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PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF THE RHIZOME OF *DRYNARIA QUERCIFOLIA* L.

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ABSTRACT

The present investigation has been carried out the physicochemical, fluorescence, histochemical and phytochemical analysis of methanol extract of *Drynaria quercifolia* rhizome. In physicochemical analysis, parameters such as moisture content (3%) water soluble ash (6%) sulphated ash (5%) alcohol soluble extractive value (7%) and water soluble extractive value (9%) of plant were determined. In fluorescence analysis, different colours of fluorescent were observed under UV and visible light. The histochemical analysis indicates the presence of lignin, flavonoids, alkaloids and polyphenol based on colours. Quantitative phytochemical analysis was also performed and the results indicates the significant amount of flavonoids (32.84 mg/g), saponin (32.74 mg/g), phenols (84.56 mg/g), tannins (45.23 mg/g) and alkaloids (6.38 mg/g) were determined. Our finding provided evidence that the methanol extract of plant contain medicinally important bioactive compounds and it justified their use in the traditional medicine.

Keywords: *Drynaria quercifolia*, Fluorescence, Methanol extract, Physicochemical, Phytochemical.

INTRODUCTION

Phytochemicals (from the Greek word "phyto" meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for human being further than those attributed to macronutrients and micronutrients [1]. They protect plants from disease and damage contributes to the plant's colour, aroma and flavor. In general, the plant chemicals that protect plants cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals [2, 3]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics [4] and 150 phytochemicals have been studied in detail.

Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important to prevent or to some common diseases. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown

phytochemicals. It is well known that plants produce these chemicals protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases [5].

Medicinal plants contain some natural products which perform definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [6]. The phytochemical screening of the plants is a preliminary for verification and then these plants may be utilized as new sources of herbal drug. Such studies have included identification and isolation of the chemical components, establishment of their biological potency both by *in vitro* and *in vivo* studies in experimental animals and through epidemiological and clinical case control studies in man. Study findings suggest that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity. Hence the present investigation has been carried out for the physicochemical and phytochemical analysis of *Drynaria quercifolia* rhizome.

Drynaria quercifolia is an epiphytic fern, is used in medicinal system by different groups of people to treat various kinds of health problems including urinary tract

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infection [7]. The rhizome paste is applied for treatment of diarrhea, typhoid, cholera, chronic jaundice, fever, and headache and skin diseases. Whole plant is anthelmintic, expectorant and tonic [8, 9]. It is also used in the treatment of chest disease, cough, hectic fever, dyspepsia, loss of appetite, chronic jaundice and cutaneous infection [10]. Tribals in kalakad, Mundanthurai Tiger Reserve India, use this rhizome of fern to cure rheumatism [11]. In the treatment of hyperthyroidism, *Drynaria* along with other drugs are used. In these conditions *Drynaria* is used externally as well as internally. *Drynaria quercifolia* along with other combination of herbs is used to treat pain from traumatic injury, such as sprains and contusions with bruising and swelling [12]. 30 bioactive compounds have been identified by GC MS analysis [13]. The rhizome is also reported to have anti fertility [14], anti inflammatory [15] and antipyretic and analgesic [16], antimicrobial [17] and antiulcer [18] properties.

MATERIALS AND METHODS

Collection of plant materials

The material used in the present study is the rhizome of *Drynaria quercifolia* L.(J) smith. The rhizome was collected from the Kolli malai, Namakkal district, Tamil Nadu, India. The rhizome is covered with small brown coloured hair like structures they were removed using sterile scalpel and washed with sterile distilled water. There were cut into small pieces and dried in shade and made into fine powder, using blender. The powder was used for the present study.

Preparation of extracts

2g of *Drynaria quercifolia* L. powder was weighed and macerated in 50 ml methanol. They were kept at the room temperature for 72 hours. The mixture was stirred every 24 hours using a sterile glass rod. Then it was filtered through the Whatmann Filter paper. The dried residue of methanol extracts was used for evaluating the phytochemical activity. They were kept in refrigerator until they use.

Determination of physicochemical parameters

Physicochemical parameters such as of moisture content [19], water soluble ash, alcohol soluble extractive, water soluble extractive, and sulphated ash were determined in rhizome of *Drynaria quercifolia* according to methods described in WHO guidelines [20].

