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Phytochemical Screening and Anti Inflammatory Activity of *Drynaria quercifolia* Rhizome

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Inflammation is the complex response of blood vessels to harmful elements like bacteria, irritants, or cells that are injured.Plant derived bioactive compounds have many biological functions including decrease of inflammation. The bioactive compounds derived from Drynaria quercifolia rhizomes possess antiinflammatory properties. The Drynaria quercifolia rhizomesample was collected from the Koyambedu market, Chennai, Tamil Nadu, India.The collected rhizomes were subjected to wet and dry weight measurementwhich results found1800g of wet rhizomes gave 440g of dry rhizome powder. The experiments were conducted usingtwo different solvents:ethanol and hot distilled water to extract phytochemicals from Drynaria quercifolia rhizome.Ethanolic extract contains alkaloids, cardiac glycosides, phenols, quinones, steroids and terpenoids. Hot distilled water extract contains the cardiac glycosides, phenols, proteins, quinones, steroids and terpenoids. The total phenolic content of Drynaria quercifolia rhizome using ethanolic extraction was about 36.731mg of gallic acid equivalent/g dry weight whereas hot distilled water extraction contained 30.296mg of gallic acid equivalent/g dry weight. The ethanolic extract exhibited a protein denaturation inhibition of 77.72% at 1600 μg/mL, which was lower than that of the standard diclofenac sodium (92.80%) at the same 1600 μg/mL of concentration.Lower level of protein denaturation was inhibited in hot distilled water extract (68.85%) at 1600 μg/mL of concentration when compared to standard drug and ethanolic extract. These experiments were triplicated using theone-way ANOVA method.IC50 values of the standard drug (Diclofenac sodium), ethanolicextract and hot distilled water extract were 69.7μg/mL, 357.28μg/mL and 402.60μg/mL respectively.

Keywords: Denaturation; Drynaria quercifolia; Inflammation,Phytochemicals; Rhizome.

Inflammation is a complex response of blood vessels to harmful factors, including bacteria, irritants, or damaged cells. It's a defensive move by the body to get rid of the harmful factors and start the recovery of the tissue.[1] However, if left uncontrolled inflammation can cause the beginning of conditions such as vasomotor rhinorrhea, rheumatoid arthritisand atherosclerosis.[2] Products made from plants have grown in popularity lately as substitutes for conventional medicines.[3-4] The plant based secondary metabolites are responsible for their regulatory effects. This category consists of lipids, carbohydrates, phenolics, alkaloids, saponins and terpenes.[5] Plant-derived phytochemicals have a variety of bioactivities including anti-inflammation.[6] The previous report

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the maximum phytochemicals were found in the *Drynaria quercifolia* rhizomes.[7] The bioactive compounds of *Drynaria quercifolia* rhizomes has varying due to their different environment and by the solvent's nature for extraction.[8] These bioactive compounds consist of anti-inflammatory, anthelmintic and astringent properties also used in the traditional treatment of joint pain, dyspepsia, Body pain, skin diseases, typhoid, diarrhoea, chronic jaundice, cholera, fever and headache.[9-10] *Drynaria quercifolia* is an indigenous plant found in India, Bangladesh, Western Australia, Malaysia, Philippines, Southeast Asia, South China, Indonesia and New Guinea. This is an epiphytic medicinal pteridophyte plant, the family belongs to the Polypodiaceae and it is widely distributed in the evergreen forests of Kerala's Western Ghats. These plants are called as different names like Asvakatri and also locally called as Marappan kizhangu or Attukal kizhangu. In tamil the *Drynaria quercifolia* refers to as "mudavaatukkal Kizhangu and the rhizome soup is used as rheumatism treatment and dietary supplement. The rhizomes were use in Indian medicine as anti-inflammatory, anodyne, arthralgia and migraine especially in Tamil Nadu at Kolli malai Hills of Native Indians used this herb as an anti-inflammatory.[11] Therefore, in this study the plant *Drynaria quercifolia* rhizome was used for the extraction of phytochemicals and anti-inflammation activity.

