COMPARATIVE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *DRYNARIA QUERCIFOLIA* (L.) J. SM LEAF

Vikram P. Masal
Associate Professor, Department of Botany
Dapoli Urban Bank Senior Science College, Dapoli, MS India

Abstract:
Drynaria quercifolia (L.) is an important medicinal fern known for its rich phytochemical composition and antioxidant properties. The present study aimed to compare the phytochemical constituents and antioxidant activity of fresh and dried leaves of Drynaria quercifolia (L.) using different solvents. Leaf extracts were prepared using chloroform, acetone, and water to evaluate the effect of leaf condition and solvent polarity on bioactive compounds. Qualitative phytochemical screening revealed the presence of flavonoids, phenolics, tannins, saponins, alkaloids, and terpenoids in both fresh and dried leaf extracts, with noticeable variation in their distribution. Quantitative analysis indicated that dried leaf extracts contained higher levels of total phenolic and flavonoid contents compared to fresh leaves. Antioxidant activity assessed through in vitro free radical scavenging assays showed that dried leaf extracts exhibited greater antioxidant potential than fresh leaf extracts. Among the solvents used, acetone and aqueous extracts demonstrated superior antioxidant activity compared to chloroform extracts. The results suggest that drying enhances phytochemical extraction and antioxidant efficiency, while solvent selection significantly influences the recovery of bioactive compounds. This study confirms the potential of Drynaria quercifolia (L.) leaves as a natural source of antioxidants and supports their therapeutic significance.

Key words: comparative analysis, *Drynaria quercifolia (L.)****, Antioxidants, Phytochemical constituents, DPPH***

INTRODUCTION

Medicinal plants have attracted increasing attention in recent years due to their immense potential to benefit human health, particularly in the fields of medicine and pharmacology. Their therapeutic efficacy is primarily attributed to the presence of phytochemical constituents that exert specific pharmacological effects on the human body. Among the most important bioactive compounds are alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and several other secondary metabolites (Edeogo et al., 2005). These phytochemicals are naturally synthesized by plants through primary and secondary metabolic pathways (Molyneux et al., 2007).

Drynaria quercifolia (L.) J. Sm., commonly known as Aswakatri, is an epiphytic medicinal pteridophyte belonging to the family Polypodiaceae. It is widely distributed in the evergreen forests of the Western Ghats of Kerala and is locally referred to as "Marappan kizhangu" or "Attukal kizhangu." Ethnomedicinal reports indicate that the rhizome of this plant is extensively used by tribal communities of Tamil Nadu and Kerala for the treatment of ailments such as dyspepsia and cough. The leaves are traditionally applied as poultices to reduce swelling, while the plant is also used to relieve body ache, headache, and rheumatic pain when administered alone

or in combination with other medicinal preparations. Furthermore, the whole plant exhibits antihelminthic, pectoral, expectorant, and toxic properties and is traditionally employed in the management of skin diseases, loss of appetite, tuberculosis, and fever (Korwar et al., 2010).

Botanically, *Drynaria quercifolia (L.)* is characterized by a short, thick, fleshy, creeping rhizome densely covered with reddish-brown, soft scales. The fronds are dimorphic in nature, comprising sterile and fertile fronds. The sterile fronds are relatively small, concave, and turn brown upon maturation, whereas the fertile fronds are long-stalked, large, pinnately lobed, and leathery, with a network of small quadrangular areoles containing sori that are numerous and typically occur in pairs within each primary areole. The rhizome is the principal plant part utilized in folk medicine.

