World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 3.347

ANTIBACTERIAL EVALUATION OF DIFFERENT EXTRACT OF *PTERIS VITTATA* AGAINST HUMAN PATHOGENS IN DOON VALLEY, UTTARAKHAND, INDIA

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Article Received on 10/07/2016 Article Revised on 31/07/2016 Article Accepted on 21/08/2016

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ABSTTRACT

Pteris vittata L. has antimicrobial activity, antitumor activity, platelet aggregation, anti-inflammatory action, hypotensive, hypogycemic, antiviral and anti bacterial activity. Ethno medicinally, the whole plant is used for healing of wounds, for the relief of glandular swelling by the folklores of Orissa and also used for phytoextraction of heavy metals. The present study was carried out on the antibacterial activity of *Pteris vittata* in Doon valley, situated in foot hills of the Himalayas. Plant material was collected, dried and grinded out a powdered form which was then subjected to soxhlet extraction using different solvents in the increasing order of their polarity. Different extract of the plants revealed its antibacterial activity against the studied pathogenic bacterial strains. Out of the seven extracts assayed the ethyl acetate extract was most active against the studied bacterial strains. Therefore, minimum inhibitory concentration (MIC) of this extract was determined against the selected bacteria showing zones of inhibition ≥ 10mm.

KEYWORDS: *Pteris vittata*, antimicrobial activity, minimum inhibitory concentration.

INTRODUCTION

For thousands of years, nature has been a source of medicinal agent from where an impressive number of modern drugs have been isolated based on their use in traditional

medicine. Historically, most of the medicinal preparations were derived from plants, the medicinal value of which lies in some chemical substances that produce a definite physiological action on the human body. Pteridophytes are vascular cryptograms which constitute ferns and ferns allies and forms a conspicuous element of vegetation all over the earth's surface.[1] The world flora consists of approximately 12,000 species of pteridophytes of which around 1000 species distributed in 70 families and 192 genera are likely to occur in India[2]**.** Most of the pteridophyte diversity is observed in the Himalayas, Eastern and Western Ghats. Though the pteridophytes occur in abundance in the tropical, sub-tropical and moist deciduous forests of India, large scale destruction of forests has drastically affected the diversity of pteridophyte species.[2] The pteridophytes possess 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.

Pteris vittata L., which is commonly known as the Chinese brake fern is a cosmopolitan pteridophyte found in crevices and in any calcareous substrate.[3] This vascular fern, *Pteris vittata* L. is reported to have antimicrobial activity[4,5], antitumor activity, platelet aggregation, anti-inflammatory action[6] as well as hypotensive, hypogycemic, antiviral and anti bacterial activity.[7] Ethnomedicinally, the whole plant is made into a paste and used for healing of wounds by applying externally.[8] Moreover, paste of fresh rhizomes and fronds of this fern is applied for the relief of glandular swelling by the folklores of Orissa.[9] *Pteris vittata* can be used for phytoextraction as it hyper accumulates arsenic(As)[3] even from low contaminated soils, can tolerate up to 1,500 mg kg^{-1} As in the soil, and can concentrate the metalloid in the fronds up to 2.3% of the plant dry weight. In view of the above literature, the present study was conducted to find a good natural antimicrobial drug for treatment of the manifestation caused by microorganisms. Different extract of plant *Pteris vittata* L were tested for their antimicrobial potential against various pathogenic bacterial strains.

MATERIAL AND METHODS

Materials

The material for the present study comprised of whole plant of Chinese Brake fern, *Pteris vittata* belongs to the family Pteridaceae. It grows on undisturbed moist and shady areas in Dehradun, Uttarakhand.

EXPERIMENTAL METHODOLOGY

Collection of plant

The fresh whole plant was collected from different places of Doon valley. The plant samples were dried in shade at 25 ^{0}C to 35 ^{0}C for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant materials were stored in paper bags.

