

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.012>

Evaluation of Anti – Inflammatory Property of Rhizomes of *Drynaria quercifolia* (Mudavattukal Kilangu)

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ABSTRACT

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness. The present study aimed to evaluate the presence of phytochemicals, antioxidant activity and anti-inflammatory property of ethanolic extract of *D. quercifolia* rhizomes. The preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids, gums & mucilages and tannins in the ethanolic extract of *D. quercifolia*. In the context of quantification of phytochemicals, alkaloids, tannins, flavonoids, phenols were assessed in ethanolic extract of *D. quercifolia* and significant quantity was present. In addition, antioxidant activity of ethanolic extract of *D. quercifolia* rhizome was tested and the radical scavenging activity of the extract showed that it exhibits increase in radical scavenging activity (ferric ion and nitric oxide radical) on increasing the concentration, suggesting the increased antioxidant activity due to the presence of essential phytochemicals. The anti-inflammatory property of ethanolic extract of *D. quercifolia* rhizome was carried out which was revealed in terms of protein denaturation inhibition, and was found to have significant anti-inflammatory property as that of standard. The study outcome concludes that the ethanolic extract of *D. quercifolia* rhizome can provide a new approach for finding a complementary and alternative remedy in inflammation and its associated inflammatory diseases.

Keywords

Rhizomes,
Phytochemicals, Anti-oxidant, Anti-inflammatory property, Ethanolic extract.

Article Info

Received:
24 August 2026
Accepted:
26 September 2026
Available Online:
10 October 2026

Introduction

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose,

improve or treat physical and mental illness (Res *et al.*, 2017). The whole plant of *D. quercifolia* is used for the treatment of anthelmintics, pectoral, skin diseases and loss of appetite (Rajan *et al.*, 1996). The plant is known to have therapeutic uses in tuberculosis and fever. The fronds are pounded and used as a poultice for swelling

because of its antibacterial and astringent properties (Rajan *et al.*, 1996). Rhizome and roots are used as tonic in typhoid fever. It is very specifically used in the treatment of migraine. Traditional use of this drug is in diarrhoea, typhoid, cholera, jaundice and syphilis (Rajan *et al.*, 1996). The plant is used by the Nicobarese to treat body ache, headache and with other drugs in rheumatic pain. The whole plant of *D. quercifolia* is anthelmintic, pectoral, expectorant and tonic, and is used to treat chest and skin diseases and loss of appetite (Dixit *et al.*, 1984). *Drynaria quercifolia* belongs to family of polypodiaceae is an epiphytic medicinal pteridophyte distributed widely in the evergreen forests of the western ghats of Kerala, locally called marappankilangu or attukalkilangu. (Figure 1 & 2) Stipules are winged at the base and lamina is not separated into distinct leaflets. Sori are round and circular in shape. Spores 37.5-55µm long, 22.5-37.5 µm wide (Dixit *et al.*, 1984). It is an epiphytic fern with a rhizome (horizontal stem) that is thick and fleshy with many scales, giving it a woolly appearance. The scales are dark brown and narrowed gradually to the tip. Its simple, lobed fronds (leaves) develop as 2 distinct types, nest and foliage fronds. A nest leaves shallowly lobed to 40cm long by 30cm wide, light green, turning light brown - reddish brown with age, persisting even after drying up completely (Res *et al.*, 2017).

A fertile fronds mid green with cream-white streaks, to 1.2m in length or occasionally shorter. The fertile and sterile frond or nest-leaves. The paleae of the five species of *Drynaria* have all filiform apex (Res *et al.*, 2017). A large species with deep pinnate leaves and the name comes from the resemblance of the leaves of the nest to oak leaves (Res *et al.*, 2017). The rhizomes are 2cm thick and woolly. The rhizomes of *Drynaria* are creeping and densely covered in brown scales and are 20-25mm long and 0.7-2.5mm wide and soft (Rajan *et al.*, 1996). The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases like dyspepsia and cough (Rajan *et al.*, 1996). *D. quercifolia* is commonly known as "Oak Leaf Fern" and is found in India, Pakistan, North America and Africa (Khan *et al.*, 2012). The rhizome is popularly used as a traditional soup condiment and analgesics by the people of Eastern Ghats, Tamil Nadu, India (Khan *et al.*, 2012).

