GC-MS ANALYSIS OF SECONDARY METABOLITES FROM THE WHOLE PLANT METHANOLIC EXTRACT OF DRYNARIA QUERCIFOLIA (L.) J. SMITH (POLYPODIACEAE)

Kalpana Devi Rajesh¹*, Vasantha Subramanian¹, Annamalai Panneerselvam¹, Nakulan Valsala Rajesh² and Nallaperumal Jeyathilakan³

¹PG and Research Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Thanjavur – 613503, TamilNadu, India.
²Veterinary University and Training Centre, TANUVAS, Ramanathapuram – 623 503, TamilNadu, India
³Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu – 614 625, TamilNadu, India

*Corresponding author E-mail: kalpanafern@gmail.com

Abstract

To investigate the secondary metabolites present in methanolic extract of Drynaria quercifolia (L.) J. Smith (Polypodiaceae). GC-MS analysis of whole fern extract were performed using a Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited. The instrument was set as follows, Injector port temperature set to 250ºC, Interface temperature set as 250ºC, and source kept at 200ºC. The oven temperature programmed as a variable, 70ºC for 2 mins, 150ºC @ 8ºC/min, up to 260ºC @ 10ºC/min. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS Nonpolar column was used whose dimensions were 0.25 mm OD x 0.25 μm ID x 30 metres length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The results of the GC-MS analysis confirmed the presence of 9 compounds. The most prevailing compounds in this study are Tetrahydroisovelleral, 7, 10, pentadecadiynoic acid (CAS), Phosphoric Acid, octyl diphenyl ester (CAS), Octicizer, Phosphoric acid, 2-ethylhexyl diphenyl ester (CAS), QUERCETIN 7, 3´, 4´ TRIMETHOXY, 1, 30-Triacetanediol, Ergost-5-en-3-ol, (3a´-(CAS), and Lucenin 2 found to have significant medicinal property. It can be concluded that the plant extract show the presence of 9 phytocompounds. The presence of various bioactive compounds justifies the use of the whole fern for various ailments by traditional practitioners.

Keywords: Drynaria quercifolia (L.) J. Smith, Secondary metabolites; GC-MS analysis; Whole fern

Note: All the figures and tables are listed in supplementary article
Introduction
Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards. GC-MS is the best technique to identify the secondary metabolite constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc. *Drynaria quercifolia* (L.) J. Smith, commonly known as the oakleaf fern, is a species of basket fern in the family Polypodiaceae. Other common names for the fern are pakpak lawin, gurar, koihin, ashvakatri, or uphatkarul. It is a large species 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. The ferns are characterized by the presence of two types of fronds, fertile foliage fronds and sterile nest fronds. The dark green foliage fronds are large, 2–4 feet (0.61–1.22 m) long, with elongated stalks. They are deeply lobed or pinnate, winged, and bear sori (structures producing and containing spores) on the bottom surfaces. The nest fronds are smaller rounded leaves basal to the foliage fronds. They do not bear sori and are persistent, not being shed after turning brown and dying. They form a characteristic 'basket' that collect litter and organic debris, hence the common name. The collected debris decompose into humus, providing the plants with nutrients it would otherwise not have received from being suspended above the ground. Both frond types grow from rhizomes typically anchored to a tree or a rock. The rhizomes of Drynaria are creeping and densely covered in brown scales. Basket ferns are epiphytic (growing on trees) or epipetric (growing on rocks). They can also sometimes be found in man-made structures like brick walls. They are found in wet tropical environments, usually in rainforests. Their native range extends from equatorial Africa to tropical South and East Asia, Southeast Asia, Australia, and Oceania. Numerous ethnopharmacological studies have been conducted into the properties of Drynaria. In agreement with their use in traditional medicine, several studies have shown that basket...
ferns (D. roosii in particular) are effective in preventing resorption of bone cells and osteoporosis, increases bone density, and have therapeutic effects on bone healing. They have also been shown to possess a wide range of antimicrobial activity [11]. Rhizoma drynariae has immune-promoting, anti-inflammatory, and neuroprotective effects [12].

Extracts from the rhizomes of some Drynaria species are used extensively in traditional medicine. In China, Taiwan, Vietnam, Thailand, and Laos, the rhizomes of Gu-Sui-Bu Drynaria roosii (more frequently cited by Asian authors by its synonym Drynaria fortunei), are commonly used to treat bone injuries. Its common name literally means "mender of shattered bones" in Chinese. Another species, the oak-leaf fern (Drynaria quercifolia) is used similarly in South Asia and Maritime Southeast Asia.

Species of Drynaria commonly used in traditional medicine like D. roosii and D. quercifolia are in danger of being overexploited. None of the species are currently cultivated for the alternative medicine industry [13]. Drynaria are also considered endangered in some areas (like in New South Wales, Australia), due to threats of habitat loss and low population numbers. Taking into consideration of the medicinal importance of this plant, the methanol extract of whole plant of Drynaria quercifolia (L.) J. Smith were analyzed for the first time using GC-MS. Perusal of literature reveals that information on the GC-MS analysis of Drynaria quercifolia (L.) J. Smith is totally lacking. Hence, the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique.

