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Comprehensive Pharmacognostical Profile of *Drynaria quercifolia* (L.) J. Sm.

Research Article

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Abstract

Pteridophytes are gaining importance as therapeutic agents due to the presence of various phytochemicals and their promising bioactivities. *Drynaria quercifolia* (L.) J. Sm., a Polypodiaceae member is endowed with numerous medicinal properties and finds wide usage in ethno as well as traditional medicines. The rhizome of *D. quercifolia* (L.) J. Sm. was subjected to macro-microscopic, physicochemical, phytochemical and HPTLC analysis to derive a standard for this drug. The microscopic detailing showed a wavy outline due to the presence of ridges and furrows and a broad ground tissue with diffusely arranged steles. The powdered drug showed trichomes, stellar tissue and silica crystals while the physicochemical and phytochemical screenings gave substantial values of different parameters. The rhizome extracts were subjected to HPTLC studies with Linomat 5 TLC applicator and diagnostic peaks were recorded under UV 254 nm, 366 nm and 620 nm. The study put forward an exclusive identity profile of this medicinal rhizome.

Key Words: *Epiphytic, Polypodiaceae, Standardization, Steles, Traditional Medicine.*

Introduction

Drynaria, an ephiphytic fern genus belong to the family Polipodiaceae is represented by fifteen species in the world. Of these, *D. quercifolia* (L.) J.Sm. (syn. *Podophyllum quercifolium*) called as 'oak leaf ferns' or a 'basket fern' is distributed in Australia, China, India, Indonesia, Malaysia, Philippines, Thailand, Singapore and Srilanka in different habitats like rock crevices, along the soil boulders and very often on the tree trunks (1). In India this fern is distributed throughout various habitats (2). It is used in traditional medicinal systems like Ayurveda and folk medicines. Known as 'Asvakatri' in Ayurvedic medicine the rhizome, possessing bitter taste, anodyne, anti-inflammatory, anti- bacterial and astringent properties, is used for the treatment of typhoid fever, dyspepsia, cephalagia, cough and phthisis (3).

This rhizome possesses immense medicinal properties and is used by various tribal communities world over. Among the tribals in South East Asia it is used in the preparation of antipyretic formulations (4). In Bangladesh it is used in treating chest pain, diabetes, debility, insanity, jaundice, malaria, spermatorrhoea, sleeping and urinary disorders (5-12). For the treatment of baldness the rhizome is used in Chinese medicines (13). In Tripura it is used for treating intestinal worms and stomachache (14). The tribals living in the ghat regions of India use this rhizome in treating ailments like bone fracture, cholera, fever, headache, jaundice, rheumatism and vomiting (15-19). *Aattukal kilangu* soup made from the rhizomes of *D.* quercifolia is served in the hill stations of Tamil Nadu (20).

As there is no comprehensive monographic standardization report of this raw drug the present study was taken up with the view to standardize the rhizome of *Drynaria quercifolia* (L.) J. Sm. with respect to macro-microscopic, physicochemical and HPTLC finger profiling for authentication and quality characterization of this highly medicinal rhizome.

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Materials and Methods

Collection and Identification of samples

The fresh rhizomes were collected from Yercaud in Salem district, Tamil Nadu during September 2019. The sample was identified and authenticated at Pharmacognosy department, SCRI, Chennai. The rhizome was cleaned and portion of it was air-dried for further studies.

Pharmacognostical Evaluation

Macroscopic characterization

The macroscopy of the rhizome was documented by Nikon COOLPIX5400 digital camera. The colour, odour and taste were also recorded (21).

Microscopic characterization

For microscopy small pieces of rhizome were hand cut into transverse sections using sharp platinum blade, stained with safranine and photographed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX5400 digital camera under bright field light. Magnifications were indicated by the scale-bars (22).

Powder characterization

A portion of the rhizome was shade dried, powdered and passed through sieve no. 60, and preserved in airtight containers for powder microscopy. The powder was mounted in glycerine on a clean microscopic slide, observed under Nikon ECLIPSE E200 trinocular microscope magnified to 400X and diagnostic characters were photographed (22).

