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Antimicrobial Activity of *Aspidium cicutarium* against Skin Infection Pathogens

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ABSTRACT

Medicinal plants and natural product derived drugs are enormously occupying a significant space in today's modern therapeutics. Medicinal plants being a natural source of herbal drugs ensure the safety, quality and effectiveness of such products. Although from ancient time plants supply food and medicine to humankind, however, systematic evaluation of herbal medicine to cure specific diseases is being initiated quite recently. As a result of which the herbal drug compound emerged as a new era of plant drug medicine to almost all diseases. In context of traditional knowledge along with modern research approaches and techniques, medicinal plants needs to be evaluated for presence of new key components to combat with an emerging concern such as antibiotic resistance or drug safety. Natural treatment is cheap and safe. It serves best raw material for production of new drugs. The aim of present study was to present antimicrobial activity of different extract like-hexane extract, ethyl acetate extract, petroleum ether extract of *Aspidium cicuarium* and their role to cure skin pathogens.

INTRODUCTION

Ayurveda, Siddha, Unani and folk system of medicine gives us knowledge of natural remedies for many diseases. It is being estimated that alternative medicines are being used by almost 60% of the world's population. These medicines not only consumed by rural masses from developing countries but are also significantly used in developed world where modern medicines dominate the market. This interest in traditional medicines is growing rapidly and scientists across the world focusing more on plant based medicine for cure of diseases like Cancer, hepatitis, rheumatoid arthritis and several of infectious diseases[1].

Aspidium cicutarium L. (Family: Dryopteridaceae) is recently documented folk herb known as *waghchavadi, bichawaor kukkutnakhi*. Roots of this plant is resembling like claw of tiger and dried resembled like legs of hen. Medicinal herbs are available in plenty in number in nature and their roots are traditionally used by many local practitioners in to cure many diseases. The medicinal information and the detail of plant information is found well documented in 'Ferns of Bombay'[2]. Herbal drug of this plant is used in many conditions as, tonsillitis, cysts, tumors, abscess mentioned in editorial published of 'Ayurved Patrika'[3]. The plant is also known with its several synonyms as [4] - *Tectaria coadunata, Aspidium coadunata, Nephrodium cicutarium, Tectaria viridifrons* and so on. Medicinal potential of this plant in cure of diseases along with the presence of an active component is not explored extensively, thus the systematic on in-vitro analysis is essential to allocate its medicinal significance and associated characteristic.[5]

The present work try to demonstrate the application of modern methods to evaluate and quantify the active ingredients present in plant material along with exploration of its antimicrobial potency specifically against skin pathogens. In the present work phytochemical analysis and antimicrobial activity of *Aspidium cicutarium* was performed with the use of three extract viz. hexane extract, ethyl acetate extract, petroleum ether extracts respectively. This study may help to validate possible role of Kukktnakhi in medicinal formulation.

Table No. 1: Significant plant part used for treatment of various diseases

Plant part	Solvent	Concentration	Activity
Root	Methanol		Antioxidant[6]
Root	Ethanol		Anti-inflammatory[1]
Root	Methanol	100g of powder extracted in methanol	Defensive weapons against predators, anti-inflammatory, anti-oxidant therapy, antibiotics, anti-arthritic drug[7]
Root	Ethanol	1:100 solution	Anti-cancer activity [8]
Rhizome	Methanol		Antioxidant, wound healing [9]
Rhizome		540mg/kg	Acute toxicity and hyperlipidemic activity [10]
Rhizome	Distilled water	300gm	Chronic human ailments[11]
Rhizome	Benzene		Anti-inflammatory, anti-cancer activity[12]

MATERIALS AND METHODS

Collection of plant material

Rhizome of *Aspidium cicutarium* plant was obtained from authentic medicinal plant material Nursery from Mumbai and authenticated from the local botanist.