Hence the present study was designed to evaluate the presence of phytochemicals, antioxidant activity and anti-inflammatory property of ethanolic extract of *D. quercifolia* rhizomes.

Materials and Methods

Drynaria quercifolia rhizome was procured from nearby area, Thiruverkadu, Chennai. The sample was authenticated by Central Institute of Siddha Research Arumbakkam, Chennai. Dried rhizome was then powdered and then sieved. Then the extraction process with ethanol, infusion, a type of maceration was utilized for the extract preparation. Then, the samples were filtered with grade 1 Whatman paper. The extract prepared was 2% and; they were stored and utilized for further analysis. Preliminary phytochemical analysis was carried out in the ethanolic extract of the rhizome and shown in Table. 1.

Quantification of Phytochemicals

Alkaloids: The extract was dissolved in 2 N of Hydrochloric acid (HCl) and then filtered. 1 ml of test extract solutions was transferred to separating funnel and added 5 ml of phosphate buffer pH 4.7 and 5 ml of bromocresol green (BCG) solution. The mixture was shaken and complex formed was extracted with 5 ml of chloroform. Chloroform layer was collected in 10 ml of volumetric flask and volume was made up to mark with chloroform. Absorbance was taken at 470 nm against blank. The alkaloid content was expressed in terms of mg of atrophine equivalents/ g of extract.

Tannins: The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80, 100 µg/ ml) were prepared as described earlier. Absorbance for test and standard solutions were measured against the blank at 720 nm with an UV/Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of gallic acid equivalents/ g of extract.

Total Phenol: The total phenolic contents in the extracts were measured using Folin-Ciocalteu reagent method. Extract of 0.02 ml, 0.05 ml and 0.1 ml were transferred into test tubes and the volume was made up to 0.5 ml with distilled water. To this solution, 0.25 ml of Folin-

Ciocalteu reagent (1N) and then 1.25 ml of 20% sodium carbonate was added, and the tubes were vortexed thoroughly. Absorbance was recorded at wavelength of 550 nm using UV- visible spectrophotometer after 40 minutes. The concentration of total phenolic compounds in the extract was calculated as Gallic acid equivalent (GAE) from standard curve. The total phenolic content was expressed as GAE/g of extract.

Total Flavonoid: Total flavonoid content of the extracts was determined according to method of Nabavi *et al.*, (2008). The ethanolic plant extract (0.5 ml) was mixed with distilled water (2 ml) and subsequently with 5% NaNO₂ solution (0.15 ml). After 6 mins of incubation, 10% AlCl₃ solution (0.15 ml) was added and then allowed to stand for 6 min, followed by addition of 1M NaOH solution (2 ml) to the mixture. Then, distilled water was added to the sample to make up the volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture's absorbance was determined at 510 nm using UV-Visible spectrophotometer. The test was performed in triplicate and the flavonoid content was expressed as mg of quercetin equivalent per g of extract (mg QE/g).

Ferric reducing antioxidant power assay: The antioxidant capacity of the extract was estimated spectrophotometrically following the procedure of Benzie and Strain.

Nitric oxide radical scavenging activity: Nitric oxide radical scavenging activity of extract was determined by Griess-Ilosvay reaction using sodium nitroprusside.

Anti-inflammatory activity: The anti-inflammatory activity was determined by evaluating the capacity of the samples to inhibit protein denaturation.

Statistical Analysis

In this study, all the experiments were carried out in three independent biological replicates, and data were reported as mean $\pm$ standard deviation for each set of conditions. Statistical significance of the data was tested through one-way analysis of variance (ANOVA) using least significant difference with $p < 0.05$, $p < 0.01$, $p < 0.001$.

