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Research Article

Qualitative phytochemical screening and extraction yield of whole plant of ethnomedicinal fern *Drynaria quercifolia* (L.) J. Smith

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Abstract

The present investigation has been carried out on the qualitative phytochemical screening and extraction yield of the whole plant of the ethno-medicinal fern *Drynaria quercifolia* (L.) J. Smith. The present work is the first study reporting information on these ethno-medicinal ferns with respect to the whole plant parts. Phytochemical screening of immature and mature fronds of *D. quercifolia* was performed, and the results indicate that alkaloids were absent in immature fronds for aqueous, ethanol, and hexane extracts, but present in methanol, ethyl acetate, and petroleum ether extracts, while absent in ethanol and hexane extracts. Alkaloids were completely absent in all extracts of both immature and mature sterile fronds. Carbohydrates, flavonoids, coumarins, phenols, proteins, amino acids, and saponins were consistently present in all solvent extracts of both immature and mature fronds, indicating their abundance and solvent-soluble nature. Flavonoids, coumarins, phenols, and proteins/amino acids were consistently present in all solvent extracts of both immature and mature rhizomes. The highest extraction yield was obtained with methanol Soxhlet extraction of Immature fronds (15.14 ± 0.05%), mature rhizome (15.40 ± 0.21%), and mature Sterile Fronds (11.53 ± 0.37%). These findings highlight that both solvent polarity and extraction method play crucial roles in optimizing phytochemical yield. Phytochemical screening and extract yield of medicinal plants are important in identifying new sources of valuable compounds having medicinal significance to make the best and thoughtful use of available natural wealth.

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Introduction

According to the World Health Organization (WHO), a large portion of the global population relies on traditional medicine for primary health care. Natural products, especially pure or standardized plant extracts, are a vast source of bioactive compounds. Plants contain a wide variety of unique chemicals; they offer many opportunities to discover new medicines (Cos et al., 2006). One of the oldest and most primitive plant groups on the planet is the ferns. The evolutionary connection between lower and higher plants is greatly facilitated by ferns. They generate a distinctive variety of secondary metabolites, many of which are not present in other plants. Due to their unusual evolutionary history and biology, ferns have been found to contain a variety of alkaloids, polyphenols, flavonoids, steroids, and terpenoids (Dong et al., 2012; Ho et al., 2012). Extraction is a key step in

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phytochemical processing to find bioactive components of plant materials and for standardizing herbal products. The yield percentage and bioactivity of the prepared extract of different extraction methods have been reported to be different in several studies (Hayouni et al., 2007). The most commonly used conventional techniques, such as maceration, percolation, infusion, decoction, Soxhlet extraction, etc., have been rapidly and constantly increasing worldwide for phytochemical processing of medicinal plants, as these techniques are reliable and used by most authors. Further, many phytochemical constituents, like alkaloids, flavonoids, phenols, and terpenoids, etc., present in plants may directly or indirectly determine the medicinal aspects of concern plants and also play an important role in bioactive compound analyses. Consequently, numerous researchers have examined how different extraction techniques affect the yield of phenolic compounds from plant sources. In recent years, advanced methods such as microwave-assisted extraction, supercritical carbon dioxide extraction, and ultra-high-pressure extraction have been employed to obtain plant extracts. These modern techniques offer several advantages; however, the efficiency of phenolic compound recovery is significantly influenced by factors such as the extraction method used, duration, and temperature conditions. Due to these variations, it remains challenging to design a single extraction process that can effectively isolate all phenolic compounds from plants (Brglez-Mojzer et al., 2016). Traditional healers usually utilize water for the extraction process for herbal medicine. Although it has been discovered that plant extracts from organic solvents provide more reliable antibacterial efficacy than water extracts (Parekh et al., 2005). Most of the plants represent a rich source of antioxidant agents. Some phytochemicals, like flavonoids and vitamins, are known to be potent antioxidant compounds. However, the literature currently contains only a limited amount of information on the phytochemicals found in ferns.

