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ANTIBACTERIAL ACTIVITY OF FOUR ETHNOMADICINAL *CHEILANTHES* SPECIES FROM NORTHERN WESTERN GHATS OF INDIA

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ABSTRACT: Objectives: To screen the antimicrobial potential of four ethno medicinal ferns in Pteridaceae family such as *Cheilanthes* sp.: *C. farinosa, C. anceps, C. tenuifolia*, and *C. albomargi nata* which is used in folk medicines in Northern Western Ghats of India against human bacterial pathogens. Methods: Antibacterial ability was performed by disc diffusion method against the pathogens viz., *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) and incubated for 24 h at 37 ˚C Results: The maximum degree of antibacterial activity was observed in *C.farinosa* followed by, *C.anceps*, C.tenuifolia, whereas C. *albomarginata* indicated comparatively low degree of antibacterial activity. The methanolic extract of *C.anceps* indicated the antibacterial activity against three pathogens viz., *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) with the inhibition zones 12 mm, 10 mm and 9 mm, individually. Hexane extracts of *C.farinosa* also indicated the antibacterial activity against two selected pathogens such as Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) with 15 mm and 12 mm of inhibition zones. Totally the three different concentrations (0.25, 0.50 & 0.75 mg/mL) of methanolic extract of *C.anceps* display the inhibitory result on the three susceptible bacteria Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) through the maximum inhibition in the top concentration (0.75 mg/mL). The methanolic and Hexane extracts of *C.tenuifolia* exhibited the antibacterial activity against only one bacterium each i.e. Staphylococcus aureus (S. aureus) (NCIM 2654) and *Pseudomonas aeruginosa* (NCIM 5032) with the maximum zone of inhibition 13 and 11 mm respectively. The methanolic and hexane extracts of *C. albomarginata* exhibited the antibacterial activity against only one bacterium i.e. *Pseudomonas aeruginosa* with the maximum zone of

inhibition 13 and 11 mm respectively. Conclusions: The present investigation revealed that the *C.farinosa, C.anceps*, *C.tenuifolia* and *C. albomarginata* are potentially noble source of antibacterial agents and determines the significance of such plants in the traditional medicines they may be used for medicinal determinations in future. To the best of our knowledge, this is the first paper presenting complete data on Antibacterial Activity of Four Ethnomadicinal *Cheilanthes* Species from Northern Western Ghats of India.
Keywords: Antibacterial, Pteridaceae, Ferns, *Cheilanthes* spp, Ethnomadicinal, folk medicines.

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1.INTRODUCTION

Pteridophytes Plants and plant products have been widely used throughout history to treat therapeutic problems. Several studies have been carried out to extract numerous natural products for screening antimicrobial activity (1-5) however attention has not been concentrated intensively on studying the combinations of these products for their antimicrobial activity. The aim of this study were to examine the antibacterial effect of four plant extracts utilized in Northern Western Ghats of India in popular medicine as well as to determine the effectiveness of some combinations of these extracts on six different species of Gram-positive and Gram-negative bacteria. In India this is necessary because traditional plant medicines often come in multi-component preparations targeted at curing quite a lot of diseases simultaneously. Plants bear plentiful active compounds, each identified with specific biological activities between which antimicrobial activity is the most essential one, which preserves the plants from susceptible state of microbial infections. Various such active compounds like secondary metabolites such as flavonoids, phenolics and polyphenols, tannins, terpenoids and sesquiterpenes [6,10], etc., and they are found to act as active antimicrobial compounds against a widespread range of microorganisms. The lower phyto group particularly the pteridophytes also have alike such active principles for which they are not infected by microbial pathogens and in future survived for more than 350 million years. [12]. Screening designed for antibiotic activity in the extracts of 114 species of pteridophytes fit in to 61 genera from 27 families was surveyed. 67 ferns and 6 fern allies, representing 64% of the samples inspected, were keenly antibiotic. The active responsive substances in most cases were antibacterial and only three influenced antifungal activities [13]. The Northern Western Ghats of India is one of the 29 biological hot spots well-known in the world and remarkable with large number of plants with unique medicinal