Physico-chemical analysis

The physico-chemical parameters like moisture content, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive were determined as per standard methods (23).

Primary phytochemical screening

The preliminary phytochemical screening of the rhizome was done to find various phytoconstituents following standard procedures (24).

High Performance Thin Layer Chromatography

One gram of powdered samples was dissolved in 10 ml ethanol and kept for cold percolation for 24hrs and filtered. 6µl and 9µl of the above samples were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in toluene: ethyl acetate (10: 1). The developed plates were visualized in UV 254, 366 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

Results

Morphology

The fresh fleshy rhizome is up to 18 cm in length and up to 8 cm in width. The dried rough rhizome is reddish brown in colour, irregular in shape and nearly flat and measures up to 12 cm x 6 cm x 2cm. It is covered by velvet like soft copper coloured scale leaves. Longitudinal wrinkles are visible and the inner cut surface is light reddish brown in colour; fracture splintery; no characteristic odour and bitter taste (Fig.1).

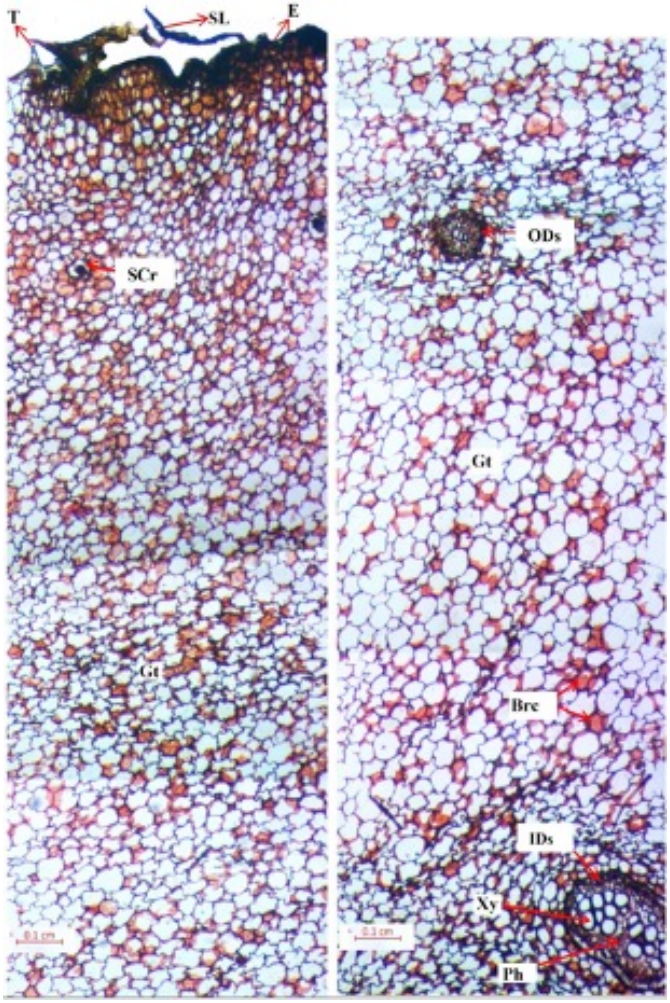
Figure 1. *Drynaria quercifolia* rhizome

