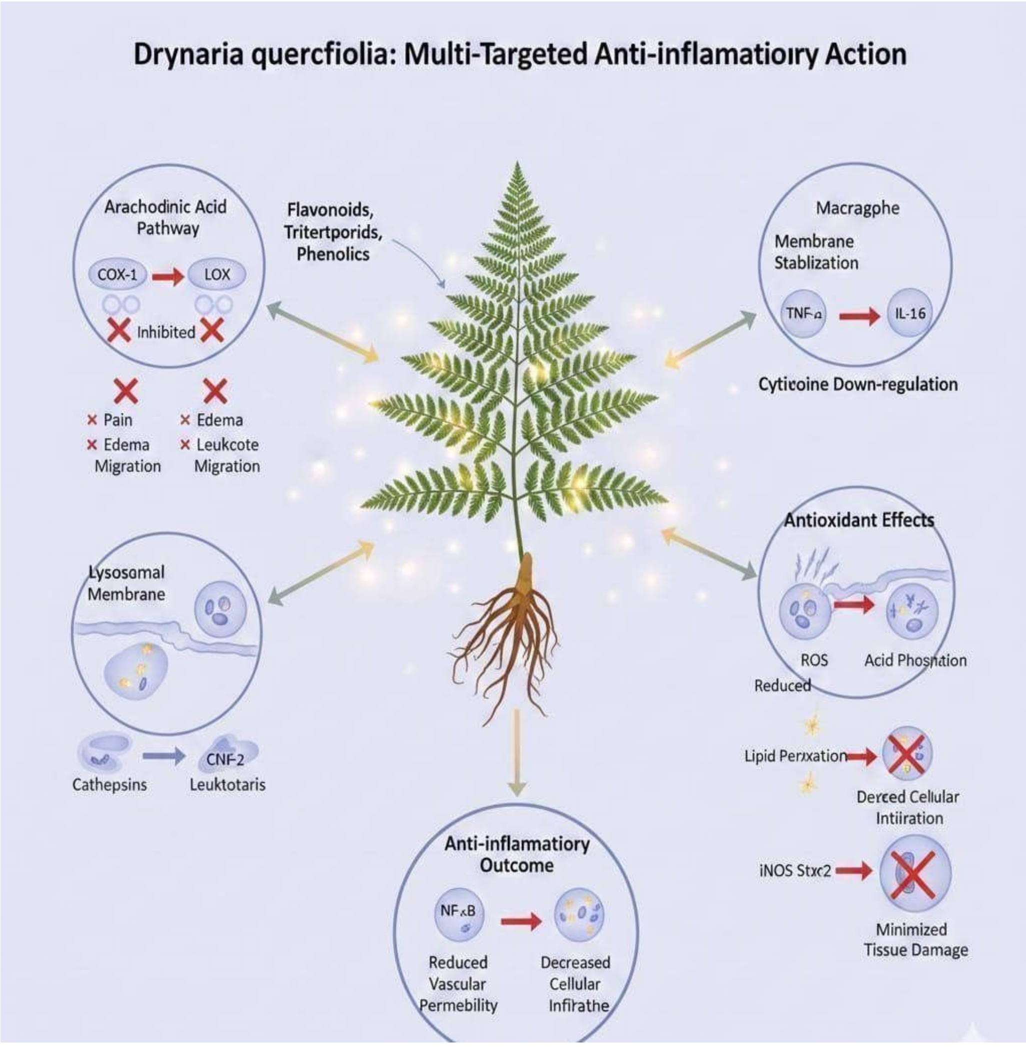
EXTRACTION ON WHOLE PLANT



MECHANISM OF ACTION FOR ANTI-INFLAMMATORY

Drynaria quercifolia whole plant exhibits significant anti-inflammatory activity through a multi- targeted mechanism involving inhibition of inflammatory mediators, modulation of immune responses, antioxidant effects, and cellular membrane stabilization. The anti-inflammatory action primarily begins with suppression of the arachidonic acid pathway, where bioactive constituents such as flavonoids, triterpenoids, and phenolic compounds inhibit cyclooxygenase (COX-1 and COX-2) and lipoxygenase (LOX) enzymes. This inhibition reduces the biosynthesis of pro-inflammatory prostaglandins and leukotrienes, which are responsible for pain, edema, fever, and leukocyte migration at the site of inflammation. In addition, *Drynaria quercifolia* down-regulates the production of key pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) by activated macrophages and neutrophils, thereby preventing amplification of the inflammatory cascade.

