Anti-dermatophytic activity of *Drynaria quercifolia* (L.) J. Smith

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Abstract

*Drynaria quercifolia*, a non flowering group of plant, is being used by the tribals against skin diseases. It is found to be growing in rain forest of Western Ghats of Maharashtra. The aim of the present study was to assay antidermatophytic activity different extract of *D. quercifolia*. Four different solvents such as ethanol, methanol, acetone, di-ethyl ether and water, were used to extract the bioactive compounds from the rhizome of *D. quercifolia*. Agar dilution and disk diffusion methods were used to screen the antidermatophytic activity against infectious disease causing pathogenic fungi such as *Trichophyton mentagrophytes*, *Microsporum canis*, *M. gypseum*, *T. rubrum* and *Epidermophyton floccosum*. The ethanol extract of the dried rhizome of *D. quercifolia* did not show inhibitory activity up to concentration of 20mg ml⁻¹. The solvents of acetone, methanol and water also did not show any efficacy for extraction from *D. quercifolia* rhizome but di-ethyl ether with semi-polarity gave clear zone to antifungal activity compounds. Also high performance thin layer chromatography studies confirmed that the ethyle acetate extracts of rhizome of *D. quercifolia* contains triterpenes and coumarins and since coumarins soluble in semi-polar di-ethyl ether solvent, may be these compounds are responsible for antidermatophytic activity of this plant.

Keywords: Antidermatophytic activity, *Drynaria quercifolia*, Dermatophyte

Introduction

*Drynaria quercifolia* (L.) J. Smith belongs to Pteridophyta, and family Polypodiaceae. The plant is an epiphytic fern with a short thick, fleshy, creeping rhizome [1]. It is found to be growing in rain forest of Western Ghats of Maharashtra, India. The rhizome paste is applied for treatment of diarrhoea, typhoid, cholera, chronic jaundice, fever, headache and skin diseases. Whole plant is anthelmintic, expectorant, tonic and used in the treatment of chest and skin diseases [2,3]. From methanolic extracts of *D. quercifolia* dried rhizome were isolated epifriedelinol, beta-amyrin, beta-sitosterol, beta-sitosterol 3-beta-D-glucopyranoside, and naringin and gave positive test for coumarins and triterpenes. Despite being the methanolic extract of *D. quercifolia* rhizome showed inhibitory activity against bacteria by the agar-well diffusion method but showed negative activity against fungi [4]. The main aim of the present investigation was to screen the crude extract of *D.
*Quercifolia* rhizome prepared in organic solvents of different polarity such as ethanol, methanol, acetone, di-ethyl ether as well as water also for *in vitro* assay and the chemical composition of responsible for antidermatophytic activity of this plant.

**Materials and Methods**

**Plant material and extractions**

The material used in the present study is the rhizome of *D. quercifolia* that it was collected from Dapoli of Ratnagiri District of Maharashtra State, India and identified by Botanical Survey of India (BSI). A voucher specimen has been deposited at the herbarium of botany department of Pune University, India. The rhizome is covered with small brown coloured hair. They were removed using sterile scalpel and washed with sterile distilled water. They were cut in to small pieces and dried in shade to avoid decomposition of chemical constituents and made into fine powder by using grinder and stored in clean and dry airtight containers for extraction of bioactive compounds.

**Preparation of plant extracts**

Ten gram of the powdered of *D. quercifolia* rhizome was added to 100ml ethanol 80% in a conical flask for maceration. Flask plugged with cotton and kept on a rotary shaker at 190-220rpm for 3×24h at room temperature [5]. The suspension was filtrated through a Buckner funnel with Whatman filter paper #1. The ethanolic extracts were evaporated to dryness in room temperature. Finally one gram of dryness extract dissolved in one millilitre dimethyl sulfoxide (DMSO). Final concentration of this extract adjusted to 1000 mg ml$^{-1}$.

Then 5-10ml different solvents with various polarities like diethyl ether, acetone, methanol and water was added to conical flasks contain dryness plant extracts. Conical flasks were stirred slowly for dissolving antifungal active fractions in those solvents. Finally, four extracts were obtained by as mentioned above solvents.

**Dermatophyte isolates and fungal inoculums preparation**

In the present study three strains of dermatophytes were obtained from the Persian Type Culture Collection (PTCC) Tehran, Iran viz. *Trichophyton mentagrophytes* PTCC5054, *Microsporum canis* PTCC5069, *M. gypseum* PTCC5070. In addition, 13 isolated of dermatophytes were collected from different lesions of patients at the department of dermatology, Imam hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Dermatophytes were identified by standard procedure [6]. These dermatophytes are follows; *M. canis* (n=2), *M. gypseum* (n=3), *T. rubrum* (n=2), *T. mentagrophytes* (n=3), and *Epidermophyton floccosum* (n=3). Sabouraud’s dextrose agar (SDA; Hi Media, India) at 25ºC was used to maintain isolates. In antifungal assays, each dermatophyte isolate was sub cultured onto SDA slants and incubated at 28-30ºC for four to five days and sub cultured every 15 days to prevent pleomorphic transformations.

