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Antibacterial efficacy of *Drynaria quercifolia* (L.) J. Smith (Polypodiaceae) against clinically isolated urinary tract pathogens

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ABSTRACT

Objective: To make scientific validation of the ethnomedicinal fern *Drynaria quercifolia* (L.) J. Smith in relation to urinary disorders. **Methods:** Antibacterial study was carried out on clinically isolated Urinary Tract Infecting (UTI) bacteria by disc diffusion method. **Results:** Among the six extracts tested against eight different UTI bacteria, acetone extract was effective against *Enterococcus faecalis* and *Streptococcus pyogenes*, while ethanol extract was effective against *Pseudomonas aeruginosa*. Preliminary phytochemical screening shows the presence of phytoconstituents viz. phenols, tannin, alkaloids, proteins, xanthoproteins, carboxylic acid, coumarins and saponins. **Conclusions:** Based on the present study, along with previous studies, the ethnomedicinal use of *Drynaria quercifolia* for the treatment of urinary infection has been scientifically validated.

1. Introduction

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. 1 in 5 women will develop UTIs in their lifetime in America. 34% of adults over 20 self-reported having at least one occurrence of a urinary tract infection in the US 1988-1994[1]. The four most common nosocomial infections (any disease contracted by a patient while under medical care) are urinary tract infections, surgical wound infection, pneumonia, and primary bloodstream infection[2]. UTI is an important cause of childhood morbidity and it has been recommended for the inclusion in Integrated Management of Childhood Illness[3]. The most common bacterial strains that cause UTIs include *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella*, *Enterococci bacteria*, and *Proteus mirabilis*. Rare bacterial causes of UTIs include Ureaplasma urealyticum and *Mycoplasma hominis*.

Although antibiotics are the first treatment choice for urinary tract infections, antibiotic-resistant strains of *Escherichia coli*, the most common cause of UTIs, are increasing worldwide. Some observational evidence shows the effectiveness of antibiotic prescribing in young women with UTIs and it also shows that between 12% and 16% of patients will return within 28 days for further treatment, irrespective of the antibiotic prescribed initially[4]. Vancomycin Resistant *Enterococci* (VRE) urinary isolates are common in the United States. In North America, approximately 75% of all vancomycin-resistant enterococci (*Enterococcus faecium* and *Enterococcus faecalis*) isolates demonstrated a VanA phenotype which is resistance to both vancomycin and teicoplanin[5]. Guzman *et al*[6] have explained that the adhesive properties are important virulence factors in the pathogenesis of UTI and endocarditis and also suggest that UTI strains showing the highest invasion and adhesive potential invade the kidneys, cause bacteremia, and, after having expressed the serum-dependent surface modification, colonize the heart.

Due to the above problems in controlling the UTIs pathogens with antibiotics, herbal medicines are used as alternative medicines. In several Asian and African countries, 80% of the population depends on traditional medicine for primary health care, including UTIs. Throughout the world there are several reports for the herbal treatment of UTIs. The most commonly used herbs are Cranberry juice (Vaccinium macrocarpon or

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Vacinnium oxycoccus) and Uva ursi (Arctostaphylos uva-ursi). *Malva verticillata* L. is used to treat urinary complaints by the local people in Kedarnath Wildlife Sanctuary in Western Himalaya[7]. Sahoo et al[8] have investigated the antibacterial activity of *Hybanthus enneaspermus* Muell. against six selected UTIs pathogens. Other important herbs used in UTIs are Goldenseal (*Hydrastis canadensis*)[9], Marshmallow root (*Althea officinalis*) [10], Buchu (*Barosma betulina*)[11], Corn silk (*Zea mays*)[12] and the pteridophyte Horsetail - Equisetum arvense[13,14]. An epiphytic fern *Drynaria quercifolia* (L.) J. Smith, commonly called Oak Leaf Fern, is used in traditional medicinal system by different groups of people to treat various kinds of health problems including urinary tract infection[15]. In Ayurvedic system of medicine, it is called 'Ashwakatri' and it is used as pectoral, expectorant and anthelmintic agent. It is also used in the treatment of chest diseases, cough, hectic fever, dyspepsia, loss of appetite, chronic jaundice and cutaneous affections[16]. Pounded fronds are used as poultice for swellings. Peeled rhizome with sugar is prescribed for urinary disorders and in spermatorrhoea[17]. Tribals in Kalakad Mundanthurai Tiger Reserve, India, used the rhizome of this fern to cure rheumatism[18]. The rhizome of this fern is one of the twelve ingredients of a drug to treat cancer[19].