Preparation and Extraction of Plant Material

Soxhlet extraction is generally use when solubility of desired compound is limited in particular solvent with less impurity. Rhizomes of plant material were dried and grind into fine powdered with the help of grinder. Hexane extract, ethyl acetate extract, petroleum ether extract was prepared by using Soxhlet method. 100 g of rhizome powder was subjected for Soxhlet extraction in “thimble” and aliquots was filtered with Whatman filter paper no. 1. This extract was subjected for further analysis.[13]

Antimicrobial activity

Microorganisms

For analysis of broad spectrum, antimicrobial activity skin infecting pathogens such as *Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis* were selected. The pathogenic strains were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, and National Centre for Industrial Microorganisms (NCIM), NCL, Pune, India. All target bacterial strains were maintained on slant and subculture on regular intervals till its subjected to the final antimicrobial assay.

Disc Diffusion Assay[14]

The antimicrobial activity of non-polar fraction was determined by disc diffusion method. Desired quantity of nutrient agar was prepared and autoclaved. This autoclaved medium was poured in sterile Petri plats. After solidification of medium 1mL of bacterial suspension was added with micropipette and spread consistently with the glass road. A sterile paper disc was placed on solidifying medium. 100 µl of the test sample fraction were added on four paper discs placed on agar. Allow the test sample to diffuse across radius almost for 15 min. at room temperature. The respective plates were incubated for 24 hr at 37 ºC temperature. After incubation period, the antimicrobial activity was determined by measuring the diameter of zone of inhibition and expressed in mm. All the test was performed in triplets. The final zone of inhibition obtained was the average of the three readings obtained.

Minimum inhibitory concentration (MIC)[15]

MIC is lowest concentration the test observed with no turbidity. MIC was performed by agar disc. The most potent and promising sample from the previous antimicrobial assay was selected for the MIC test. Nutrient agar medium plates with target pathogens were subjected with petroleum ether extract only being the most promising extract in earlier analysis. Various concentration of petroleum ether extract ranging from 50 µl/ml to 300 µl/ml. were subjected for MIC analysis. Test extract allowed to diffuse on seeded nutrient agar plates for 15 min at room temperature. Plates were kept for incubation for 24 h at 37ºC temperature. The MIC was determined by observing least concentration resulting in inhibition of microbial growth measure in terms of zone of inhibition (ZOI)in mm against each test pathogen.

Minimum bactericidal concentration (MBC)[16, 17]

MBC is the lowest concentration at which complete bacterial growth inhibition is observed. This was determined with broth dilution assay. Test pathogens along with various concentrations (500– 1000 µl/ml) of the petroleum ether extract was subjected for determination of minimum bactericidal concentration. After incubation of 24 h turbidity was observed and 1ml of each test sample was plated on nutrient agar medium to confirm the bactericidal effect. All plates incubated at 37ºC temperature for 24 h. Results for MBC against each test pathogen was noted.

Preliminary Phytochemical Screening

The phytochemical investigation of all the obtained test extracts of rhizomes of *Aspidium cicutarium* were performed using available standard protocol[18].

Test for Alkaloids

Mayer's test

Two drops of Mayer's reagent were added along the sides of test tube into the obtained plant extracts. The presence of alkaloids is indicated by a white creamy precipitate[19].

Wagner's test

A few drops of Wagner's reagent were added into the obtained plant extracts and a reddish brown precipitate confirmed the presence of alkaloids[19].

Dragendroff's test

The addition of few drops of Dragendroff's reagent into the extracts gives red precipitate if alkaloids are present in the respective sample[20].

Hager's test

A small amount of Hager's reagent was added to the test extracts. The formation of yellow precipitate indicates the presence of alkaloids[20].

Test for phenolic and Tannins compound

A few drops of ferric chloride were added in a respective plant extract. Bluish black colour development confirms the presence of phenols. 10% lead acetate solution was added in each test extract. The white precipitation confirms the positive result[20].

Test for Flavonoids[19-20]

Alkaline reagent test

A few drops of sodium hydroxide were added in small amount of all obtained test extracts when dark yellow colour solution changes to colourless on addition of dilute acids confirms the test is positive.

