

**PHYSICOCHEMICAL STANDARDIZATION AND METAL ANALYSIS
OF DRYNARIA QUERCIFOLIA (L.) J. SM.****Vikas V. Vaidya, Saurabh H. Patil*, Deepal D. Ghadge and Hardik R. Shah**

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Corresponding Author*Saurabh H. Patil**Department of Chemistry,
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Mumbai, Maharashtra, India.**ABSTRACT**

Drynaria quercifolia (L.) J. Sm., commonly known as Oak-leaf fern is a medicinal pteridophyte belonging to the family Polypodiaceae. It is traditionally used by the tribal communities to treat various ailments. Several workers have reported its uses against body ache, headache, phthisis fever, dyspepsia, tuberculosis, cholera, typhoid fever, haemoptysis, gonorrhea, rheumatic pain and inflammatory disorders. In the present study, physicochemical parameters and metal content in the fronds and rhizome of *D. quercifolia* was analyzed. Physicochemical parameters in plants give valuable information and help to access quality of the sample. The ash values, extractive values,

loss on drying, moisture content in the fronds and rhizome samples were determined as per the WHO guidelines. Heavy metals are a matter of concern in the herbal drugs, especially as certain plants have the tendency of storing heavy metals from the soil, polluted water and atmosphere. Micronutrients are very essential for plant growth and regulation. Hence the presence of heavy metals; cadmium (Cd), chromium (Cr) and microelements; Copper (Cu), iron (Fe) and manganese (Mn) was determined using atomic absorption spectroscopy. The obtained results revealed that the content of heavy metals was within the permissible levels and hence the plant was safe to be utilized in herbal drug formulations.

KEYWORDS: *Drynaria quercifolia* (L.) J. Sm., Polypodiaceae, Physicochemical standardization, Heavy metal content, Atomic absorption spectroscopy (AAS).

INTRODUCTION

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed

from them. Herbal medicines are promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally herbal formulations involve the use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety and efficacy of the herbal product.

Drynaria quercifolia (Oak Leaf Fern) is a large species of fern with deeply pinnatifid foliage fronds. The nest fronds resemble the leaves of oaks, hence the common name. The sori are either scattered or arranged in two regular rows in between the secondary veins.^[1] It is native to India, Southeast Asia, Malaysia, Indonesia, the Philippines, New Guinea, and Australia, found in low down in the mountains, on trees (epiphytic) or rocks (epipetric).^[2,3] *D. quercifolia* usually grows in low fertile land with humid conditions. This also grows in coastal areas of India including coastal Western Ghats of Maharashtra^[3]. Several workers have reported its uses against phthisis fever, dyspepsia, tuberculosis, cholera, typhoid, haemoptysis and gonorrhea.^[4]

The process of evaluation of the quality and purity of crude drugs by means of various parameters like morphological, microscopical, physical, chemical and biological observations is called standardization.^[5] The increased use of herbal drugs, and concerns over their safety and efficacy have certainly augmented the need of standardization of these herbal drugs. WHO has set up guidelines for standardization of these drugs, which are used as a standard by the majority of countries. The process of standardization can be achieved by stepwise pharmacognostic studies.^[6] Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect.^[7] Determination of extractive values, ash residues, moisture content and active components (saponin, alkaloids & essential oil content) plays a significant role for standardization of the indigenous crude drugs.^[8]

Heavy metals are significant and extremely persistent environmental pollutants and their toxicity is a problem of increasing significance for ecological, nutritional, and environmental reasons. The Heavy metals cause metabolic disturbance and the excess produce serious consequences on human health. It's widely acceptable that the metals may react directly with DNA and produces cross links between the DNA strands as was observed after exposure.^[9] Men, animals and plants through air, water and food take up these metals from the environment. The plants are widely used as raw materials for pharmaceutical preparations and as a supplement for dietetic products and especially for 'self-medication' in the general

population.^[10] There is little information available about the safety of herbal plants and their products in respect to heavy metals contamination. Due to the use of enormous amount of herbal medicine, it is important to know the toxic metal contents in these products. The objective of this work is to investigate the magnitude of heavy metals; cadmium (Cd), chromium (Cr) contamination and microelements; Copper (Cu), manganese (Mn) and iron (Fe) in *D. quercifolia*. In order to ensure safety and quality we have developed a sensitive, flexible and rapid method for the quantification of metals using atomic absorption spectroscopy.

MATERIALS AND METHODS

Collection of the plant

Whole plants of *Drynaria quercifolia* were collected from Kanakeshwar, 10 km away from Alibag, Maharashtra, India in the month of August. The plant was authenticated and voucher specimen no. SP-1 was deposited in The Botanical Survey of India, Pune, India.

Preparation of plant material

The collected *D. quercifolia* whole plants were washed with tap water. The plants were air-dried thoroughly under shade at room temperature for 2 weeks to avoid direct loss of phytoconstituents from sunlight. The shade dried materials were powdered using grinder and sieved through an ASTM 80 mesh. It was then homogenized to fine powder and stored in an air-tight container for further analysis.

