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ANTI BACTERIAL ACTIVITY OF LEAVES EXTRACT OF POLYPODIUM DECUMANUM

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ABSTRACT

Plants used in traditional medicines contain a vast array of substances that can be used to treat chronic and infectious diseases and the present study was carried out with the same intent to appraise the possible medicinal value of Polypodium decumanum a medicinal plant found mainly in Sikkim, Kamau region and in Western Ghats found. The antimicrobial assay was carried out by Disc diffusion method. The crude plant extracts of leaves part exhibited antimicrobial activity on majority of test organisms. The antimicrobial results revealed that the Ethyl acetate, Ethanol & Aqueous leaves extract of Polypodium decumanum among the selected reference bacterial strains tested, the zone of inhibition was seen in concentration dependent manner on the two different Gram positive and two-gram negative strains.

Keywords *– Polypodium decumanum, Gram positive, Gram negative, Bacteria*

1. INTRODUCTION

Polypodium decumanum is an herbal medicinal plant belong to family *Polypodiaceae*. Medicinal plants contain chemical substances and have been reported to possess many useful properties including anti- inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity, anti-allergic activity, antioxidant activity vascular activity and cytotoxic anti-tumor activity [1,2].

These plants can serve as a possible source for new antimicrobial to which pathogen strain are not resistant. Traditional medicine is an important source of product for developing countries in treating common infection bacteria [3]. The emergence of multiple drug resistant infectious bacteria, high cost of synthetic compounds as well as undesirable side effect of certain drugs insists on pharmaceutical companies to look for new therapeutic agents from other alternative sources including medicinal plants [4].

Polypodium is a genus of between 75-100 species of true ferns, widely distributed throughout the world, with the highest species diversity in the tropics. Polypodies have use in herbalism, but are today most important in horticulture where several species, hybrids, and their cultivars like Polypodium. Green Waves are commonly used as ornamental plant for shady location. The Polypody family contains 3 quarters of all ferns over 6,000 species of plants, mostly native to the tropics of both hemispheres [5,6]. There are 75 species of plants in the Polypodium genus, many of which have been used medicinally for centuries. Polypodium has the ability to regulate and support the growth of various cells in the body including the skin and brain. It's most commonly used in traditional medical systems to treat skin disorders and upper respiratory infections (especially those with a

cough), however, newer research has discovered an even more useful ability. That is to protect the brain cells from damage. Other uses for Polypodium includes treatment for vitiligo, kidney disease, cleansing the blood, an addition to detox formulations, improving and modulating the immune system, arthritis, skin disorders, and cancer [7,8].

Poplypodium contains flavonoids, alkaloids and lipids. It is a rich source of lipids and fatty acids. The main plant chemicals identified in Poplypodium includes adenosine, alkaloids, arachidonic acid, arabinopyranosides, calagualine, ecdysone, ecdysterone, eicosapentaenoic acid, elaidic acid, juglanin, kaempferols, linoleic acids, linolenic acids, melilotoside, oleic acid, ferulic acid, polypodaureine, ricinoleic acid, rutin, selligueain, and sulphoquinovosyl diacyl glycerols[9].

The aim of this research was to identify the antibacterial effect of the Polypodium decumanum plant with different extracts i.e., Ethyl acetate, Ethanol & Aqueous extract obtained from the leaves of the plant.

2. MATERIALS AND METHODS

2.1 Plant material

The fresh leaves of Polypodium decumanum used in this study was procured from the forest of Sikkim and was authenticated from the Regional Research Institute, Bangalore.

2.2 Chemicals

All the chemicals and reagents used were from CDH, New Delhi & Mumbai. Glass wares used were from borosil.

2.3 Microorganisms

The reference bacterial strains used in the study were obtained from MTCC, Chandigarh.