Extraction

The dried plant material powder was subjected to successive Soxhlet extraction with different solvents in increasing order of polarity (i.e. Petroleum ether < Benzene < Chloroform < Ethyl acetate < Acetone <Distilled water).

About 50 gm accurately weighed dry plant sample powder was taken in thimble and about 250 ml of solvent taken in round bottom flask (RBF) and it was fitted with thimble and condenser on a heating mantle and was extracted for 24 hrs. On completion of extraction of the plant sample was taken out of the thimble and dried is shed. Then the residue was extracted with other solvents successively in the same manner. The aqueous extract of the plant left was obtained by infusion method that is by soaking plant power in 250 ml of distilled water for 24 hrs. The crude extract was then taken in a 100 ml beaker and the solvent was evaporated on water bath and it was finally reduced to dryness to get dry extract. The extract was then transferred to previously weigh air tight container, (weighed on an electronic balanced) and stored in refrigerator until they were screened for the antibacterial activity.

Source of bacterial strains

The antibacterial assay of different extracts was performed. All bacterial strains were procured from the department of Biotechnology, Sri Guru Ram Rai Institute of Technology and Science, Dehradun, Uttarakhand, India.

Evaluation of the antibacterial potential of plant extract

A total of six bacterial strains i.e. two gram positive and four gram negative bacteria viz. *Staphylococcus epidermides* MTCC-5615(Sw-3), *Staphylococcus aureus* MTCC737(Sw-6), *E. coli* ATCC-433(G-001),*Pseudomonas aerogenosa* ATCC-424(G-004), *Salmonella typhi* ATCC-733(G-008), *Klebsiella pneumonae* ATCC-109(G-0110) were taken to evaluate anti bacterial potential of the different extracts of *Pteris vittata.* All the crude extracts were first screened for preliminary test with the concentration 0.5mg/ml to know whether they were active against the particular bacteria or not. The sensitivities against standard drug

Erythromycin (15mcg) was also observed. Only extracts with good activity were then assayed further at different concentration for MIC test.

Antibacterial assay method

The antibacterial assay performed by the disc diffusion method using sterilized disc made from whatmann filter paper of diameter 6mm.[10] The disc diffusion assay was used to screen out antimicrobial activity of the plant extract. It was accomplished by dipping the pre sterilised filter paper disc in a known concentration of the extract for 2-3 hours. The activated bacterial culture (100ul) was introduced to solid surface of agar media with the help of micropipette it was then spread across the surface of solid agar media by means of a sterile spreader and kept at room temperature for15 min or absorption to occur. The pre sterilized discs dipped in different extract were then placed on the surface of the agar media. The Petri dish was then incubated in BOD incubator for 24 hrs at temperature 37^0C. After incubation the degree of sensitivity was determined by measuring the zone of inhibition around the disc in mm. The absence of bacterial growth around the disc containing the extracts, indicate the plant extract containing antimicrobial activity.

Minimum Inhibitory Concentration (MIC) analysis

The MIC value of the extract was determined only against those bacterial strains which showed high sensitivity during the preliminary antibacterial testing, MIC analysis performed by the serial dilution of the active concentrated extract in the pure DMSO to achieve a decreasing concentration range of 1000mg/ml to 31.25mg/ml. By using different concentration of the active extract i.e. the growth around the disc with lowest concentration to which the organism is susceptible would be determined as MIC of the extract against the particular organism.

RESULT AND DISCUSSION

The available literature about *Pteris vittata* L. revealing its phytochemical, ethno botanical, antimicrobial activity, antitumor activity, platelet aggregation, anti-inflammatory action as well as hypotensive, antiviral and anti bacterial activity.[4,5,6,8] Therefore, the experimental methodology that has been adopted for the present study includes successive soxhlet extraction using different solvents in increasing order of polarity, concentration of the extracts followed by antimicrobial screening and the determination of the MIC value of the active extract against various pathogenic microbe. The findings of the present study were described under the following heads.