The various authors reported that *Drynaria quercifolia* (L.) J. Smith is a valuable source of natural antioxidants. Drynaria quercifolia is an ethnomedical fern distributed in most of the world. It is native to Southeast Asia, Western Australia, the Philippines, Indonesia, Malaysia, New Guinea, Africa, and India. It belongs to the family Polypodiaceae of pteridophytes (Prasanna and Anuradha, 2015). It is an important ethno-medicinal plant used in the traditional medicinal system to cure health-related problems of human beings. Selvi et al. (2016) reported the fluorescence, histochemical, and photochemical analysis of the methanol extract of the rhizome of *Drynaria quercifolia*. This is a well-recognized medicinal fern, traditionally valued for its therapeutic properties. However, comprehensive information on the phytochemical composition of the entire plant remains limited and largely underexplored. The present study aims to look into and analyse the phytochemical constituents of the whole plant of *D. quercifolia*. This investigation is anticipated to offer important information that can support future pharmacological and biochemical studies on this species.

Materials and Methods

Collection of Specimens

The fern *Drynaria quercifolia* was collected from the Darjeeling district of West Bengal, India, from April 2022 to August 2023. The identification of *Drynaria quercifolia* has been confirmed by referring to standard literature and also to Lubos and Amorosa (2011). The voucher specimen has been deposited in the Herbarium of the Department of Botany, University of North Bengal (Accession number 01, dated 20/06/2023).

Extraction and sample preparation

The collected different plant parts of *D. quercifolia,* such as immature fronds, mature fronds, immature sterile fronds, mature sterile fronds, immature rhizomes, and mature rhizomes (Figure 1), were washed under running tap water and then rinsed with distilled water and shade-dried at normal room temperature. After drying, plant parts were finely powdered with the help of an electric grinder. 5 gm of powder of different plants was extracted with 500 mL of each solvent, namely water, methanol, ethanol, ethyl acetate, petroleum ether, and n-hexane, using a Soxhlet apparatus for 18 hours at the boiling point temperature of the mentioned solvent.

The solvent extracts were concentrated and dried in an incubator and immediately transferred to desiccators. After drying, all products were stored in a refrigerator (4 ± 2°C), and the same was used for the phytochemical test. The extraction yields of fronds (immature and mature), rhizomes (immature and mature), and sterile fronds (immature and mature) of *D. quercifolia* were evaluated using three different techniques (Soxhlet, maceration, and reflux) using water, methanol, ethanol, ethyl acetate, petroleum ether, and n-hexane. The

extraction yield of the plant sample was calculated by using the following formula:

$$Sample\ Yield\ (\%) = \frac{Weight\ of\ wet\ extract}{Weight\ of\ dried\ extract} \times 100$$