MATERIALS AND METHODS

Sample collection

The *Drynaria quercifolia* rhizome samples were collected from Koyambedu market, located in Chennai, state of Tamil Nadu, India. The sample was collected in the month of February 2023 and photographed separately. The wet weights of the collected rhizomes were measured. The sample was transported to Biotechnology laboratory at Apollo arts and Science College and washed using running tap water and distilled water to remove undesirable compounds. The rhizomes skin were peeled off and cut into small pieces and dried at room temperature upto 15 days. Finally, the dry weight was measured and powdered using a pestle and mortar.

Extraction of phytochemicals

The experiments were used two types of solvents specifically ethanol and hot distilled water to extract phytochemicals from *Drynaria quercifolia* rhizome. For extraction,20g of *Drynaria quercifolia* rhizome powder was mixed with 250 mL (1:12.5) of ethanol and hot distilled water and incubatedat room temperature for 72 hours (3 days). After three days collected the extraction, filtered with muslin cloth whatman filter paper and evaporated using rotary evaporator at 40°c (8 hours/100ml) and stored in airtight container for analysis of qualitative and quantitative.

Analysis of Qualitative phytochemical

The collected extractions from the ethanolic and hot distilled water were subjected to qualitative phytochemical analysis.[12]

Quantitative analysis phenolic content

The estimation of total phenolic in test samples was carried out following method.[13-14] 1000 µg/ml of the test samples (ethanol extraction and distilled water extraction) were mixed with the 0.5 ml of water and 0.2 ml of the Folin-Ciocalteu's phenol reagent (1:1) and kept for 5 min. Then added 1 mL of the saturated sodium carbonate solution (8% w/v in water) to the mixture and the volume was made up to 5 mL with the distilled water. Then the reactionmixtures were kept under the dark condition for 30 min. After that presence of the blue colored from different samples were measured at 765 nm. The total phenolic content was calculated as the Gallic acid equivalents gallic acid equivalent/g of the dry plant material based on the standard curve of the Gallic acid (2-64 µg/ml) $y = 0.0144x - 0.0106$, $R^2 = 0.9948$. These experiments were made triplicated and the statistical was analyzed with one-way ANOVA.

Denaturation Protein Assay

This experimentation was carried out by previousreport[15] with minor modification. The different concentrations of test samples (50, 100, 200, 400, 800 and 1600 µg/mL)along with the standard diclofenac sodium and a control (without standard and test sample) were preparedthe volume up to 4 mL of the phosphate buffer solution (0.2 M, pH 7.4). 1 mL of 1mM albumin solution in the phosphate buffer was mixed with the all tubes and kept at 37 °C in an incubator for 15 min. The denaturations were induced while keeping the reaction mixture in water bath at 60 °C for 15 min. Finally reaction mixtures were cooled, calculated the turbidity at the 660 nm and inhibition percentage of the denaturations were calculated by

the following formula and repeated the experiment upto three times and the statistical was analyzed with one-way ANOVA.

% Inhibition = [(OD of test - OD of control)/OD of test] x 100

RESULTS

This study the *Drynaria quercifolia* plant rhizomes were used to extract the phytochemicals and which was collected from Koyambedu local market, Chennai, Tamil Nadu, India. Collected rhizomes were subjected to remove the microbes and dust particles and wet and dry weights were measured which result obtained from1800g of wet rhizomes gave 440g of dry rhizome (Table 1 and Fig.1).

Phytochemical screening

Phytochemicals were extracted from *Drynaria quercifolia* rhizome powder using ethanol and hot distilled water as solvents. This extractions were analyzed for phytochemicals and which results confirmed the consisting of bioactive compounds (Table 2).The result of phytochemicals in *Drynaria quercifolia* plant rhizome was showed in this study the ethanol extract contains alkaloids, cardiac glycosides, phenols, quinones, steroids and terpenoids whereas in hot distilled water extract contains cardiac glycosides, phenols, proteins, quinones, steroids and terpenoids. Protein was absent in ethanolic extract when compared to the solvent hot distilled water extract and Alkaloids was absence in the hot distilled water extract compared to the ethanolic extraction (Table 2).