The whole plant extract also stabilizes lysosomal membranes, restricting the release of proteolytic enzymes such as cathepsins and acid phosphatase that contribute to tissue damage during inflammation. Furthermore, its strong antioxidant potential plays a crucial role in anti- inflammatory activity by scavenging reactive oxygen species (ROS), reducing lipid peroxidation, and inhibiting oxidative stress-induced activation of nuclear factor-κB (NF-κB), a key transcription factor regulating inflammatory gene expression. By preventing NF-κB activation, the extract suppresses the expression of inducible nitric oxide synthase (iNOS), COX-2, and other inflammatory mediators. Collectively, these mechanisms act synergistically to reduce vascular permeability, cellular infiltration, and tissue damage, supporting the therapeutic potential of *Drynaria quercifolia* whole plant as a natural anti-inflammatory agent.



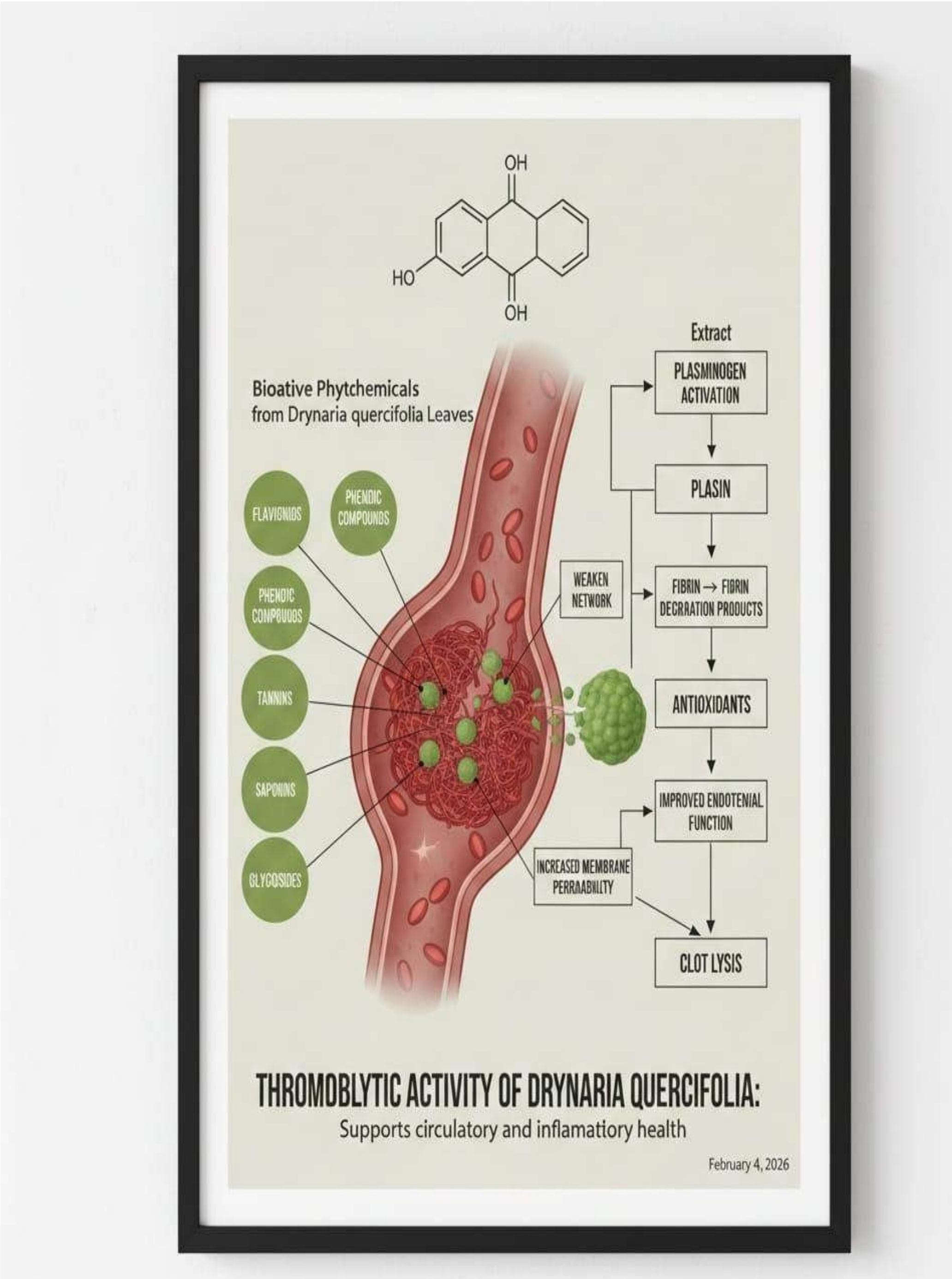
MECHANISM OF ACTION OF ANTI-INFLAMMATORY

