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EVALUATION OF ANTI-MICROBIAL ACTIVITY OF *DRYOPTERIS COLCHEATA* RHIZOME

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Keywords:

Dryopteris cochleata, Antibacterial activity, Disc diffusion method, Gram-positive bacteria, Gram-negative bacteria

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ABSTRACT: Herbal drugs have gained more importance and popularity in recent years because of their safety, efficacy, and low cost. The pteridophytes have an important role in folklore medicine, although neglected in modern days. These plants have been successfully used in different systems of medicines like Ayurvedic, Unani, Homeopathic, and other systems of medicines. Antimicrobial drugs are the greatest contribution of the present century to therapeutics. *Dryopteris cochleata*, family Dryopteridaceae which is popularly called as Jatashankari exhibits various therapeutic values to treat many diseases such as, epilepsy, leprosy, wounds, ulcers, diabetes, *etc*. was selected for the present research work to screen for antibacterial properties. The antimicrobial activity of ethanolic extract of *Dryopteris cochleata* was screened against two gram-positive bacteria (*B. subtilis*, *S. mutans*) and three-gram negative bacteria (*E. coli*, *P. aeruginosa*, *K. pneumonia*) and antifungal activity against *Candida albicans* and *Aspergillus flavus* by disc diffusion method. The extract showed significant antimicrobial activity against *B. subtilis*, *S. mutans*, *E. coli*, *P. aeruginosa*, *Candida albicans* but did not show antimicrobial activity towards *Klebsiella pneumonia* and *Aspergillus flavus*. Ciprofloxacin and fluconazole were used as standards for antibacterial and antifungal activity, respectively.

INTRODUCTION: Antimicrobial resistance (AMR) is becoming the major threats for human and animal health that has accelerated the search for new antimicrobial agents to be used from nature. Initial research projected that a continued rise in antimicrobial resistance by 2050 would lead to 10 million people dying every year [1]. From time immemorial, the phenomena of nature having healing properties are of different kinds.

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They have been the providers of precise and particular spheres of action and performance pertaining to Biology. This is in pursuance of a formal statement of the theorem that indispensable all the natural products have specific obligatory receptor binding power [2].

Herbal drugs have been the foundation for treatment of different disorders of the body and physiological conditions in traditional methods exercised by the practitioners of Ayurveda, Unani and Siddha handed down from generation to generation [3]. However, the use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community [4]. Pteridophytes also are known to have antibacterial, antifungal, antiseptic, anthelmintic and detergent properties [5]. In order to help the progress of the appropriate application of the herbal drugs and to ascertain their core capabilities as origins for new medicines, it is necessary to carefully and closely examine the herbs having curative properties with fabulous fame in traditional beliefs and legends [6].

A considerable amount of special attention to a very careful and conscientious study of the natural phenomena as origins of new medicines to combat against the microbial substances has been noticed during the last two decades [7]. And, as a matter of fact, antimicrobial abilities of a fair number of herbal medicines have already been ratified by the appropriate authority. A number of recent reviews can be referred for obtaining an exhaustive list of herbals with anti-microbial activity [8-9]. Although several successes have been achieved in the field of research, an uninterrupted and intensive investigation of herbage with extensive efforts are really required to find out new compounds combating against microbial elements.

They ought to be accompanied by several chemical organisms and a new process of protection against manifesting contagious diseases of recent times [10-11]. Bacteria and fungi cause some important human diseases, particularly in persons who are under medical treatment and not having enough of immunity. Despite the presence of powerful and efficacious preventatives of bacteria and funguses, the past 30 years have an exciting growth of manifold microbes antagonistic to antimicrobial substances resulting in the reiterated application of antibiotics and inadequate influence over ailments. Consequently, there has been an absolute and inevitable necessity of permanency in the investigation, followed by the fruitful gradual unfolding of new drugs [12].

In every part of the history of mankind, multifarious medicinal herbs have been applied to cure a good number of contagious diseases. Apart from ancient times, even in many progressive parts of the modern world, many kinds of healing herbage are used at the initial stage as medicines concerned with curing diseases [13]. In an endeavor to find out new pharmaceutical compounds, a great number of eager research-workers are now more and more attracted to indigenous traditional drugs with the view of obtaining new causal compounds to produce new reactive remedies to be used against various contagions of microbes [14].