Lead acetate Test

On addition of few drops of lead acetate solution to each respective test extract, formation of yellow colour indicates positive test.

Shinoda's Test

Separately dissolved all extracts in alcohol and then mixed in portion of magnesium with dropwise addition of conc. hydrochloric acid. Magenta colour development indicates positive test.

Test for Steroids[19-20]

Salkowski Tests

Addition of concentrated sulphuric acid solution in extract with slight intermittent shaking, if develop red colour then test recorded as positive test for steroids.

Lieberman-Burchard tests

1 ml concentrated sulphuric acid was added from the side of test tube and add few drops of acetic anhydride in each of the test extract. Reddish ring formed at the interface of 2 layers confirmed the presence of steroids.

RESULTS AND DISCUSSION

1. The results of antimicrobial activity

The results of antimicrobial activity of *Aspidium cicutarium* against the target skin pathogens i.e. *S. aureus, S. epidermidis, E. coli, P. mirabilis* have shown that the petroleum ether extract of the plant inhibited the growth of majority of the pathogen. The zone of inhibition (ZOI) as represented in Table No. 2 confirmed the highest zone of inhibition obtained with petroleum ether extract against *S. aureus* with 15.16 ± 0.4 mm ZOI, followed by ethyl acetate extract against *S. epidermidis* with ZOI of almost 13.63 ± 0.5mm and again followed by petroleum ether extract against *S. epidermidis* with 13.08 ± 0.1 mm ZOI respectively. Test pathogen *P. mirabilis* was showed susceptibility only to the petroleum ether extract with ZOI of almost 10.11 ± 0.3 mm. The graphical representation in Figure No. 1 represents the antimicrobial potency of the target test samples against of major skin pathogens. The antimicrobial test confirms the highest antimicrobial potency of petroleum ether extract followed by ethyl acetate extract and hexane extract respectively.

Table No. 3 and 4 reveals the results obtained after the MIC and MBC assays performed with the most potent and promising petroleum ether extract against all four selected bacterial pathogens. The MIC of the extract of petroleum was observed as 75 µl/ml against *S. aureus* and *S. epidermidis* whereas against pathogens *E. coli* and *P. mirabilis* it was recorded as 100 µl/ml. The MBC results obtained confirmed the complete microbial growth inhibition for *S. aureus* at concentration of 700 µl/ml, followed by *S. epidermidis* at concentration of 900 µl/ml. The bactericidal concentration for remaining two pathogens was not achieved at the highest concentration set for the experiment (1000 µl/ml).

2. The results of Preliminary phytochemical analysis

Preliminary phytochemical analysis of *Aspidium cicutarium* of hexane and petroleum ether extract shows presence of Alkaloids, Phenols, Tannins, Flavonoids and Sterols. While Ethyl acetate extract show presence of phenols, tannins and flavonoids respectively. Maximum of the phytochemicals were observed in petroleum ether extract. This extract was found to be potent as compare with the other extract under the present study. This extract was used for further analysis. The results are reported in Table No 5. Antimicrobial properties of this nonpolar extracts may be associated with the presence of these secondary metabolites with antimicrobial mechanism.

Earlier studies on preliminary phytochemical analysis of the rhizome of *Aspidium cicutarium* in methanolic and aqueous extracts revealed the presence of secondary metabolites such as Alkaloids, Phenols, Tannins, Flavonoids and Steroids and saponins which also confirmed that alkaloids were present only in methanolic extracts and not in aqueous extract.[21]

Present study reveals the antimicrobial potency of the petroleum ether *rhizome* extracts of *Aspidium cicutarium* against skin pathogens in particular. Secondary metabolites confirmed in preliminary phytochemical assessment as Alkaloids, Phenols, Tannins, Flavonoids and Sterols may be held accountable for the antimicrobial potency of the extract.

Further chemical characterization of these potent extract is essential by applying more sophisticated separation and purification techniques. It is also necessary to find out the exact chemical compounds and their association with the pharmacological activity.

Table No. 2: Antimicrobial activity of three extract of *Aspidium cicutarium*.