Table 1. The Wet weight and Dry weight of *Drynaria quercifolia* Rhizomes

Sample	Wet Weight (g)	Dry Weight (g)
Drynaria quercifolia Rhizome	1800	440

Total Phenolic Content of *Drynaria quercifolia* Rhizome

The total amount of phenolic content in the*Drynaria quercifolia* rhizome using ethanolic extraction was about 36.731mg of gallic acid equivalent/g dry weight of the extract whereas in hot distilled water extraction contained 30.296mg of gallic acid equivalent/g dry weight of the extract (Table 3 and Fig.2).This experiment was made triplicated by the one-way ANOVA method.

Protein denaturation

In this studythe protein denaturation was performed with minor modification and triplicated using one-way ANOVA method. The maximum percentage of protein denaturation inhibition was found in standard diclofenac sodium (92.80%) at 1600 µg/mL of concentration, in ethanolic extraction contained 77.72% in 1600 µg/mL of

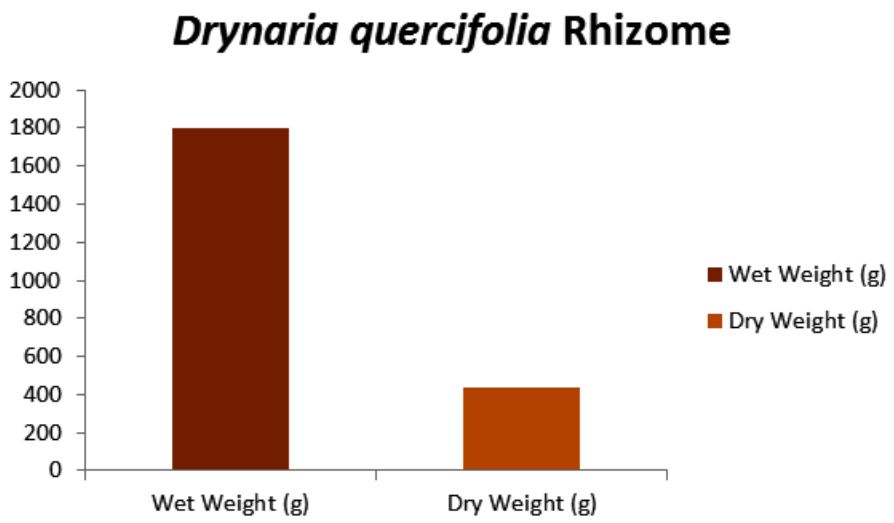

Fig. 1. The Wet weight and Dry weight of *Drynaria quercifolia* Rhizomes

Table 2. Phytochemicals from *Drynaria quercifolia* Rhizome

Sample	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Cardiac glycosides	Steroids	Terpenoids	Quinones	Proteins
Ethanolic Extraction	+	-	-	-	+	+	+	+	+	-
Hot Distilled water Extraction	-	-	-	-	+	+	+	+	+	+

concentration which was lesser than the standard drug. The lower percentage of protein denaturation inhibition was found in hot distilled water extract (68.85%) at the concentration of 1600 µg/mL while compared to the ethanolic extract and standard drug (Table 4 and Fig.3).IC_{50} value of the Diclofenac sodium (standard), ethanolic extract and hot distilled water extract were 69.76 µg/mL, 357.28 µg/mL and 402.60 µg/mL respectively (Table 5 and Fig.4).

DISCUSSION

The process of extracting possible phytochemicals relies on the polarity of the solvents because it allows for easy extraction using of polar solvents.[16] Therefore, the study used the polar solvents ethanol and hot distilled water to extract

Table 3. Total phenolic content of *Drynaria quercifolia* rhizome

S. No	Sample	Total Phenolic Content of *Drynaria quercifolia* rhizome (mg) (gallic acid equivalent/g)
1	Ethanolic Extract	36.731
2	Hot Distilled Water Extract	30.296

Table 4. Percentage of Protein Denaturation inhibition of *Drynaria quercifolia* Rhizome