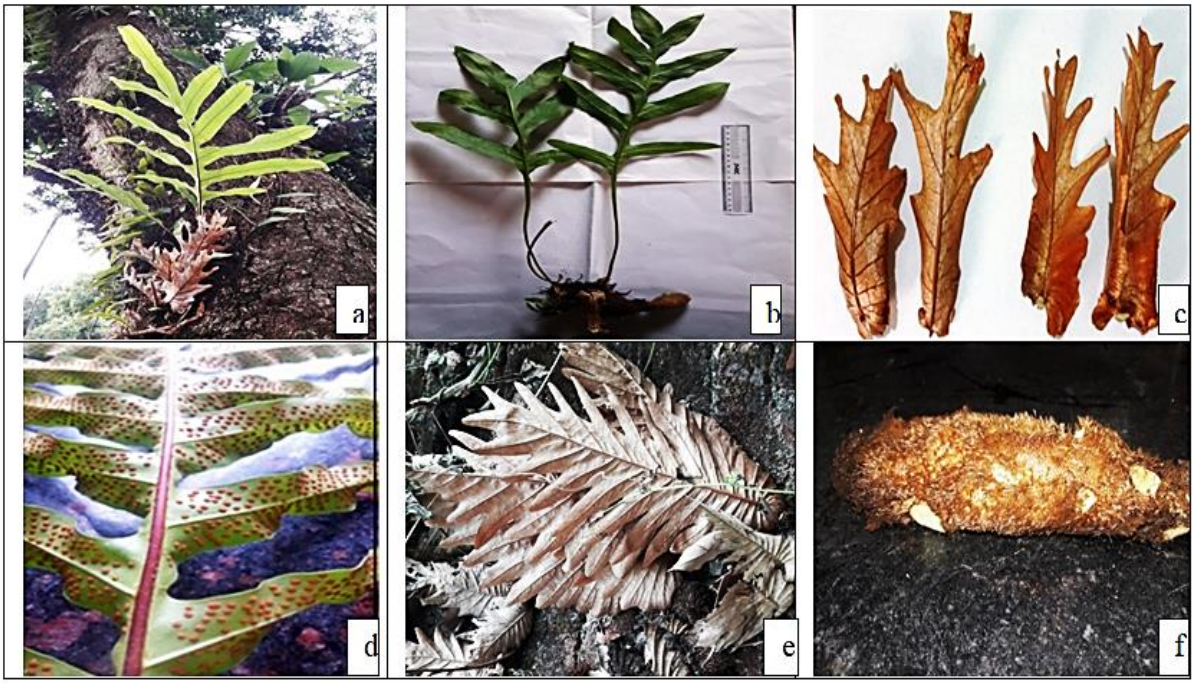

Figure 1. Photography of *Drynaria quercifolia (L.) J. Smith* a) Natural habitat, b) Immature frond (without sorus), c) Immature sterile fronds, d) Mature frond (with sorus), e) Mature sterile fronds, f) Rhizome

Preliminary qualitative phytochemical screening

A preliminary qualitative phytochemical test was performed for alkaloids, phenols, tannins, reducing sugars, saponins, flavonoids, carbohydrates, proteins, and steroids following the methodology of Harborne et al. (1984) and Kokate et al. (2003) with minor modifications.

Result and discussion

Numerous plant-derived compounds make plants valuable sources of novel, physiologically active compounds with antibacterial properties. The successful extraction of a biologically active compound from plant material depends heavily on the type of solvent used. Since the final extraction product contains residual solvent, the solvent must be non-toxic and must not interfere with the bioassay (Ncube et al., 2008). Preliminary phytochemical screening of different solvent extracts of immature and mature fronds of *Drynaria quercifolia* revealed the presence of diverse bioactive compounds. The extracts tested included aqueous (A), methanol (M), ethanol (E), ethyl acetate (EA), petroleum ether (PE), and n-hexane (H).

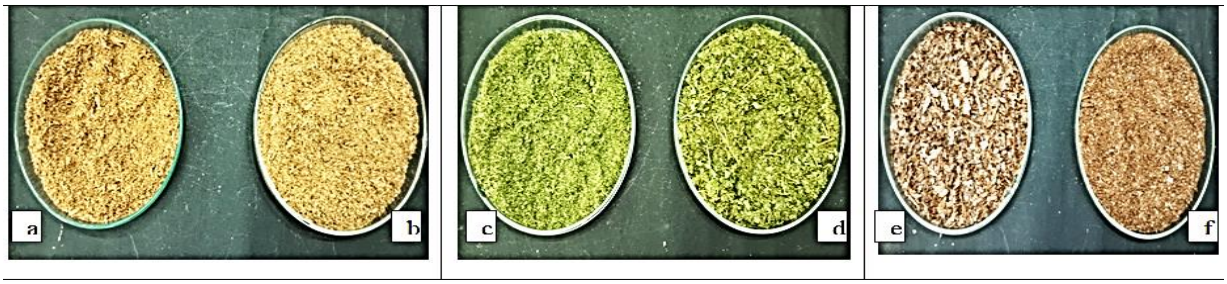

Figure 2. Powdered form of different parts of *Drynaria quercifolia* a) Immature rhizome, b) Mature rhizome, c) Immature frond (without sorus), d) Mature frond (with sorus), e) Immature sterile fronds, f) Mature sterile fronds.

