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HPTLC Finger Printing Profile Of *Drynaria Quercifolia* (L.) J. Sm. With Biomarker Naringin

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ABSTRACT: -

Drynaria quercifolia (L.) J. Sm. is a common and safe medicinal fern of tropical climate, used by Ayurvedic physicians of Kerala and tribal peoples in different parts of the country for various ailments with inflammation. But, to get a desired therapeutic effect the drug must be genuine with good quality. Authentication and quality of plants can be accurately done by doing HPTLC fingerprints with biomarker. Development of HPTLC finger printing profile of aqueous extract of rhizome of the plant with biomarker naringin was the aim of this study. Precoated silica gel 60 F_{254} was the stationary phase and ethyl acetate, formic acid, acetic acid, water in the ratio -1.5:1.1:1.1:0.1 was the mobile phase used for this purpose. After derivatization with methanolic sulphuric-10%, TLC plates were viewed at UV 366 nm and in visible light and densitometric profile were developed at 590 nm. At 590 nm, chromatogram of the aqueous extract of the drug showed 4 peaks and the 3rd peak was found to be homologous to the chromatogram of naringin. Thus the study revealed the identification as well as genuineness of the plant and has shown the potency of the drug as an anti-inflammatory agent.

Key Words: HPTLC, *Drynaria quercifolia*, biomarker, Ayurveda, naringin, inflammation

Research Article.

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INTRODUCTION

Drynaria quercifolia (L.) J. Sm. is a medicinal fern indigenous to tropical areas of India, Sri Lanka, Malaysia, Indonesia, Philippines and Oceania. It is found as an epiphytic, epipetric or terrestrial fern in open forests, rain forest margins and in dry rain forest. It has a creeping fleshy rhizome with a dense covering of reddish brown soft scales. Fertile foliage fronds and sterile nest fronds arises from this rhizome. Large sized and winged foliage fronds bear sori on its bottom surface. The persisting stiff nest fronds seen basal to foliage fronds forms a characteristic 'basket' that collects litter and organic debris. So it is known as Basket fern in English.[1], [2], [3]

Hortus Malabaricus, a compilation work on plant wealth of Kerala elaborately describes about this fern with its medicinal uses in diseases such as blackening aphthous, burning sensation of viscera, etc.[4] References from the renowned Malayalam medical text books of Kerala such as *Chikitsamanjari* and *Yogamrutam* also shows the therapeutic uses of this rhizome in diabetic carbuncle, [5] abscess, [5] scrofula, [6] fissure in ano [6] etc. *Ellumnisadi choorna* is a very popular formulation among the Ayurvedic physicians in Kerala for management of painful swelling in knee associated with rheumatism.[5] Tribal use of this rhizome in swelling, fever, intestinal worms etc. is also reported. All these shows the anti-inflammatory potential of this rhizome. A number of in-vitro and in-vivo studies of this rhizome demonstrated its anti-inflammatory, anti-pyretic, anti-arthritic, anti-microbial and anti-ulcer activities. The rhizome of this plant contains a large number of phytoconstituents. Among them the specific chemical constituents within the plant used to verify the identity or potency of this plant is naringin.[1]

High Performance Thin Layer Chromatography (HPTLC) helps in separation of various components of the plant extracts acts as a tool for identification of the plants and to check its purity and quality. A chromatographic fingerprint shows the chromatographic pattern of the pharmacologically active chemical constituents in a plant extract.[7]So chromatographic fingerprints with biomarker helps in accurate authentication as well as quality of the plants.[8]

Even though high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) methods of extracts from the rhizome of this plant is reported in the literature, there is no HPTLC method of aqueous extract with marker compound naringin.[3], [9]Thus the present study is aimed at development of HPTLC finger printing profile of aqueous extract of rhizome of *Drynaria quercifolia* (L.) J. Sm. (DQ) along with biomarker naringin.

MATERIALS AND METHODS

Collection of plant material

The plant used for this study were collected from Vadakara village, Ernakulam District, Kerala during the month of July 2019. The identification was done in Pharmacognosy unit, Department of Dravyaguna vijnanam, Government Ayurveda College, Tripunithura, Ernakulam, Kerala.

Preparation of plant

The fronds and outer hair on the rhizome were removed and the rhizome was cleaned thoroughly. It was cut into small pieces and dried under shade. It was then powdered and passed through a sieve of 120 mesh size. 11. 11 gm of this powder was kept in 50 ml water for 24 hours for maceration. From this, 0.067 ml of the residue were taken and made up to 1 ml with ethanol.