Table 1: List of bacterial strains used

Gram Positive Bacteria	Reference No.
Staphylococcus aureus	MTCC 389
Bacillus subtilis	MTCC 1924
Gram Negative Bacteria	**Reference No.**
Escherichia coli	MTCC 40
Pseudomonas aeruginosa	MTCC 424

2.4 Preparation of Extracts

Grange has reported that antibacterial activity of *Polypodium decumanum* shows the maximum zone of inhibition at 200 mg/ml (Grange et. al., 1990) [10]. So 100 mg, 150 mg and 200 mg of each extract was weighed and dissolved in 1ml of dimethyl sulfoxide (DMSO) to obtain the concentration of 100 mg/ml, 150 mg/ml and 200 mg/ml. 0.1 ml of this solution was used for measurement of zone of inhibition. (Pereira et. al., 2003)[11].

2.5 Preparation of Standard

50 mg of the Ampicillin was dissolved in the 100 ml of sterile water (Mellion et. al., 2007)

Microorganisms used

Cultures of *Staphhylococcus aureus* (MTCC 389)*, Bacillus subtilius* (MTCC 1924)*, Pseudomonas aeruginosa* (MTCC2 242) and *Escherichia coli* (MTCC 40) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The microorganisms were identified by various staining techniques and bio-chemical reactions. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

2.6 Preparation of standard inoculum

Mc Farland Constants: A chemically induced precipitation reaction can be used to approximate the turbidity of a bacterial suspension which is produced by the interaction of barium chloride with sulfuric acid (Bailey et. al., 1990) [12].

Procedure

a) Ten test tubes of equal size were set up.

b) 1% chemically pure sulphuric acid solution was prepared.

c) 1.175 % aqueous solution of barium chloride ($BaCl_2$) was prepared.

d) Slowly with constant agitation the designated amount of the two solutions were added to the tubes as given in the table to make a total of 10 ml per tube.

e) The tubes were sealed and the suspended barium sulfate ($BaSO_4$) precipitate corresponds approximately to homogenous cells densities per ml.

f) The McFarland standard tubes were stored in the dark at room temperatures, as they are stable for six months.

Table 2: McFarland Concentration

Tube no.	0.5	1	2	3	4	5	6	7	8	9	10
$Bacl_2$ (ml)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
H_2SO_4 (ml)	9.95	9.9	9.8	9.7	9.6	9.5	9.4	9.3	9.2	9.1	9.0
Cell density (1×10^8/ ml)	1.5	3	6	9	12	15	18	21	24	27	30

Table 3 Preparation of Assay Media*

Sl. No.	**Ingredients**	**Weight (g)**
1.	Beef extract	4.0
2.	Peptone	5.0
3.	Agar	20.0
4.	Distilled water	q. s. 1000 ml

***pH 7.4 was maintained for the assay media.*

The above mentioned quantities of different ingredients were accurately weighed and dissolved in appropriate amount of distilled water. The prepared media was sterilized by autoclaving at 121^0C for 15 minutes.

The Petri dishes were thoroughly washed and sterilized in hot air oven at 160^0C for one hr. Inoculum was added to 30 ml of sterile nutrient agar medium and was poured into sterile Petri dishes for solidifying. Bores were made on the medium using sterile borer. 0.1ml of test solution was added to the respective bores, 0.1ml of the Ampicillin at a concentration of 100 μg/ 0.1 ml was taken as standard reference. A control having only DMSO in the cup was maintained in each plate.

The Petri dishes were kept in the refrigerator at 4^0C for 45 minutes for diffusion to take place. After diffusion, the Petri dishes were incubated at 37^0C for 24 hrs and zones of inhibition were observed and measured using a scale.

Antibacterial activity of all the compounds was carried out against all four microorganisms. The same media was used both for sub culturing and for estimating antibacterial activity. All the reading was taken in triplicate and is reported in Standard Error Mean (± SEM).

3. RESULTS AND DISCUSSION

The antimicrobial activity of the different extracts i.e., Ethyl acetate, Ethanol & Aqueous leaves extract of Polypodium decumanum were studied in different concentrations (mg/ml) (100, 150, 200) against four reference bacterial strains which includes two Gram-positive (*S. aureus*: MTCC 389, Bacillus subtilis: MTCC 1924,) and two Gram-negative (E. coli: MTCC 40, P. aeruginosa: MTCC 424).