Micro morphology

Anatomy

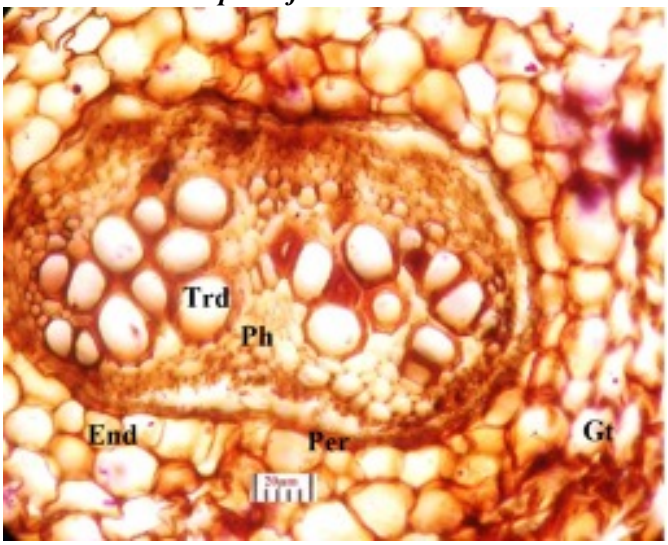
Detailed TS of rhizome is wavy with ridges and furrows. An outermost single layered wavy epidermis covered with winged scales arising from the furrows and few multicellular trichomes are present. Scales are long, lanceolate with an elongated tapering apex. The epidermal cells are rectangular and filled with brownish contents. It is followed by a wide zone of ground tissue composed of thin walled compactly arranged rectangular parenchyma cells. The cells in the ground tissue also contain brownish content and steles are arranged diffusely in addition to the presence of few starch grains. The highly dissected protostele gives rise to numerous heteromorphic meristeles. Each stele is covered by a distinct layer of endodermis and pericycle. The meristeles have isolated elements of xylem surrounded by phloem. Xylem is composed of thick walled angular endarch metaxylem and exarch protoxylem elements surrounded by smaller phloem elements (Fig. 2 and 3).

Figure 2. TS of *Drynaria quercifolia* rhizome



Brc- Brownish content; E- Epidermis; Gt- Ground tissue; IDs- Inner dictyostele; ODs- Outer dictyostele; Ph- Phloem; SCr- Silica crystal; SL- Scale leaves; T- Trichome; Xy- xylem

Figure 3. Enlarged Dictyostele of *Drynaria quercifolia* rhizome

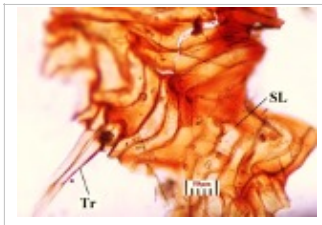


End- Endodermis; F- Fibre; Gt- Ground tissue; Per- Pericycle; Ph- Phloem; Trd- tracheid

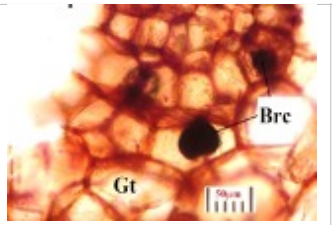
Powder microscopy

The powdered rhizome is coffee brown in colour with no characteristic odour and a bitter taste. The microscopic investigation of powdered rhizome revealed the presence of epidermal cells of scale leaves, numerous trichomes, parenchyma cells with brownish inclusions and silica crystals, pitted parenchyma cells, normal and pitted fibres, scalariform and reticulate tracheids (Fig 4).

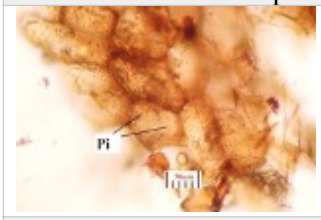
Figure 4. Powder microscopy of *Drynaria quercifolia* rhizome



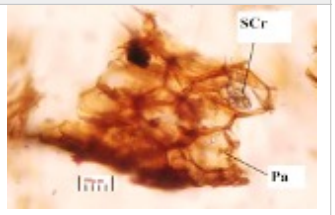
Scale leaves with trichome and oil drops



Outer parenchyma cells with brown contents



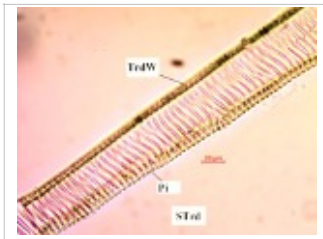
Pitted parenchyma cells with starch grains



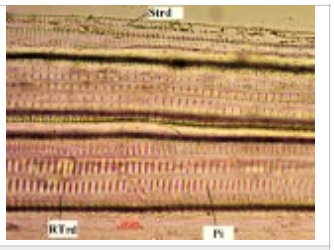
Parenchyma cells with Silica crystals

Inner Parenchyma cells filled with tannin

Simple pitted fibre-tracheids



Scalariform tracheid



Reticulate tracheids

Brc- Brownish content; FTw- Fibre tracheid wall; Gt- Ground tissue; Pa- parenchyma; Pi- pits; RTrd- Reticulated tracheid; SCr- Silica crystal; SL- Scale Leaves; STrd- Scalariform tracheid; Tr- Trichome; TrdW-Tracheid wall

Physico chemical analysis

Decrease in the weight of air-dried sample was noticed on drying and was found to be 9.527 at 105° C. The estimation of the total inorganic content was 8.713. The water soluble ash value was determined and found to be 5.785 and the acid insoluble ash was calculated to be 0.349. Water soluble extractive was estimated to be 12.595 as compared to 6.429 for ethanol (Table 1).

