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COMPARATIVE STUDY ON THE *INVITRO* ANTIOXIDANT AND ANTI-ARTHRITIC POTENTIAL OF AQUEOUS AND ETHANOLIC EXTRACTS OF *DRYNARIA QUERCIFOLIA* RHIZOME

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ABSTRACT

Back ground: The present study investigates the *invitro* antioxidant and anti-arthritic activities of aqueous and ethanolic extracts of *Drynaria quercifolia* (L.) J. Sm. rhizome, a medicinal fern traditionally used for the treatment of bone fractures, rheumatism, and inflammatory disorders. **Materials and Methods:** The rhizomes were shade-dried, powdered, and extracted successively with water and ethanol using the Soxhlet method. The extracts were subjected to the antioxidant potential of both extracts was evaluated using phosphomolybdenum and reducing power assay, while the anti-arthritic property was assessed through the inhibition of protein denaturation method. Ascorbic acid and diclofenac sodium were used as standard reference drugs for antioxidant and anti-inflammatory comparisons, respectively. **Results:** Both aqueous and ethanolic extracts exhibited concentration-dependent antioxidant and anti-inflammatory activities. The ethanolic extract demonstrated higher antioxidant activity (38.65%) and reducing power (37.59%) compared to the aqueous extract, indicating the presence of potent antioxidant constituents. Similarly, the ethanolic extract showed significant inhibition of protein denaturation (63.25%), comparable to the standard diclofenac sodium (65.33%). **Conclusion:** The results suggest that *D. quercifolia* rhizome possesses bioactive compounds with strong antioxidant and anti-inflammatory potential, supporting its traditional medicinal use. The enhanced activity of the ethanolic extract may be attributed to its higher content of phenolic and flavonoid compounds. Further *in vivo* and pharmacological investigations are recommended to isolate, characterize, and validate the active principles responsible for these effects, paving the way for its development as a natural anti-arthritic agent.

KEYWORDS: *Drynaria quercifolia*, antioxidant activity, anti-arthritic activity, phosphomolybdenum, reducing power, protein denaturation, flavonoids.

INTRODUCTION

Oxidative stress and inflammation are interrelated biological processes involved in the onset and progression of various degenerative disorders such as arthritis, diabetes, cardiovascular diseases, and cancer.[1] Free radicals, particularly reactive oxygen species (ROS), are generated during normal cellular metabolism; however, excessive production can overwhelm the endogenous antioxidant defense system, leading to oxidative damage of biomolecules such as lipids, proteins, and DNA[2] To counteract oxidative stress, the use of natural antioxidants derived from medicinal plants has gained significant attention due to their safety, efficacy, and affordability[3]

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by synovial inflammation, cartilage degradation, and joint deformity, leading to functional disability and pain.[4] The pathogenesis of arthritis involves the generation of free radicals and pro-inflammatory mediators such as cytokines and prostaglandins, which amplify tissue injury.[5] Current therapeutic agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide symptomatic relief but are associated with

adverse effects upon long-term use.[6] Therefore, natural anti-arthritic agents with antioxidant potential are being explored as alternative or complementary remedies.

Medicinal plants are rich sources of bioactive phytoconstituents such as flavonoids, phenolics, tannins, and terpenoids, which possess strong antioxidant and anti-inflammatory activities.[7] These compounds exert their effects by scavenging free radicals, chelating metal ions, and inhibiting oxidative enzymes, thereby preventing cellular and tissue damage.[8]

Drynaria quercifolia (L.) J. Sm., commonly known as the oak-leaf fern, belongs to the family Polypodiaceae and is widely distributed in tropical and subtropical regions of Asia, including India, Malaysia, and Sri Lanka.[9] Traditionally, the rhizome of *D. quercifolia* has been used in Ayurvedic and folk medicine for the treatment of bone fractures, rheumatism, cough, fever, and skin ailments.[10] Phytochemical investigations of *D. quercifolia* have revealed the presence of flavonoids, phenolic acids, glycosides, and triterpenoids, which contribute to its pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective effects.[11,12]