Appearance and yield of crude extracts

50 gm of the powdered plant material was subjected to successive solvent extraction. The extract was concentrated on water bath and it was finally reduced to dryness to get dry extract. Various extracts obtained showed different colour appearance. It varies from green, yellow to dark brown. Total percentage yield of crude extract varies from 0.96 to 14.1. Out of the seven extract, the ethyl acetate crude extract showed the highest yield (14.16%) while the aqueous showed the lowest (0.96%) yield (Table-1).

Table.no.1. Yield and appearance of crude extracts from different solvent.

S. N.	Solvent used	Quantity of plant material (gm)	Weight of Extract (gm)	Percentage Yield	Appearance
1.	Petroleum ether	50 gm	2.4	4.8	Dark Green
2.	Benzene	50 gm	2.05	4.1	Light Green
3.	Chloroform	50 gm	1.05	2.1	Blackish Green
4.	Ethyl acetate	50 gm	7.08	14.16	Dark yellow
5.	Acetone	50 gm	3.03	6.06	Light Green
6.	Ethanol	50 gm	0.77	1.54	Reddish Brown
7.	Aqueous	50 gm	0.48	0.96	Reddish brown

Preliminary antibacterial assay

The pteridophytes which constitute ferns and ferns allies are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes.[11] A systematic survey of the antimicrobial activity of pteridophytes has been scarcely undertaken by several workers. Khare (1996)[12] and Vasudeva (1999)[13] had discussed the traditional uses of some potential *Adiantum* species for the treatment of various infectious diseases. Antimicrobial activity of some ethnobotanically important ferns against gram positive and gram negative bacterial pathogen was studied by Mandal *et al.*, (2011).[14] *In vitro* antibacterial activity of leaf extracts of 12 pteridophytes harvested at Rajasthan against four gram-negative and one gram positive human and plant pathogenic bacteria were studied by Parihar *et al.*, (2010)[15], Singh *et al.*, (2008).[5]

All the seven solvent extract i.e. petroleum ether, benzene, chloroform, ethyl acetate, acetone, ethanol, and aqueous extract of *Pteris vittata* were subjected for their preliminary antibacterial screening at 0.5mg/ml concentration against 2 gram positive and 4 gram negative bacterial strains. Different extract of *Pteris vittata* showed antibacterial activity against all the studied bacterial strains. Among the studied assayed extract, ethyl acetate exhibited the maximum activity against all the bacterial strains (Table-2, Figure-1). On the

other side, benzene, acetone extract of *Pteris vittata* exhibited their activity against some bacteria such as *Staphylococcus epidermides* MTCC 5615, *Staphylococcus aureus* MTCC 737, *Escherichia coli* ATTC 433, *Pseudomonas aerogenosa* ATTC 424, *Salmonella typhi* ATTC 733, *Klebsiella pneumonae* ATTC 109, respectively. In contrast to this chloroform, alcohol, petroleum ether, and aqueous extract showed negligible activity against all bacterial strains. Ready to use antibiotics impregnated disc i.e. Erythromycin (15mcg) were used as a positive control in order to check the sensitivity of the bacterial cultures. All of them showed clear zones of inhibition around the disc interpreting their high sensitivity towards antibiotic. In contrast to this, DMSO (99%pure) was used as a negative control. Imperato *et al*.,(2000)[16] studied antimicrobial activity and phytochemical profile of *P. vittata*, especially against gastrointestinal (GI) pathogens and reported the presence of flavanoid. The antimicrobial activity of some polypodiaceous ferns such as *Microsorium alternifolium, Leptochillus decurrens, Polypodium irioides, Pyrrosia mannii* and *Phymatodes ebenipes*. Nine species of *Selaginella* represented on Moorea has shown bioactivity like cytotoxic activity, while the other exhibit antiviral, anti- inflammatory, antifungal, antimicrobial, and antioxidant properties.[3]

Table.2: Zone of inhibition (mm) against the bacterial strains.