MECHANISM OF ACTION FOR THROMBOLYTIC ACTIVITY

The thrombolytic activity exhibited by the whole plant of *Drynaria quercifolia* is believed to result from the integrated and synergistic actions of its phytochemical constituents, including flavonoids, phenolic acids, saponins, triterpenoids, glycosides and phytosterols. These compounds collectively modulate key processes involved in thrombosis and fibrinolysis. Flavonoids and phenolic compounds play a central role by enhancing endogenous fibrinolytic mechanisms, particularly through the stimulation of plasminogen activators such as tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Activation of these enzymes promotes the conversion of plasminogen into plasmin, a serine protease responsible for the degradation of fibrin, the principal structural component of blood clots.

The presence of saponins and triterpenoids may further facilitate thrombolysis by interacting with fibrin polymers, weakening fibrin cross-linking and reducing clot density, thereby improving the accessibility of plasmin to fibrin strands. In addition, the antioxidant potential of D. quercifolia, primarily attributed to its phenolic constituents, plays a crucial indirect role in thrombolysis by mitigating oxidative stress within the vascular endothelium. Oxidative stress is known to enhance fibrin stabilization and impair fibrinolytic enzyme activity; therefore, antioxidant- mediated protection helps preserve plasmin activity and maintains endothelial function necessary for balanced hemostasis. Furthermore, flavonoids present in the plant extract exhibit antiplatelet activity by inhibiting platelet aggregation pathways, including suppression of thromboxane A_2 synthesis and modulation of intracellular calcium signaling, leading to reduced platelet activation and clot propagation. This antiplatelet effect limits further thrombus growth and renders existing clots more susceptible to enzymatic degradation. The combined influence of fibrin destabilization, enhanced plasmin-mediated fibrinolysis, antioxidant protection of vascular and enzymatic components and inhibition of platelet aggregation suggests that the thrombolytic effect of *Drynaria quercifolia* arises from a multifactorial mechanism rather than a single target action. These findings support the therapeutic potential of D. quercifolia as a natural thrombolytic agent and warrant

Further investigation through bioassay-guided isolation of active constituents and in vivo validation of its antithrombotic efficacy. (M Chithra *..et.,al*).