S. No	Sample	% of Protein Denaturation Inhibition at 1600 µg/mL
1	Diclofenac Sodium (Standard Drug)	92.8%
2	Ethanolic Extract	77.72%
3	Hot Distilled Water Extract	68.85%

Table 5. IC_{50} Valueof *Drynaria quercifolia* Rhizome

S. No	Sample	IC_{50}Value (µg/mL)
1	Diclofenac Sodium (Standard Drug)	69.76
2	Ethanolic Extract	357.28
3	Hot Distilled Water Extract	402.60

phytochemicals from the *Drynaria quercifolia* rhizomes.According to previous reports, the highest phytochemical content was found in *Drynaria quercifolia* rhizomes[7].This study also confirmed the presence of maximum phytochemicals in *Drynaria quercifolia* rhizome. Protein denaturation involves the breakdown of proteins, leading to the loss of their complex three-dimensional shape and the arrangement of their secondary structures. This occurs due to the introduction of external forces or substances, including harsh acids or bases, highly concentrated inorganic salts, organic solvents, or elevated temperatures. The majority of natural proteins lose their ability to perform their biological roles upon denaturation. The process of denaturation is among the well-understood factors that lead to inflammation.[17] This study found that *Drynaria quercifolia*rhizome

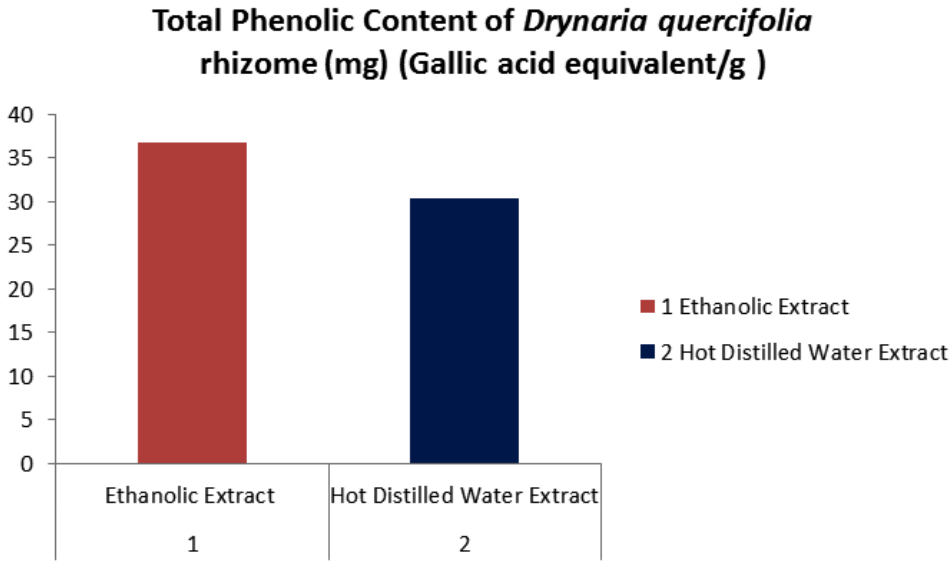

Fig. 2. The total phenolic content of *Drynaria quercifolia* Rhizome
(1 – Ethanolic extract, 2 – Hot Distilled water extract)