The ethnomedicinal uses of the fern *Drynaria quercifolia* (L.) J. Smith have been pharmacologically confirmed by several workers. Anthelmintic activity and antipyretic activity have been studied by Kulkarni *et al*[20] and Khan *et al*[21] respectively. Antibacterial and antidermatophytic activities have been evaluated by Ramesh *et al*[22], Shokeen *et al*[23], Kandhasamy *et al*[24] and Nejad and Deokule[25]. Pharmacognostical study on the rhizome has been carried out by Irudayaraj and Senthamarai[26]. Recently, anti-inflammatory and analgesic effects have been confirmed in this fern by Anuja *et al*[27].

Although this fern is being ethnomedicinally used to treat urinary tract infection, there is no specific study on antibacterial activity against the pathogens causing urinary tract infection. The present investigation was aimed to confirm the ethnomedicinal use of this fern to treat urinary tract infections by studying the antibacterial activity in rhizome of *Drynaria quercifolia* against clinically isolated urinary tract pathogens.

2. Materials and methods

Drynaria quercifolia (L.) J. Smith (Polypodiaceae) is an epiphytic fern growing from sea level to medium altitude. It is a large herb with densely scaly woody rhizome. Small, sessile nest leaves are present on the rhizome. Fronds are large and pinnatifid with reticulate venation. On the under surface of each pinna two rows of superficial exidusiate sori are present. For the present study, materials were collected from Kattakkada village, Trivandrum district, Kerala, India.

5 g of fresh rhizome was washed 2 - 3 times with tap water and distilled water and then surface sterilized with 90% ethanol. Subsequently, the plant materials were grounded in 50 mL of distilled water and the organic solvents such as acetone, benzene, methanol, and pet ether, separately. The macerates prepared from organic solvents were kept for 24 h at room temperature to evaporate the solvents. In the remaining residue, 50 mL of DMSO (Dimethyl sulfoxide) was added. Macerates were squeezed through double-layered muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 10 000 rpm for 20 min at room temperature. The supernatants were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 μm disposable filters. The extracts (10%) thus, obtained were used for the phytochemical studies. For screening the phytochemical constituents standard methodologies given by Harborne[28] were adopted.

2.1. Collection of urine and isolation of UTI pathogens

Urine samples were collected from clinically diagnosed cases of Urinary Tract Infection from Scudder Microbiology Laboratory, Nagercoil, Tamilnadu, India. Before collecting a sample, the women were instructed to swab the vulvae and men to retract the foreskin and cleanse the glans penis. Mid stream urine was collected in a sterile wide mouthed container (Hi-Media). For the isolation of UTI bacterial strains, loop full of urine samples were streaked in to the nutrient agar, Mac Conkey agar, blood agar and Choclate agar plates and incubated at (37±2 ℃) for 24 h. Next day individual colonies were selected and identified on the basis of morphological characteristics, gram staining, and biochemical characters.