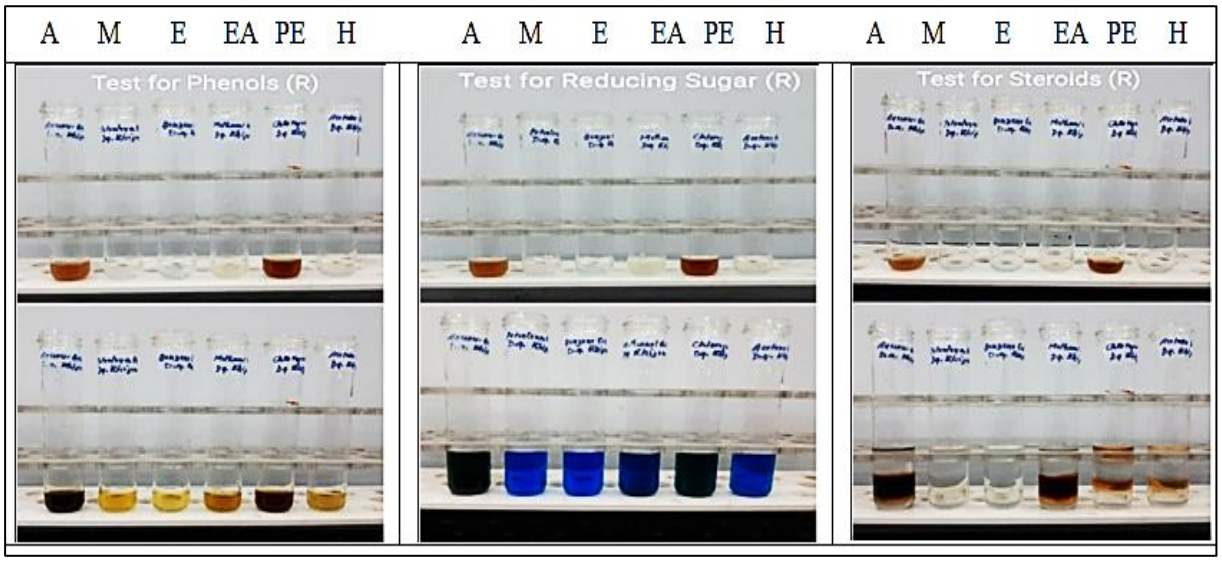

Figure 3. Phytochemical screening of different solvent extracts of the immature rhizome of *Drynaria quercifolia* (A = Aqueous; M = Methanol; E = Ethanol; EA = Ethyl acetate; PE = Petroleum ether; H = n-Hexane)

Table 1. Phytochemical screening of different solvent extracts of *D. quercifolia* (A = Aqueous; M = Methanol; E = Ethanol; EA = Ethyl acetate; PE = Petroleum ether; H = n-Hexane)