METHODS:

Successive Solvent Extraction: The rhizome part of *Dryopteris cochleata* was collected from Amarkantak forest district Bilaspur, Chattisgarh, and was authenticated by the Botanical Department, Saifia Science College, Bhopal, Madhya Pradesh, voucher specimen numbers 476/Bot/Saf/13. The rhizomes were washed with tap water prior to distilled water, shade dried and coarsely powdered using a cutter mill, then stored in an air-tight, light-resistant container for further use. The shade dried plant material was subjected to extraction with petroleum ether (60-80 ºC) in a Soxhlet apparatus. The extraction was continued until the defatting of the material had taken place. The marc from the central compartment was removed and dried 100 g. the dried material was exhaustively extracted with ethanol and using continuous hot percolation for 16 h. The extract was evaporated above their boiling points. Finally, the percentage of yields were calculated for the dried extracts.

Anti-microbial Activity: For the studies of antimicrobial effect of phytochemicals obtained from *Dryopteris cochleata* rhizome extract a medicinally important plant, there was 7 microorganism successfully procured from a microbial culture collection, National Centre for Cell Science, Pune, Maharashtra, India.

The lyophilized cultures of bacterial strains upon culturing in nutrient broth for 24-48 h at 37 ºC in an incubator resulted in a turbid suspension of activated live bacterial cells ready to be used for the microbiological study. Five bacterial and two fungal species with suitable codes used in the antimicrobial studies. The standard antimicrobics used for bacterial strains was Ciprofloxacin, and that for fungal strains Fluconazole. From the broth of respective revived cultures of bacteria loop full of inoculums is taken and streaked on to the nutrient agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on to the surface of the agar culture that are successfully stored in refrigerated conditions at 4 ºC as stock culture to be used for further experimentation.

The well diffusion method was used to determine the antibacterial activity of the extract prepared from the *Dryopteris cochleata* rhizome using a standard procedure (Bauer, 1966). There was 3 concentrations used, which are 25, 50, and 100 mg/ml for each extracted phytochemicals in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37 ºC for 24 h and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Statistical Analysis: The data are expressed as mean ± SEM (n=6). Statistical significance was determined by two way ANOVA followed by Bonferroni post-tests and one way ANOVA followed by Tukey's post-tests with using Graph Pad Prism 5.0 (San Diego, CA, USA) and Microsoft Excel 2007 (Roselle, IL.USA).

TABLE 1: ANTIMICROBIAL ACTIVITY OF STANDARD DRUGS ON DIFFERENT MICROBES

S. no.	Name of drug	Name of microbes	Zone of inhibition		
			10 µg/ml	**20 µg/ml**	**30 µg/ml**
1	Ciprofloxacin	*Bacillus subtilis*	8 ± 0.15	10 ± 0.18	15 ± 0.14
		Streptococcus mutans	11 ± 0.12	16 ± 0.32	18 ± 0.45
		Escherichia coli	9 ± 0.19	12 ± 0.13	14 ± 0.15
		Pseudomonas aeruginosa	15 ± 0.08	18 ± 0.12	19 ± 0.09
		Klebsiella pneumonia	12 ± 0.14	17 ± 0.22	25 ± 0.37
2		*Candida albicans*	8 ± 0.04	9 ± 0.09	11 ± 0.11
	Fluconazole	*Aspergillus flavus*	9 ± 0.21	10 ± 0.13	14 ± 0.14

TABLE 2: ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT ON DIFFERENT MICROBES

S. no.	Name of microbes		Zone of inhibition		
			25 mg/ml	**50 mg/ml**	**100 mg/ml**
1	*Bacillus subtilis*	Gram +ve	9 ± 0.14	10 ± 0.11	16 ± 0.15
2	*Streptococcus mutans*	Gram +ve	13 ± 0.12	16 ± 0.41	18 ± 0.14
3	*Escherichia coli*	Gram -ve	9 ± 0.11	10 ± 0.15	14 ± 0.12
4	*Pseudomonas aeruginosa*	Gram -ve	8 ± 0.08	9 ± 0.15	11± 0.14
5	*Klebsiella pneumonia*	Gram –ve	6 ± 0.00	6 ± 0.00	6 ± 0.00
6	*Candida albicans*	Fungus	8 ± 0.08	9 ± 0.21	11 ± 0.23
7	*Aspergillus flavus*	Fungus	6 ± 0.00	6 ± 0. 00	6 ± 0.00