Results and Discussion

The present study analyzed the preliminary phytochemicals, antioxidant activity and anti-inflammatory property of ethanolic extract of *D.*

quercifolia. The phytochemical screening showed the presence of alkaloids, carbohydrates, phenols, flavonoids, sterols, diterpenoids, gums & mucilages and tannins in ethanolic extract of *D. quercifolia*. Phytochemical evaluation helps in the determination of quality and purity of any drug. For qualitative analysis previous researchers used petroleum ether, chloroform, ethyl acetate, methanol, and water extracts of rhizome. They determined the presence or absence of alkaloids, flavonoids, saponins, carbohydrate, proteins, phenol, steroids, tannins, terpenoids, fixed oil and fat, gum and mucilage, coumarins, glycosides, lignins, and cholinergic acids.

In another study (Prasanna and Chitra, 2014), petroleum ether and methanol were used as the solvent for qualitative analysis of phytochemicals. The phytochemical compound such as tannins, saponins, flavonoids, steroids, coumarins, alcohol, glycosides, carbohydrate, phytosterol, fixed oils, phenol, protein and amino acids and cholinergic acids were found in methanolic extract. Tannins, saponins, coumarins, glycosides, carbohydrate, fixed oils and phenols were present in petroleum ether extract. These compounds may be responsible for several medicinal activities.

The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases like dyspepsia and cough which is supported by phytochemical analysis results exhibiting the presence of all essential phytochemicals. Coherent to our study results, the phytochemical analysis of ethanolic extract of *Drynaria quercifolia* shows the presence of glycosides, tannins, saponins, flavonoids, steroids, gum and mucilage carbohydrates, amino-acids, polyphenols and fixed oil and fats.

In the context of quantification of phytochemicals, alkaloids, tannins, flavonoids, phenols were assessed in ethanolic extract of *D. quercifolia*. Figure 3 depicts the significant presence of alkaloids, tannins, flavonoids, phenols in ethanolic extract of *D. quercifolia*. Quantitative estimation of phytochemicals such as flavonoids, saponins, phenols, tannin and alkaloids were done by Padma Selvi *et al.*, 2016 using the methanolic extract of the rhizome.

Similarly, Jannatul and coworkers, 2013, evaluated the total phenolic content in the *D. quercifolia* leaves extract prepared with petroleum ether, carbon tetrachloride, chloroform and aqueous solution; and found significant presence of phenol in all extracts.

Table.1 Preliminary Phytochemical Screening

S. NO.	EXPERIMENTS	OBSERVATIONS
1	ALKALOIDS WAGNER'S TEST A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube.	A reddish- brown precipitate
2	AMINO ACID NINHYDRIN TEST Two drops of ninhydrin solution was added to 2 ml of aqueous filtrate.	Appearance of purple colour
3	CARBOHYDRATE MOLISCH' S TEST To 2ml of extract, two drops Molish's reagent was added. The mixture is shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of test tube BENEDICT' S TEST To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 minutes	A violet ring A red precipitate
4	PHENOLIC COMPOUNDS FERRIC CHLORIDE TEST The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added	A dark green colour
5	FLAVONOIDS ALKALINE REAGENT TEST An aqueous solution of the extract was treated with 10% ammonium hydroxide solution.	Appearance of yellow fluorescence
6	STEROLS SALKOWSKI'S TEST Filtrate+ few drops of Conc. H ₂ SO ₄ (Shaken well and allowed to stand)	Appearance of red color (in lower layer)
7	PROTEINS BIURET TEST 2 ml of filtrate was treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets	Pink colour
8	GUM AND MUCILAGES The extract (100 mg) was dissolved in 10 ml of distilled water and to this 2 ml of absolute alcohol was added with constant stirring.	White or cloudy precipitate
9	DITERPENES Plant extract was dissolved in distilled water + 3-4 drops of copper acetate solution	Emerald green color
10	TANNINS 10% NaOH TEST 0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion
12	QUINONES CONC. HCL TEST Plant extract + Conc. HCl	A green color
13	GLYCOSIDES BORNTRAGER'S TEST To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it.	Pink colour

Figure.1 & 2 *Drynaria quercifolia* (Plant & Rhizome)



Figure.3 Quantification of alkaloids, tannins, flavonoids, phenols in ethanolic extract of *D. quercifolia*.