Given the ethnomedicinal significance and phytochemical richness of *Drynaria quercifolia*, the present study was designed to evaluate the *invitro* antioxidant and anti-arthritic activities of the aqueous and ethanolic extracts of its rhizome. The antioxidant potential was assessed using the phosphomolybdenum assay and ferric reducing antioxidant power (FRAP) method, while the anti-arthritic effect was determined by the inhibition of protein denaturation assay. This study aims to scientifically validate the traditional claims and establish the therapeutic potential of *D. quercifolia* rhizome as a source of natural antioxidant and anti-inflammatory compounds.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh rhizomes of *Drynaria quercifolia* (L.) J. Sm. were collected from the Kolli Hills region, Namakkal District, Tamil Nadu, India, during the month of December 2024. The plant was authenticated by a taxonomist from the Department of Botany, D.G. Government Arts College for Women, Mayiladuthurai. A voucher specimen (DQ-2024-BCH01) was deposited in the departmental herbarium for future reference.

Preparation of Plant Extracts

The collected rhizomes were thoroughly washed with running tap water to remove adhering soil and debris, then shade-dried at room temperature for 10–15 days until a constant weight was obtained. The dried rhizomes were pulverized into coarse powder using a mechanical grinder and stored in airtight containers.

Aqueous Extract

About 50 g of powdered rhizome was extracted with 500 mL of distilled water using a Soxhlet apparatus for 8 hours. The extract was filtered through Whatman No.1 filter paper and concentrated using a rotary vacuum evaporator at 40°C. The concentrated extract was then dried to obtain a solid residue and stored in a refrigerator at 4°C until further use.[13]

Ethanolic Extract

Similarly, 50 g of powdered rhizome was extracted with 95% ethanol (500 mL) using the Soxhlet extraction method for 8 hours. The extract was filtered, concentrated, and dried as described above.[13]

Invitro Antioxidant Activity

Phosphomolybdenum Assay

The total antioxidant capacity (TAC) of the extracts was evaluated by the phosphomolybdenum method described by Prieto *et al.*[14] An aliquot of 0.1 mL of extract solution (20–100 µg/mL) was combined with 1 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard antioxidant.

$$\text{Antioxidant Effect (\%)} = \frac{A_{Sample} - A_{Control} \times 100}{A_{Sample}}$$

Where, A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control. The concentration of extract at which 50% inhibition is observed (IC_{50}) were calculated in µg/ml.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing power of the extracts was determined according to Oyaizu.[15] Various concentrations of extract (20–100 µg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5 mL of 10% trichloroacetic acid. After centrifugation, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm. Higher absorbance indicates greater reducing power.

Invitro Anti-Arthritic Activity

Inhibition of Protein Denaturation Assay

The anti-arthritic activity of the extracts was evaluated using the inhibition of albumin denaturation method described by Mizushima and Kobayashi with slight modifications.[16] The reaction mixture consisted of 0.45 mL of bovine serum albumin (5% aqueous solution) and 0.05 mL of plant extract (20–100 µg/mL). The pH was adjusted to 6.3 using 1N HCl. Samples were incubated at 37°C for 20 minutes, then heated at 57°C for 3 minutes.

After cooling, the turbidity was measured at 660 nm using a UV–Vis spectrophotometer. Diclofenac sodium was used as a standard drug.

The percentage inhibition of protein denaturation was calculated as:
Percentage inhibition = (Abs Control -Abs Sample) X 100/ Abs control
Where Abs control = absorbance of control and Abs sample = absorbance of test sample.

Statistical Analysis
All experiments were carried out in triplicate. Results were expressed as mean ± standard deviation (SD).[17]

RESULTS AND DISCUSSION
The antioxidant activity of aqueous and ethanolic extracts of *D. quercifolia* rhizomes was evaluated using various *invitro* assays, including total antioxidant activity, reducing power assay, and antiarthritic activity by protein denaturation assay. The results were compared with standard ascorbic acid and diclofenac sodium.

