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IN VITRO ANTI-BACTERIAL SCREENING OF *DRYNARIYA QUERCIFOLIA*

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Abstract

Purpose: To make scientific validation of antibacterial activity of ethnomedical fern *Drynaria quercifolia*. **Method and results:** The rhizome extracts were prepared using different solvents like benzene, ethyl acetate, chloroform, ethanol & water. The percentage yield was 7, 3, 2, 3, 3.1 & 6.4 respectively. Preliminary phytochemical screening revealed the presence of maximum constituents in the ethyl acetate extract. The anti-bacterial activity was screened against both gram-positive and gram-negative bacteria, and the zone of inhibition was measured. The results were compared with standard Ampicillin (250µgm) and ethyl acetate was found to be most effective against *Bacillus subtilis*. **Conclusion:** In the antibacterial study ethyl acetate extract showed good antibacterial activity even in microgram quantities and is found to be very effective against *Bacillus subtilis*. Based on the studies it can be concluded that *Drynaria quercifolia* can be effectively used as an anti-bacterial agent.

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Introduction

Drynaria is an epiphytic or pterophilous fern widely distributed throughout Asia to North-East Australia and Africa. Four species are reported to occur in India. *Drynaria quercifolia* alone is distributed in South India, in the plains or very low down in mountains, on trees or rocks.

The rhizomes are short thick fleshy creeping, up to 3 cm in diameter, adpressed to the substratum tightly, densely clothed with paleae all over. Paleae linear- lanceolate or lanceolate, peltate with a short stalk, red brown, non-clathrate, gland-tipped, gland deciduous, margins dentate-ciliate.

The fronds are seasonal, dimorphic, coriaceous or sub coriaceous of two types; The sterile fronds become brown on ageing and are small and somewhat concave. They vary in size from 7.5 to 30 cm (l) and 18-20 cm(w), cordate oval, lobate - pinnatifid sessile, lateral veins prominent and closely covering rhizome.

Taxonomical Classification	Regional Names/ Vernacular names
Domain: Eukaryote Kingdom: Plantae Sub kingdom: <i>Viridaeplantae</i> Phylum: <i>Tracheophyta</i> Sub phylum: <i>Pteridophytina</i> Infraphylum: <i>Filices</i> Class: <i>Filicopsida</i> Order: Filicales Family: <i>Polypodiaceae</i> Sub family: <i>Vaccinioideae</i> Tribe: <i>Androcdeae</i> Botanical name: <i>Drynaria quercifolia</i> Synonym: <i>Polypodium quercifolium</i>	Hin: Asvakatri, Katikapan Mal: Matilpanna, Pannakkilannu San: Asvakatri, Tam: Pannakkilanku



Image 1: *Drynaria quercifolia*



Image 2: *Drynaria quercifolia* rhizome

Medicinal uses

Anti-bacterial, Anti-fungal, Antipyretic, Anthelmintic, Anti-inflammatory, Hepatoprotective, Antiulcer, Anticancer, Anti-diabetic etc.

Chemical constituents

Phytoconstituents isolated from the plant include friedelin, epifriedelinol, β -sitosterol, β -amyrin, 3,4-dihydroxybenzoic acid, β -sitosterol 3- β -D-glucopyranoside, naringin, naringenin and acetyl lupeol.

Materials and methods

Analytical parameters

Determination of total ash values

2 gm of rhizome powder was separately weighed and taken in a pre-weighed silica crucible. The powder was incinerated at a temp above 450°C until free from carbon and then cooled in a desiccator. The weight of total ash was taken and the percentage yield was calculated with reference to air dried sample.

Determination of Acid insoluble ash value

The total ash obtained was boiled with 25ml of 2 N HCl for 5 minutes. The insoluble matter was collected on an ashless filter paper and washed with hot water until it is neutral. The filter paper is then incinerated in a crucible and cooled in a desiccator and weighed. Percentage of acid insoluble ash with reference to air dried drug was calculated.

Determination of Water-soluble ash value

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ashless filter paper and washed with hot water until it is neutral. The filter paper is then incinerated in a crucible and cooled in a desiccator and weighed. Percentage of acid insoluble ash with reference to air dried drug was calculated.

Sl.no	Ash Value	Percentage ash (w/w)
1.	Total ash value	8%
2.	Acid insoluble ash value	12.5%
3.	Water soluble ash value	50%

Determination of extractive values

Determination of water-soluble extractive value

5gm of the coarsely powdered drug was accurately weighed and macerated with 100 ml of chloroform water in a stoppered flask for 24 hours shaking frequently for 6 hours and allowing to stand for 18 hours. The mixture was filtered and 25 ml of the filtrate was evaporated to dryness in a flat-bottomed shallow dish, dried at 105°C, cooled in desiccator and weighed. The percentage w/w of water-soluble extract with reference to the air-dried drug was calculated.

