



RESEARCH ARTICLE

**PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF
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Abstract

Drynaria quercifolia, (L.) J.Smith a non-flowering group of plant found to be growing in rain forest of Western Ghats of Maharashtra. The rhizome paste is applied for treatment of diarrhea, typhoid, cholera, chronic jaundice, fever, headache and skin diseases. In this present study the bioactive compounds from *Drynaria quercifolia* was extracted using different solvents –ethanol, methanol, chloroform and petroleum ether. Preliminary screening for the phytochemical compounds in the extracts were performed and the extracts were then examined for antibacterial and antifungal activity by disc diffusion method. Compounds like saponin, coumarin, terpenoids were detected in ethanol extract. Similarly ethanol extract showed greater antibacterial and antifungal activity, followed by methanol extract.

Keywords: *Drynaria quercifolia*, rhizome paste, bioactive compound, antibacterial and antifungal activity.

Introduction

Medicinal plants are the most important source of life saving drugs for the majority of world's population. Over centuries cultures around the world have learned how to use plants to fight illness and maintain health. In India, around 20,000 medicinal plants have been recorded however traditional communities are using only 7,000 -7,500 plants for curing different diseases. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.

Ferns play an important role in folklore medicine. A systematic survey of medicinal use of fern has been scarcely undertaken. Chopra and his colleagues (1933) and Kirtikar and his colleagues (1975) worked on 44 and 27 species of ferns

respectively and reported on the medicinal uses of these Pteridophytic plants.

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 rhizome paste is applied for treatment of diarrhea, typhoid, cholera, chronic jaundice, fever, headache and skin diseases. Whole plant is anthelmintic, expectorant and tonic (Viswanathan, 1995; and Chopra RN, Nayar SL, Chopra IC, 1956). An epiphytic fern *Drynaria quercifolia*, commonly called Oak leak Fern, is used in medicinal system by different groups of people to treat various kinds of health problems including urinary tract infection (Sen & Ghosh, 2011). It is also used in the treatment of chest disease, cough, hectic fever, dyspepsia, loss of appetite, chronic jaundice and cutaneous infections (Khare, 2007).

Tribals in kalakad, Mundanthurai Tiger Reserve India, use this rhizome of this fern to cure rheumatism (Sutha *et al*, 2010).The rhizome of this fern is one of the twelve ingredients of a drug to treat cancer (Saetung *et al*,2005).

In this present study the bioactive compounds from *Drynaria quercifolia* was extracted using different solvents and preliminary screening for the phytochemical compounds in the extracts were performed. The extracts were then examined for antibacterial and antifungal activity.

Materials and Methods

Description of the Plant

Drynaria quercifolia (L.) J.Smith

This Plant belongs to Pteridophyta and family Polypodiaceae. It is an epiphytic fern. The rhizome is thick, fleshy covered with small brown coloured hairs. The plant has small sterile fronds and long stalked fertile fronds. The rhizome is used as medicine by the tribals.

Plant Collection and Processing

The Rhizome of *Drynaria quercifolia* was collected from Kollimalai, Namakkal District, Tamilnadu. The plant is identified as *Drynaria quercifolia*.(J.)Sm using the Herbarium Specimen at Ranipat Herbarium (RPT), St.Joseph College, Trichirapalli.

The hairs were removed and the rhizome was washed with sterile distilled water. It was cut into small pieces and air-dried in shade. The dried material was powdered using grinder and stored in air tight containers.

Plant Extract Preparation

25 g of powdered rhizome sample of *Drynaria quercifolia* was weighed and macerated with respective solvents. It was kept at room temperature for 72 hrs with stirring for every 24 hours. The suspension was filtered through Whatman filter paper # 1. The extracts were dried by evaporating in room temperature. The dried residue was stored in refrigerator and used for further studies. Finally four extracts were obtained with solvents methanol, ethanol, chloroform, petroleum ether respectively.

Microbial Culture

Bacteria

Organism used were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella typhi*, *Vibrio cholera*.

Fungi

Candida albicans, *Cryptococcus neoformans*

Media Used

Nutrient agar, Mueller – Hinton agar, SDA medium (Hi media).

Preparation of Antibiotic Disc

20 mg of crude extract was dissolved in 1 ml of 20% DMSO. From this stock solution 10-20 µl of respective solvent extract is added to the disc separately in aseptic condition. Each disc now contains 0.2 mg of the extract. The disc were dried at room temperature and used for study of antibacterial activity.

Phytochemical Screening

Chemical tests were carried out on ethanol, methanol, methanol-chloroform, chloroform extracts of *Drynaria quercifolia*, using standard procedures to identify the constituents as described by Sofowara (1993) Trease and Evans (1989), Harborne (1973) and E1.Tawil, (1983).

Alkaloids

About 0.2 g of the extracts was warned with 2% H₂SO₄ for two minutes. It was filtered and few drops of Dragencloffs reagent were added. Orange red precipitate indicates the presence of alkaloids.

Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

Saponins

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing

(appearance of creamy mass of small bubbles) shows the presence of saponins.

Flavonoids

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

Steroids

2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H₂SO₄. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Coumarins

In a test tube 1 gm of plant sample was placed and covered with filter paper moistened with dil. NaOH, then heated on water bath for few minutes. The filter paper was examined under UV light, yellow fluorescence is indicative for the presence of coumarins.