Reactive oxygen species (ROS) are generated as natural byproducts of normal cellular oxygen metabolism and play a significant role in the pathogenesis of various acute and chronic human disorders, including diabetes, atherosclerosis, aging, immune suppression, and neurodegenerative diseases. The involvement of free radical reactions in disease progression is well established. Plants are rich sources of natural antioxidants, containing diverse free radical scavenging molecules such as phenolic compounds (including phenolic acids, flavonoids, tannins, quinones, coumarins, lignans, and stilbenes), nitrogen-containing compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and other endogenous metabolites with potent antioxidant activity. Due to their ability to neutralize free radicals, natural antioxidant agents have garnered considerable scientific interest. Consequently, evaluating the antioxidant potential of medicinal plants used in herbal medicine is essential, either to elucidate their pharmacological mechanisms or to provide scientific validation of their therapeutic applications (Prasanna et al., 2015).

MATERIALS AND METHODS

Preparation of plant extract

The fresh leaves were collected, cleaned with water to get rid of any unwanted material, dried at room temperature in the shade, and then ground into a powder using a grinder. After being finely dried, the powder is kept in airtight vials and used throughout the extraction process.

The coarse powder was extracted using chloroform, acetone and water. and fresh leaves were soaked in respective solvent overnight to get the extracts. 25g of fresh and dried material was extracted in 100 mL solvents. The extracts were further used for phytochemical screening and antioxidant assays. Preliminary phytochemical screening is conducted by different tests and the antioxidant assays are carried out by DPPH assay.

Phytochemical Screening

Phytochemical analysis was conducted using the method described by Roghini and Vijayalakshmi (6) slightly modified to determine the presence of secondary metabolites in *Ixora coccinea*.

Saponin: In a test tube, 1 ml of plant extract was diluted with distilled water and agitated for 15 minutes. A 1 cm layer

of foam formed when saponins were present.

Flavonoids: 2-3 drops of sodium hydroxide solution were added to 1 mL of plant extract. The presence of flavonoids was revealed by the production of an acute yellow color.

Phenols: 1 ml of the extract was mixed with 1 ml distilled water and a few drops of 10% ferric chloride. The presence of phenols was revealed by the formation of a blue tint.

Tannins: 2 ml ferric chloride (5%) was added to 1 ml of extract. Tannins were detected by the formation of a dark blue black.

Alkaloids: 2 mL strong hydrochloric acid was applied to 2 mL extract. The Salkowski reagent was then added in three drops. The presence of alkaloids was revealed by the presence of white precipitate.

Glycosides: 2 mL extract, 3 mL chloroform, and 10% ammonia solution were added to 2 mL extract. The inclusion of glycosides was shown by the formation of a pink color.

DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated following the method described by Shekhar et al. (2014). The free radical scavenging potential of different ethanolic extracts of rhizome and leaves was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH). A 0.1 mM DPPH solution was prepared in ethanol, and 1 mL of this solution was mixed with 3 mL of each extract at varying concentrations (20, 40, 60, 80, and 100 µg/mL). Only ethanol-soluble extracts were used, and the required concentrations were prepared by the dilution method.

The reaction mixtures were shaken vigorously and incubated at room temperature for 30 minutes in the dark. Absorbance was then measured at 517 nm using a UV–Visible spectrophotometer (Shimadzu). All experiments were performed in triplicate. The IC_{50} value, defined as the concentration of extract required to scavenge 50 % of DPPH radicals, was calculated from the logarithmic dose–inhibition curve. A decrease in absorbance of the reaction mixture indicated higher free radical scavenging activity.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = $[(A_0 - A_1) / A_0] \times 100$,

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

RESULTS

Table. 1. Preliminary qualitative analysis of phytochemicals present in *Drynaria quercifolia (L.)*

Chemical Components	Chloroform		Acetone		Water	
	Fresh Leaves	Dried Leaves	Fresh Leaves	Dried Leaves	Fresh Leaves	Dried Leaves

Steroids	+	+	ND	+	ND	+
Alkaloids	ND	+	+	ND	ND	+
Tannins	ND	+	ND	ND	ND	+
Glycosides	ND	+	+	+	+	+
Saponins	+	ND	ND	+	+	+
Flavonoids	+	+	+	+	+	ND
Triterpenes	+	ND	+	+	+	ND
Carbohydrates	+	+	+	+	+	ND