properties [14, 17]. These plants are primarily utilized by the local folkloric people inhabiting in this area [18,19].Constructed on these reports it is assumed that pteridophytes constitute, a good source of antimicrobial compounds among which the species four *Cheilanthes* spp.: *C farinosa, C.anceps, C.tenuifolia,* and *C. albomarginata* was screened for evaluation of the antibacterial activity. As these plants are in an endangered and vulnerable state due to habitat destruction as well as cutting down of forest species in which these cultivate as epiphytes, it pressed its comparative bioactivity study on *in vivo* and *in vitro* developed plant parts locally used as medicinal. The other significant reason for screening of its antibacterial activity is to place restriction in increasing failure of chemotherapeutics as well as antibiotic resistance discovered by microbial pathogens therefore establishing its antimicrobial potency [20,22] ultimately providing herbal source with less cost effective as well as no side effects. Therefore all the above four plants are being used by the Tribal peoples of Northern Western Ghats of India to cure mostly the pathogenic diseases. In future, in the current investigation, the antimicrobial potential of these four plants (*C. farinosa, C. anceps, C.tenufolia* and *C.albomarginata*) have been evaluated against selected human bacterial pathogens.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

Plant materials of four *Cheilanthes* species were obtained from different localities of Northern Western Ghats of India (*Cheilanthes farinosa* (Forssk.) Kaulf. — Molem locality, GPS: 15^0 22^l09^{ll} N′,74^0 12l 44^{ll} E; *Cheilanthes anceps Sw*, — Mahabaleshwar locality, GPS: 17^0 55^l 31^{ll} N, 73^0 39^l 45^{ll} E; *Cheilanthes tenuifolia (Burm.f.) Sw*, — Gaganbawada locality, GPS: 16^0 31^l 58^{ll} N′,73^0 49^l 5^{ll} E; *Cheilanthes albomarginata* Clarke — Amboli locality, GPS: 15^0 57^l 42^{ll} N, 73^0 59^l 48^{ll} E;). Specimens were authentically identified with help of Dr.Manisha kale (Associate Professor Department of Botany, Jaysingpur College Jaysingpur, Maharashtra, India. The Whole Plant along with rhizome of *Cheilanthes* sp. was collected from Northern Western Ghats, India. The *Cheilanthes* species were cleaned and separated into dry powder form. The CSWPR was stored in a freezer (−20°C) until further analysis.

2.2 Methanol and Hexane extraction

10 g of each plant powder was added to 100 ml of methanol and Hexane in a conical flask as well as plugged with cotton wool. The supernatant was collected after 24 hours and the solvent was evaporated to make the crude extract and stored at 4 ℃Methanolic and Hexane extract were prepared from powder materials and the extract was used for antimicrobial studies. Antimicrobial studies was carried out by disc diffusion method [20] against the pathogens viz., *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032)

3. RESULTS AND DISCUSSION

The methanolic and hexane extracts were tested for antibacterial activity against three human bacterial pathogens by using three differ concentrations viz., 0.25, 0.50 and 0.75 mg/mL. The maximum degree of antibacterial activity was observed in *C.farinosa*, *C. anceps*, *C.tenufolia* as well as *C. albomarginata* indicated comparatively low degree of antibacterial activity. The antibacterial activity of extract and their potency was quantitatively assayed by presence of inhibitory zone. The extract differed in its antibacterial activity against different microorganisms which was extracted by using methanolic and hexane solvents. The all extracts showed antibacterial activity against different organism (Figure 1, 2, 3). Among them methanol and hexane extract showed maximum antimicrobial activity and it was chosen for further study (Table 1). The methanol and hexane extract showed best antimicrobial activity against *Staphylococcus aureus Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) in present study.