Physicochemical investigations

Fronds and rhizome powder of *D. quercifolia* were subjected for determination of physicochemical parameters such as ash values, aqueous and alcoholic extractive values, loss on drying, moisture content were carried out according to the methods recommended by the World Health Organization.^[11]

Determination of total ash

Two grams powder of fronds and rhizomes of *D. quercifolia*, was weighed separately in a tarred silica crucible accurately. Dried material was spread in an even layer in the crucible and incinerated completely in a muffle furnace at 500–600°C for 3 hours until the ash became white in colour, indicating the absence of carbon. Ash was cooled in a desiccator and weighed. Total ash content was calculated in percentage.

Acid-insoluble ash

Twenty five ml of 2M hydrochloric acid was added to the crucible containing the total ash. It was covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. It was allowed to cool, and filtered through Whatman filter paper no. 41. The residue was then washed with hot water till washings were free from chloride (no white precipitate with AgNO_3 solution). The filter paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the temperature not exceeding than 450°C in a muffle furnace for 3 hours. The residue was allowed to cool in a desiccator for 30 minutes, and then weighed without delay. The percentage of acid insoluble ash was calculated with reference to the air-dried sample.

Water-soluble ash

Twenty five ml of distilled water was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes. Insoluble matter was collected on Whatman filter paper no. 41. The residue was washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding than 450°C in a muffle furnace. The residue was allowed to cool in a desiccator for 30 minutes and weighed. The water-soluble ash was calculated by difference in weight of this residue and that of the total ash. Finally, the percentage of water soluble ash was calculated with reference to the air-dried sample.

Determination of water and ethanol extractable matter

Four grams of coarsely powdered air-dried material accurately weighed in a glass-stoppered conical flask, macerated with 100 ml of distilled water and ethanol separately for 6 hours, shaken frequently, and allowed to stand for 18 hours. It was then filtered rapidly and transferred 25 ml of the filtrate to a tared flat-bottomed dish and evaporated to dryness on a water-bath, dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter was calculated in percentage.

Loss on drying

Two grams of *D. quercifolia* powder was weighed in a wide mouth stoppered weighing bottle. The bottle was placed (with lid open) in hot air oven maintained at $100\text{--}105^\circ\text{C}$ for 2 hours. The bottle was then transferred to desiccators. The bottle was cooled to room temperature and weighed. Loss of weight was calculated in percentage.

Moisture content

Karl-Fisher titrimetric method was used to determine the moisture content in *D. quercifolia* plant powder. Reaction vessel was rinsed thoroughly with methanol; magnetic stirring rotor was inserted in the vessel and placed in a proper position. The large rubber cork was removed and some Karl-Fisher grade methanol was added through funnel just enough to submerge the metal wires of sensors in the reaction vessel. The cork was replaced immediately. The Karl-Fisher reagent and methanol bottles were placed in position. The instrument was turned on and the speed of the magnetic stirrer was adjusted. Methanol in the reaction vessel was neutralized and the titer factor was determined by calibrating the Karl-Fisher reagent. This was done by adding 10 μ l of distilled water with the help of a micropipette in the reaction vessel and completing the titration. The calibration of the reagent was done in triplicate. The readings were noted and the titer factor was calculated. This titer factor was used to calculate the moisture content.

Titer factor = mg. of water added (wt.) / reading in ml (vol.)

100 mg of the plant powder was weighed and transferred to the titration vessel and the titration was allowed to go for completion. Percentage moisture was calculated using the formula;

$$\text{Moisture percentage} = [(\text{titer factor} \times \text{reading}) / \text{weight of sample (in mg)}] \times 100$$

Metal Analysis

Analysis of the metals in selected plant samples were performed on Shimadzu AA-7000F atomic absorption spectrophotometer (AAS). Measurements were made using a BCG-D₂ lamp mode for cadmium, chromium, copper, iron and manganese at wavelengths of 228.8 nm, 357.9 nm, 324.8 nm, 248.3 nm and 279.5 nm respectively. For each of the selected metals a standard linear calibration curve of various concentrations was analysed by AAS. Method parameters used for the analysis of metals are summarized in table no. 1.