+ presence; ND- Not detected

Table. 2. DPPH free radical scavenging potential of *Drynaria quercifolia (L.)*

Concentration (ug/ml)	Ascorbic acid	Chloroform		Acetone		Water	
		Fresh Leaves	Dried Leaves	Fresh Leaves	Dried Leaves	Fresh Leaves	Dried Leaves
50	46.77	7.22	9.98	7.98	8.64	7.12	8.09
100	61.98	19.34	21.27	21.32	24.45	20.15	21.23
150	72.33	18.30	22.18	29.09	35.43	24.67	33.42
200	89.67	29.67	34.56	32.12	42.46	36.41	38.97
400	93.12	47.87	54.13	63.68	68.87	71.24	74.32
800	95.56	72.54	78.82	75.64	81.23	73.45	79.09
1000	96.24	82.98	90.12	84.87	92.76	86.08	88.54

DISCUSSION

The qualitative phytochemical screening of fresh and dried leaves using chloroform, acetone, and aqueous solvents revealed the presence of a wide range of bioactive compounds, with noticeable variations depending on the extraction solvent and the leaf condition (fresh or dried).

Steroids were detected in chloroform extracts of both fresh and dried leaves, while acetone extracts showed their presence only in dried leaves. In aqueous extracts, steroids were absent in fresh leaves but present in dried leaves, indicating that drying may enhance the extractability of steroids in polar solvents.

Alkaloids were not detected in chloroform extracts of fresh leaves but appeared in dried leaves. In acetone extracts, alkaloids were present only in fresh leaves, whereas aqueous extracts showed alkaloids exclusively in dried leaves. This variation suggests that both solvent polarity and drying influence alkaloid extraction.

Tannins were observed only in dried leaf extracts obtained using chloroform and water, while they were absent in all fresh leaf extracts and acetone extracts, indicating that tannins are more readily

detected after drying and in non-acetone solvents.

Glycosides were predominantly present across most extracts. They were absent only in the chloroform extract of fresh leaves, while both fresh and dried leaf extracts prepared with acetone and water showed positive results, highlighting their wide distribution and solubility in moderately polar to polar solvents.

Saponins were detected in chloroform extracts of fresh leaves and acetone extracts of dried leaves, as well as in both fresh and dried aqueous extracts. Their consistent presence in water extracts reflects their polar nature.

Flavonoids were widely present in chloroform and acetone extracts of both fresh and dried leaves, and in the aqueous extract of fresh leaves, but were absent in the aqueous extract of dried leaves, suggesting possible degradation or reduced solubility of flavonoids upon drying.

Triterpenes were detected in chloroform extracts of fresh leaves and acetone extracts of both fresh and dried leaves, as well as in aqueous extracts of fresh leaves. However, they were absent in chloroform extracts of dried leaves and aqueous extracts of dried leaves, indicating sensitivity to drying or solvent specificity.

Carbohydrates were consistently present in all chloroform and acetone extracts of both fresh and dried leaves, as well as in the aqueous extract of fresh leaves, but were not detected in the aqueous extract of dried leaves.

Overall, the results demonstrate that both the physiological state of the plant material (fresh vs. dried) and the choice of solvent significantly influence the qualitative phytochemical profile of *Drynaria quercifolia (L.)* leaves. The dried leaf extracts, particularly in acetone and water, generally showed a broader range of detectable phytoconstituents, which may contribute to enhanced biological activity.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavanoids, glycosides, phenols, saponins, steroids etc (Sofowara, 2013).

In both rhizome and leaves of *Drynaria quercifolia (L.)*, proteins are completely absent. In leaves saponins are present but it is absent in rhizome. In rhizome tannins, flavanoids, and glycosides are present but they are absent in leaves.

The DPPH radical scavenging activity of chloroform, acetone, and aqueous extracts of fresh and dried leaves was evaluated at concentrations ranging from 50 to 1000 µg/mL and compared with the standard antioxidant, ascorbic acid. All extracts exhibited a concentration-dependent increase in free radical scavenging activity.