Antioxidant Activity
Total antioxidant capacity
The total antioxidant activity of the aqueous and ethanolic extracts of *D. quercifolia* increased in a concentration-dependent manner (Fig. 1). The percentage inhibition ranged from 3.08% to 35.11% for the aqueous extract and 3.47% to 38.65% for the ethanolic extract across the tested concentrations (20–100 µg/mL). The ethanolic extract showed higher antioxidant potential than the aqueous extract, though both were lower than the standard ascorbic acid (14.12%–40.92%). The observed antioxidant activity suggests that *D. quercifolia* possesses compounds capable of donating hydrogen atoms to neutralize free radicals. The enhanced activity of the ethanolic extract may be attributed to the higher solubility of phenolic compounds in ethanol, consistent with findings by Saha *et al*. and Kumar *et al*., who reported that ethanolic extracts of ferns often yield stronger radical scavenging activity than aqueous counterparts.[18,19]

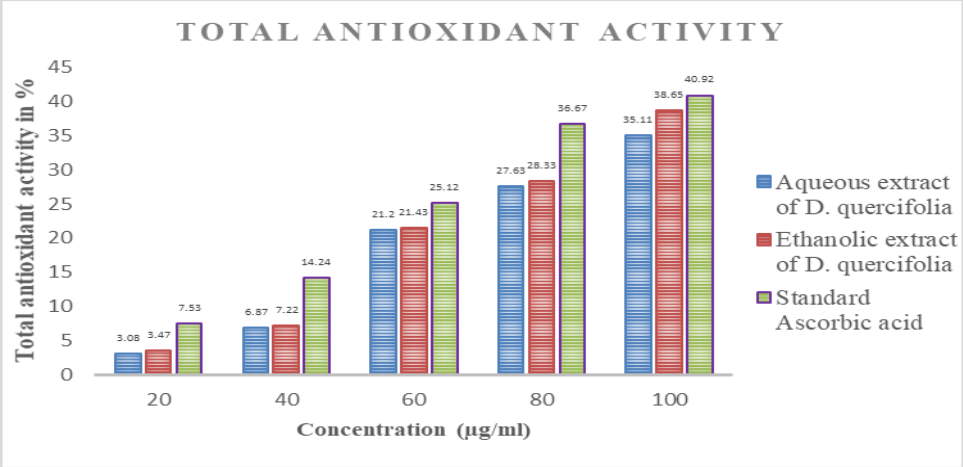

Fig. 1: *Invitro* antioxidant anctivity of aqueous and ethanolic extracts of *D. quercifolia.*

The IC_{50} value of aqueous and ethanolic extracts were 140 µg/ml and 122 µg/ml respectively. Ascorbic acid was used as a reference standard with IC_{50} value of 143µg/ml. The standard have the IC_{50} value higher to aqueous and ethanolic extracts corresponds to high antioxidant activity.

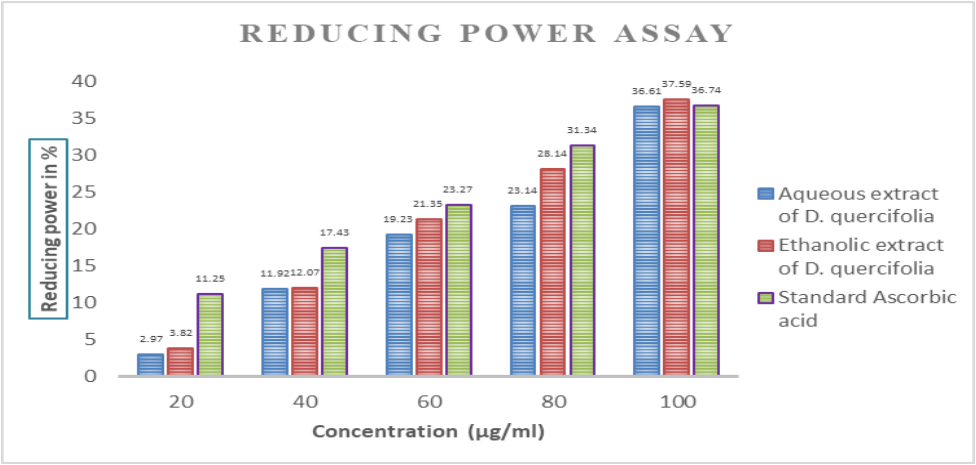

Fig. 2: Free radicals reducing power of aqueous and ethanolic extracts of *D. quercifolia.*

Reducing Power Assay

The reducing power of *D. quercifolia* extracts also increased with concentration (Fig. 2). The aqueous extract exhibited activity ranging from 11.92% to 36.14%, while the ethanolic extract ranged from 12.07% to 37.59%, compared to ascorbic acid (17.43% to 36.74%). The reducing power correlates with the presence of reductants, which exert antioxidant action by breaking free radical chains through hydrogen donation. The ethanolic extract again displayed superior performance, likely due to higher concentrations of polyphenols and flavonoids. This agrees with Rahman *et al*., who demonstrated that phenolic-rich extracts from medicinal ferns show significant reducing potential.[20]

