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ANTIMICROBIAL STUDIES ON *DRYNARIA QUERCIFOLIA* (L.) J. Sm. LEAF AND RHIZOME EXTRACT

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ABSTRACT

The results of the preliminary screening experiments proved that Drynaria quercifolia (L.) J. Sm., exhibited considerable antibacterial activity. The plant was selected for detailed study because, the plant had been ethnobotanically relevant, non-toxic, easily available and a widely spread vegetation. A single species for the genus Drynaria was reported according to the biodiversity documentation of Kerala, Pteridophyes by (Easa, 2003). The acetone extract of Drynaria rhizomes shows anti-bacterial property against Vibrio cholerae and antifungal property against Aspergillus sp. The acetone extract of rhizome indicates anti-bacterial property by producing maximum zone of inhibition by 3 cm in diameter. The water extract of rhizome shows negative result with absence of anti-bacterial property. The acetone extract of leaf was less when compared with rhizome extract. The anti-fungal activity of Drynaria rhizome was also high in acetone extract than in water extract. The rhizome extract prevented the fungal growth. The rhizome can use effectively for preventing the growth of the fungus than the leaf because the extract shows less anti-fungal property. So the present study clearly indicates the anti-microbial efficiency of rhizome and leaf extract of Drynaria quercifolia (L.) J. Sm.

KEY WORDS

Anti-microbial activity, Drynaria quercifolia (L.) J. Sm., Pathogenic microorganisms.

INTRODUCTION

Many drug resistant bacterial strains were developed due to the increased use of a number of anti-bacterial drugs (Kandhasamy et al., 2008). It also created the problem in controlling the growth of infectious disease-causing pathogenic bacteria. Moreover, synthetic drugs produce side effects to the users. Many anti-bacterial drugs were discovered from plants. Use of substance with anti-microbial propertiesis known to have been used for at least 2000 years. anti-microbial substances derived from plants have received considerable attention in recent years. Even though numbers of plant-derived antibiotics were identified, the scientific evaluations of plant derived antibiotics still remain in an area of intensive investigation (Cutter, 2000). Ancient Egyptian and ancient Greeks use specific molds and plant extract to treat infection. The studies by Samir shows that good antimicrobial activity of three different pteridophytes indicating the presence of good amount substances like phenolic compounds, glycosides, flavonoids and alkaloids. *Drynaria quercifolia* (L.) J. Sm., commonly known as basket ferns, is a genus of ferns in the family Polypodiaceae. It contains around 16 species and one natural hybrid. Basket ferns are epiphytic and are native to tropical Africa, South Asia, East Asia, Southeast Asia, Australia, and Oceania. Most of the current studies of Drynaria involve the flavonoids, either as a complete extract or isolating individual components. Among the flavonoids of Drynaria, naringin (Flavanone-7-o-glycoside) which has the best-known source in grapefruit, and epiafzelechin, which is currently under investigation as an anti-inflammatory agent and inhibitor of herpes simplex virus.

MATERIALS AND METHODS

Plant collection and processing

The plant is collected from our campus, Little Flower College Mammiyoor, Kerala. Basket ferns are epiphytic plants. The rhizome was covered with small brown coloured hair like structures. They were removed using sterile scalpel and washed with sterile distilled water. They were cut in to small pieces and dried in shade and made into fine powder, using blender. The powder was used for extraction of bioactive compounds.

Extraction procedure

25g of powder of rhizome of *Drynaria quercifolia* (L.) J. Sm. was weighed and macerated in respective solvents namely acetone and water, individually in the ratio of 1:6. They were kept at the room temperature for 72 h. Each mixture was stirred every 24h using a sterile glass rod. Then it was filtered through the Whatmann No: 1 filter paper. Extracting procedure was done further twice for complete extraction of the bioactive compounds. The obtained filtrate was combined together and concentrated in vacuum using rotary evaporator. The dried residue of respective solvent extract was used for evaluating the antibacterial activity. They were kept in refrigerator until they use.

Preparation of antibiotic discs

Sterile empty antibiotic discs (6 mm diameter) were purchased from Hi-Media. 20 mg of dried crude extract was dissolved in 1 ml of 20% DMSO (Dimethyl Sulphoxide). From this stock solution, 10µl of respective solvent of extract of *D. quercifolia* was added to the disc (0.2 mg/disc) individually and aseptically. Each disc contained 0.2 mg of extract. Then the disc allowed drying at room temperature. After drying they were used for screening the antibacterial activity.