Preparation of standard

Commercially available Naringin standard

Chromatographic conditions and procedure

TLC plate consists of 5×10 cm precoated silica gel 60 F_{254} (E MERCK K Ga A) without prewashing. DQ was spotted on the stationary phase at 15 mm from the edge of the plate and the standard was spotted 20mm away from DQ using CAMAG Linomat 5 sampler. Application rate was 150 nl per seconds. The mobile phase used was ethyl acetate: formic acid: acetic acid: water (1.5:1.1:1.1:0.1). TLC plate was prepared on saturated twin through glass chamber of 20×10 cm size and the solvent front position was 80 mm. Plates were dried at 60ºC for 5 minutes and transferred to CAMAG visualizer under UV 254nm and UV 366nm. Post chromatographic derivatization was done using methanolic sulphuric-10% and the plates were dried in oven at 120ºC for 20 minutes. The slit dimension for densitometry was 10×0.2 mm and the scanning speed was 20mm per seconds. Then densitometric profile was recorded at 590nm.

RESULTS

At 590 nm, HPTLC chromatogram of DQ showed 4 peaks and naringin showed a single peak. The Rf values, area and area percentage of DQ and naringin are given in Table 1 and Table 2 respectively.

Table 1: Peak and area of aqueous extract of *Drynaria quercifolia* (L.) J. Sm. at 590 nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.07	-0.01	0.02	3781.7	29.57
2	0.03	0.08	0.15	7457.5	58.31
3	0.22	0.25	0.30	673.6	5.27
4	0.38	0.42	0.48	876.2	6.85

Table 2: Peak and area of Naringin at 590 nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	0.18	0.23	0.27	924.9	100

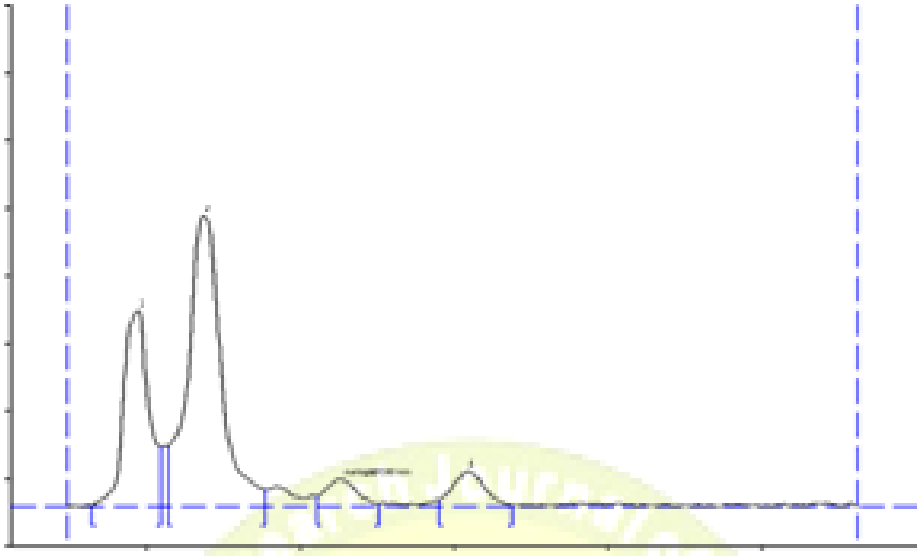

Figure 1: Chromatogram of aqueous extract of *Drynaria quercifolia* (L) J. Sm. at 590 nm