Fig. 1a

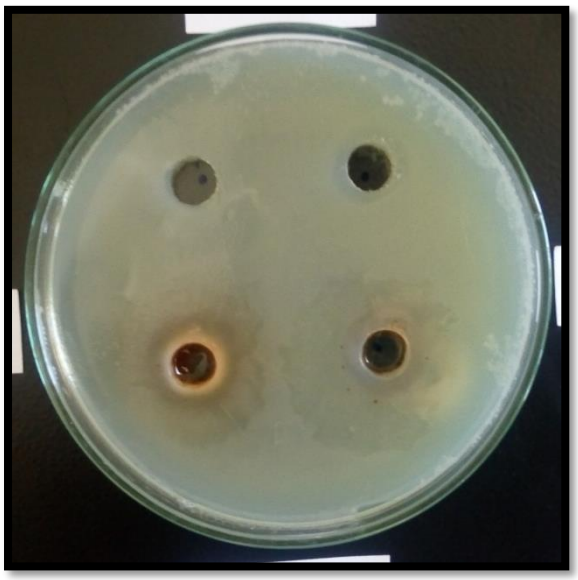

Fig. 1b

Fig.1 Antimicrobial activity of crude extract dissolved in; **Fig-1a** Methanol and Hexane, **Fig.-1b** Methanol and Hexane against *Salmonella typhimurium.*

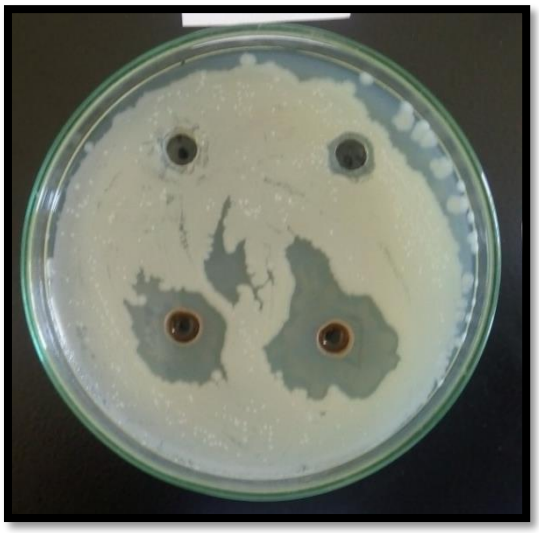

Fig. 2a

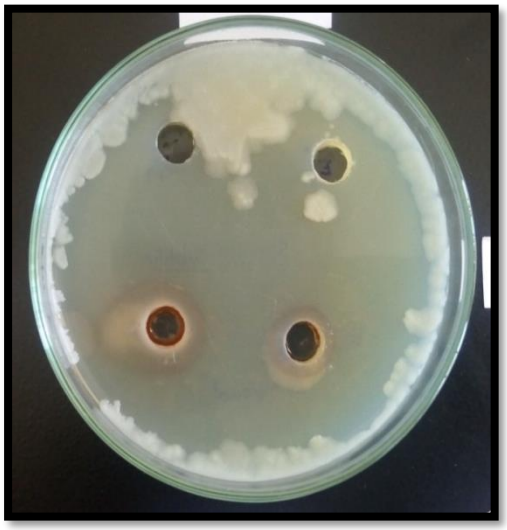

Fig. 2b

Fig.2 Antimicrobial activity of crude extract dissolved in; **Fig-2a** Methanol and Hexane, **Fig.-2b** Methanol and Hexane against *Staphylococcus aureus*

Fig. 3a

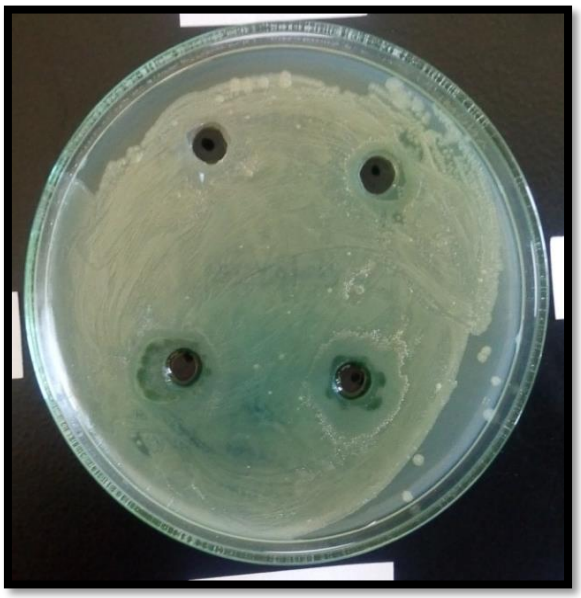

Fig. 3b

Fig.3 Antimicrobial activity of crude extract dissolved in; **Fig-3a** Methanol and Hexane, **Fig.-3b** Methanol and Hexane against *Pseudomonas aeruginosa* .