Tannins

Small quantity of extract was mixed with water and heated on a water bath. The mixture filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Terpenoids (Salkowski test)

0.2 g of the extract of the whole plant sample was mixed with 2 ml of chloroform (CHCl₃) and concentrated H₂SO₄. (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Antibacterial Susceptibility test

Disc diffusion method

Kirby-Bauer method was followed for disc diffusion assay. In vitro microbial activity was screened using Mueller – Hinton agar (MHA) .The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 mins and 0.1 ml of the inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 mins. The discs loaded with the plant extract were placed on the

surface of the medium and the compound was allowed to diffuse for 5 mins. The plates were then incubated at 37° C for 24 hours. Negative control was prepared using respective solvents. Gentamycin (10 µg/disc) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in duplicate.

Antifungal susceptibility test

The fungistatic activity the extract was also evaluated by disk diffusion method. 100 µl of fungal suspension. (10⁵ CUF ml-1) was pipette onto petri dishes containing SDA and spread uniformly. Discs impregnated with 20 µl of extracts were placed on the surface of solid agar petri dishes. The plates were incubated for 48-72 hrs and the diameter of zone inhibition around each disc was measured in millimeters

Results and Discussion

Preparation of plant extract

Drynaria quercifolia rhizome were collected from Kollimalai, Nammakal. The air dried sample was powdered and extracted with four different solvents -ethanol, methanol, chloroform and petroleum ether.

Phytochemical screening of *Drynaria quercifolia*

The results of phytochemical screening tests for 9 compounds are given in Table-1. Alkaloids were not present in any of the four extracts. Similarly glycosides and phenolics were not detected in extracts with all the four solvents. Trace amount of flavanoids is detected from ethanol extract but absent in methanol, chloroform and petroleum ether. Trace amount of steroids is indicated all the extracts. Saponin and Coumarin is present in ethanol and methanol extract. Abundant tannin is indicated in ethanol extract. Terpenoids are also present in ethanol and methanol extract. Collectively more number of compounds are present in the ethanol extract of rhizome of *Drynaria quercifolia* . The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). This extract may be used for the detailed study of the medicinally active compounds in *Drynaria quercifolia* .

Table 1. Phytochemical analysis of extract from *Drynaria quercifolia*

S.No	Phytochemical	Solvent used			
		Ethanol	Methanol	Chloroform	Petroleum ether
1.	Alkaloids	-	-	-	-
2.	Glycosides	-	-	-	-
3.	Saponins	++	++	-	-
4.	Flavanoids	++	-	-	-
5.	Steroids	++	++	++	++
6.	Coumarin	++	++	-	-
7.	Tannins	+++	++	++	++
8.	Terpenoids	+++	++	-	-

Table 2. Antibacterial activity of rhizome extracts of *Drynaria quercifolia* in different solvents

Organism	Zone of inhibition(mm)				
	Ethanol	Methanol	Chloroform	Petroleum ether	Control
<i>S. aureus</i>	12	10	9	8	12(G)
<i>Bacillus cereus</i>	12	7	-	-	25 (C)
<i>E.coli</i>	11	10	10	-	11(G)
<i>K.pneumoniae</i>	8	8	-	-	13(C)
<i>P.aeruginosa</i>	8	11	-	-	13 (C)
<i>Salmonella typhi</i>	10	-	-	-	13(Cp)
<i>Vibrio cholera</i>	11	9	-	-	12(T)
<i>Proteus mirabilis</i>	12	11	9	-	15(Cp)

Control:G –Gentamycin, Cp-Ciprofloxacin,T – Tetracycline,C- Chloramphenicol

Table 3. Antifungal (yeast) activity of rhizome extracts of *Drynaria quercifolia* in different solvents

Organism	Zone of inhibition(mm)			
	Ethanol	methanol	Chloroform	Petroleum ether
<i>Candida albicans</i>	12	11	9	--
<i>Cryptococcus neoformans</i>	10	11	--	--

Antibacterial and antifungal activity of *Drynaria quercifolia*

The results for test for antibacterial activity of rhizome extract of *Drynaria quercifolia* with different solvents by disc diffusion method is listed in Table-3. From the results it is shown that ethanolic extract of the rhizome has greater inhibitory effect against both gram positive and gram negative bacteria. Ethanolic extract is followed by extract with methanol in its antibacterial activity. Chloroform and petroleum ether extract showed least effect. This result was similar to studies carried out by Kandhasamy et al., (2008). It was observed that ethanolic and methanolic extracts of the rhizome of *D. quercifolia* inhibited the growth of pathogenic bacteria 80% and 90% respectively (Kandhasamy et al., 2008). Among the bacteria tested *E. coli* and *Proteus mirabilis* showed high susceptibility to all the different extracts. The extracts were not active against *Bacillus cereus* and *Salmonella typhi*. Among the Gram positive bacteria tested *Staphylococcus aureus* was sensitive to all four extracts-Table 2. High antibacterial activity of the rhizome of *Drynaria quercifolia* towards *S. aureus*, which is methicillin resistant, may be due to the presence of glycoprotein, which exhibited high antibacterial activity (Fik et al., 1997; Janouska et al., 2003; Kawalski and Kedzia, 2007). *Bacillus cereus* showed susceptibility only to methanol extract. Among the 16 extracts of medicinal plants tested 10 extracts were not showing antibacterial activity against *B. subtilis* (Janovska et al., 2003). This result reveals that the ethanolic extract of rhizome can be used in medicine. The broad spectrum antibacterial activity of *Drynaria quercifolia* may be due to the active compounds like terpenoids, saponins (Singh and Gupta, 2008). From Table - 3 it is shown that ethanol and methanol extract show considerable inhibitory effect on both *Candida albicans* and *Cryptococcus neoformans*. From the above results it can be confirmed that *Drynaria quercifolia* possess strong medicinal properties. Further studies are being carried out on the identification and isolation of phytochemical compounds from ethanol and methanol extract.

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