TABLE 3: RESULTS OF ANTIMICROBIAL ACTIVITY OF PHYTOCHEMICAL EXTRACT

S. no.	Codes bacteria	Microbial strains		Activity
1	Bact-1	*Bacillus subtilis*	Gram +ve	Yes
2	Bact-2	*Streptococcus mutans*	Gram +ve	Yes
3	Bact-3	*Escherichia coli*	Gram -ve	Yes
4	Bact-4	*Pseudomonas aeruginosa*	Gram -ve	Yes
5	Bact-5	*Klebsiella pneumonia*	Gram –ve	No
6	Fungus-1	*Candida albicans*	Fungus	Yes
7	Fungus-2	*Aspergillus flavus*	Fungus	No

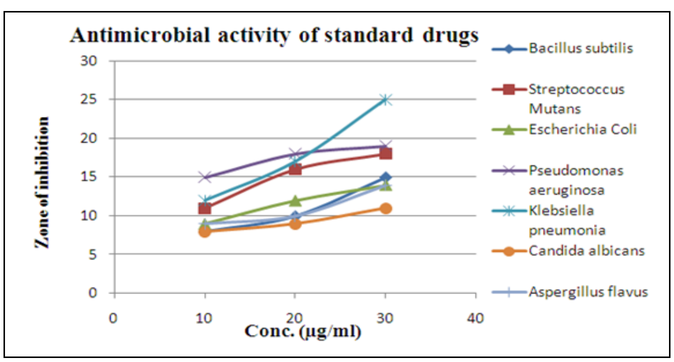


FIG. 1: ANTIMICROBIAL ACTIVITY OF STANDARD ON DIFFERENT MICROBES ZONE OF INHIBITION *vs.* CONCENTRATION

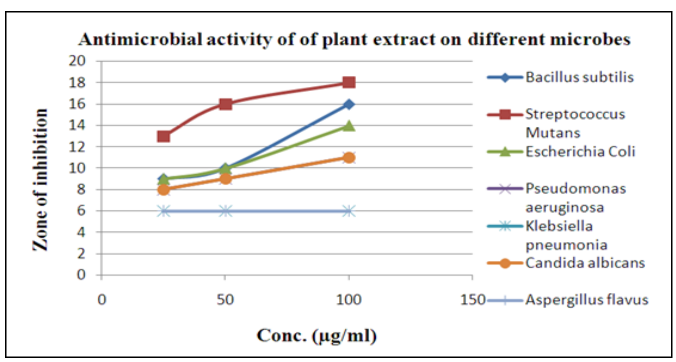


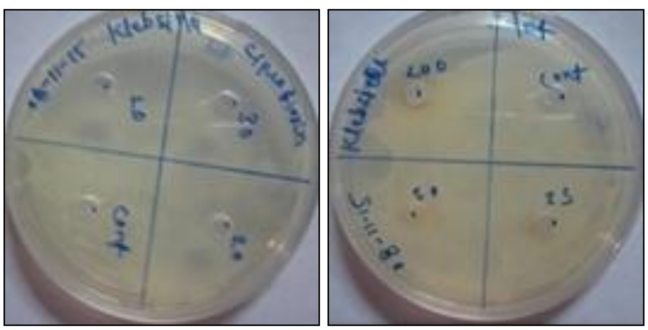
FIG. 2: ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS ON DIFFERENT MICROBES ZONE OF INHIBITION *vs.* CONCENTRATION

PHOTOPLATES OF ANTBACTERIAL ACTIVITY OF CIPROFLOXACIN AND PLANT EXTRACT ON *BACILLUS SUBTILIS*

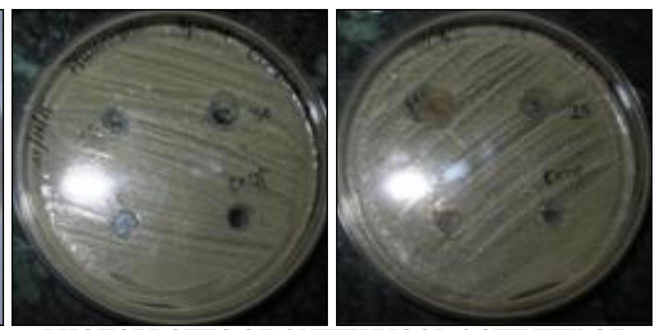
PHOTOPLATES OF ANTBACTERIAL ACTIVITY OF CIPROFLOXACIN AND PLANT EXTRACT ON *STREPTOCOCCUS MUTANS*