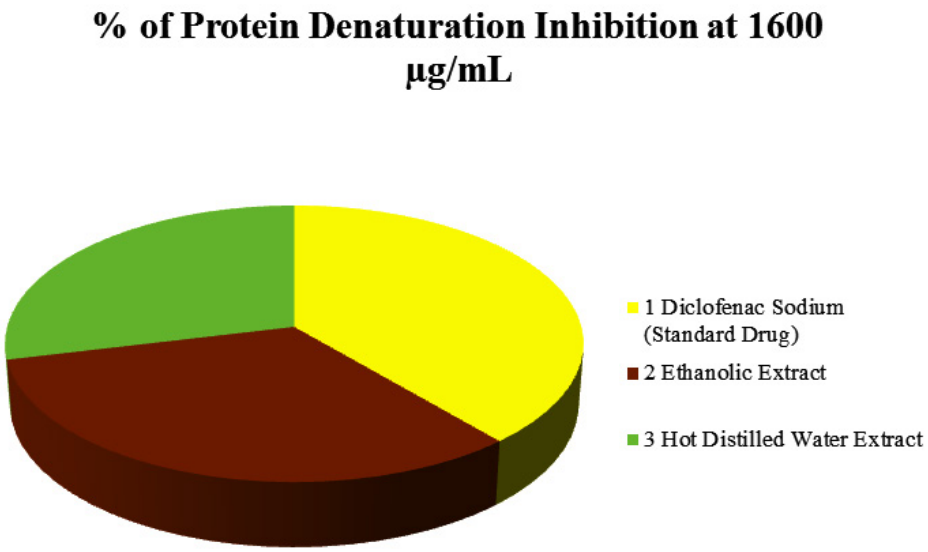

Fig. 3. Percentage of protein denaturation Inhibition of *Drynaria quercifolia* Rhizome

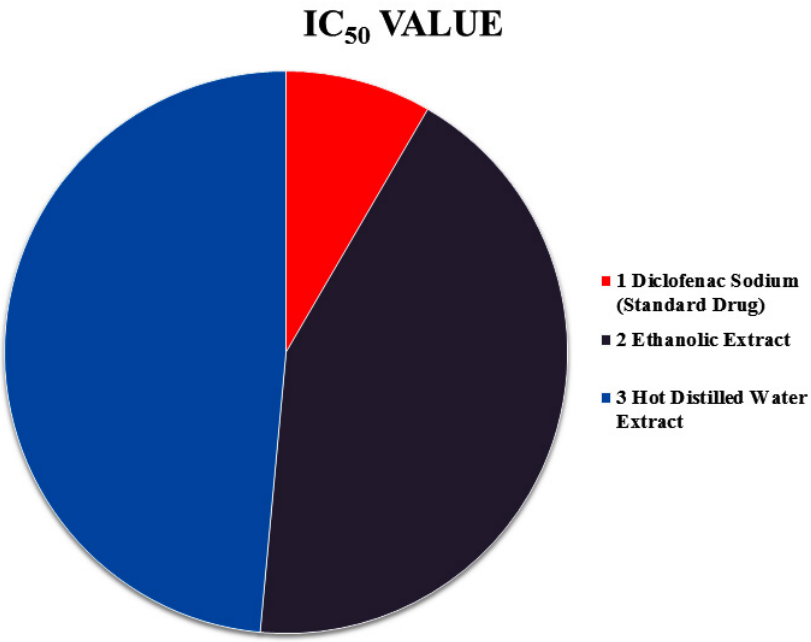

Fig. 4. IC_{50} Valueof *Drynaria quercifolia* Rhizome

extract exhibited a high percentage of protein denaturation inhibition,though slightly lower than the standard drug. The plant *Drynaria quercifolia* rhizomes were consisting of anti-inflammatory, anthelmintic, and astringent properties.[9-10]*Drynaria quercifolia* (L.) J. Sm has numerous traditional uses along with utilized in many folk treatments. Based on pharmacological report the plant also has anti-inflammatory, antimicrobial, anti-cancer properties and antioxidant activity.[18] Therefore, the phytochemicals extracted from the *Drynaria quercifolia* rhizome can able to use for the anti-inflammatory activity.

CONCLUSION

Drynaria quercifolia is naturally found in many countries, particularly in hilly regions. In this preliminary study of the phytochemical screening of *Drynaria quercifolia* rhizome wascontain various major bioactive compounds in the ethanol and hot distilled water extractions. Based on the preliminary findings, the phytochemicals in *Drynaria quercifolia* rhizomes exhibit promising anti-inflammatory properties, warranting further investigation. The rhizomes of *Drynaria quercifolia* are readily available, and the extraction procedure is simpler compared to synthetic drugs.

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Conflict of interest

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Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human

participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable.

Author Contributions

Geethashankar Jegan: Supervision, Work Plan, Project Administration; Alagumurugan Kaavya: Data Collection, Analysis, Results Writing; Elumalai Keerthana: Methodology, Review and Editing; Srinivasan Sneha: References writing and Alignment

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