A standardized inoculum was prepared by counting the microconidia microscopically. A suspension of conidia was prepared by using sterile distilled water or 0.85% physiological saline solution. The suspension was added to the slant tube culture and gently swabbing the colony surface with a sterile bent glass rod to dislodge the conidia from the hyphal mat. The suspension was transferred to a sterile centrifuges tube and the volume was adjusted to 5-10ml with sterile normal saline. The final suspension of conidia was counted with a hemocytometer cell counting chamber. The inoculums of cell or spore suspensions were obtained according to reported procedure of [7] and adjusted to $10^4$-$10^5$ Cells/spores with colony-forming units (CFUml$^{-1}$).

**Antifungal susceptibility testing**

The fungistatic activity of the rhizome extract of *D. quercifolia* was evaluated by the agar dilution method according to
procedures of Souza et al. [8]. In addition, the fungistatic activity of extract was also evaluated by the disk diffusion method [5]. 100µl of dermatophytic suspension of \(10^5\) CFU/ml was pipetted onto petri dishes containing SDA and uniformly spreading by using a sterile bent glass rod. Sterile filter blank disks (7mm diameter) were impregnated with 10-20µl obtained extracts with as mentioned various polarity solvents. Disks were placed on the surface of solid agar petri dishes that were inoculated with the dermatophyte suspension. The plates were incubated at 35°C. Following an incubation period of 48-72h the diameter of the zone of inhibition around each disk was measured in millimetres (Fig. 1).

**High performance thin layer chromatography**

One gram of the powdered rhizome of *D. quercifolia* was added to 10ml of ethyl acetate. Mixture was kept on a rotary shaker at 150 rpm for 3h at room temperature and centrifuged at 10,000 rpm for five minutes. Supernatant was collected and concentrated from 100ml to 20ml. Then 5-10µl of concentrated supernatant was used for high performance thin layer chromatography (HPTLC). For the separation of compounds of herbal extract, 5µl of the ethyl acetate of extract was analyzed by thin layer chromatography (TLC) by using aluminum-backed TLC plates (silica gel 60 F 254, E. Merck). The TLC plates were developed with the mobile phase system of chloroform. The mobile phase was removed from the plate by drying in the room temperature. The developed plates were sprayed with Iodine reagent to check presentation of spots and observed in UV light using TLC Scanner [9].

**Results and Discussion**

The ethanolic extract of *D. quercifolia* rhizome did not show any inhibition in the concentration of up to 20mg ml\(^{-1}\) by agar dilution method. In addition, the solvents of acetone, methanol and water did not also show any efficacy by disk diffusion method. However di-ethyl ether extract with semi-polarity showed high affection against *T. mentagrophytes* that it was been inactive with ethanolic extract (inhibition zone of 25 mm diameter).

Results on HPTLC indicated that the ethyl acetate extract of *D. quercifolia* rhizome contains coumarins [9]. It was revealed as a prominent quenching zone (blue fluorescence of the coumarins) in UV-366nm before derivation and in UV-254nm with nine peaks (Figs. 2,3). In UV-366nm after derivation with vanillin sulphuric acid it was revealed with seven peaks (Figs. 4,5). Also the ethyl acetate extract of *D. quercifolia* rhizome according [9] contains triterpenes that it was revealed a blue fluorescent zone of triterpene in UV-366 nm before derivation and in UV-254 nm with six peaks (Figs. 6,7). In UV-366nm after derivation with anisaldehyde sulphuric acid it was revealed 13 peaks and a violet zone at visible (Figs. 8,9).

Review of literature revealed that this kind of study has not been reported so far. This is the first report on the antifungal activity of the ethanolic extract of *D. quercifolia* rhizome on dermatophytic species. But as per the earlier reports methanolic extract of *D. quercifolia* rhizome showed inhibitory activity by the agar-well diffusion method against all tested bacteria.
like *Kelebsiella pneumoniae*, *Salmonella typhi*, *Vibrio cholerae*, *S. aureus* and *Bacillus subtilis*. But no activity was observed against fungi tested like *Aspergillus flavus*, *A. niger* and *Candida albicans* [4].

In conclusion, since, coumarins only dissolve in semi-polar solvent like di-ethyl while terpenoids dissolve in solvents like water, ethanol and methanol [10]. Hence, coumarins can be responsible probably for antidermatophytic activity of this plant.

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**Fig. 2**: Coumarins before derivation (In UV-366 nm)

**Fig. 3**: Estimation of coumarins at 254 nm from *D. quercifolia* rhizome

**Fig. 4**: After derivation with vanillin sulphuric acid (In UV-366 nm)

**Fig. 5**: Estimation of coumarins at 366 nm after derivation from *D. quercifolia* rhizome
Fig. 7: Triterpenes before derivation (In UV-366nm)

**Fig. 7:** Estimation of triterpen at 254 nm from *D. quercifolia* rhizome

Fig. 8: Image at visible after derivation with anisaldehyde sulphuric acid

**Fig. 9:** Estimation of triterpen at 366 nm after derivation from *D. quercifolia* rhizome

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