2.2. Antibacterial assay

Solutions of known concentration (10%) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4 ℃) for 24 h to allow maximum diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37 ℃ for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

3. Results

The present study was conducted to evaluate the antimicrobial efficacy of the rhizome extracts of *Drynaria quercifolia* against the clinically isolated human pathogens from infected urinary tract. The gram positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus pyogenes* and gram negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas*

Table 1
Phytochemical study of the rhizome of Drynaria quercifolia (J.) Smith

Compounds	Extracts					
	Acetone	Benzene	Chloroform	Water	Ethanol	Petroleum Ether
Alkaloids	−	−	−	−	−	−
Phenols	+++	+++	++	+++	++	++
Flavonoids	−	−	−	+++	+++	−
Saponins	−	++	−	−	−	++
Proteins	−	−	−	−	−	−
Quinones	−	−	−	−	−	−
Steroids	−	−	++	+++	+++	++
Tannins	++	++	++	+++	+++	++
Xanthoprotiens	−	−	−	−	−	−
Carboxylic acids	−	−	−	−	−	−
Coumarins	−	−	−	−	−	−
Carbohydrates	+	+	+++	−	−	−
Total	3	4	4	4	4	4

aeruginosa, *Acinetobacter* sp. and *Proteus mirabilis* were selected for the present study. The commercially available antibiotic Amikacin was used as control to compare the efficacy of plant extracts against the microorganism studied. Among six extracts tested against eight UTI pathogens, two extracts were found to be the effective against the microorganism, which cause urinary tract infection. All the extracts were ineffective against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter* sp. and *Proteus mirabilis*.

The maximum zone of inhibition (22 mm in acetone extract and 23 mm in Amikacin treated control) was obtained against *Streptococcus pyogenes*, followed by 8 mm against *Enterococcus faecalis* in the same extract. Seven millimeter of inhibiton zone was observed in ethanol extracts against the pathogenic organism *Pseudomonas aeruginosa*. No inhibition zone was observed for other extracts. Thus, the acetone extract of the rhizome of *Drynaria quercifolia* is more active, almost equivalent to the antibiotic amikacin, against the bacterium *Streptococcus pyogenes*. The same extract also has moderate activity against the bacterium Enterococcus faecalis. From the present study it is concluded that the acetone extract from the rhizome of *Drynaria quercifolia* shows inhibitory effect on two gram positive bacteria *Streptococcus pyogenes* and *Enterococcus faecalis* which are invariably connected with the UTIs.

Preliminary phytochemical study on the rhizome of *Drynaria quercifolia* indicates the presence of phenols and tannin in all the six extracts tested. Flavonoids show positive result only in aqueous and ethanol extracts. The phytochemicals such as alkaloids, proteins, xanthoproteins, carboxylic acid and coumarins gave negative result for their presence in any of the six extracts tested. Saponin was present only in benzene and petroleum ether extracts (Table 1).

4. Discussion

The acetone and ethanol extracts from rhizome of *Drynaria quercifolia* shows antibacterial effect on clinically isolated bacterial pathogens of urinary tract such as *Streptococcus pyogenes*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. *Streptococcus pyogenes* is a major human pathogen, causing diseases ranging from mild superficial infections of the skin and pharyngeal mucosal membrane, up to severe systemic and invasive diseases and autoimmune sequelae[29]. *Enterococcus faecalis* is a frequent cause of urinary tract infection in hospitalized patients. Recent reports have suggested that the organism may frequently be acquired by cross-infection from other patients[30]. Most of these infections occur after surgery of the abdomen or a puncturing trauma, but can also be linked to the increased use of IV's and catheters, which are considered compromising devises. It is also responsible for urinary tract infections, bacterimia, endocarditis, meningitis, and can be found in wound infections along with many other bacteria. Treatment of *Enterococcus faecalis* consists of a synergistic combination of aminoglycoside and cell wall-active antibiotics[31].

Previous studies on phytochemistry and antibacterial activity of the rhizome of *Drynaria quercifolia* shows the presence of various antibacterial agents with high degree of antimicrobial activity against different pathogenic bacteria and fungi. Kandhasamy *et al*[24] have confirmed that the ethanolic and methanolic extracts of the rhizome of *Drynaria quercifolia* showed wide range of antibacterial activity. They have found nil activity in all the ten tested bacteria with Petroleum ether extract and Hexane extract. Benzene and chloroform extracts have shown mild activity. Irudayaraj and Senthamarai[26] have observed high degree of antimicrobial activity in ethanol extract of the rhizome against *Candida albicans*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with the inhibition zone range from 12-29 mm. They have reported the presence of steroid, phenolic groups, catechin and tannin with the very good positive result for catechin. Chunmey *et al*[32] confirmed the inhibitory effect of catechin on *E. coli* in a dose-dependent manner starting from the concentration of 0.05 to 1.6 mg/mL. Catechin (−)-Epigallocatechin gallate (EGCg), a major component