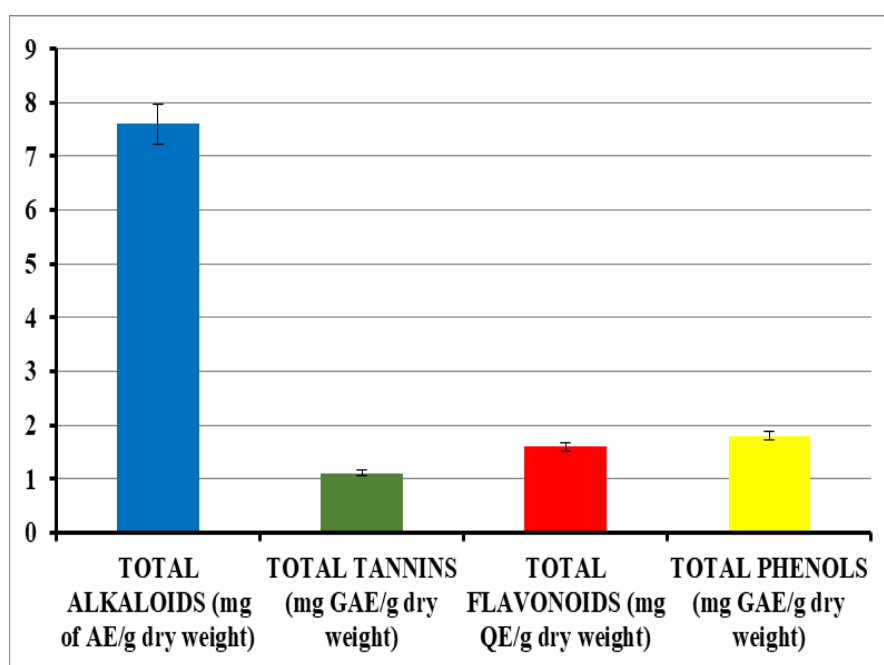


Figure.4 & 5 Depicts the radical scavenging activity of *D. quercifolia* extract which showed that it exhibits increase in radical scavenging activity (ferric ion and nitric oxide radical) on increasing the concentration.

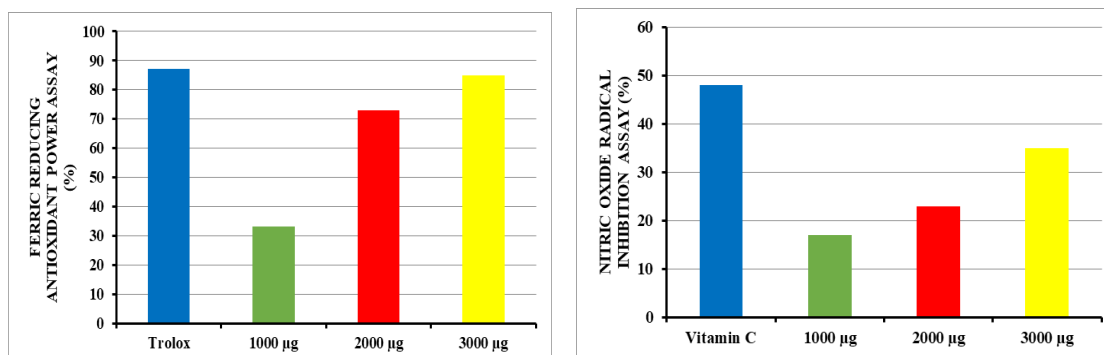
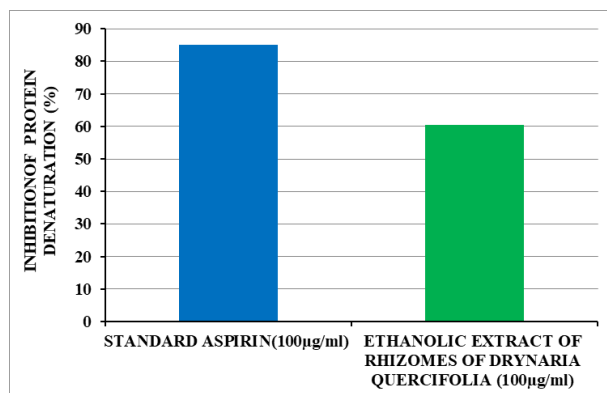