Antibacterial activity of different extracts i.e. Ethyl acetate, Ethanol & Aqueous leaves extract of Polypodium decumanum against gram negative bacteria & gram positive bacteria:

The antibacterial results revealed that different extracts i.e. Ethyl acetate, Ethanol & Aqueous leaves extract of Polypodium decumanum at different concentrations (mg/ml) (100, 150, 200) were found to exhibit significant antibacterial activities against all tested bacterial strains.

Among the two Gram negative bacteria tested with different extracts i.e., Ethyl acetate, Ethanol & Aqueous leaves extract of *Polypodium decumanum* at different concentrations (mg/ml) (100, 150, 200). We found that the Ethyl ether extract of *Polypodium decumanum* at concentration mg/ml (100.150,200) shows greater zone of inhibition on both gram-negative bacteria i.e., E. coli & *P. aeruginose* as compared to Ethanolic & Aqueous extract of Polypodium decumanum at concentration mg/ml (100,150,200) as shown in table 2 & graph 1 & graph 2.

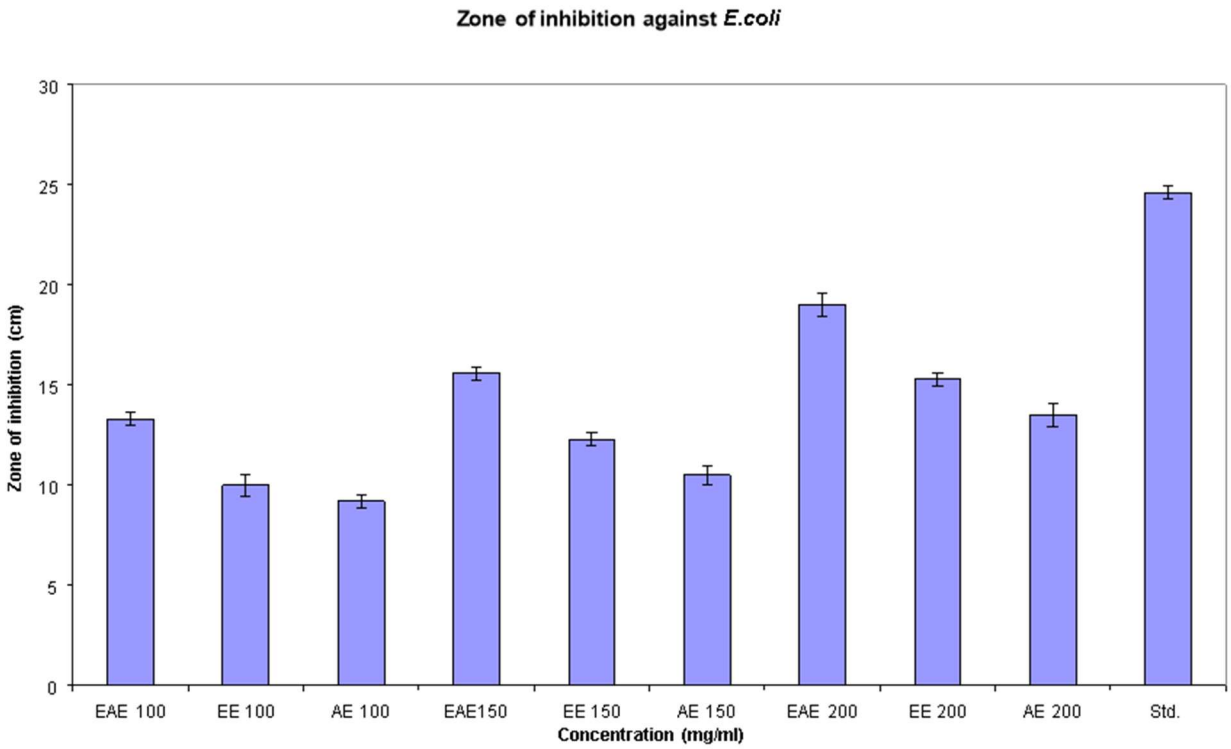
Among the two Gram positive bacteria tested with different extracts i.e., Ethyl acetate, Ethanol & Aqueous leaves extract of *Polypodium decumanum* at different concentrations (mg/ml) (100, 150, 200). We found that the Ethyl ether extract of *Polypodium decumanum* at concentration mg/ml (100.150,200) shows greater zone of inhibition on both gram-positive bacteria i.e., *S. aureus* & *B. subtilis* as compared to Ethanolic & Aqueous extract of Polypodium decumanum at concentration mg/ml (100,150,200) as shown in table 2 & graph 3 & graph 4.