MECHANISM OF ACTION FOR THROMBOLYTIC ACTIVITY

EVALUATION OF THROMBOLYTIC ACTIVITY

Overview of Thrombolytic Screening (streptokinase-induced thrombolytic assay)

Thrombolysis refers to the enzymatic degradation of fibrin clots, a critical process in the management of thromboembolic disorders such as myocardial infarction, ischemic stroke, and deep vein thrombosis. The formation of stable fibrin clots involves cross-linking of fibrin monomers by activated Factor XIII and subsequent entrapment of platelets and erythrocytes. Medicinal plants containing bioactive phytoconstituents such as flavonoids, saponins, tannins, and phenolics may facilitate endogenous fibrinolysis by enhancing plasminogen activation, destabilizing fibrin networks, and reducing oxidative stress. *Drynaria quercifolia* , traditionally used for circulatory and inflammatory disorders, was selected for thrombolytic evaluation to substantiate its therapeutic potential. (Kamalakkannan Mani..*et., al* 2013) (Shinde *et al*., 1999).

Principle of In-Vitro Clot Lysis Assay

The in-vitro clot lysis assay is predicated on quantifying the dissolution of preformed blood clots upon exposure to a test sample. Human blood, upon standing, coagulates to form a fibrin mesh entrapping red blood cells, thereby generating a stable clot. The addition of thrombolytic agents or bioactive plant extracts initiates fibrinolysis, leading to partial or complete dissolution of the clot. The extent of clot lysis is determined gravimetrically by comparing the clot weight before and after treatment. This assay provides a direct measure of fibrinolytic efficacy and allows correlation with the phytochemical profile of the extract.

The assay also indirectly assesses the antioxidant potential of phytoconstituents, as oxidative stress can increase fibrin cross-linking, making clots more resistant to degradation. Hence, compounds with both anti-inflammatory and antioxidant properties, such as those present in *Drynaria quercifolia*, may synergistically enhance thrombolytic activity. (Mounnissamy *et al.,* 2008).

IN VITRO CLOTLYSIS ASSAY (STREPTOKINASE-INDUCED THROMBOLYTIC ASSAY)

Preparation of Streptokinase (SK)

Commercially available lyophilized streptokinase was reconstituted by adding 5 ml of sterile distilled water and mixed gently to obtain a homogeneous solution. This preparation was used as a stock solution. From the stock, 100 µl containing 30,000 IU of streptokinase was used as a positive control for the in vitro thrombolysis assay.

EXPERIMENTAL PROCEDURE

a) Blood Collection and Clot Formation:

Fresh venous blood was collected aseptically from healthy, consenting adult volunteers. Approximately 500 µL of blood was dispensed into sterile pre-weighed microcentrifuge tubes and incubated at 37°C for 45–60 minutes to allow clot formation. The formation of a stable, adherent clot was confirmed visually.

b) Removal of Serum:

Following clot formation, the serum was carefully aspirated without disturbing the clot. The initial weight of the clot was determined by subtracting the pre-weighed tube mass from the total mass of clot-containing tube.

c) Treatment with Test Samples:

A measured volume (e.g., 100 µL) of *Drynaria quercifolia* extract at different concentrations was added to each tube containing the clot. Control tubes were treated with an equal volume of normal saline, while positive control tubes received a standard thrombolytic agent (streptokinase).

d) Incubation for Clot Lysis:

The tubes were incubated at 37°C for 90–120 minutes to allow interaction between the extract and the fibrin network. Visual observations of clot dissolution were recorded.

e) Gravimetric Analysis:

Post-incubation, the lysed fluid was carefully removed, and tubes were re-weighed to determine residual clot mass.

This quantitative evaluation allows assessment of thrombolytic efficacy at multiple concentrations and comparison with the standard agent.

f) Standard Thrombolytic agent

Streptokinase was used as the reference thrombolytic agent due to its well-established mechanism of activating plasminogen to plasmin, which enzymatically cleaves fibrin. The percentage clot lysis produced by *Drynaria quercifolia* extracts was compared to streptokinase to assess relative efficacy. Extracts demonstrating clot lysis values approaching or exceeding 50% of streptokinase activity were considered pharmacologically significant.

g) Collection of Blood

Whole blood was collected from healthy human volunteers who had no history of oral contraceptive use or anticoagulant therapy. About 1 ml of blood was transferred aseptically into previously weighed sterile Eppendorf tubes. The blood samples were allowed to clot. (Kamalakkannan Mani..*et., al* 2013).