Phytoconstituents	**Immature frond**							**Mature frond**					
	A	M	E	EA	PE	H		A	M	E	EA	PE	H
Alkaloids	-	+	-	+	+	-		+	+	-	+	+	-
Carbohydrate	+	+	+	+	+	+		+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+		+	+	+	+	+	+
Coumarins	+	+	+	+	+	+		+	+	+	+	+	+
Phenols	+	+	+	+	+	+		+	+	+	+	+	+
Glycosides	-	-	-	+	-	-		-	+	-	+	-	-
Fixed oil and fats	-	-	-	-	-	-		-	-	-	-	-	-
Protein and aminoacids	+	+	+	+	+	+		+	+	+	+	+	+
Tannins	+	-	+	-	-	-		+	-	+	-	-	-
Saponins	+	+	+	+	+	+		+	+	+	+	+	+
Phytosterols	-	-	-	-	+	+		-	-	-	-	+	+
Phytoconstituents	**Immature rhizome**							**Mature rhizome**					
	A	M	E	EA	PE	H		A	M	E	EA	PE	H
Alkaloids	-	-	-	+	-	-		-	+	+	+	-	-
Carbohydrate	+	+	+	+	-	+		+	+	+	+	-	+
Flavonoids	+	+	+	+	+	+		+	+	+	+	+	+
Coumarins	+	+	+	+	+	+		+	+	+	+	+	+
Phenols	+	+	+	+	+	+		+	+	+	+	+	+
Glycosides	-	+	+	+	+	-		-	+	+	+	+	+
Fixed oil and fats	-	-	-	+	+	+		-	-	-	+	+	+
Protein and aminoacids	+	+	+	+	+	+		+	+	+	+	+	+
Tannins	+	+	+	-	-	-		+	+	+	-	-	-
Saponins	+	+	+	+	+	-		+	+	+	+	+	-
Phytosterols	-	-	-	-	+	+		-	+	-	+	+	+
Phytoconstituents	**Immature sterile fronds**							**Mature sterile fronds**					
	A	M	E	EA	PE	H		A	M	E	EA	PE	H
Alkaloids	-	-	-	-	-	-		-	-	-	-	-	-
Carbohydrate	+	+	+	+	+	+		+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+		+	+	+	+	+	+
Coumarins	+	+	+	+	+	-		+	+	+	+	+	-
Phenols	-	+	+	+	+	+		-	+	+	+	+	+
Glycosides	-	+	+	+	+	-		-	+	+	+	+	-

Fixed oil and fats	-	-	-	-	-	-		-	-	-	-	-	-
Protein and aminoacids	+	+	+	+	+	+		+	+	+	+	+	+
Tannins	-	+	+	-	+	-		+	+	+	+	+	-
Saponins	+	+	+	+	+	+		+	+	+	+	+	+
Phytosterols	-	+	-	+	-	-		-	+	-	+	-	-
+ Positive (present); - Negative (absent)													

Phytochemical screening of immature and mature fronds of *D. quercifolia*

Alkaloids were absent in immature fronds for aqueous, ethanol, and hexane extracts, but present in methanol, ethyl acetate, and petroleum ether. In mature fronds, alkaloids were more widely distributed, being detected in aqueous, methanol, ethyl acetate, and petroleum ether extracts, while absent in ethanol and hexane. Carbohydrates, flavonoids, coumarins, phenols, amino acids, and saponins were consistently present in all solvent extracts of both immature and mature fronds, indicating their abundance and solvent-soluble nature. Tannins were detected in aqueous and ethanol extracts of both immature and mature fronds, while absent in other solvents, suggesting selective solubility. Glycosides were mostly absent, except in the ethyl acetate extracts of immature fronds and in ethyl acetate and methanol extracts of mature fronds, showing limited occurrence. Phytosterols were detected only in petroleum ether and hexane extracts for both immature and mature fronds, reflecting their lipophilic nature. Fixed oils and fats were completely absent in all extracts of immature and mature fronds of *D. quercifolia* (Table 1).

Phytochemical screening of immature and mature rhizomes of *D. quercifolia*

In immature rhizomes, Alkaloids were present only in the ethyl acetate extract. In mature rhizomes, alkaloids were present in methanol, ethanol, and ethyl acetate extracts, but absent in the rest. Carbohydrates were present in aqueous, methanol, ethanol, ethyl acetate, and n-hexane extracts of both immature and mature rhizomes, but were absent in petroleum ether extracts. Flavonoids, coumarins, phenols, and proteins/amino acids were consistently present in all solvent extracts of both immature and mature rhizomes, indicating their abundance and easy solubility across different polarities. Glycosides were present in mature rhizomes, being present in methanol, ethanol, ethyl acetate, petroleum ether, and n-hexane extracts, while in immature rhizomes they were absent in aqueous and n-hexane extracts. Fixed oils and fats were absent in most polar extracts but appeared in ethyl acetate, petroleum ether, and n- hexane extracts of both immature and mature rhizomes, suggesting their lipophilic nature. Tannins were restricted to aqueous, methanol, and ethanol extracts in both immature and mature rhizomes and were absent in less polar solvents. Saponins were generally present in aqueous, methanol, ethanol, ethyl acetate, and petroleum ether extracts of both immature and mature rhizomes but were absent in n-hexane extracts. Phytosterols were absent in immature rhizomes except in petroleum ether and n-hexane extracts, whereas in mature rhizomes they were more widespread, being detected in methanol, ethyl acetate, petroleum ether, and n-hexane extracts.