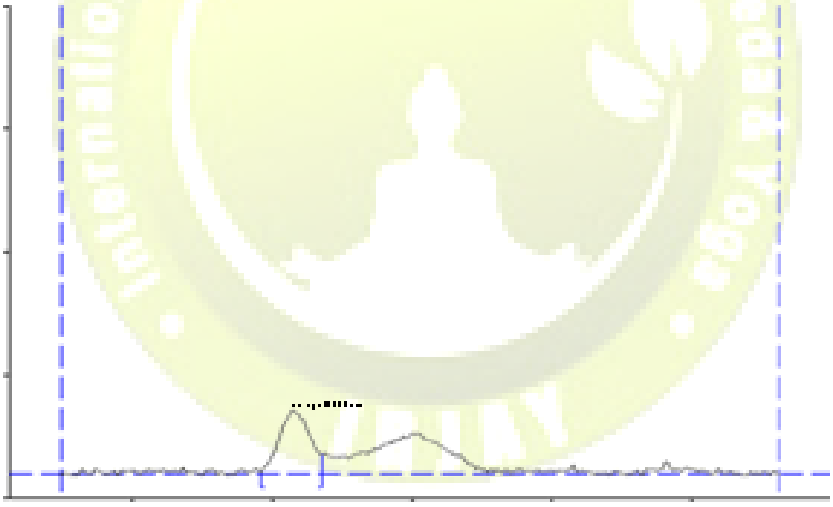

Figure 2: Chromatogram of Naringin at 590 nm

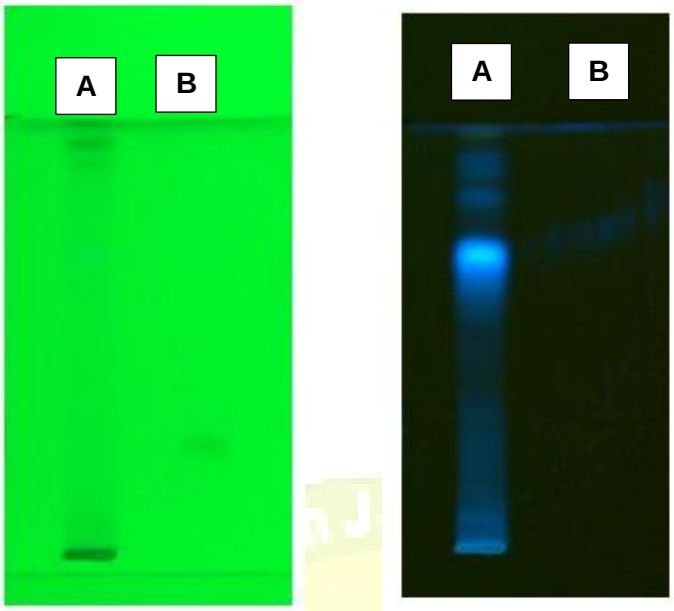

Figure 3: TLC plate view before derivatization
(A: *Drynaria quercifolia* (L.) J. Sm. B: Naringin)

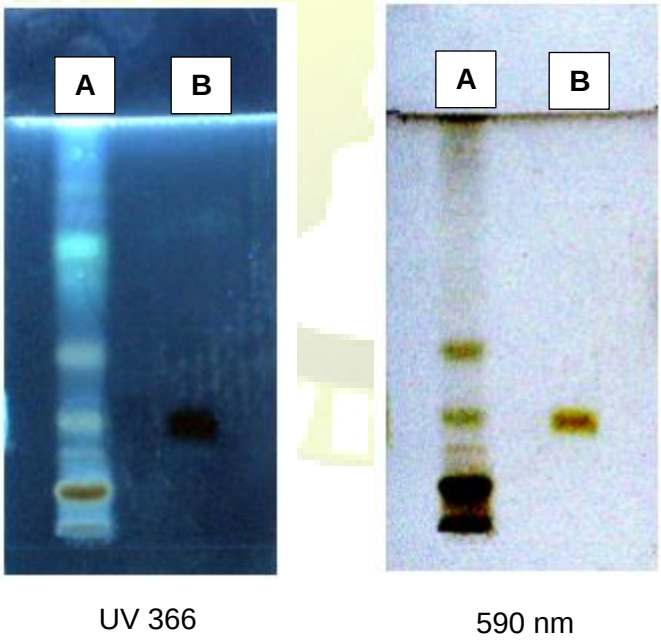

Figure 4: TLC plate view after derivatization
(A: *Drynaria quercifolia* (L.) J. Sm. B: Naringin)

DISCUSSION

Drynaria quercifolia (L.) J. Sm. is an easily available fern in tropical climatic conditions, which has no toxic effect. Its rhizome is used by Ayurvedic physicians in Kerala for the preparation of various kinds of medicinal formulations. In order to get the best therapeutic

effect of a formulation, all the ingredients used must be genuine with good quality. So assurance of quality of raw drugs is an important factor in pharmaceutical industry. HPTLC fingerprinting profile with biomarker is an important tool for the identification and the standardization of medicinal plants.[10] Owing to its high separation power, performance and reproducibility, it is considered superior to classical TLC.

Naringin is a flavanone glycoside formed from the flavanone naringenin and the disaccharide neohesperidose, [11] is identified as a marker phytochemical in the plant *Drynaria quercifolia* (L.) J. Sm.[1] The literature survey on the drug as well as naringin revealed that both possesses anti-inflammatory, antioxidant, anti-ulcer, anti-carcinogenic and anti-osteoporotic properties.[1], [11]

On HPTLC finger printing profile of DQ at 590 nm, 4 peaks were found. The third peak in chromatogram had an Rf start at 0.22 and end at 0.30 and the chromatogram of the naringin at 590 nm had an Rf start at 0.18 and end at 0.27. This showed that the third peak of DQ was homologous to naringin. That means DQ contained the Active phytoconstituent naringin.

Plants contains a large number of phytoconstituents in it. Development of HPTLC finger-printing profile of the pharmacologically active specific chemical constituent of herbal medicines plays key role in correct identification and to confirm the quality as well as purity of the drug. In the present study, the HPTLC fingerprinting profile of aqueous extract of rhizome of *Drynaria quercifolia* (L.) J. Sm. with biomarker naringin showed the identification as well as genuineness of the plant and revealed the potency of the drug as an anti-inflammatory agent. The present HPTLC fingerprinting profile can be used as a diagnostic tool to identity and to determine the quality and purity of this drug in future studies.

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Conflict Of Interest: NIL
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