of green tea extracts, has been reported to be biologically active and interacting with membranes[33]. Ramesh et al[22] have isolated Friedelin, epifriedelinol, β-amyrin, β-sitosterol, β-sitosterol 3-β-D-glucopyranoside, and naringin from the dried rhizome of *Drynaria quercifolia*. The methanol extract showed broad and concentration-dependent antibacterial activity. Clinical studies have confirmed the beneficial effects of beta-sitosterol in patients with prostate enlargement. The phytochemical decreases post-void residual urinary volume and increases urinary flow rate in these patients[34]. The triterpenoid Friedelin has also been proved to be of active antimicrobial agent against both gram positive and gram negative bacteria[35]. Bioassay-guided investigations on rhizome of *Drynaria quercifolia* led to the isolation of 3,4-dihydroxybenzoic acid which has shown significant antibacterial activity against four gram-positive and six gram-negative bacteria[36]. It is to be noted that fifteen antioxidant benzoic and phenolic acids, including 2, 4 Dihydroxy benzoic acid, have been isolated from American cranberry fruits which is the effective herb for the treatment of UTIs. Based on the present study along with the previous studies on phytochemistry and antimicrobial activities, it is concluded that the rhizome of *Drynaria quercifolia* contains various bioactive compounds with high degree of antimicrobial activity against various pathogens, including bacterial pathogens of Urinary Tract Infections. The traditional use of rhizome of this fern to cure UTIs is thus confirmed from the present study along with previous reports. The reasons for different kinds of results with different researchers on the same part of the same species may be due to the difference in methodology, particularly the extraction procedures, concentrations of the extracts used. The different strains of microorganisms used for the experiment may also be responsible for the different results. In majority of the studies on antimicrobial activities, the same pure cultured strains are used repeatedly for longer time for different experiments. But several microbes, particularly the UTI bacteria develop resistance to different antibiotics and drugs. In such condition, as in the present study, the use of isolated pathogens from freshly collected clinical samples will give confirmed results on the resistant strains. From the present study along with previous studies, it is also clear that mixture of different active compounds will give more effect synergistically, than pure compounds since the treatment of UTI pathogens require mixture of different antibiotics with different mode of action. The isolation of active compounds and discovery of new formulation with different combinations of different active compounds from the rhizome of *Drynaria quercifolia* and other herbs will be the best challenge to develop potential drug to cure the resistant strains of UTI pathogens. Unlike other herbs, *Drynaria quercifolia* is highly adopted species which withstand dry condition with the presence of special bracket leaves. Like angiosperms, pteridophytes are also associated with different species of Vesicular and Arbuscular Mycorrhizal fungi including the hyphomycetes. The live and dead tissues, and trapped leaf litter by the epiphytic fern Drynaria quercifolia yielded 37 species of water-borne conidial fungi on bubble chamber incubation. Dead bracket leaves of this fern possess the highest species as well as conidia. As stable epiphyte, Drynaria exposed to wet and dry regimes in tree canopies of west coast and Western Ghats likely to serve as host for perfect states of water-borne hyphomycetes. Their ability to colonize, decompose, and use substrates and to interact with or parasitize other organisms is a result of the enzymes, antibiotics, toxins, and other metabolites they produce, coupled with wide genetic diversity. With the association of such important fungi on the rhizome and bracketed leaves even during stress condition, there is more chance for the accumulation of different kinds of antibiotics and secondary metabolites in the woody rhizome of Drynaria quercifolia. It may also be one of the reasons for the presence of variety of bioactive compounds, including antimicrobial agents as confirmed from the present study along with previous studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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