Determination of alcohol soluble extractive value

This was determined by the method described under water-soluble extractive value using 100 ml of 90% alcohol instead of chloroform water taking adequate precautions to avoid loss of alcohol during filtration.

Nature of extract	Percentage (w/w)
Water soluble extractive value	2.2%
Alcohol soluble extractive value	1.4%

Extraction procedures

Collection & Processing of *Drynaria quercifolia*

The rhizomes are collected from Idukki and identified in botany department of Nirmala College, Muvattupuzha. They are cleaned properly, cut into pieces and dried in shade. The fine powder prepared by blender is used for extraction.

Method of extraction

31g were accurately weighed & extracted in Soxhlet apparatus using solvents benzene (2.7), ethyl acetate (4.3), chloroform (4.4), ethanol (5.2) and water (9) in the increasing order of polarity. Each time before extracting with the next solvent the powdered material was dried.

Percentage Yield of *Drynaria quercifolia*

Sl. No	Extracts	Weight in grams	%yield (w/w)
1.	Benzene	2.17g	7%
2.	Ethyl acetate	0.971g	3%
3.	Chloroform	0.713g	2.3%
4.	Ethanol	0.991g	3.1%
5.	Water	2.01g	6.4%

Preliminary Phytochemical Analysis of *Drynaria quercifolia* extracts

All extracts of *Drynaria quercifolia* rhizome were subjected to qualitative chemical tests for the identification of various phytoconstituents.

	Benzene	Ethyl acetate	Chloroform	Ethanol	Water
Steroids	-	+	-	-	-
Alkaloids	-	-	+	-	-
Glycosides	-	+	-	-	-
Flavonoids	-	+	-	-	-
Carbohydrates	+	+	+	+	-
Fats&oils	+	+	+	+	-
Proteins	-	-	+	+	+
Saponins	+	+	-	-	-

Identification of Active Constituents by Thin Layer Chromatography Procedure

The benzene, ethyl acetate, chloroform, ethanol, water was used to dissolve the samples.

Solvent system: 15% methanol in chloroform

Detection technique: -U. V

The TLC plates (7.6 x 2.5cm) were prepared with silicagel G. A slurry of adsorbent was prepared by triturating it with water in a mortar. The slurry was then spread uniformly over the glass plates and thin layer was allowed to set. The plates after spreading were air dried and then activated by heating in an oven at 100-110°C for 30-60 minutes. Then the various extracts were deposited as a spot with the help of capillary tubes. After drying the chromatogram were developed in solvent system Methanol: Chloroform (15:85, vol: vol) by placing in a developing tank which was paper lined so that the atmosphere inside was saturated with solvent phase. The plates were removed when the solvent front reaches 3/4 of the plate. The solvent front was marked and the plates were allowed to dry. The spots were detected by UV.

The extracts were separated into different spots. The Rf values were calculated by measuring the distance travelled by the solute and the solvent.

$$\text{Rf Value} = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent front from the origin}}$$

Rf Value of Different Compounds Isolated from *Drynaria quercifolia* Rhizome

Sl. No.	Name of the extract	Distance travelled by solute in cm	Colour of the spot	Number of spots	Distance travelled by solvent front in cm	Rf value
1.	Benzene	4.2	Yellow	1	5.4	0.77
2.	Chloroform	4	Yellowish green	1	5.5	0.72
3.	Ethyl acetate	1.5 3 4.3	Yellow Blue blue	3	5.7	0.26 0.52 0.75

Evaluation of Antibacterial Activity

Preparation of Plant Extract

Various extract of *Drynaria quercifolia* prepared with the help of Soxhlet apparatus. 250 mcg/ml solutions were prepared by dissolving the various extracts in the corresponding solvents.

Preparation of Media

Each ingredient (Beef extract-10gm, peptone -10gm, sodium chloride- 5gm) except agar was dissolved in the appropriate volume of distilled water. The pH of the fluid medium is determined with a pH meter and adjusted to pH 7 by using 1N HCl or 1N NaOH. Agar powder was added and the medium was heated to dissolve the agar to form a clear liquid. The medium was dispensed into flasks, plug the flasks containing medium by using nonadsorbent cotton. The media was sterilized at 121° C at 15 lbs pressure for 15 mins in an autoclave. Allow the flask to cool up to 50° C and pour the medium quickly into sterile petriplates (sterilized by hot air oven by heating at 160° C for hr) under aseptic conditions. Allow the medium to cool and to produce solid agar plates.