Ascorbic acid showed the highest scavenging activity at all tested concentrations, increasing from 46.77% inhibition at 50 µg/mL to 96.24% at 1000 µg/mL, confirming its strong antioxidant potential and validating the assay.

Among the plant extracts, relatively low scavenging activity was observed at lower

concentrations (50–100 µg/mL). At 50 µg/mL, the percentage inhibition ranged from 7.12% to 9.98% across the extracts, indicating weak antioxidant activity at minimal doses. However, a gradual and consistent increase in activity was noted with increasing concentration.

At moderate concentrations (150–200 µg/mL), acetone and aqueous extracts showed higher scavenging activity compared to chloroform extracts. Notably, acetone extract of dried leaves exhibited 35.43% inhibition at 150 µg/mL, while the aqueous extract of dried leaves showed 33.42%, suggesting better extraction of antioxidant compounds in these solvents after drying.

At higher concentrations (400–800 µg/mL), a marked increase in antioxidant activity was observed in all extracts. The aqueous extract of dried leaves demonstrated the highest scavenging activity among the extracts, showing 74.32% inhibition at 400 µg/mL and 79.09% at 800 µg/mL. Acetone extracts of dried leaves also exhibited strong activity, reaching 81.23% inhibition at 800 µg/mL.

At the maximum concentration tested (1000 µg/mL), all extracts showed substantial DPPH scavenging activity. The acetone extract of dried leaves recorded the highest inhibition (92.76%), followed by chloroform extract of dried leaves (90.12%) and aqueous extract of dried leaves (88.54%). In general, dried leaf extracts demonstrated higher antioxidant activity than fresh leaf extracts across all solvents.

Overall, the results indicate that *Drynaria quercifolia (L.)* leaf extracts possess significant antioxidant potential in a dose-dependent manner, with acetone and aqueous extracts—particularly from dried leaves—exhibiting superior DPPH radical scavenging activity. This enhanced activity may be attributed to a higher concentration or better extractability of antioxidant phytochemicals in dried plant material.

The higher FRAP value was observed at high concentration of plant extract indicates the greater antioxidant activity (G.Prasanna etal, 2015). Comparing the solvent activity, acetone extract is better than water in exposing the antioxidant activity of rhizome and in leaves, water extract showed the better result than acetone extract.

CONCLUSION

The present study demonstrates that *Drynaria quercifolia (L.)* leaves possess a rich phytochemical profile and significant antioxidant potential, which are strongly influenced by the extraction solvent and the physical state of the plant material. Qualitative phytochemical analysis revealed the presence of various bioactive compounds such as steroids, alkaloids, tannins, glycosides, saponins, flavonoids, triterpenes, and carbohydrates in different solvent extracts of fresh and dried leaves. Overall, dried leaf extracts, particularly those obtained using acetone and water, exhibited a broader range of phytoconstituents, suggesting that drying enhances the extractability or stability of certain secondary metabolites.

The DPPH radical scavenging assay further confirmed the antioxidant efficacy of *D. quercifolia* leaf extracts in a concentration-dependent manner.

Although all extracts showed lower activity compared to the standard ascorbic acid, a substantial increase in free radical scavenging activity was observed at higher concentrations. Among the tested extracts, acetone and aqueous extracts of dried leaves demonstrated comparatively higher antioxidant activity than fresh leaf extracts and chloroform extracts.

Taken together, the findings indicate a positive correlation between the presence of antioxidant-related phytochemicals—such as flavonoids, phenolic compounds, and glycosides—and the observed DPPH scavenging activity. The superior performance of dried leaf extracts highlights their potential as a valuable source of natural antioxidants. These results support the traditional use of *Drynaria quercifolia (L.)* and suggest that dried leaves extracted with acetone or water may be promising candidates for further quantitative phytochemical analysis and development of plant-based antioxidant formulations.

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