Fluorescence and histochemical analysis

Fluorescence analysis of entire rhizome of *Drynaria quercifolia* has been carried out in daylight and under UV light. Fluorescence analysis was carried out by the treatment of different chemical reagents such as methanol, H₂SO₄, HCl, HNO₃, NaOH, acetone, hexane, chloroform and distilled water. The powders were observed in normal daylight and under short (245 nm) and long U.V. light (365 nm) [21]. For histochemical analysis, the powder of *Drynaria quercifolia* rhizome was treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope for the

identification of chemical components.

Phytochemical screening

Quantitative analysis was performed in methanol extract of plant to quantify the phytoconstituent such as Phenols [6], Alkaloids [22], Tannin [23], Saponin [24], Flavonoid [25].

RESULTS AND DISCUSSION

Medicinal plants are inextricably intertwined with the rich history, culture, and culinary tradition of India. It is reported that 4639 ethnic communities who lived in different regions of India use locally available medicinal plants to treat various ailments, based on their rich and varied folk knowledge. Similarly, medicinal plants are also used by the codified systems of medicine such as Ayurveda, Siddha, Tibetan, and Unani. In most preparations, the medicinal plants being used very often are in powder or paste forms of the crude herbs, which contain both organic and inorganic constituents. Vitamins and minerals are present in a variety of plants utilized as important components of both human and animal's diets [26]. In the present study, Physicochemical, fluorescence, histochemical, and phytochemical analysis have been carried out in the methanol extract of *Drynaria quercifolia* rhizome. The results were tabulated and discussed in this chapter.

Physicochemical analysis

Table 1 shows the physicochemical analysis of rhizome of *Drynaria quercifolia*. The result revealed the moisture content, water soluble ash, sulphated ash, alcohol soluble extractive value and water soluble extractive value of plant. The determination of moisture content is important for the plant drugs because insufficient drying may lead to possible enzymatic deterioration of active principles [27]. This parameter is therefore essentially used to control the quality of crude drugs and/or herbal drugs/drug products. The purity of crude drugs could also be evaluated by the determination of ash values which represent the content of foreign matter such as inorganic salts or silica present as a form of adulterant in the drug sample. The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. These ash values are important pharmacognostic tool to standardize the crude drugs. In the present study 3% of moisture and 6% of water soluble ash and 6% of sulphated ash were observed.

The extracts obtained by exhausting plant materials with specific solvents are indicative of approximate measures of their chemical constituents extracted with those solvents from a specific amount of air-dried plant material. This parameter is employed for materials for which as yet no suitable chemical or biological assay exists [28]. Successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of good solvent in plant

extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate [29]. As the end product in extraction will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay [30]. The choice will also depend on the targeted compounds to be extracted. Initial screening of plants for possible antimicrobial activities typically begins by using the crude or alcohol extractions and can be followed by various organic solvent extraction methods. In the present study, highest extractive value obtained from water (9%) followed by alcohol (7%).

Fluorescence and Histochemical analysis

Fluorescence is the phenomenon exhibited by various chemical constituents present in the organo gel. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by the application of different reagents [31, 32]. Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [33]. In the present study, Fluorescence analysis of *Drynaria quercifolia* rhizome was analyzed (Table 2). The result showed that in visible light, the plant powder exhibit various shades of green and brown fluorescence and various shades of green, blue and brown were found in under UV light.

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues; it is a powerful tool for localization of trace quantities of substances present in biological tissues [34]. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major compounds such as proteins, lipids, starch, phytin and minerals like

calcium, potassium and iron [35]. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. In the present study, histochemical analysis shows pink, yellow, reddish brown and bluish green colour were observed which indicate the presence of lignin, flavonoids, alkaloids and polyphenol, respectively (Table 3).

Phytochemical analysis

Table 3 shows the phytochemical analysis of methanol extract of *Drynaria quercifolia* Rhizome. The result revealed the presence of tannins, terpenoids, alkaloids, anthroquinone, glycosides, phlobatannins, saponins, flavonoids, steroids, triterpenoids, carbohydrate, aminoacid and polyphenol. Collectively more number of compounds are present in the methanol extract of rhizome of *Drynaria quercifolia*.