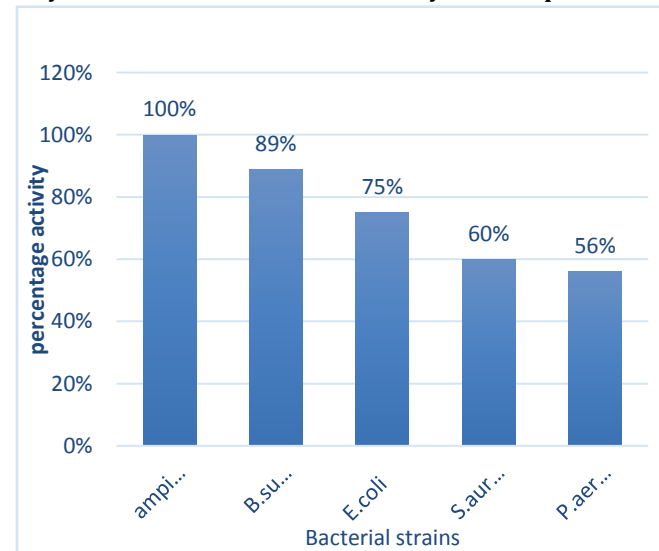
Procedure

Invitro antibacterial screening was carried out against two Gram positive. [*Bacillus subtilis*, *Staphylococcus aureus*] and two Gram negative [*Escherichia coli*, *Pseudomonas aeruginosa*] bacteria.

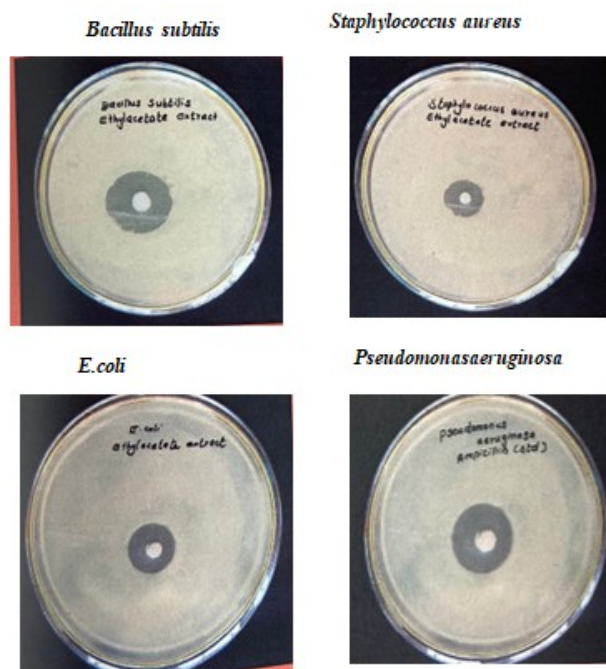
Disc diffusion method, was used for antibacterial screening, 20 ml quantities of nutrient agar were plated in petridish with 0.1 ml of each bacterial culture. Filter paper discs (6 mm in diameter) impregnated with respective samples [Benzene, Ethyl acetate, Chloroform, Ethanol and Water fraction] were placed on the test organism seeded plates. Benzene, Ethyl acetate, Chloroform, Ethanol and Water were used to dissolve the samples and were completely evaporated before application on the test organism seeded plates. Blank disc impregnated with

solvents Benzene, Ethyl acetate, Chloroform, Ethanol, Water followed by drying off were used as negative control. The activity was determined after 18 hrs of incubation. The zone of inhibition produced by samples was then compared with the standard antibiotic (Ampicillin).

Percentage inhibition of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* on ethyl acetate extracts of *Drynaria quercifolia*.



Antibacterial Activity of Ethyl Acetate Extract of Rhizomes of *Drynaria quercifolia* Against *Bacillus subtilis*.



Results and Discussion

The percentage yield of Benzene, Ethyl acetate, Chloroform, Ethanol, Water extracts of *Drynaria quercifolia* were found to be 7, 3, 2.3.3.1 and 6.4 respectively. Analytical parameters like Ash value and Extractive values were determined as a mode of identification of the fern. The presence of maximum active constituents like steroids, glycosides, flavonoids, carbohydrates, fats and saponins in ethyl acetate extract was confirmed by phytochemical screening and is decided to scientifically validate its antibacterial activity. The TLC of

Benzene, Ethyl acetate and Chloroform extracts were performed using solvent system, Methanol: Chloroform (15:85). The three different spots were detected using UV light and their Rf values were 0.75, 0.52 and 0.26 respectively. The antibacterial activity of the ethyl acetate extract of *Drynaria quercifolia* showed a percentage activity of 89 %, 75 %, 60 % & 56 % against *B. subtilis*, *E. coli*, *S. aureus* & *P. aeruginosa* respectively.

Conclusion

The antibacterial study ethyl acetate extract shows good antibacterial activity even in microgram quantities and is found to be very effective against *Bacillus subtilis*. So *Drynaria quercifolia* can be used effectively as an anti-bacterial agent.

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Conflict of interest

Authors are declared that no conflict of interest.

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