Materials and Methods

Collection and Identification

The whole plant of Drynaria quercifolia (L.) J. Smith was collected during the month of January 2015, from the Kothayar region, Western Ghats, Tamil Nadu. The fern was identified taxonomically and authenticated by Department of Botany, Scott Christian College, Nagercoil and a voucher specimen for herbarium was preserved at A.V.V.M Sri Pushpam College, Thanjavur, Tamil Nadu, India. The ferns were shaded dried and pulverized to powder in a mechanical grinder and stored in polypropylene air-tight containers under proper conditions for further uses.

Preparation of the fern extract

Preparation of the fern extracts was assessed by following method as described by [14]. One gram of dried powder of plant materials were extracted with 20 mL aqueous for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-vator at 40 °C to a constant weight and then dissolved in
respective (methanol) solvents. The dissolving rate of the crude extracts was approximately 100%. The solution was stored at 18 °C until use.

**Extraction of active compounds by column chromatography**

The concentrated extract in aqueous extract was separated and analysed by Column chromatography technique as per standard methods. Silica gel (100 - 200 mesh - Fisher Scientific – India) were washed thoroughly using methanol solvent for 3 times. The cleaned silica gel, 10gm of silica gel was dissolved with 20 ml of Double distilled water; the slurry of semisolid / liquid silica gel carefully poured to column without any air bubbles. Concentrated sample plant extract (10mg /ml) was carefully transferred on to the upper surface of silica gel. The Mobile phase used for extraction was methanol: chloroform (2:1) ratio. The eluent is slowly passed through the column. For visualization of phenolic compound, Folin-cioclaute reagent was used as the spraying agent.

**GC–MS Analysis**

GC-MS analysis was performed in separated fraction of fern extract having high anti-oxidant potential (Fraction VIII) under column chromatography. Based on the anti-parasitic assay, the most effective fraction of the methanolic extracts of the fern (F8) was further used for the identification of bioactive constituents by GC– MS analysis, at The South India Textile Research Association (SITRA), Coimbatore, Tamil Nadu, India. The Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited, were engaged for analysis\textsuperscript{[15]}. The instrument was set as follows, Injector port temperature set to 250°C, Interface temperature set as 250°C, and source kept at 200°C. The oven temperature programmed as a variable, 70°C for 2 mins, 150°C @ 8°C/min, up to 260°C @ 10°C/min. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS Nonpolar column was used whose dimensions were 0.25 mm OD x 0.25 μm ID x 30 metres length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200°C and < 40 motor vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

**Identification of compounds**

Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology NIST4 and WILEY9\textsuperscript{[16]}. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.
**Results**

The compounds present in the methanol extract of whole plant of *Drynaria quercifolia* (L.) J. Smith were identified after separation of fraction under column chromatography by GC-MS analysis (Fig.1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area % in the methanol extract of whole fern of *Drynaria quercifolia* (L.) J. Smith are presented in Table 1. The prevailing compounds in methanol extract of whole plant were Tetrahydroisovelleral, 7, 10, pentadecadiynoic acid (CAS), Phosphoric Acid, octyl diphenyl ester (CAS), Octicizer, Phosphoric acid, 2-ethylhexyl diphenyl ester (CAS), QUERCETIN 7, 3’, 4’-TRIMETHOXY, 1, 30-Triacotanediol, Ergost-5-en-3-ol, (3a´- (CAS), and Lucenin 2 (Fig 2-7) shows mass spectrum and structures of various secondary metabolites present in *Drynaria quercifolia* (L.) J. Smith. Table 2 listed the major phyto-components and its biological activities obtained through GC-MS study of *Drynaria quercifolia* (L.) J. Smith.

**Discussion**

Authentication of medicinal plants as genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations. For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. In plant taxonomy, during this molecular era, the morphological characters also play a vital role in plant systematic study and used as a tool for the classification of a taxon. In recent times, in addition morphological markers, anatomical, cytological, biochemical, and molecular markers are also being used to classify the organisms. Gas Chromatography-Mass Spectrometry (GC-MS) is a valuable tool for reliable identification of phytocompounds [17] [18]. In the present study, 9 compounds have been identified from the methanol extract of the whole plant of *Drynaria quercifolia* (L.) J. Smith by Gas Chromatography-Mass Spectrometry analysis. Among the identified phytochemicals, QUERCETIN 7, 3’, 4’-TRIMETHOXY, is a flavonoid derivatives had significant antioxidant activity and are used as anthelmintic drugs and anti-microbial drugs [19]. Ergost-5-en-3-ol, (3a´- (CAS) is a steroid had anti-inflammatory, analgesic and antipyretic property [20]. Lucenin 2 is used as an antibacterial, anthepatotoxic, antioxidant and anti-microbial and agents[21]. Thus this type of GC-MS analyses is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study. Further investigation into the pharmacological of *Drynaria quercifolia* (L.) J. Smith and their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.
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References

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