Phytochemical screening of immature and mature sterile fronds of *D. quercifolia*

Alkaloids were completely absent in all extracts of both immature and mature sterile fronds. Carbohydrates were abundantly present in all solvent extracts of both stages, suggesting their universal distribution and solubility. Flavonoids were consistently detected in all solvent extracts of both immature and mature fronds, indicating high abundance. Coumarins were present in aqueous, methanol, ethanol, ethyl acetate, and petroleum ether extracts of both immature and mature fronds, but were absent in n-hexane extracts. Phenols were absent in aqueous extracts but consistently present in methanol, ethanol, ethyl acetate, petroleum ether, and n-hexane extracts of both immature and mature fronds. Glycosides were detected in methanol, ethanol, ethyl acetate, and petroleum ether extracts of both frond stages, while absent in aqueous and n-hexane extracts. Fixed oils and fats were absent in all solvent extracts of immature and mature sterile fronds. Proteins and amino acids were uniformly present in all solvent extracts of both stages, confirming their essential role in metabolic activity. Tannins showed variable distribution: in immature fronds, they were present in methanol, ethanol, and petroleum ether extracts, while in mature fronds, they were more widely distributed, appearing in aqueous, methanol, ethanol, ethyl acetate, and petroleum ether extracts. Saponins were consistently detected in all solvent extracts of both immature and mature fronds. Phytosterols were

selectively present in methanol and ethyl acetate extracts of both frond stages, while absent in the rest.

Extraction yield/percentage

The extraction yields of fronds (immature and mature), rhizomes (immature and mature), and sterile fronds (immature and mature) of *D. quercifolia* (Fig. 4) were evaluated using three different techniques (Soxhlet, maceration, and reflux) across six solvents of varying polarity. The results indicated clear differences in solvent efficiency and extraction method. The highest yield was obtained with methanol Soxhlet extraction of Immature frond (15.14%), mature frond (15.80%), immature rhizome (14.48%), mature rhizome (15.40%), immature Sterile Fronds (10.66%), and mature Sterile Fronds (11.53%), followed closely by aqueous Soxhlet extraction. Maceration and reflux extractions produced comparatively lower yields across all solvents, indicating reduced efficiency of these methods as compared with the Soxhlet method of extraction. Reflux with n-hexane resulted in the lowest yield in all solvents used, indicating the limited efficiency of this combination. The highest yields were exhibited with polar solvents (methanol, aqueous) and the lowest yields with non-polar solvents (petroleum ether, n-hexane) across all three methods.