Inoculum preparation

Pure cultures of bacterial pathogens were removed nutrient agar slant and transferred to tryptone broth and incubated at 37°C for 24 h. The turbidity was adjusted to that of standard level by adding sterile tryptone broth.

Assay of antimicrobial activity

The tested bacterial strains were inoculated in to nutrient agar medium. The bacteria used for culture was then swabbed on nutrient agar. Using micro pipette, the extract was taken in different concentrations and loaded on to the disc. The plates where incubated at 37^0c for 24hrs. For *Vibrio cholera* incubation was performed at microaerophilic conditions. This experiment is continued for every extract of Drynaria plant part (leaf and rhizome extract using acetone and water), and diameter of zone of inhibition is measured in each case.

Anti fungal property

The rot causing fungi were isolated from the infected vegetable, tomato (*Lycopersicam esculentum*). The materials were collected from the local market in Guruvayoor. The specimen was washed in tap water followed by distilled water to remove dust and debris present. Then the specimen is wipe with alcohol, care was taken to avoid the margins of infected area so as to avoid saprophytes. The fungus was then inoculated in to petridish containing fungal growth medium potato dextrose agar (PDA). Amended PDA medium was used for the assay of fungal activity. A small portion of test fungi grown on PDA medium was taken by inoculation needle and inoculated in the center of solidified medium for different concentrations of plant extract. These plates were inoculated at room temperature and observations were recorded after 72 hours. The diameter of the colony growth was then measured.

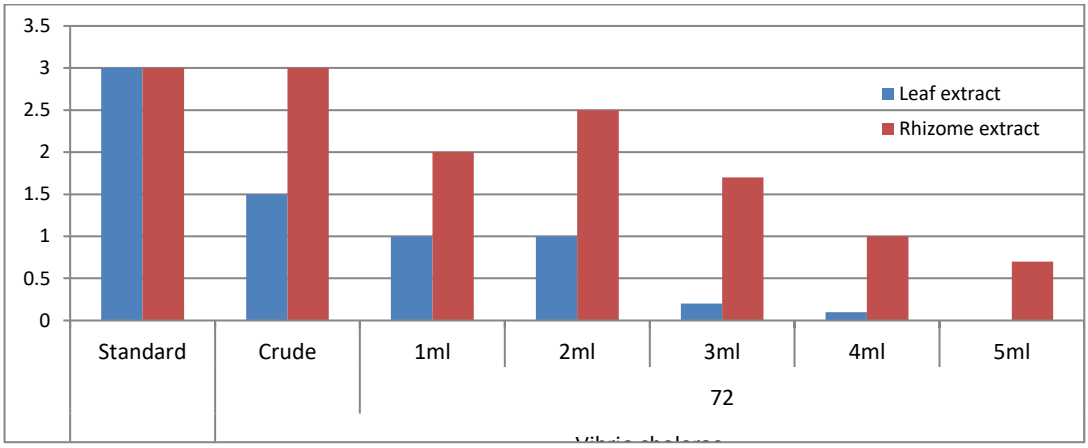
RESULTS

The antibacterial activity results are shown in table 1. Different extracts show varying degree of inhibitory effect. The inhibitory effect of extracts was directly proportional to increasing concentration of leaf and rhizome extracts. The rhizome extract inhibits the growth of pathogenic microorganisms than leaf extract. Maximum inhibitory zone was obtained in 1ml exract compared to standard. Minimum inhibitory zone was observed in 5ml concentration.

Table 1. Effect of extract of *Drynaria quercifolia* on *Vibrio cholerae*

Test Bacteria	Incubation period	Concentration (ml)	Inhibition in diameter (cm)	
			Leaf extract	Rhizome extract
Vibrio cholerae	72	Standard	3	3
		Control	0	0
		Crude	1.5	3
		1ml	1	2
		2ml	1	2.5
		3ml	0.2	1.7
		4ml	0.1	1
		5ml	-	0.7

Figure 1:- Graphical representation of Effect of extract of *Drynaria quercifolia* on *Vibrio cholera*



The antibacterial activity results are shown in table 1& fig.1. When the concentration of the extract solution was increased, the reduction in the growth of the fungal mycelium was observed. The maximum inhibition of mycelial growth was observed at 0.1ml concentration. The medium inhibition of mycelial growth was observed at 0.01ml concetration. The minimum inhibition of mycelial growth was observed at 0.001ml concentration.