S. N.	Bacterial strains	Zone of inhibition in mm								
		I_1	I_2	I_3	I_4	I_5	I_6	I_7	Ery. (15mcg)	DMSO
1.	*Staphylococcus epidermides* MTCC 5615(Sw-3)	8	14	-	15	7	8	-	11	-
2.	*Staphylococcus aureus* MTCC 737(Sw-6)	12	14	-	17	11	7	9	13	-
3.	*Escherichia coli* ATTC 433(G-001)	20	21	18	22	16	17	18	08	-
4.	*Pseudomonas aerogenosa* ATTC 424(G-004)	8	8	5	18	9	4	13	10	-
5.	*Salmonella typhi* ATTC 733 (G-008)	5	11	-	13	9	7	7	14	-
6.	*Klebsiella pneumonae* ATTC 109 (G-011)	11	15	5	15	8	12	7	07	

Note: (I_1 =Petroleum ether, I_2 =Benzene, I_3 =Chloroform, I_4 =Ethyl acetate, I_5 =Acetone, I_6 =Alcohol, I_7 =Aqueous). Ery.-erythromycine

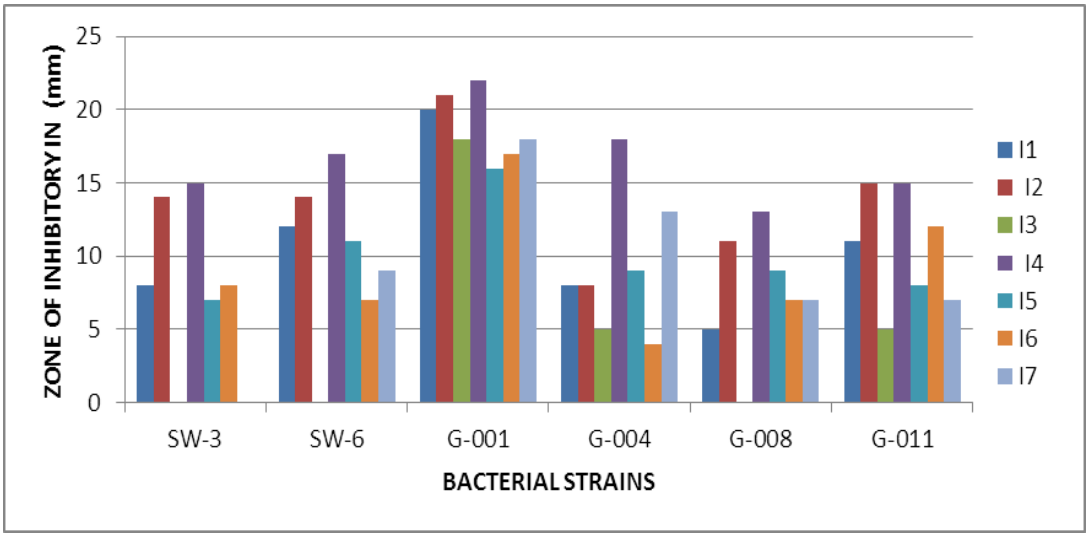

Fig.1: Graph of preliminary antibacterial assay.

Minimum inhibitory concentration (MIC) analysis

Minimum Inhibitory Concentration is important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent.[17] An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.[18]

MIC analysis was performed by serial dilution of the concentrated ethyl acetate extract in pure DMSO to achieve a decreasing concentration range of 1000mg/ml to 31.25mg/ml. On performing MIC for the ethyl acetate extract of *Pteris vittata*, the results revealed that all bacterial strains i.e., *Escherichia coli* ATCC 433 (G-001), *Staphylococcus epidermides* MTCC 5615 (SW-3), *Staphylococcus aureus* MTCC 737 (SW-6), *Pseudomonas aerogenosa* ATCC 424(G-004), *Klebsiella pneumonae* ATCC 109 (G-011) were sensitive against the 125mmg/ml concentration of the extract, there by exhibiting 125mg/ml as their MIC value. No zone of inhibition was obtained around the disc impregnated with 62.5 mg/ml and 31.25 mg/ml concentration interpreting that all bacterial strains could resist this concentration of the extract (Table-3, Figure-2).