The antibacterial activity of both Gram negative i.e. (E. coli & P. aeruginosa) & Gram positive (S. aureus & B. subtilis) & bacteria tested with different extracts i.e., Ethyl acetate, Ethanol & Aqueous leaves extract of *Polypodium decumanum* at different concentrations (mg/ml) (100, 150, 200) shows lesser zone of inhibition compared with standard i.e., Ampicillin as shown in table 2.

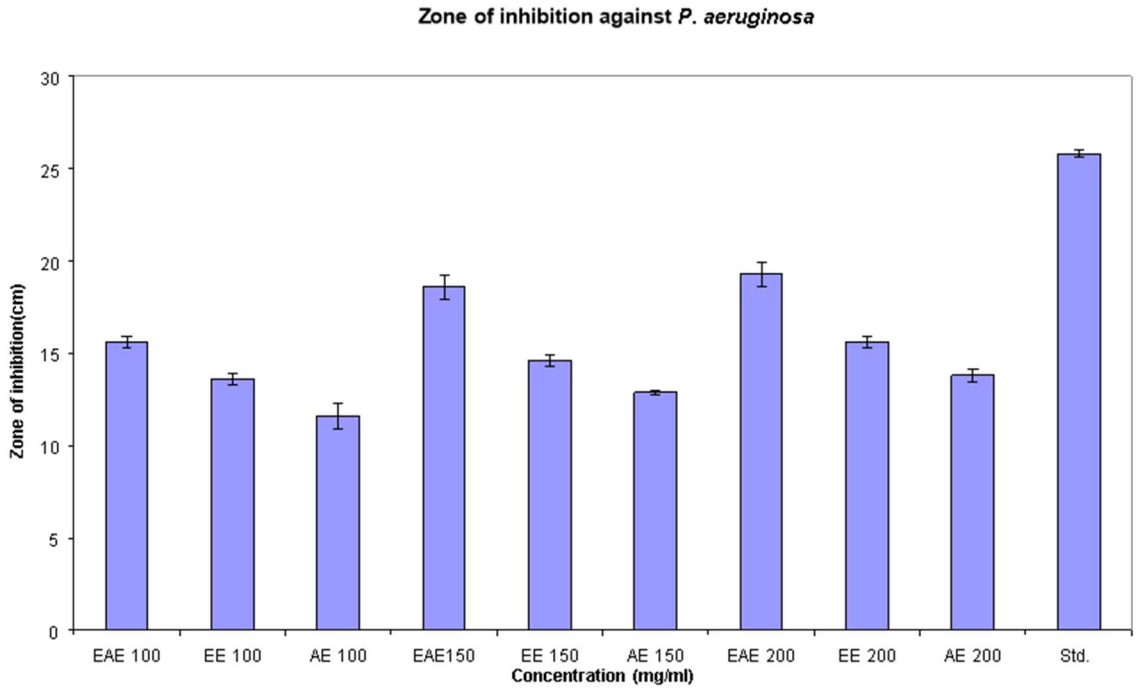
Table 2: Evaluation of antibacterial activity