Determination of Thrombolytic Activity

The Eppendorf tubes containing blood samples were incubated at 37 °C for 45 minutes to ensure complete clot formation. After incubation, the serum was carefully removed without disturbing the formed clot.

Each tube was then weighed again to determine the clot weight using the following formula:

Clot weight = Weight of clot-containing tube − Weight of empty tube

To each tube containing a pre-weighed clot, 100 µl of the plant sample (*Drynaria quercifolia* , DQ) at different concentrations was added separately. For comparison, 100 µl of streptokinase solution was added to the positive control tube, while 100 µl of sterile distilled water was added to the negative control tube to assess non-thrombolytic activity.

All the tubes were incubated at 37 °C for 90 minutes to allow clot lysis. After incubation, the released fluid was carefully removed, and the tubes were reweighed to determine the residual clot weight.

The difference in clot weight before and after treatment was used to calculate the percentage of clot lysis

% Haemolysis= Test OD/ Control OD x 10

ANTI-INFLAMMATORY ACTIVITY

Human Red Blood Cell (HRBC) Membrane Stabilization Assay The anti-inflammatory activity of the sample *Drynaria quercifolia* (DQ) was evaluated by the Human Red Blood Cell (HRBC) membrane stabilization method, as described by Shinde et al. (2019). This method is widely used to assess the ability of test samples to stabilize biological membranes under stress conditions. (Mounnissamy *et al.,* 2008).

PRINCIPLE

Inflammation is associated with the destabilization of lysosomal membranes, leading to the release of inflammatory mediators such as proteases and phospholipases. Human Red Blood Cell (HRBC) membranes closely resemble lysosomal membranes in composition and structure. When HRBCs are exposed to hypotonic solutions, osmotic stress causes swelling and rupture of the cell membrane, resulting in haemolysis and the release of haemoglobin into the surrounding medium (Feirrali et al., 2018). Substances that can prevent or reduce

HRBC membrane lysis under these conditions are considered capable of stabilizing lysosomal membranes, thereby inhibiting the release of inflammatory mediators. (Mounnissamy *et al.,* 2008).

REAGENTS

Alsever's solution Dextrose – 2%

Sodium citrate – 0.8%

Citric acid – 0.05% Sodium

chloride – 0.42%

(Prepared in distilled water and used as an anticoagulant)

Isosaline – 0.85%

HRBC suspension medium – Isosaline (10% v/v)

Phosphate buffer – 0.15 M (pH 7.4)

Hyposaline – 0.36% NaCl solution

Standard drug – Diclofenac sodium

MEMBRANE STABILIZATION (MS) ASSAY:

a) Preparation of Human Red Blood Cell (HRBC) Suspension

Fresh human blood was collected from healthy volunteers with prior consent. The collected blood was immediately mixed with an equal volume of sterilized Alsever's solution to prevent coagulation. The mixture was centrifuged at 3000 rpm for 5 minutes, and the supernatant was discarded. The packed red blood cells were washed three times with isosaline (0.85%, pH 7.2) to remove plasma and leukocytes. Finally, a 10% v/v HRBC suspension was prepared using isosaline and used for the assay.

b) Experimental Procedure

The assay mixture consisted of the following components: Phosphate buffer (0.15 M, pH 7.4) – 1.0 ml Hyposaline (0.36%) – 2.0 ml HRBC suspension – 0.5 ml Test sample (DQ) at various concentrations (200, 400, 600, 800, and 1000 µg/ml) – 1.0 ml for the control, 2.0 ml of double distilled water was used instead of hyposaline to produce complete haemolysis.