Table.no.3: Maximum inhibitory concentration (MIC) of ethyl acetate extract.

S. N.	Bacterial strains	Zone of inhibition in mm					
		1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.25 mg/ml
1.	*Staphylococcus epidermides* MTCC 5615(Sw-3)	19	15	12	9	-	-
2.	*Staphylococcus aureus* MTCC 737(Sw-6)	9	7	6	-	-	-
3.	*Escherichia coli* ATTC 433(G-001)	14	11	9	7	-	-
4.	*Pseudomonas aerogenosa* ATTC 424(G-004)	12	9	7	-	-	-
5.	*Klebsiella pneumonae* ATTC 109 (G-011)	11	9	6	-	-	-

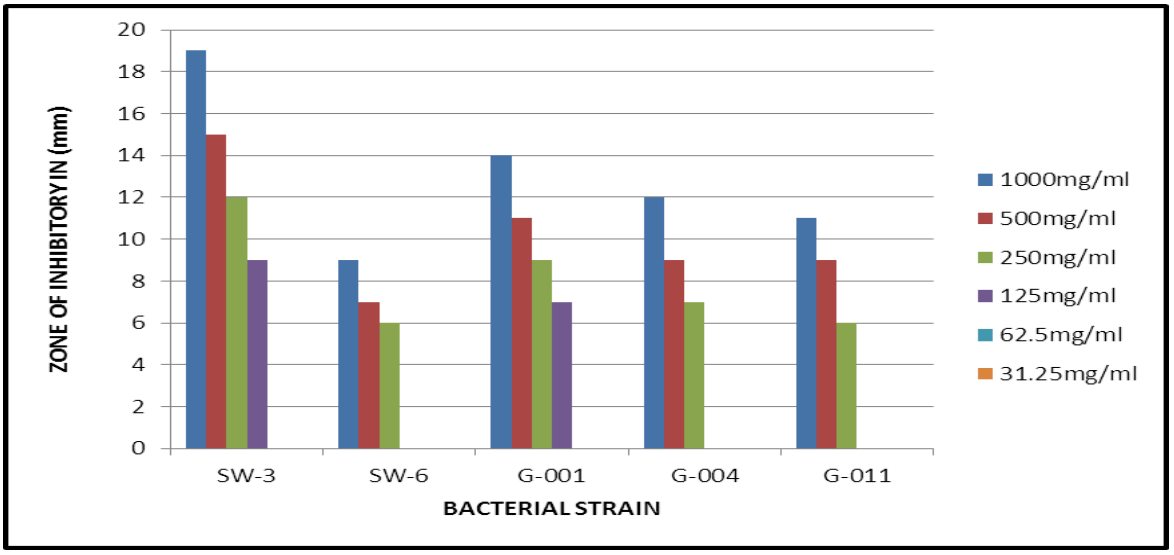

Fig.no.2: Graph of MIC of ethyl acetate.

CONCLUSION

The present studies revealed that ethyl acetate extract of *Pteris vittata* possess good antibacterial activity against pathogenic bacterial strains that causes infection like food poisoning, fever, diarrhoea. Therefore, the presence of triterpenoids, phenols and glycosides in lower vascular plants like *Pteris vittata* can be recommended in future for various biological activities such as antibacterial, antioxidant, ant diabetic, anti-inflammatory.

ACKNOWLEDGEMENT

Authors are grateful and express sincerely thanks to Prof. (Dr.) Preeti and Dr. Manoj Gahlot, SGRRITS, Patel Nagar, Dehradun for their kind support and suggestions.