Table 1. Physicochemical Analysis of *Drynaria quercifolia* rhizome

Parameters	Results n = 3 % w/w Average ± SD
Loss on Drying at 105° C	9.527 ± 0.05
Total Ash	8.1713 ±0.01
Acid insoluble Ash	0.349 ± 0.07
Water soluble Ash	5.785 ±0.15
Alcohol soluble extractive	6.429 ±0.03
Water soluble extractive	12.595 ±0.50

Preliminary phytochemical analysis of the sample revealed the presence of alkaloids, carbohydrates, coumarins, flavonoids, tannins and terpenoids while the other phytochemicals were absent (Table 2).

Table 2. Quantitative analysis of phytochemical in *Drynaria quercifolia* rhizome

Tests	Color if positive	Inference
Alkaloids		
Dragendrof's test	Orange precipitate	Present
Wagners test	Red precipitate	
Mayers test	Dull white precipitate	
Hagers test	Yellow precipitate	
Steroids		
Liebermann-buchard test	Bluish green	Absent
Salkowski test	Bluish red to cherry red	
Carbohydrate		
Molish test	Violet ring	Present
Fehlings test	Brick red precipitate	
Benedicts test	Red precipitate	
Tannin		
With $FeCl_3$	Dark blue or green or brown	Present
Flavonoids		
Shinoda's test	Red to pink	Present

Saponins		
With $NaHCO_3$	Stable froth	Absent
Triterpenoids		
Tin and thionyl chloride test	Pink	Present
Coumarins		
With 2 N NaOH	Yellow	Present
Phenols		
With alcoholic ferric chloride	Blue to blue black, brown	Absent
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	Absent
Resin		
With aqueous acetone	Turbidity	Absent
Quinone		
5% NaOH	Pink/purple/red	Absent

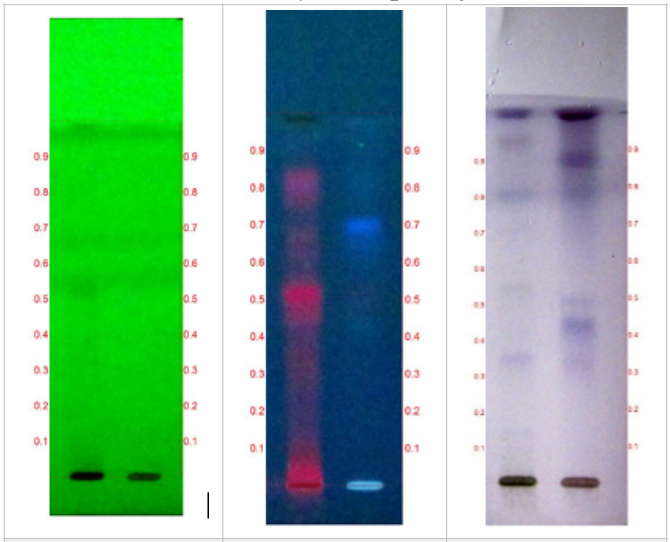
TLC

TLC finger print profile of ethanol extract of *Drynaria quercifolia* rhizome revealed two bands with R_f 0.52 and 0.69 (light green) under short UV (254nm); 5 spots with R_f 0.44 (fluroscent green), 0.50, 0.65, 0.70 and 0.83 (fluorescent blue) under long UV (366nm); five spots with R_f 0.33, 0.43 (dark purple), 0.50 (light purple), 0.79, 0.87 (dark purple) under white light (post derivatization) as seen in Figure 5. Successive densitometric scan showed two bands with R_f 0.63 and 0.76 under short UV; three bands with R_f 0.51, 0.60, 0.80 under long UV and seven bands with R_f 0.02, 0.16, 0.38, 0.48, 0.55, 0.88, 0.97 respectively (Fig.5 and Table 3).