Antiarthritic Activity of *D. quercifolia*

Protein Denaturation Inhibition Assay

Protein denaturation is one of the major causes of inflammation. The ability of *D. quercifolia* extracts to inhibit protein denaturation was compared with standard diclofenac sodium (Fig. 3). The aqueous extract exhibited inhibition ranging from 11.08% to 36.14%, whereas the ethanolic extract showed 12.15% to 63.25%. Diclofenac sodium, the standard anti-inflammatory agent, recorded inhibition between 13.15% and 65.33%.

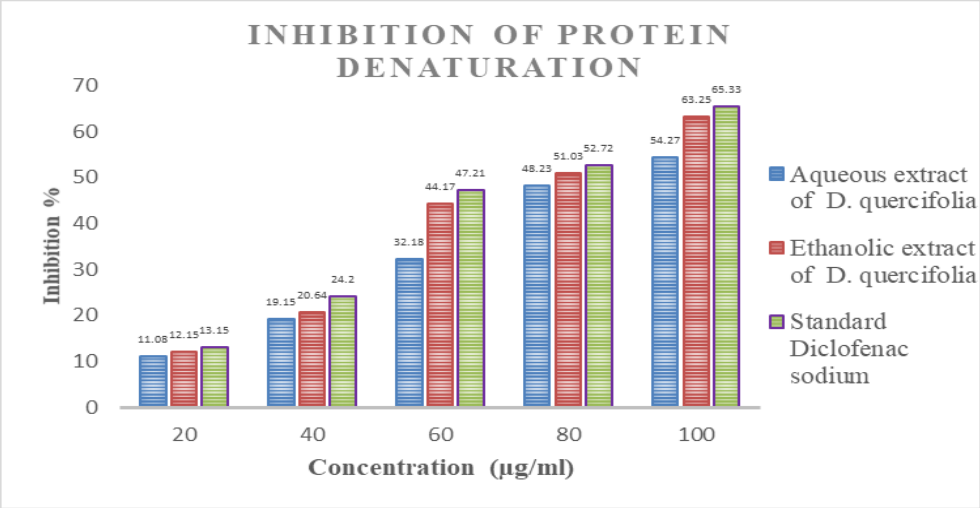

Figure 3: *Invitro* antiarthritic effect of aqueous and ethanolic extracts of *D. quercifolia.*

The ethanolic extract exhibited a strong, dose-dependent inhibition of protein denaturation, comparable to the standard at higher concentrations. This suggests potential anti-inflammatory properties, likely due to the presence of flavonoids and tannins known to stabilize protein structures and prevent denaturation.[21,22]

The results demonstrate that both aqueous and ethanolic extracts of *D. quercifolia* possess potent antioxidant and anti-inflammatory activities. The ethanolic extract consistently outperformed the aqueous extract across all assays, indicating that ethanol is a more efficient solvent for extracting bioactive compounds such as phenolics, flavonoids, and terpenoids.

Previous studies support these findings. Sinha *et al.* reported that *D. quercifolia* contains high levels of naringin, quercetin, and kaempferol-flavonoids known for their antioxidant and anti-inflammatory effects. Similarly, Gupta and Tandon demonstrated that fern extracts with high phenolic content show strong DPPH radical scavenging and protein inhibition properties.[23,24]

The ability of *D. quercifolia* to inhibit protein denaturation comparable to diclofenac sodium indicates its therapeutic potential as a natural anti-arthritic agent. Therefore, *D. quercifolia* could serve as a promising candidate for the development of plant-based antioxidant and anti- arthritic formulations.

CONCLUSION

The present study scientifically validates the antioxidant and anti-arthritic properties of *D. quercifolia* rhizome. The ethanolic extract, in particular, shows strong potential as a natural source of therapeutic compounds that may be developed into herbal formulations for the prevention and treatment of arthritis and other inflammatory diseases. However, further *in vivo* studies, isolation and characterization of active phytoconstituents, and toxicological evaluations are recommended to confirm safety, mechanism of action, and pharmacological efficacy, thereby providing a solid foundation for its future development as a plant-based anti-arthritic agent.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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