Plant	Treatment	Dose (mg/ml)	Zone of inhibition(mm) (Mean±SEM)			
			Gram negative bacteria		Gram positive bacteria	
			E.coli	*P.A*	*S. aureus*	*B. subtilis*
Polypodium decumanum	Ethyl acetate extract	100	13.3±0.33*	15.6±0.33*	15.6±0.33*	12.6±0.66*
		150	15.6±0.33*	18.6±0.66*	18.6±0.66*	15.6±0.33*
		200	19±0.57*	19.3±0.66*	20±0.57*	18±1.00*
	Ethanol Extract	100	10±0.57*	13.6±0.33*	13.6±0.33*	10±0.57*
		150	12.3±0.33*	14.6±0.33*	17±0.57*	12±0.57*
		200	15.3±0.33*	15.6±0.33*	22±1.00*	13.6±0.33*
	Aqueous Extract	100	9.22±0.33*	11.6±0.66*	11.9±0.43*	10.3±0.33*
		150	10.5±0.45*	12.9±0.12*	12.4±0.51*	9.15±0.57*
		200	13.5±0.57*	13.81±0.33*	15.66±0.56*	12.6±0.33*
	Standard (Ampicillin)	25µg/ml	24.6±0.33*	25.8±0.20*	24.9±0.35*	25.6±0.20*

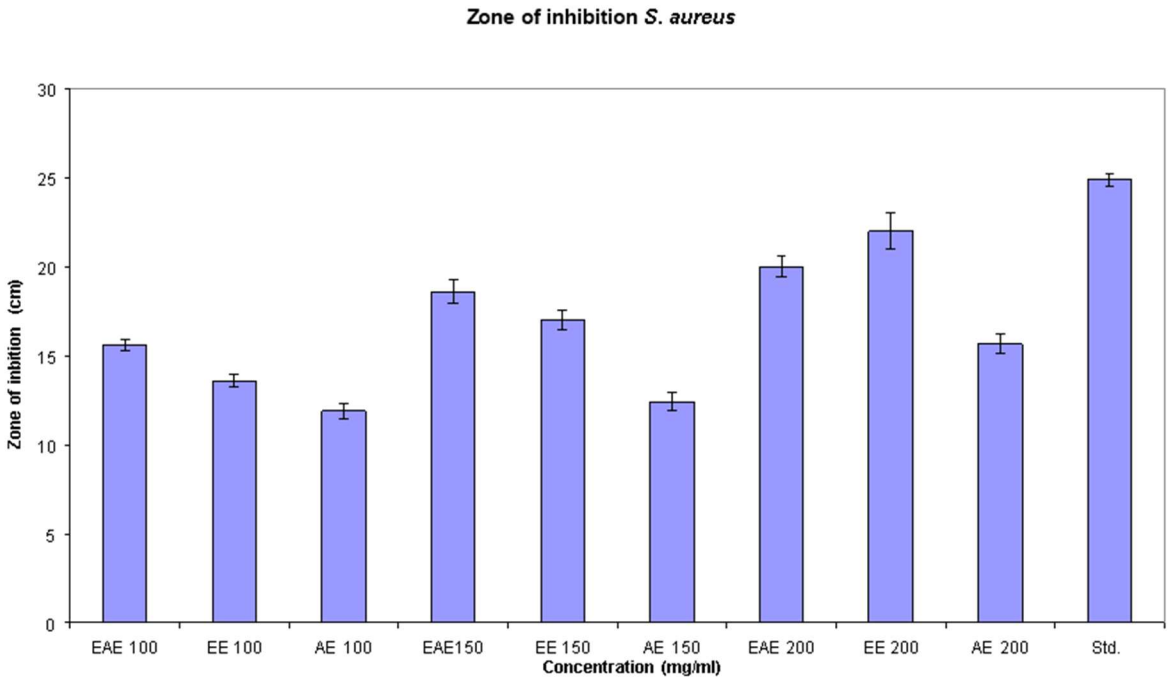
Each value represent Mean±SEM, n=5. One-way ANOVA followed by Dunnet test through Instat software, compare all vs. standard applied. Statistically significant at **P<0.01, *P<0.05.