All reaction mixtures were incubated at 37°C for 30 minutes, followed by centrifugation at 3000 rpm for 5 minutes. The absorbance of the supernatant, representing released haemoglobin, was measured at 560 nm using a UV–Visible spectrophotometer.

Diclofenac sodium was used as the standard anti-inflammatory drug for comparison. Calculation of Percentage Haemolysis The extent of haemolysis was calculated using the formula: Lower absorbance values indicate reduced haemolysis and greater membrane stabilization. (Haripriya..*et.,al.,* 2016).

RESULT AND DISCUSSION

The whole plant of *drynaria quercifolia* (l.) j. sm. was collected and the same was authenticated as *drynaria quercifolia* (l.) j. sm. (family: polypodiaceae) by dr. v. aravindhan, assistant professor, department of botany, kongunadu arts and science college, coimbatore – 641 029.

EXTRACTIVE VALUE OF WHOLE PLANT OF DRYNARIA QUERCIFOLIA

CALCULATION

- Weight of crude drug taken = 5 g
- Total volume of filtrate = 100 mL
- Volume of filtrate taken for evaporation = 25 mL

EXTRACTIVE VALUES

SOLVENT	WEIGH OF RESIDUE(g)	EXTRACT VALUE
Petroleum ether	0.040	$\frac{0.040 \text{ X } 100 \text{ X } 100}{5 \text{ X } 25} = 3.5\%$
chloroform	0.055	4.4%
Ethyl acetate	0.070	5.6%
Ethanol	0.150	12.0%
Methanol	0.125	10.0%
Water	0.165	13.0%

RESULT OF EXTRACTIVE VALUE

From the above result, we concluded that water and ethanol are more extractive value. In this we prefer ethanolic extraction above water as a solvent because water has more chance for contamination.

LOSS OF DRYING TABLE LOSS

OF DRYING

S. NO	DESCRIPTION	WEIGHT
1	Weight of empty evaporating dish	50.00 g
2	Weight of dish + sample before drying	60.00 g
3	Weight of sample taken	10.00 g
4	Weight of dish + sample after drying (at 105° C)	58.80 g
5	Weight of sample after drying	8.80 g
6	Loss in weight	8.80 g

CALCULATION

LOD (% w/w) = Loss in weight / Weight of sample ×100

=1.20 / 10 × 100

=12%

LOSS OF DRYING RESULT

Loss on Drying of Drynaria quercifolia whole plant powder = 12% w/w

1.1 FOREIGN ORGANIC MATTER

CALCULATION FOR FOREIGN ORGANIC MATTER

Formula

$$\text{Percentage of Foreign Organic Matter} = \frac{\text{Weight of Foreign Matter}}{\text{Total Weight of Sample}} \times 100$$