REFERENCES

1. Benniamin A, Irudayaraj V and Manickam, VS. How to identify rare and endangered ferns and fern allies. Ethnobotanical Leaflets, 2008; 12: 108 – 117.
2. Dixit RD. Conspectus of Pteridophytic diversity in India. Indian Fern Journal, 2000;17: 77 – 91.
3. Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelley ED, A fern that hyper-accumulates arsenic, Nature, 2001; 409: 579.
4. Singh M, Malhotra K and Singh C. Phytochemical evaluation and antioxidant activity of different samples of *Pteris vittata* in Doon valley, Uttarakhand region .Int. J. Pure App. Biosci, 2015; 3(4): 296-304.
5. Singh M, Raghavan G, Singh AK, Prem B. Antimicrobial flavonoids rutin from Pteris vittata L. against pathogenic gastrointestinal micro flora, Am Fern J, 2008; 98(2): 98-103.
6. Gong XL, Chen ZH, Liang NC. Advances in study on chemical constituents and pharmacological activities of plants of genus *Pteris*, Zhongguo zhong yao za zhi, 2007; 32(14): 1382-1387.
7. Kumari P, Otaghvari AM, Govindapyari H, Bahuguna YM, Uniyal PL, Some ethnomedicinally important pteridophytes of India, Int. J Med Arom. Plants, 2011; 1(1): 18-22.
8. Karthik V, Raju K, Ayyanar M, Gowrishankar K, Sekar T. Ethnomedicinal uses of Pteridophytes in Kolli Hills, Eastern ghats of Tamil Nadu, India, J Nat Prod Plant Resour, 2011; 1(2): 50-55.
9. Rout SD, Panda T, Mishra N. Ethnomedicinal studies on some pteridophytes of Similipal Biosphere Reserve, Orissa, India. Int J Med Medic Sci, 2009; 1(5): 192-197.
10. Dulger B & Ugurlu E. Evaluation of antimicrobial activity of some endemic Scrophulariaceae members from Turkey. Pharmaceutical Biology, 2005; 43(3) 275-279.
11. Sharma P C, Murti K S, Bhat A V, Narayanappa D, Kishore, P. Medicinal folk lore of Orrisa-II. Gastrointestinal disorders. Bull Med Ethnobot, 1986; Res 7: 26-40.
12. Khare P. B. Ferns and fern allies their significance and fantasies. In: Applied Botany Abstracts, 1996; vol. 16, no. 1. EBIS, NBRI, Lucknow.
13. Vasudeva S. M. Economic importance of pteridophytes. Indian Fern J., 1999; 16(1): 2130-152.

14. Mandal A, Mondal A K. Studies on antimicrobial activities of some selected ferns and lycophytes in Eastern India with special emphasis on ethno-medicinal uses, African Journal of Plant Science, 2011; 5(7): 412-420.
15. Parihar P, Parihar L, Bohra A. In vitro antibacterial activity of fronds (leaves) of some important pteridophytes, Journal of Microbiology and Antimicrobials, 2010; 2(2): 19-22.
16. Imperato F. The new flavone ester Apigenin-7-O-oxy-p-hydroxyhenzoate and 3-Di-C-glycosyl flavones from *Pteris vittata*. Amer. Fern. J., 2006; 96(2): 62-65.
17. Andrews J M. Determination of Minimum Inhibitory Concentrations. Journal of Antimicrobial Chemotherapy, 2001; 48(1): 5-16.
18. Turnidge JD, Ferraro MJ, Jorgensen JH. Susceptibility test methods: general considerations. In P.R. Murray, E.J. Baron, J.H. Jorgensen, M.A. Pfaller, R.H. Yolken. Manual of Clinical Microbiology. 8th Ed. Washington. American Society of Clinical Microbiology., 2003; 1103.