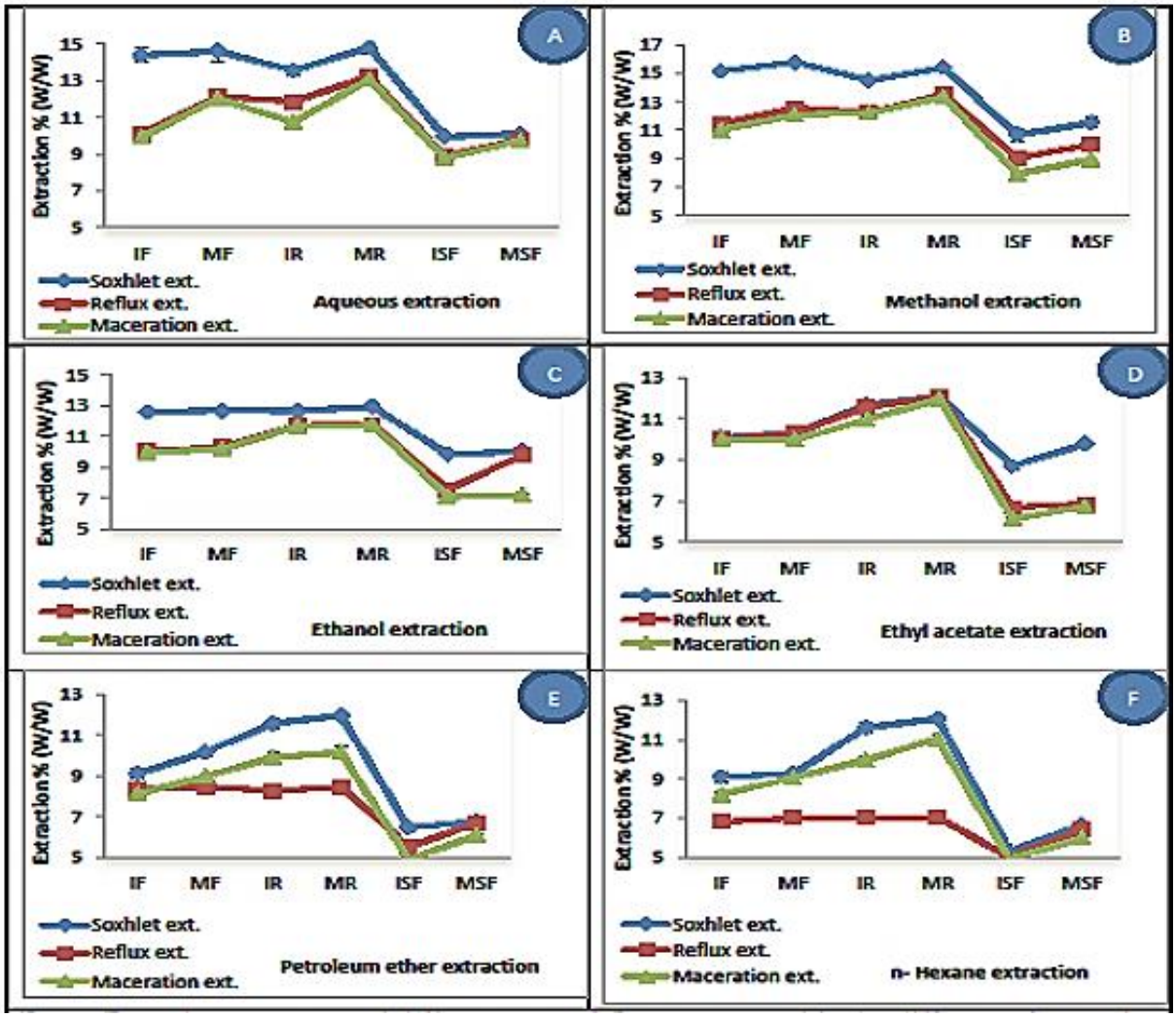

Figure 4. Extraction percentage of different parts of *Drynaria quercifolia* by different solvents using Soxhlet, Ruflux, and Maceration extraction methods. IF = Immature frond (without sorus), MF = Mature frond (with sorus), IR = Immature rhizome, MR = Mature rhizome, ISF = Immature sterile fronds, MSF = Mature sterile fronds. Means ± SE, n=3; ($p<0.05$).

Conclusion

In recent years, bioactive substances from medicinal plants with pharmacological properties have received a great deal of attention as potential sources of pharmaceuticals. *Drynaria quercifolia* (L.) J. Smith is an important ethnomedicinal plant. The present study is the first study reporting information on these ethno-medicinal ferns with

respect to the whole plant parts. The phytochemical investigation of medicinal plants plays a vital role in understanding their therapeutic potential. It is also of great commercial importance to research institutions and pharmaceutical companies. Such studies help in identifying bioactive compounds that can be used to develop new herbal medicines for treating various plant and human diseases. Through detailed phytochemical analysis, scientists can identify natural substances that may lead to safer, more effective, and affordable drug formulations.

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Conflict of interest: The authors declare that they have no conflict of interest.

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