HPTLC

The fingerprint profile of ethanol extract under λ 254 nm revealed the presence of only two peaks one at R_f 0.63with an area of 44.43% followed by the second at R_f 0.76 with an area of 55.57%; under λ 366 nm, three peaks appeared at R_f 0.51 with an area 15.1%, second major peak at R_f 0.60 with an area 32.4% followed by R_f 0.80 with an area of 52.6%; under white light after derivatization, seven peaks were noted viz. at R_f 0.02 with area 9.03%, R_f 0.16 with area 1.20%; R_f 0.38 with area 21.64%, R_f 0.48 with area 45.59, R_f 0.55 with area 12.92%, R_f 0.88 with area 7.73% and R_f 0.97 with area 1.89% (Fig. 6).

Figure 5. Photo documentation of TLC profile of ethanol extract of *Drynaria quercifolia* rhizome



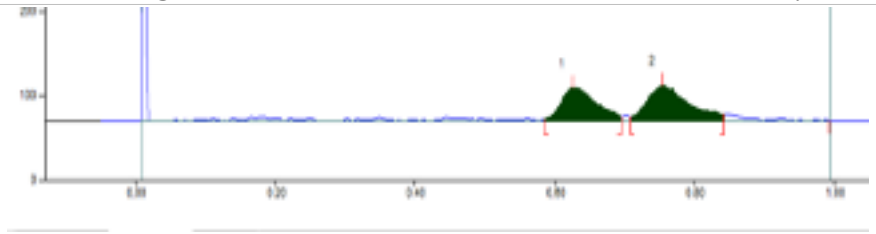
At 254nm | **At 366 nm** | **After derivatisation**

Table 3. R_f values for TLC profile of ethanol extract of *Drynaria quercifolia* rhizome

At 254 nm		At 366 nm		After derivatisation	
Colour	**R_f values**	**Colour**	**R_f values**	**Colour**	**R_f values**
-	-	-	-	D purple	0.33
-	-	FL green	0.44	D purple	0.43
-	-	FL blue	0.50	L purple	0.50
L green	0.52		-	-	-
	-	FL blue	0.65	-	-
L green	0.67		-	-	-
-	-	FL blue	0.70	-	-
-	-		-	D purple	0.79
-	-	FL blue	0.83		-
-	-		-	D purple	0.87

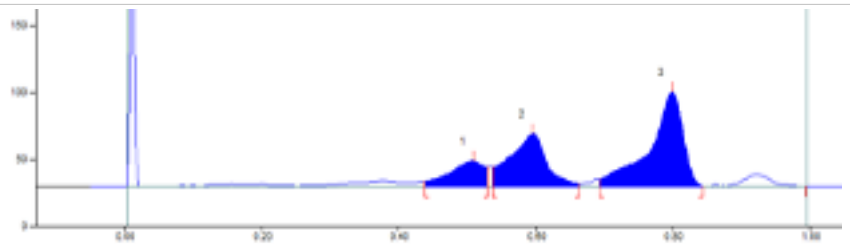
D – dark; F – fluorescent; L – light (Toluene : Ethyl acetate – 10:1)

Figure 6. Densitometric scans of ethanol extract of *Drynaria quercifolia* rhizome



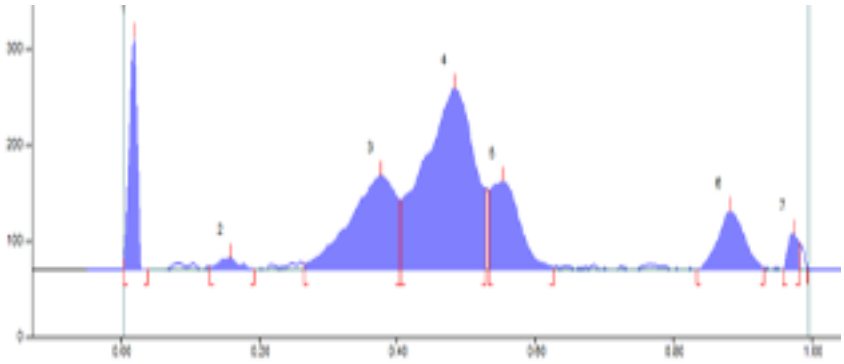
Peak	R_f	Area %
1	0.63Rf	44.43
2	0.76Rf	55.57