Total weight of sample taken = 10 g

Weight of foreign organic matter separated = 0.1 g

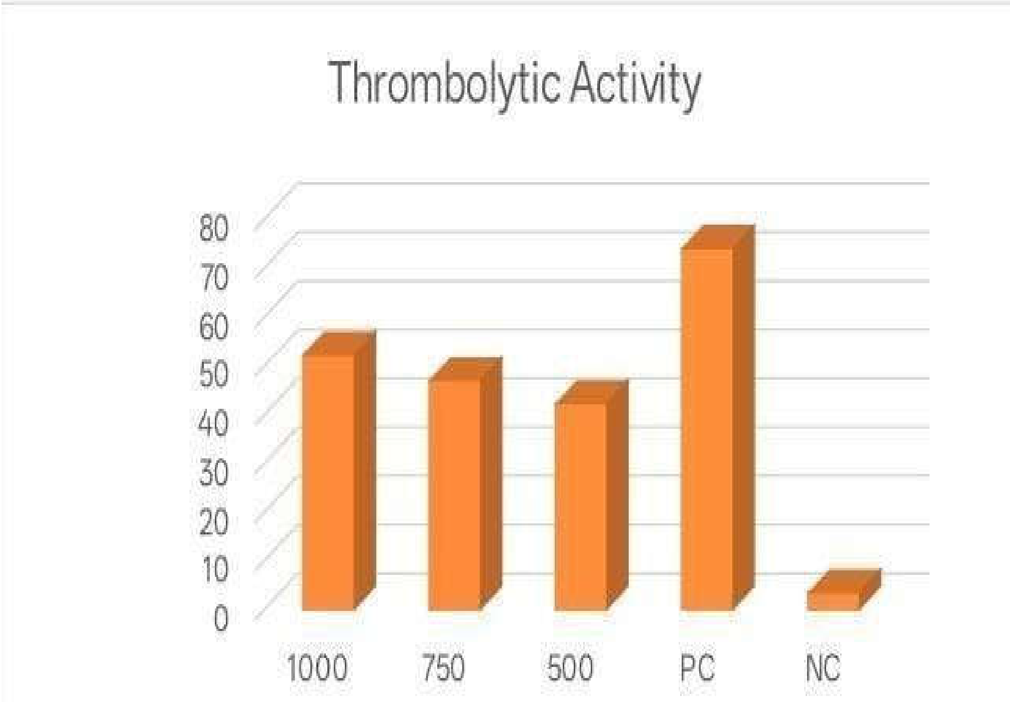
Percentage of Foreign Organic Matter=10 / 0.1 ×100 = 1%

FOREIGN ORGANIC MATTER RESULT

The percentage of foreign organic matter present in the whole plant powder of Drynaria quercifolia was found to be 1% w/w

THROMBOLYTIC ACTIVITY

The X-axis represents the concentrations of the test sample (1000, 750, and 500 µg/mL) along with the Positive Control (PC) and Negative Control (NC). The Y-axis indicates the percentage of clot lysis, reflecting thrombolytic activity. At 1000 µg/mL, the sample exhibits approximately 55–58% clot lysis, indicating strong thrombolytic activity. At 750 µg/mL, the activity slightly decreases to around 50–52%. At 500 µg/mL, the clot lysis further decreases to approximately 45–48%, showing a gradual reduction in activity with decreasing concentration. The Positive Control (PC) demonstrates the highest thrombolytic activity, around 75–80%, confirming the validity and effectiveness of the assay. In contrast, the Negative Control (NC) shows minimal clot lysis (about 5–8%), indicating negligible thrombolytic effect. Conclusion: The graph clearly demonstrates dose-dependent thrombolytic activity of the test sample, with higher concentrations producing greater clot lysis. Although the activity is lower than that of the positive control, the sample shows significant thrombolytic potential compared to the negative control.



THROMBOLYTIC ACTIVITY

TABLE 6.3 PERCENTAGE OF CLOT LYSIS OF EXTRACT

S. NO	CONCENTRATION (µg/ml)	PERCENTAGE OF CLOT LYSIS
1	1000	51.90
2	750	46.64
3	500	41.92
4	Positive control	73.71
5	Negative control	3.1

THROMBOLYTIC RESULT

The sample *Drynaria quercifolia* (DQ) exhibited dose-dependent thrombolytic activity. The highest clot lysis was observed at 1000 µg/ml (51.90%), followed by 750 µg/ml (46.64%) and 500 µg/ml (41.92%). The positive control showed maximum clot lysis (73.71%), while the negative control showed negligible activity (3.1%).

ANTI-INFLAMMATORY

MEMBRANE STABILIZATION (MS) ASSAY:

The X-axis shows the concentrations of the sample DQ (200, 400, 600, 800, and 1000 µg/ml) along with the standard drug.

