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Full Length Research Paper

Drynaria quercifolia (L.) J.Sm: A potential resource for antibacterial activity

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Six different organic solvents such as ethanol, methanol, petroleum ether, hexane, benzene and chloroform were used to extract the bioactive compounds from the rhizome of *Drynaria quercifolia* to screen the antibacterial activity against infectious disease causing bacterial pathogens such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Salmonella marscence, Staphylococcus aureus* and *Bacillus subtilis* by agar diffusion method. The ethanolic extract of *D. quercifolia* was more active against 80% of the organisms tested. It was followed by methanolic extract (70%), benzene (50%) and chloroform extract (40%) in inhibiting the growth of the organisms tested. Petroleum ether and hexane extract of *D. quercifolia* did not show any antibacterial activity against any of the pathogenic bacteria tested. Among the bacteria tested, gram-negative bacteria were more susceptible to the crude extracts compared to gram-positive bacteria. Among gram-positive bacteria, *S. aureus* was susceptible to ethanolic, methanolic and chloroform extracts whereas *B. subtilis* was susceptible to methanolic extract of *D. quercifolia* alone. In the present study ethanolic and methanolic extracts of rhizome of *D. quercifolia* showed high efficiency of antibacterial activity and gram-negative bacteria were more susceptible to all the extracts tested.

Key words: *Drynaria quercifolia,* rhizome, crude extract, antibacterial activity.

INTRODUCTION

Many drug resistant bacterial strains were developed due to the increased use of a number of antibacterial drugs. It also created the problem in controlling the growth of infectious disease causing pathogenic bacteria. Moreover synthetic drugs produce side effect to the users (Tomin and Tomasz, 1986). To circumvent this problem, scie-tists are more interested to develop new antibiotics from unicellular organisms, fungi, algae, and higher plants. Among them, higher plants play an important role, by producing large number of organic compounds as secon-dary metabolites, which can be used as self-defense. They act as bioactive compounds, chemothera-peutic, bactericidal, and bacteriostatic agents (Evans et al., 1986; Purohit and Bohra 1998). As a result, anti- microbial substances derived from plants have received conside-rable attention in recent years. Even though numbers of plant-derived antibiotics were identified, the scientific evaluations of plant-34.derived antibiotics still remain an area of intensive investigation (Cutter, 2000).

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. 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. 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 antibacterial activity of some ferns has been studied (Kumar and Kausik, 1999; Parihar and Bohra, 2000a and b; 2003). Based on the information, the fern *D. quercifolia* was selected to evaluate its antibacterial activity.

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MATERIALS AND METHODS

Description of the plant

The plant used in the present study belongs to Pteridophyta, and family Polypodiaceae. The plant is an epiphytic fern with a short thick, fleshy, creeping rhizome and densely clothed with reddish brown soft scales. The fronds are two types. The sterile fronds are small, somewhat concave and become brown on aging. The fertile fronds are long stalked, large pinnately lobed, leathery having network of small quadrangular areoles with or without free vein sori. They are small, numerous and two in each primary areoles. The plant part used in folk medicine was rhizome. Rhizome is used for bitter, anodyne, constipating, anti-inflammatory, and is used as tonic, which is useful in typhoid fever, phthiriasis, dyspepsia, cough, arthralgia, cephalalgia, diarrhea, foul ulcers and inflammation. It is very specifically used in the reatment of migraine.

Plant collection and processing

The material used in the present study is the rhizome of *Drynaria quercifolia (J.) Sm*. The rhizome was collected from the Kolli malai, Namakkal district, Tamil Nadu, India. The plant is identified as *D. quercifolia (J.) Sm* using the Herbarium specimen at Rapinat Herbarium (RPT), St. Joseph College, Trichirapalli. The rhizome is 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.

Solvents used

Organic solvents such as ethyl alcohol, methanol, petroleum ether, hexane, benzene, and chloroform were used for the extraction of the bioactive compounds.