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance to make the best and judicious use of available natural wealth. A number of medicinal plants have been investigated [36, 37] for chemical substances that have a definite physiological action on the human body. The most important of the bioactive constituents of plant are alkaloids, tannins, flavonoids, saponin and phenolic compounds [38]. These phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. In the present study, qualitative analysis of phytochemicals were also studied in this plant extract in order to determine the concentration of flavonoids (32.84 mg/g), saponin (32.74 mg/g), phenols (84.56 mg/g), tannins (45.23 mg/g) and alkaloids (6.38 mg/g) which were presented in table 4. Among the phytochemicals, a phenol was present in high concentration followed by tannins, flavonoids and saponins. Trace amount of alkaloids was identified.

Table 1. Physicochemical analysis of *Drynaria quercifolia* rhizome powder

S.No.	Tests	Amount (%)
1.	Moisture content	3±0.21
2.	Sulphated ash	5±0.45
3.	Water soluble ash	6±0.49
4.	Alcohol soluble extractive	7±0.49
5.	Water soluble extractive	9±0.63

Table 2. Fluorescence behavior of *Drynaria quercifolia* rhizome

S. No.	Tests	Visible light	Short UV	Long UV
1.	Plant powder	Green	Pale green	Brown
2.	Plant powder treated with distilled water	Light brown	Green	Sky Blue
3.	Plant powder treated with hexane	Brown	Green	Sky blue
4.	Plant powder treated with chloroform	Pale Brown	Green	Dark Brown
5.	Plant powder treated with methanol	Light Brown	Green	Dark Brown
6.	Plant powder treated with acetone	Dark Brown	Green	Brown
7.	Plant powder treated with in sodium hydroxide in water	Pale Brown	Greenish	Dark Brown
8.	Plant powder treated with hydrochloric acid	Light brown	Green	Dark brown
9.	Plant powder treated with sulphuric acid with an equal	Light brown	Light green	Brown

	volume of water			
10.	Plant powder treated with nitric acid diluted with an equal volume of water	Dark brown	Greenish	Dark brown

Table 3. Histochemical studies of *Drynaria quercifolia* rhizome

S.No	Phytochemicals	Amount(mg/gm)
1.	Flavonoids	36.84±0.25
2.	Saponins	32.74±0.23
3.	Phenols	84.56±0.58
4.	Tannin	45.23±0.28
5.	Alkaloids	6.38±0.04

+ indicates present

Table 4. Quantitative analysis of phytochemicals of *Drynaria quercifolia* rhizome

S.No	Characterization	Colour	Result
1.	Lignin	Red/pink	+
2.	Flavonoids	Yellow	+
3.	Alkaloids	Reddish brown	+
4.	Polyphenol	Bluish green	+

CONCLUSION

Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. The present studies revealed that the physicochemical, florescence, histochemical and phytochemical analysis are the essential analytical aspect

for the study of identity, purity, quality, safety and efficacy of rhizome of *Drynaria quercifolia* for its use as potential drug candidate. Overall, *Drynaria quercifolia* is the rich source of phytochemicals which are important for health preservation and disease prevention.

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The authors declare that they have no conflict of interest.