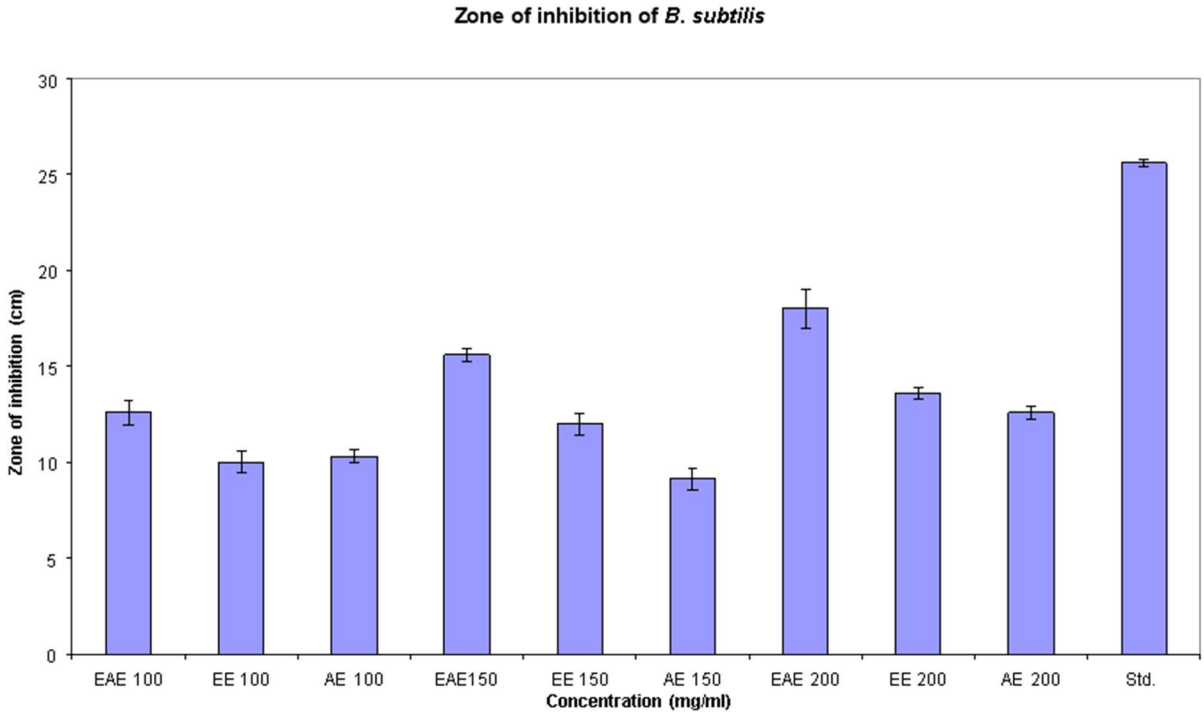
Graph 1 – Graphical representation of Antibacterial activity against *E. coli.*



Graph 2 – Graphical representation of Antibacterial activity against *P. aeruginosa*



Graph 3 – Graphical representation of Antibacterial activity against *S. aureus*



Graph 4 – Graphical representation of Antibacterial activity against *B. subtilis*

4. CONCLUSION

In the present study, comparative analysis of Polypodium decumanum at different extracts i.e. Ethyl acetate, Ethanol & Aqueous leaves extract of Polypodium decumanum at different concentrations (mg/ml) (100, 150, 200) were found to exhibit significant antibacterial activities against all tested bacterial strains. In the study it was found that the two Gram negative & Gram positive bacteria tested with different extracts i.e. Ethyl acetate, Ethanol & Aqueous leaves extract of Polypodium decumanum at different concentrations (mg/ml) (100, 150, 200). We found that the Ethyl ether extract of *Polypodium decumanum* at concentration mg/ml (100.150,200) shows greater zone of inhibition on both gram negative i.e. (*E. coli* & *P. aeruginosa*) & Gram

positive (*S. aureus* & *B. subtilis*) bacteria i.e., as compared to Ethanolic & Aqueous extract of Polypodium decumanum at concentration mg/ml (100,150,200) as shown in table 2.

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