Figure.6 Depicts the anti-inflammatory property of ethanolic extract of *D. quercifolia* rhizome



In addition, antioxidant activity of ethanolic extract of *D. quercifolia* rhizome was tested. Figure 4 & 5 depicts the radical scavenging activity of *D. quercifolia* extract which showed that it exhibits increase in radical scavenging activity (ferric ion and nitric oxide radical) on increasing the concentration, suggesting the increased antioxidant activity due to the presence of essential phytochemicals. Similarly, Louis and coworkers, 2020 determined the antioxidant property of the rhizome and leaves by DPPH and FRAP assay; which showed the significant radical scavenging activities for both rhizome and leaves extract.

In agreement to our results, Arunkumar *et al.*, 2010 revealed effective antioxidant activity for methanolic extract of *D. quercifolia* extract on comparison to the other extracts such as aqueous, petroleum ether, and chloroform; and has significant activity compared to the standard drug. coherently.

Elakkiya and Prasanna, 2012 described that the ferric ion radical scavenging by the extracts was due to the quenching free radicals or reduction of Fe^{3+} to Fe^{2+} , which can be attributed to the presence of number of polyphenolics such as flavonoids, anthocyanins, etc.

The outcome of the experiment in the current research was similar with DPPH radical scavenging action of rhizome of *D. quercifolia* experimental earlier reported by Jinu *et al.*, (2014).

In the context of anti-inflammatory property, Figure 6 depicts the anti-inflammatory property of ethanolic extract of *D. quercifolia* rhizome which reveals that in terms of protein denaturation inhibition, anti-inflammatory property was tested and found a significant

anti-inflammatory property as that standard. Janaranjani B *et al.*, screened the anti-inflammatory activity of ethanolic extract of DQR against carrageenan-induced paw oedema rats using various concentrations (100, 200 & 500mg/kg) of extract; The extract at 500 mg/kg dose showed a greater reduction of carrageenan-induced and histamine-induced paw oedema in rats (71.76% & 66.67%). Test results were compared with the standard indomethacin 10 mg/kg (88.24% & 68.89%) respectively in both cases. Coherent to our study, in vitro and in vivo anti-inflammatory property of water and methanol extract of DQR was reported against the albumin denaturation, carrageenan-induced paw oedema and membrane-stabilizing methods. Protein denaturation and membrane stabilization tests are efficient methods to screen the potency of drug molecules as well as herbal extracts as potent anti-inflammatory agents. It is suggesting that this anti-inflammatory property may be attributed to the presence of various bioactive components in ethanolic extract of *D. quercifolia* rhizomes. So far various studies explored the anti-inflammatory property using diverse solvent extracts. However, ethanolic extract of *D. quercifolia* rhizomes is not so far tested in vitro for anti-inflammatory property through protein denaturation inhibition protocol.

Hence, in the present study, it was carried out and observed a significant anti-inflammatory property as that of standard aspirin revealed from inhibition of protein denaturation. From the present study, the results suggest that *Drynaria quercifolia* rhizome will be useful as a potential source for pharmaceutical application in term of antioxidant drug to cure various ailments. Further studies regarding the role of the plant-extract and its phyto-compounds are required to explore on the gene and protein-level expression of a broad spectrum of

inflammatory biomarkers, as it may provide a clearer view on the anti-inflammatory aspects of the plant. The study outcome concludes that the ethanolic extract of *D. quercifolia* rhizome can provide a new approach for finding a complementary and alternative remedy in inflammation and its associated inflammatory diseases.

Author Contributions

B. Thiripurasundari: Designed the work plan and protocol of the project and supervision. Snekalatha G: Post graduate student executed the work plan and protocol and preparation of manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Snekalatha G. and Thiripurasundari B. 2026. Evaluation of Anti – Inflammatory Property of Rhizomes of *Drynaria quercifolia* (Mudavattukal Kilangu). *Int.J.Curr.Microbiol.App.Sci.* Special Issue-13: 94-101.

doi: <https://doi.org/10.20546/ijemas.2026.sp13.012>