PHOTOPLATES OF ANTBACTERIAL ACTIVITY OF CIPROFLOXACIN AND PLANT EXTRACT ON *ESCHERICHIA COLI*

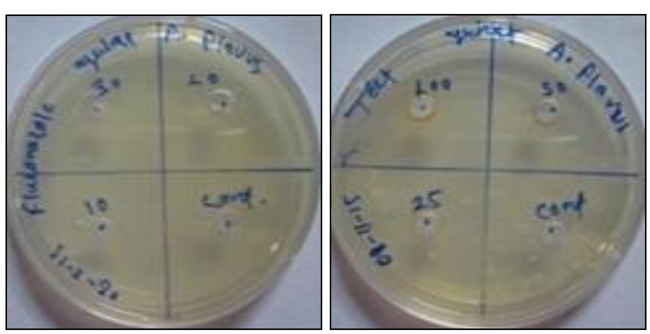
PHOTOPLATES OF ANTBACTERIAL ACTIVITY OF CIPROFLOXACIN AND PLANT EXTRACT ON *PSEUDOMONAS AERUGINOSA*



PHOTOPLATES OF ANTBACTERIAL ACTIVITY OF CIPROFLOXACIN AND PLANT EXTRACT ON *KLEBSIELLA PNEUMONIA*



PHOTOPLATES OF ANTIFUNGAL ACTIVITY OF FLUCONAZOLE AND PLANT EXTRACT ON *CANDIDA ALBICANS*



PHOTOPLATES OF ANTIFUNGAL ACTIVITY OF FLUCONAZOLE AND PLANT EXTRACT ON *ASPERGILLUS FLAVUS*

FIG. 3: PHOTO PLATES OF ANTIMICROBIAL STUDY PLANT EXTRACTS ON DIFFERENT MICROBES

RESULTS AND DISCUSSION: The lawn cultures were prepared with all the microbes used under the present study and sensitivity of bacteria and fungus towards the various phytochemicals extracts obtained from the *Dryopteris cochleata* rhizome. The fresh, pure 100% extracts obtained from the plant used to suitably dilute up to the concentrations of 25, 50, and 100 mg /ml and applied on to the test organism using well diffusion method. The extracts were compared to the standard antibiotics. The standard antibacterial agent used was Ciprofloxacin and while the standard antifungal agent used as Fluconazole. The current study was initiated because of the increasing resistance to antibiotics, including bacteria and fungi. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents. The antimicrobial activity of ethanolic extract of *Dryopteris cochleata* was screened against two gram-positive bacteria (*B. subtilis*, *S. mutans*) and three-gram-negative bacteria (*E. coli*, *P. aeruginosa*, *Klebsiella pneumonia*) and antifungal activity against *Candida albicans* and *Aspergillus flavus* by disc diffusion method. Ciprofloxacin and fluconazole were used as standards for antibacterial and antifungal activity, respectively. The results of antimicrobial activity are presented in **Table 1**, **2**, **3**, **Fig. 1**, **Fig. 2,** and **Fig. 3**.

The extract showed significant antimicrobial activity against *B. subtilis*, *S. mutans*, *E. coli*, *P. aeruginosa*, *C. albicans* but did not show anti-micobial activity towards *Klebsiella pneumonia* and *Aspergillus flavus*. The antimicrobial potential of ethanol extracts of *Dryopteris cochleata* rhizome against tested micro-organisms conformed that various phytoconstituents present in the extract has antimicrobial activity. Earlier study proves that *Dryopteris cochleata* possesses antibacterial principles, soluble in acetone, which inhibit the growth and multiplication of some multidrug-resistant bacterial strains [15].

CONCLUSION: The present research has opened the new outlook for the discovery of cost-effective and safe bioactive molecules from *Dryopteris cochleata*. Further, work, especially bioassay-guided fractionation, is warranted in order to isolate and characterize the active constituents responsible for the antimicrobial activity.

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CONFLICTS OF INTEREST: The author(s) confirm that this article content has no conflicts of interest.

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