Table 1. Antibacterial activity of Methanolic and Hexane extracts of *C. farinosa, C.anceps, C. tenuifolia and C. albomarginata* against pathogens.

Sr. No	Solvents	Concentration (mg/ml)	Zone of inhibition (mm)											
			C. farinosa			*C.anceps*			*C. tenuifolia*			*C.albomargina*		
			St	Sa	Pa	St	Sa	Pa	St	Sa	Pa	St	Sa	Pa
1	Methanol	0.25	5	4	5	6	5	6	5	5	5	5	5	5
2		0.50	7	7	7	14	7	13	7	7	8	9	11	9
3		0.75	11	8	12	13	12	15	12	13	14	12	15	14
4	Hexane	0.25	5	6	7	5	4	5	5	5	4	5	5	5
5		0.50	7	10	12	7	7	7	7	7	7	8	7	7
6		0.75	12	13	16	12	12	12	12	12	10	13	12	12

The methanolic extract of *C.farinosa* indicated that antibacterial activity against three pathogens viz., *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) with the inhibition zones 12 mm, 11 mm and 8 mm respectively (Table 1). Hexane extracts of *C.farinosa* also showed the antibacterial activity against two selected pathogens viz., *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) with 12 mm, 13 mm and 16 mm of inhibition zones to hexane extracts of C.farinosa (Table 1). All the three different concentrations (0.25, 0. 50, 0 .75 mg/mL) of methanolic extract of *C.farinosa* show the inhibitory effect on the three susceptible bacteria *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) with the maximum inhibition in the highest concentration (0.75 mg/mL). In contrast, the different concentrations of hexane extract shows inhibitory effect on only two bacteria P. vulgaris and P. mirabilis with the maximum inhibition in the highest concentration (0.75 mg/mL). The methanolic and hexane extracts of C. anceps exhibited the antibacterial activity against only one bacterium each i.e. Staphylococcus aureus (S. aureus) (NCIM 2654), and *Pseudomonas aeruginosa* (NCIM 5032) with the maximum zone of inhibition 15 and 12 mm respectively (Table 1). The methanolic and hexane extracts of *C. tenuifolia* exhibited the antibacterial activity against three bacterium i.e. *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) (Table 1) with the maximum zone of inhibition 14,13 and 12 mm respectively (Table 1).The methanolic and

hexane extracts of C. albomarginata exhibited the antibacterial activity against only one bacterium each i.e. *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032) with the maximum zone of inhibition 14, 15 and 13 mm respectively (Table.1)