Table 2: - Effect of rizhome extract of *Drynaria quercifolia* (L.) J. Sm. on *Aspergillus sp.*

Test fungi	Hour	Concentration (ml)	Growth inhibition diameter in (cm)
Aspergillus sp.	72	0.1	10.63
		0.01	7.81
		0.001	5.62

Figure 2: - Graphical representation of Effect of *Drynaria quercifolia* (L.) J. Sm. on *Aspergillus sp.*

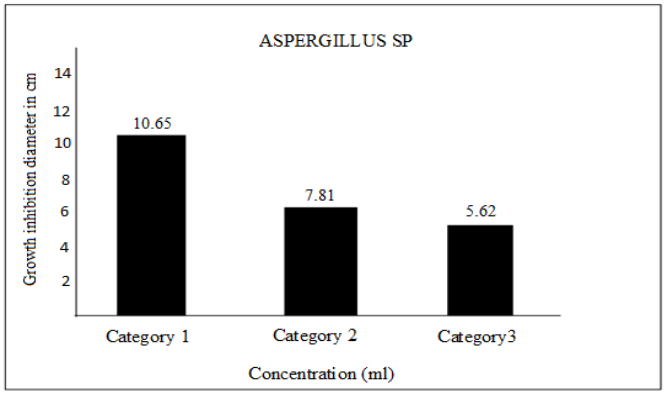
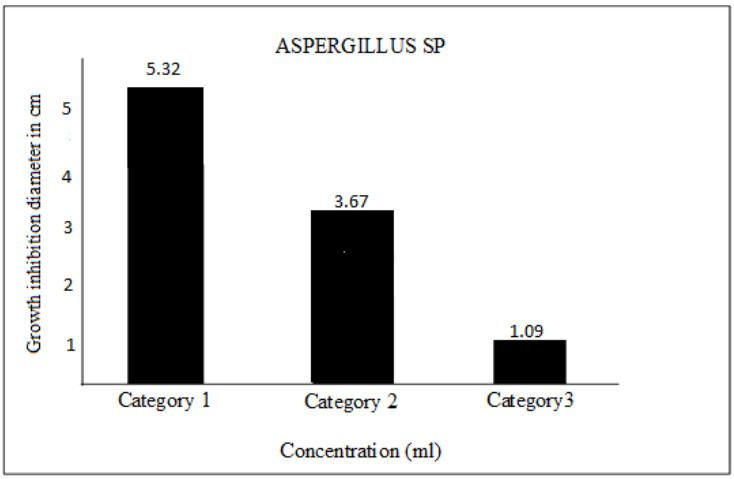


Table 3. Effect of extract of *Drynaria quercifolia* on *Vibrio cholerae*

Test fungi	Hour	Concentration (ml)	Growth inhibition diameter in (cm)
Aspergillus sp.	72	0.1	5.03
		0.01	3.67
		0.001	1.09

Figure 3: - Graphical represention of Effect of *Drynaria quercifolia* on *Vibrio cholera*



DISCUSSION

Drynaria quercifolia (L.) J. Sm. shows antimicrobial activity against pathogenic microorganisms. Based on the concentrations of extracts, zone of inhibition was changed. Disc diffusion method using nutrient agar was effective against Vibrio cholerae. Amended PDA medium shows anti-fungal property. Acetone was suitable solvent for the extraction. Whereas the water extract has no effect on reducing the micelial growth of the fungus.

Several plants are used in folk medicine and other traditional medicine as aseptic agents throughout the world. Among them ferns are also used in different traditional medicinal systems of India. Ferns play an important role in folklore medicine. 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. Medicinal uses of fern species were also described by Nadkarni (1954) and Nayar (1959). They also reported that 29 species of ferns were used in preparation of medicine. May (1978) published a detailed review of various ferns and their medicinal values. The anti-bacterial activity of some ferns has been studied (Kumar and Kausik, 1999; Parihar and Bohra, 2000a and b; 2003).

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