The Y-axis indicates the percentage of membrane stabilization, which reflects anti-inflammatory activity. Sample DQ exhibits a dose-dependent increase in membrane stabilization:

200 µg/ml: 43.55%

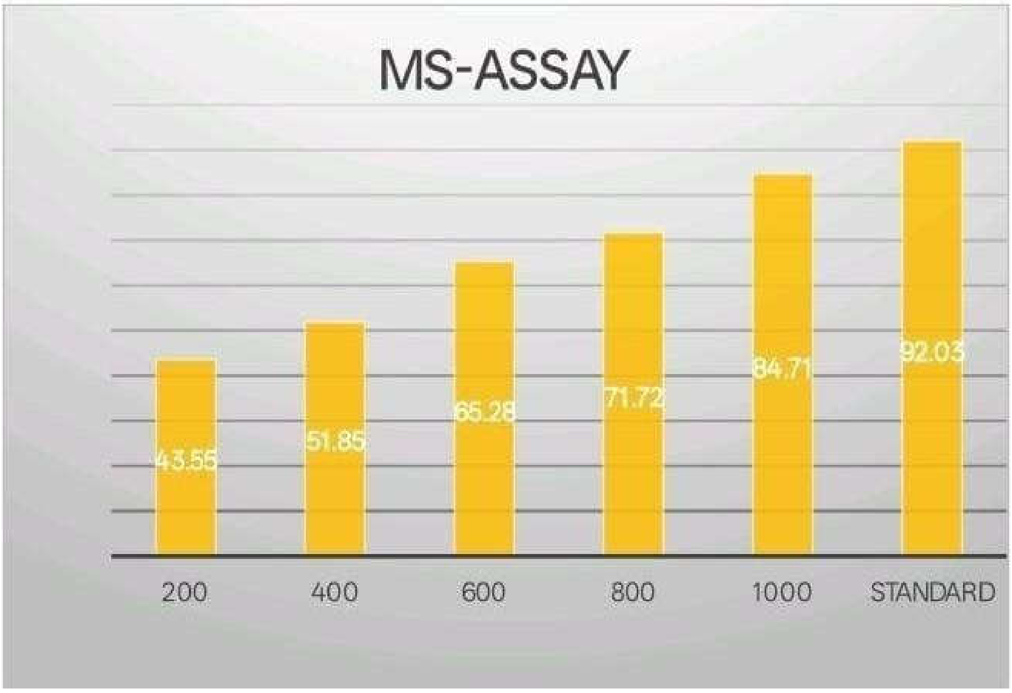
400 µg/ml: 51.85%

600 µg/ml: 65.28%

800 µg/ml: 71.72%

1000 µg/ml: 84.71%

The standard drug shows the highest activity with 92.03% membrane stabilization.



MEMBRANE STABILIZATION (MS) ASSAY

TABLE 6.4 MEMBRANE STABILIZATION PERCENTAGE

CONCENTRATION	OD (560 nm)	% STABILIZATION OF HRBC MEMBRANE
200	0.399	43.55
400	0.475	51.85
600	0.598	65.28
800	0.657	71.72
1000	0.776	84.71
Standard	0.843	92.03
Control	0.916	-

ANTI -INFLAMMATORY RESULT

The sample *Drynaria quercifolia* (DQ) showed significant dose-dependent anti-inflammatory activity I n the HRBC membrane stabilization assay. Maximum membrane stabilization was observed at 1000 µg/ml (84.71%), which was comparable to the standard drug (92.03%), while lower concentrations showed moderate activity.

SUMMARY AND CONCLUSION

The findings of this study confirm that drynaria quercifolia whole plant possesses promising anti- inflammatory and thrombolytic properties, supporting its traditional medicinal use. the pharmacological effects are mainly attributed to phytochemicals such as flavonoids, phenolics, tannins, saponins, and terpenoids, which stabilize cell membranes, inhibit inflammatory mediators, reduce oxidative stress, and enhance fibrinolysis. although the extract showed lower thrombolytic activity compared to standard drugs, it demonstrated significant in vitro potential and may act as a supportive natural therapeutic agent in inflammatory and thrombotic conditions. however, further studies, including isolation of active compounds, in vivo experiments, and clinical trials, are necessary to confirm its safety and effectiveness as a natural drug candidate.

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