Microorganisms used in the present study

Bacteria causing infectious diseases both in animals and humans were used in the present study. They were both gram negative and gram negative. Eight gram negative bacteria were *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcesence* and two gram positive bacteria *Bacillus subtilis and Staphylococcus aureus* were used in the present study. They were collected from school of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu India.

Extraction procedure

25 g of powder of rhizome of *Drynaria quercifolia* was weighed and macerated in respective solvent, individually in the ratio of 1:6. They were kept at the room temperature for 72 h. Each mixture was stirred every 24 h 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 Company, Chennai. 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.

Culture medium

Muller-Hinton agar medium was used to study the antibacterial activity of the crude extract of rhizome of *D. quercifolia*.

Inoculums 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 antibacterial activity

Antibacterial assay was carried out by agar diffusion method (Bauer et al., 1966). 0.1 ml (containing 10^5 CFU/ml) of 24 h old culture of bacterial pathogen was placed Muller-Hinton agar medium and spread throughout the plate by spread plate technique. The sterile disc containing respective solvent extract was placed on the surface of the medium at equidistance. The plates were kept at room temperature for 30 min, which helps to diffuse the extract on the medium. Later plates were incubated at 37°C for 24 h to determine the antibacterial activity of the respective solvent extraction of *D. quercifolia.* Chloramphenical antibiotic discs (30 mcg/disc) were used as positive control and disc with respective solvent (10 µl) was used as negative control. Each extract was tested in triplicate for calculation of mean value and standard deviation.

RESULTS AND DISSCUSSION

The antibacterial activities of different solvent extracts of the rhizome of *D. quercifolia* are presented in Table 1. Frequent uses of antibiotics make the organisms to become resistant to such antibiotics (Sydney et al., 1980). Because of this reason new antibiotics are discovered to control the infectious disease causing pathogens. In this regard higher plants play an important role by providing antibiotic compounds. Higher plants are rich in active principles, which are used as therapeutic drugs (Evans et al., 1986) . In India variety of medicinal plants are used to control a number of diseases in folk medicine. But only few of them were studied for their antimicrobial activities (Gehlot and Bohra, 2000, 2001).

The efficacy of different extracts of *D. quercifolia* on antibacterial activity is shown in the Table 1. Ethanolic and methanolic extracts of the rhizome of *D. quercifolia* exhibited broad spectrum of antibacterial activity. It was observed in the present study that the ethanolic and methanolic extracts inhibited the growth of pathogenic bacteria 80 and 70% respectively. The broad spectrum of antibacterial activity of these extracts was due to the presence of active principle present in the extracts. The

Table 1. Inhibitory properties (inhibition zone diameter in mm) of rhizome extracts of *Drynaria quercifolia* on different pathogenic bacteria

Test Organisms	Inhibition zone diameter in mm (Mean ± SD)						
	Different solvents extracts						
	Positive control Chloramphenical	Ethanol	Methanol	Pet.ether	Hexane	Benzene	Chloroform
E. coli	14 ± 0.0	10 ± 0.2	10 ± 0.2	ND*	ND*	10 ± 0.2	8 ± 0.2
K. pneumoniae	9 ± 0.0	8 ± 0.2	8 ± 0.2	ND*	ND*	ND*	ND*
P. mirabilis	15 ± 0.0	13 ± 0.4	11 ± 0.2	ND*	ND*	ND*	8 ± 0.4
P. aeruginosa	11 ± 0.0	8 ± 0.2	10 ±0.2	ND*	ND*	11 ± 0.4	8 ± 0.4
S. typhi	11 ± 0.0	11 ± 0.4	ND*	ND*	ND*	11 ± 0.4	ND*
S. paratyphi A	10 ± 0.0	9 ± 0.3	ND*	ND*	ND*	7 ± 0.3	ND*
S. paratyphi B	10 ± 0.0	11 ± 0.5	11 ± 0.5	ND*	ND*	8 ± 0.2	ND*
S. marscence	12 ± 0.0	ND*	ND*	ND*	ND*	ND*	ND*
S. aureus	10 ± 0.0	11 ± 0.4	10 ± 0.6	ND*	ND*	ND*	13 ± 0.6
B. subtilis	12 ± 0.0	ND*	8 ± 0.4	ND*	ND*	ND*	ND*

Crude extract used (10µl),
Inhibition Zone diameter includes the diameter of the disc (6mm),
ND*- Antibacterial activity not detected.

active principle may be polar compounds like saponins (Singh and Gupta, 2008) responsible for broad spectrum of antibacterial activity than other extracts (Kafaru, 1994). Similar observation was also made by Okeke et al., 2001.