In the current research, in vitro antibacterial effectiveness of the crude extracts of four plants was quantitatively evaluated on the basis of zone of inhibition. All the plants considered in the current study revealed fluctuating degree of inhibitory result against the selected bacterial human pathogens. Eloff J.N was stated that methanol is the maximum effective solvent for plant extraction than water and hexane. In the current study we used methanol and hexane for extraction. The current study set the Eloff interpretations with maximum activity [23].The current study point out that anti-bacterial activity of the four plants against the nominated strains of human pathogenic bacteria diverges depends upon the solvent medium used for extraction. C. farinosa displays highest activity against the bacterial pathogens followed by C. anceps and C.tenufolia. Based on the earlier literature on these four plants, there is no experimental study on C. anceps. There are some studies on Phytochemistry and pharmacology[24, 25], but there is no report on antimicrobial activity as well as the [26-35] Hence, the current study displays the presence of antibacterial activity in C. anceps and C. farinosa for the first time. In the case of C.tenufolia, in addition to the earlier observation, the current study discovered and enhanced the antibacterial activity against the bacterial pathogen *Pseudomonas aeruginosa.* The presence of antimicrobial activity in a specific amount of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc. [36]. In recent times, a number of plants have been stated for antimicrobial properties through the world [37, 38, 39]. In the current study, four ethno medicinal plants from Northern Western Ghats of India have been screened for antimicrobial potential. As point out earlier, the whole plant along with rhizome C. anceps are used to treat asthma, bronchitis, cold, cough, stomach ache, Irregular menstruations as well as used as Tonic. Between the two susceptible bacteria Staphylococcus aureus is known to cause urinary tract infections and wound, *Salmonella typhimurium* is recognized to reason fever and food borne illness. In the current study the methanolic extract and hexane extract of C. anceps display the inhibitory activity against the above two bacteria. Therefore the current study confirms the traditional medical practice of the Tribes of Northern Western Ghats. Ever since the above two bacteria are susceptible to similar extract (*Pseudomonas aeruginosa* for methanol extract and *Salmonella typhimurium* for hexane extract), it is clear that the active compound may be of completely diverse ones. Base on the current results, it is recommended that the same plant might be used to treat water borne diseases, Hepatitis, Urinary tract infections, etc. Current study on C. farinosa discovered the high degree of antibacterial activity against four different bacteria namely *Salmonella typhimurium* (NCIM 2501), Staphylococcus aureus (S. aureus) (NCIM 2654), *Pseudomonas aeruginosa* (NCIM 5032). In

overall the above three bacteria are recognized to cause gastroenteritis, food borne illness, urinary tract infections, neonatal meningitis, nosocomial infections, wound, septicemia, pneumonias and from minor superficial skin infections to life-threatening systemic diseases. The Tribes of Northern Western Ghats, without having any scientific knowledge, for numerous decades they are positively using the plant C.farinosa to treat numerous pathogenic diseases such as diarrhea, wounds, scabies and chicken pox. The current experimental study approves the traditional practice and supplement to treat other health problems such as urinary tract infections, neonatal meningitis, nosocomial infections, septicemia and pneumonias. In time being the whole plants along with rhizome C.farinosa were also found to contain high concentration of saponins [40] which may be responsible for the antibacterial activity against several bacteria.The outcomes of the current study supplement the folkloric practice of the studied plants which have several identified and unidentified bioactive compounds with antibacterial properties. Complete isolating and identifying these bioactive compounds innovative drugs might be formulated to treat several communicable diseases. Additional phytochemical and pharmacological studies on the lesser known plants, *C.farinosa* and *C.anceps* are necessary to apply these ethnomedicnally significant plants positively the existence of intraspecific difference in *C.albomarginata* and *C.tenufolia* with the presence of more differentiated chemicals with different bioactivities which are still not to be studied.

4. CONCLUSION

In this study we first time report the presence of antibacterial activity from the Four Ethnomedicinal Cheilanthes Species such as *C.farinosa, C.anceps*, *C.tenuifolia* and *C. albomarginata* shows antibacterial activity against Gram positive and Gram negative pathogenic organisms such as *Salmonella typhimurium*, Staphylococcus aureus and *Pseudomonas aeruginosa.* So this outcome could be used as an active pharmaceutical constituent in the treatment of diseases which are caused due to antibiotic resistant bacteria like *Salmonella typhimurium*, Staphylococcus aureus, *Pseudomonas aeruginosa*. The present investigation revealed that the *C.farinosa, C.anceps*, *C.tenuifolia* and *C. albomarginata* are potentially noble source of antibacterial agents and determines the significance of such plants in the traditional medicines they may be used for medicinal determinations in future as well as could be used as an active pharmaceutical ingredient in the treatment of diseases which are caused due to antibiotic resistant bacteria like *Salmonella typhimurium*, Staphylococcus aureus, *Pseudomonas aeruginosa.* To the best of our knowledge, this is the first paper presenting complete data on Antibacterial Activity of Four Ethnomedicinal Cheilanthes Species from Northern Western Ghats of India

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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