Sr. No	Microorganism	Zone of inhibition in mm		
		Hexane extract	Ethyl acetate extract	Petroleum ether extracts
1	*S. aureus*	10.99 ± 0.2	12.38 ± 0.1	15.16 ± 0.4
2	*S. epidermidis*	0 ± 0.0	13.63 ± 0.5	13.08 ± 0.1
3	*E. coli*	9.81 ± 0.3	9.81 ± 0.3	10.87 ± 0.6
4	*P. mirabilis*	0 ± 0.0	0 ± 0.0	10.11 ± 0.3

* Each value was expressed as the mean ± SD. (n=3)

Table No. 3: MIC values of petroleum ether extract of *Aspidium cicutarium*

Sr. No	Microorganism	MIC with zone of Inhibition in mm								
	Concentrations (µl/ml)	50	75	100	125	150	175	200	225	300
1	*S. aureus*	-	10.22	13.80	13.86	13.89	14.32	14.54	14.66	15.02
2	*S. epidermidis*	-	9.11	13.24	13.66	13.80	13.84	13.88	13.92	13.92
3	*E. coli*	-	-	10.66	10.80	10.80	10.86	10.90	10.90	10.98
4	*P. mirabilis*	-	-	10.12	10.12	10.12	10.12	10.68	10.68	10.68

Table No. 4: MBC values of three extract of *Aspidium cicutarium*

Sr. No	Microorganism	Extract concentration (µl/ml)
1	*S. aureus*	700
2	*S. epidermidis*	900
3	*E. coli*	>1000
4	*P. mirabilis*	>1000

Table No. 5: Phytochemical screening of hexane, ethyl acetate and petroleum ether extracts of *Aspidium cicutarium*

Phytochemical constituents	Test	Hexane extract	Ethyl acetate extract	Petroleum ether extracts
Alkaloids	Mayer's reagent	+	-	+
	Dragendorff's reagent	+	-	+
	Wagner's reagent	+	-	+
	Hager's reagent	+	-	+
Phenolic and Tannins compounds	Ferric chloride test	+	+	+
	Lead acetate test	+	+	+
Flavonoids	Lead acetate test	+	+	+
	Ferric chloride test	+	+	+
	Sodium Hydroxide test	+	+	+
	Shinoda test	+	+	+
Sterols	Salkowski reaction	+	-	+
	Liebermann's test	+	-	+

(+ :Present; -: Absent)

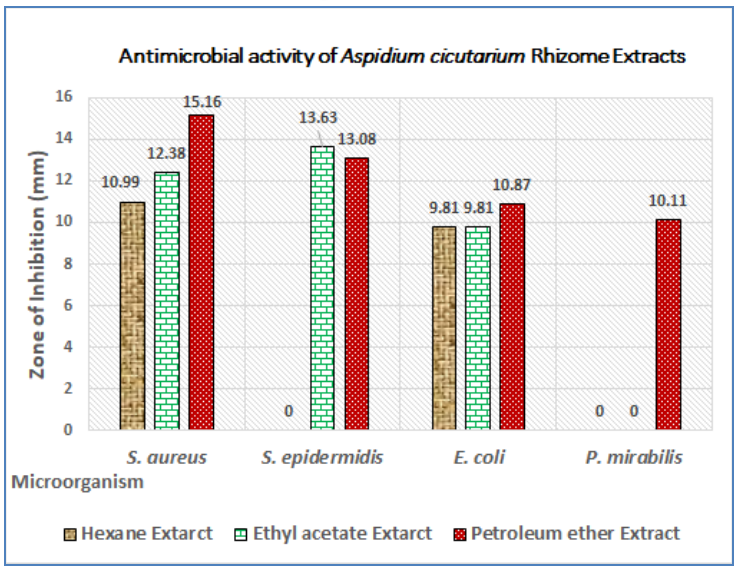

Figure No. 1: Graph - Antimicrobial activity of *Aspidium cicutarium Rhizome Extracts*

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