Benzene and chloroform extracts of the rhizome of *D. quercifolia* exhibited least antibacterial activity against the bacterial pathogens tested (Table 1), whereas petroleum ether and hexane extracts did not inhibit the growth of the bacterial pathogens tested in the present study. Studies of Rani et al. (2003) revealed that petroleum ether extract of the rhizome of *Acorous calamus* (L.) showing least antibacterial activity and inhibited the bacterial growth at the concentration of 2 mg/ml.

In the present study, it was observed that gram-negative bacteria were more sensitive to most of the extracts tested compared to gram-positive bacteria. *S. typhi, S. paratyphi A* and *S. marcesence* were sensitive to ethanolic, benzene and chloroform extracts of the rhizome of *D. quercifolia, where as K. pneumonia* was susceptible to ethanolic and methanolic extracts of *D. quercifolia*. Among gram-negative bacteria, *E. coli* and *P. aeruginosa* were sensitive to ethanolic, methanolic, benzene and chloroform extracts. The present investigation was well coincided with the studies of Ashour and Kheralla (1995) and Azu et al. (2007) . According to their studies, *P. aeruginosa* and *E. coli* were resistant to ethanolic, methanolic benzene and chloroform extracts of the fruit of Banalitis and the extracts of *Allium cepa* and *Zingifer officinale* res-pectively. But general observation was that *P. aeruginosa* was more resistant to most of the antibiotics commonly used in clinical practices (Tanira et al., 1994).

In general, gram- negative bacteria were more resistant to antibiotics than gram-positive bacteria (Paz et al., 1995; Chowdhury and Islam, 2004). The resistance is due to the differences in their cell wall composition. In gram -negative bacteria the outer membrane acts as a great barrier to many environmental substances including antibiotics (Tortora et al., 2001). Presence of thick murine layer in the cell wall prevents the entry of the entry of the inhibitors (Martin, 1995). But the present study revealed a controversy report that gram-negative bacteria were more susceptible to the crude extracts than gram-positive bacteria. It may be due to the presence of broad spectrum of antibiotic compounds present in the rhizome of *D. quercifolia*.

The present study also showed that *Serratia marcescense* being resistant to all the extracts tested (Table 1). The results of the study were well corbarroated with the studies of Meyer and Afolayan (1995).

Among the gram-positive bacteria tested *S.aureus* was sensitive to the ethanolic (11 mm), methanolic (10 mm), and chloroform (13 mm) extracts of *D. quercifolia* (Table 1). High antibacterial activity of the rhizome of D. quercifolia towards *S. aureus*, which is methicillin resistant, may be due to the presence glycoprotein, which exhibited high antibacterial activity (Fik et al., 1997; Janovska et al., 2003; Kawalski and Kedzia, 2007). But *B. subtilis* was susceptible only for the methanolic extract (8 mm) of the rhizome of *D. quercifolia* (Table 1) . Among the 16 extracts of medicinal plants tested for the antibacterial activity 10 extracts were not showing antibacterial activity against *B. subtilis* (Janovska et al., 2003). Similarly Chowdhury and Islam also reported that extracts of *T. urientalis* were not active against the gram positive bacteria.

From this study it can be concluded that ethanolic and methanolic extracts of the rhizome of *D. quercifolia*

showed wide range of antibacterial activity. So it can be used and administered in the ethnomedical practice. Bacterial pathogens that were used in the present study especially *P. aeruginosa* showed varying susceptibility against the crude extracts of the rhizome of *D. quercifolia*.

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