Table no. 1. Parameters used for the analysis of metals

Parameter	Cadmium (Cd)	Chromium (Cr)	Copper (Cu)	Iron (Fe)	Manganese (Mn)
Instrument model no.	Shimadzu A-7000F	Shimadzu A-7000F	Shimadzu A-7000F	Shimadzu A-7000F	Shimadzu A-7000F
Lamp mode	BCG-D ₂	BCG-D ₂	BCG-D ₂	BCG-D ₂	BCG-D ₂
Slit width (nm)	0.7	0.7	0.7	0.2	0.2
Wavelength (nm)	228.8	357.9	324.8	248.3	279.5
Fuel gas flow rate	1.8	2.8	1.8	2.2	2.0

(L/min)					
Support gas flow rate (L/min)	15.0	15.0	15.0	15.0	15.0
Flame type	Air-C ₂ H ₂	Air-C ₂ H ₂	Air-C ₂ H ₂	Air-C ₂ H ₂	Air-C ₂ H ₂

Sample Preparation

One gram air dried and fine powder from fronds and rhizomes of *D. quercifolia* was placed in different beakers. Five ml of concentrated nitric acid was added to it and kept overnight. The solution was evaporated to dryness on sand bath. Then 1 ml perchloric acid was added to it, evaporated to dryness and allowed to cool. Distilled water was added and filtered through Whatman filter paper no. 41 into a volumetric flask and made up the volume to 25 ml with distilled water.

RESULTS AND DISCUSSION

Physicochemical investigations

The powdered fronds and rhizomes of *Drynaria quercifolia* were subjected to evaluate its total ash, acid-insoluble ash, water-soluble ash value, water and ethanol soluble extractive values, loss on drying and moisture content. Total ash content in frond (9.20%) and rhizome (8.37%) indicates that the fronds are relatively rich in mineral elements. Acid insoluble ash in frond and rhizome was 4.33% and 3.48% respectively. Water soluble ash in frond and rhizome was 4.95% and 4.13% respectively. Water and ethanol soluble extractive value of frond was 8.24% and 4.86% respectively and that of the rhizome was 7.78% and 6.11%. Loss on drying in frond and rhizome was 14.72% and 11.18% respectively. The air dried sample of frond and rhizome powder contains 11.52% and 13.19% moisture respectively. The low moisture content of the leaf would hinder the growth of microorganism and storage life would be high.^[12]

Table no. 2. Physicochemical analysis of *Drynaria quercifolia*

Parameter	% Content	
	Frond	Rhizome
Total ash	9.20	8.37
Acid insoluble ash	4.33	3.48
Water soluble ash	4.95	4.13
Water soluble extractive	8.24	7.78
Ethanol soluble extractive	4.86	6.11
Loss on drying	14.72	11.18
Moisture content	11.52	13.19

Metal Analysis

The AAS parameters were optimized by considering the wavelength, fuel gas as well supporting gas flow. The wavelength for cadmium (228.8 nm), chromium (357.9 nm), copper (324.8 nm), iron (248.3 nm) and manganese (279.5 nm) was found to be suitable for the detection of metals. The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each compound. The heavy metals; cadmium, chromium and micronutrients; copper, iron and manganese were analysed at their particular wavelengths. The study revealed that no resultant spectral peaks of cadmium and chromium in frond and rhizome were observed (Table no. 3). Hence, neither fronds nor rhizomes of *D. quercifolia* show the presence of Cd and Cr. Heavy metal toxicity is frequently the result of long term low level exposure to pollutants common in our environment.^[13] Cadmium is absorbed by roots of many plants, cannot be removed by washing and is concentrated particularly in the kidneys, liver, blood forming organ and the lungs.^[14] Although chromium is found to be a stimulant for plant growth, several investigators reported its toxic effect. Micronutrient content in rhizome was considerably more than in fronds. Concentration of micronutrients; Cu, Fe and Mn in fronds was found to be 0.68 ppm, 1.14 ppm and 0.40 ppm respectively. In rhizome, Concentration of Cu, Fe and Mn was 10.38 ppm, 11.69 ppm and 9.82 ppm respectively (Table no. 3). Copper, iron and manganese are very essential for plant growth and regulation. Copper is necessary for carbohydrate and nitrogen metabolism and, inadequate copper results in stunting of plants. Iron is involved in the production of chlorophyll. Iron is also a component of many enzymes associated with energy transfer, nitrogen reduction and fixation, and lignin formation. Manganese is necessary in photosynthesis, nitrogen metabolism and to form other compounds required for plant metabolism.

Table no. 3. Concentration of metals in *Drynaria quercifolia*

Metal	Concentration in Fronds	Concentration in Rhizome
Cadmium (Cd)	BDL	BDL
Chromium (Cr)	BDL	BDL
Copper (Cu)	0.68 ppm	10.38 ppm
Iron (Fe)	1.14 ppm	11.70 ppm
Manganese (Mn)	0.40 ppm	9.82 ppm

* BDL: below detection limit

CONCLUSION

Present work is taken up in the view to standardize the plant of *Drynaria quercifolia* in accordance to parameters of World Health Organization (WHO) Guidelines. In the present study fronds and rhizomes of *D. quercifolia* was investigated for its physicochemical characters and metal content to analyze their quality, safety and standardization for their safe use. The results revealed that the content of heavy metals; cadmium and chromium was within the permissible levels and hence the plant is safe to be used in herbal drug formulations. The generated information of the present study provides data which is helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

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