REFERENCES

- Hasler CM, Blumberg JB. Symposium on Phytochemicals: Biochemistry and Physiology. *Journal of Nutrition*, 129, 1999, 756S-757S.
- Gibson EL, Wardel J, Watts CJ. Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. *Appetite*, 31, 1980, 205-228.
- Mathai K. Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump, 271, 2000, 274-275.
- Meagher E, Thomson C. Vitamin and Mineral Therapy. In Medical Nutrition and Disease, 2nd ed., G Morrison and L Hark, Malden, Massachusetts. *Blackwell Science Inc*, 1999, 33- 58.
- Narasinga Rao. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. *Asia Pacific Journal of Clinical Nutrition*, 12(1), 2003, 9-22.
- Edeoga OH, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol*, 4, 2005, 685-688.
- Sen A, Ghosh PD. A note on ethanobotanical studies of some Pteridophytes in Assam. *Indian J. Traditional Knowledge*, 102, 2011, 292-295.
- Viswanathan MB. Industry meets cum seminar on biodiversity and information on medicinal and aromatic plants. *NISCOM*, New Delhi, 1995, 60.
- Chopra RN. Indigenous drugs of India and their economic aspects. Calcutta. Art Press, Calcutta, 1993.
- Khare DP. Indian medicinal plants: an illustrated dictionary. New delhi: Springer-Verlag Berlin/ Heidelberg, 2007.
- Sutha S, Mohan VR, Kumaresan S, Murugan C, Athiperumalsamy T. Eynomedicinal plants used by the tribals of kalakad – Mundanthurai Tiger Reserve KMTR, Western Ghats, Tamil Nadu for the treatment of rheumatism. *Indian J. Traditional Knowledge*, 93, 2010, 502-509.
- Holtttum RE. A Revised Flora of Malaya and Ferns of Malaya. Government Singapore, 2, 1997, 275-279.
- Prasanna, G and Chitra, M. Pytochemical screening and GC-MS analysis of *Drynaria quercifolia* rhizome. *American Journal of Advanced Drug Delivery*, 3(1), 2015, 72-78.
- Rajendran A. and Rajan S. *Drynaria quercifolia*- an antifertility agent. *Ancient Science of Life*, 15(4), 1996, 286-187.
- Irudayaraj V, Senthamarai R. Pharmacognostical studies on a medicinal fern, *Drynaria quercifolia* (L.) J.Sm. (Polypodiaceae:Pteridophyta). *Phytomorphology*, 54, 2004, 193-200.

16. Prasanna G, Chitra M and Janaranjani B. Antiinflammatory and antipyretic activities of *Drynaria quercifolia* rhizome in rats. *In J of Pharmaceutical Sciences Rev. Research*, 29(1), 2014, 57-61.
17. Prasanna G, Chitra M, Suvitha N. *In Vitro* antimicrobial activity of *Drynaria quercifolia* L. Rhizome. *Asian J Bioc Pharmac Res*, 4, 2014, 342-349.
18. Soni DK, Jagan Mohan A, Sai Goud, Mantena VR, Krishna R. Antiulcer activity of ethanolic extract of *Drynaria quercifolia* Linn. Leaves. *J Pharm Res*, 5, 2012, 117-119.
19. Anonymous. Quality Control Methods for Medicinal Plant Materials (*An authorized publication of World Health Organization, Geneva*). New Delhi.
20. World Health Organization, Quality control methods for medicinal plant materials, WHO Library, 1998, 110-115.
21. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of Identification. *J American Pharmaceutical Sciences*, 38, 1969, 324.
22. Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd, 1973, 49-188.
23. Van Buren JP, Robinson WB. Formation of complexes between protein and tannic acid. *J. Agric. Food Chem*, 17, 1981, 772-777.
24. Obadomi B, Ochuko P. Phytochemical studies comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci*, 8, 2001, 203-208.
25. Boham BA and Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Science*, 48, 1974, 458-463.
26. Okwu DE. Phytochemicals and Vitamin content of indigenous spices of South Eastern Nigeria. *Sustain Agric Environ*, 6, 2004, 30 – 34.
27. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Pune, India, Nirali Prakashan, 2007.
28. Anonymous. The Useful Plants of India. NISCAIR, New Delhi, 1992, 392.
29. Hughes I. Isoprenoid compounds and phenolic plant constituents. *Elsevier, New York, N.Y. Sci. Afr. Mag*, 9(56), 2002, 502.
30. Ncube N, Afolayan SAJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol*, 7(12), 2008, 1797-1806.
31. Gupta MK, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation of *Grewia asiatica* fruits. *Int J Plant Sci*, 1(2), 2006, 249-251.
32. Ansari SH. Essentials of Pharmacognosy. New Delhi, India, Birla Publications Pvt. Ltd, 2006.
33. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra violet radiation. *J Am Pharm Assoc*, 47, 1958, 715-717.
34. Krishnamurthy KV. Methods in plant histochemistry, Chennai, 1998.
35. Krishnan S, Ebenezer GAI and Dayanandan P. Histochemical localization of storage components in caryopsis of rice (*Oryza sativa* L.). *Curr. Sci*, 80, 2001, 567-571.
36. Ambasta SP. The useful plants of India. New Delhi, PID, CSIR, 1986, 51.
37. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan, New Delhi, 1994, 107-109.
38. Hill AF. Economic Botany. A textbook of useful plants and plant products. McGraw Hill Book Company Inc. New York, 1952, 205.