At 254 nm



Peak	R_f	Area %
1	0.51Rf	15.1
2	0.60Rf	32.4
3	0.80Rf	52.6

At 366 nm



Peak	R_f	Area %
1	0.02Rf	9.03
2	0.16Rf	1.20
3	0.38Rf	21.64
4	0.48Rf	45.59
5	0.55Rf	12.92
6	0.88Rf	7.73
7	0.97Rf	1.89

At 620 nm

(Toluene : Ethyl acetate – 10:1)

Discussion

Evaluation of herbal drugs which confirms its identity and determines its purity has underwent systematic changes over the decades. Due to the variation in the sources of crude drugs, their morphological, biological and chemical nature, different standardization techniques needs to be incorporated for their identification. Rhizomes are functionally perennation organs and contain reserves in parenchyma cells. Macro-microscopy is an important identity determining test in pharmacognostical studies. The transversely cut rhizome surface which is characteristic of every botanical raw drugs aids in its botanical identification (24).

The morphology and anatomy of vascular bundles in fern rhizome remains conserved with a very minimal environmental effects (25). Stelar anatomy probably has a higher some taxonomic significance and could provide information useful to support or further refine details of the recently proposed classifications. The first account of morphological and anatomical studies of *Drynaria* was carried out by Nayar and Kachroo in1953 (26) and the taxonomic details of *Drynaria* and *Pseudo drynaria* were given by Nayar in 1961 (27). Only a fewer pharmacognostical studies has been carried out in *Drynaria quercifolia* (28, 29) which are not as comprehensive as this.

The phytochemical studies carried out by Padmaselvi *et al.,* in 2016 (30) showed the presence of lignins, alkaloids, polyphenols and flavonoids the same has been observed by testing positive for phenols in the present study. The total ash value 9.93%, acid insoluble ash value 4.49% water soluble ash value 6.69% alcohol extractive value 9.87% and water extractive value was 13.94% was determined by Prakash *et al.,* (2010) (31), during their study which can be correlated to the present study with a minimal variations.

The anatomical sections showed the presence of scales, diffused dictyostele, broad ground tissue which is in similarity with the study of Nayar and Kachroo (26). The powder microscopic analysis of the drug showed comparatively more diagnostic features when compared to the study of Janarthan *et al.* (29).

TLC of *D. quercifolia* carried out in the present study gave similar results to the previous work (31). The HPTLC profiling of the *D. quercifolia* rhizome revealed the presence of blue fluorescent band at 366 nm before derivatisation which can be accredited to the presence of triterpenes and can be very well related to the previous studies of Nejad and Deokule in 2009 (16).

Prasana and Chithra have reported the GC MS analysis of *D. quercifolia* rhizome (32). Medico folklore studies incorporating the different therapeutic usage of this fern have been carried out by few workers (33, 34). Earlier studies on antibacterial and antifungal (35), antioxidant (36), anti-inflammatory (37), anti-diabetic (38), anti-dermatophytic (16), anti-urolithiatic (39), and wound healing (40) activities has also confirmed the important bioactivities and medicinal attribute of this fern. Even the present study substantiated the presence of phyto constituents like alkaloids, coumarins, tannins, terpinoids and flavonoids which imparts the bioactivities.

Conclusion

The non-flowering plants are endowed with immense medicinal properties. The rhizome of *Drynaria quercifolia* is used in the treatment of various ailments and the present comprehensive pharmacognostic study revealed the morphological,

microanatomical and phytochemical aspects of this medicinal rhizome. Thus, the study provides an inclusive distinctive pharmacognostical profile of this pteridophytic rhizome.

Acknowledgements

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