Pharmacological investigation of leaves of *Polypodium decumanum* for anti-bacterial activity against gram-positive and gram-negative bacteria

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**ABSTRACT:** The widespread use of antibiotics has led to an increase in multidrug-resistant bacteria, which represent a serious risk of infection, in view of this problem the aim of this research was performed to characterize the antibacterial effect of ethanolic and petroleum ether extract of *Polypodium decumanum* leaves against three gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilius*, and *Streptococcus pyogenes*) and three gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Pseudomonas alcaligenes*) in vitro by disc-diffusion method. In this study, we have taken 3 concentrations of *Polypodium decumanum* plant extract (ethanolic and petroleum ether) viz. 50 mg/ml, 100 mg/ml, and 200 mg/ml, and observed that the ethanolic extract of *Polypodium decumanum* of the concentration of 200 mg/ml showed higher antibacterial effect compare to other concentration against all 3 gram-positive bacteria and all 3 gram-negative bacteria, but not higher than standard drug Ampicillin.

**INTRODUCTION**

Medicinal plants contain chemical substances and have been reported to possess many useful properties including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity[1,2], anti-allergic activity, antioxidant activity vascular activity and cytotoxic anti-tumor activity[3]. These plants can serve as a possible source for new antimicrobial to which pathogen strain are not resistant[4,5]. Traditional medicine is an important source of product for developing countries in treating common infection bacteria. 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[6].

Recently there has been a renewed interest in improving health and fitness using more natural products. Herbs and spices are an important part of the human diet. Indian subcontinent is a rich territory of medicinal plants which is use in medicinal treatment and forms a rich source of knowledge[7,8]. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plants to treat different ailments[9].

*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. 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 additional to detox formulations, improving and modulating the immune system, arthritis, skin disorders, and cancer.

Polypodium contains flavonoids, alkaloids and lipids[10]. It is a rich source of lipids and fatty acids. The main plant chemicals identified in Polypodium includes adenosine, alkaloids, arachidonic acid, arabinopyranosides, calagualine, eddysone, ecdysterone, eicosapentaenoic acid, elaidic acid, juglanin, kaempferols, linoleic acids, linolenic acids, melilotoside, oleic acid, ferulic acid, polypodaureine, ricinoleic acid, rutin, seiliguean, and sulphoquinovosyldiacylglycerols[11].

Many of existing synthetic drugs cause various side effects. Hence, drug development from plant based compounds could be useful in meeting this demand for newer drugs with minimal side effect. Antibacterial active principles isolated from higher plants appear to be one of the most important alternative approaches to contain antibiotic resistance and the management of diseases[12]. Plant metabolites appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides.

The aim of this research was to identify the antibacterial effect of the Polypodium decumanum plant extract obtained from the leaves of the plant.

MATERIALS AND METHODS

The Polypodium decumanum used in this study was procured from local market in Indore and authenticated at College of Agriculture, ICAR division Indore.

Preparation of Extract

In the continuous hot extraction method, the plant leaves extracted in Ethanol in 3 regular days at the temperature of 78 80°C. The mixture was subsequently filtered and concentrated under reduced pressure at 40°C. The extract yield was 26% w/w.

Chemicals

All the chemicals and reagents used were from CDH, New Delhi. Glass wares used were from borosil. The media and broth used for chemical used for microbial culture were from MTCC, Chandigarh.

Culture media and Strain

The media used for antibacterial test was nutrient agar media of HI media Pvt. Bombay, India and potato dextrose agar/broth of HI media Pvt. Bombay, India. Pathological strains i.e. Staphylococcus aureus (MTCC 389), Bacillus subtilus(MTCC 121), Streptococcus pyogenes (MTCC 1924), Pseudomonas aeruginosa (MTCC 424), Escherichia coli (MTCC 390), and Pseudomonas alcaligenes (MTCC 493) were tested for antibacterial activity. These cultures of strain were collected form Microbial Tissue Culture Collection (MTCC) Chandigarh, India.

Disc-Diffusion method

The antibacterial activity of the extract was determined by disc diffusion method (NCCLS, 1997) in petri disc[13]. The paper discs (6 mm in diameter) were separately impregnated with 15 L of extract placed on the agar which had previously been inoculated with the selected test microorganism. Ampicillin was used as a positive reference for bacteria. Discs without samples were used as a negative control. The petri discs were kept in the refrigerator at 4°C for 45 minutes for diffusion to take place. The plates were incubated at 37°C for 24 h for bacteria. Antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organism comparing to the control[14,15].

Determination of Antibacterial activity

The extracts were individually tested against a panel of microorganism selected. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA)[16,17]. The paper disc (6 mm in diameter) was separately impregnated with 15 L extract placed on the agar which had previously been inoculated with the selected test microorganism. The diameters of zone of inhibition observed are measured[18,19].

Determination of MIC

The antibacterial plant extract then after evaluated to determine MIC value. The broth dilution method was adopted by using N-saline for diluting the plant extract and was incubated for 48h. The minimum dilution of the plant extract that inhibits the growth of the organism was taken as MIC[20,21,22].

RESULTS AND DISCUSSION

The antibacterial activity observed by measuring the diameter of zone of inhibition recorded. The different extract of the plant Polypodium decumanum were found to have significant antibacterial activity. The results obtain in the evaluation of the antibacterial activity of the plant extract against gram positive bacteria Staphylococcus aureus, Bacillus subtilus, Streptococcus pyogenes and gram-negative bacteria Pseudomonas aeruginosa, Escherichia coli, Pseudomonas alcaligenes are shown in Table1.
Table 1: Antibacterial activity of *Polypodium decumanum* plant extract

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract used/standard drug</th>
<th>Conc.(mg/ml)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>Ethanolic extract</td>
<td>50</td>
<td>5.4±0.15</td>
</tr>
<tr>
<td>2</td>
<td>Ethanolic extract</td>
<td>100</td>
<td>11.2±0.17</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract</td>
<td>200</td>
<td>16.7±0.17</td>
</tr>
<tr>
<td>4</td>
<td>Pet. ether extract</td>
<td>50</td>
<td>7.3±0.12</td>
</tr>
<tr>
<td>5</td>
<td>Pet. ether extract</td>
<td>100</td>
<td>9.4±0.12</td>
</tr>
<tr>
<td>6</td>
<td>Pet. ether extract</td>
<td>200</td>
<td>11.7±0.19</td>
</tr>
<tr>
<td>7</td>
<td>Ampicillin</td>
<td>20mg/10ml</td>
<td>23±0.00</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

All values represent Mean ± SEM, n = 3 in each group, values are significantly differ from reference standard (Ampicillin), *p < 0.05,* **p<0.01**

In this study, we found that the ethanolic extract of *Polypodium decumanum* concentration of 200 mg ml\(^{-1}\) showing higher effect against all gram-positive bacteria as well as gram negative bacteria. We also found that the effect of plant extract of *Polypodium decumanum* (ethanolic and petroleum ether) showing higher antibacterial effect against gram positive bacteria i.e. *Staphylococcus aureus*, *Bacillus subtilus*, and *Streptococcus pyogenes* compare to gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, and *Pseudomonas alcaligenes*.

**CONCLUSION**

It is reported that *Polypodium decumanum* contain phenol, alkaloids, flavonoids, rutin, calagualine etc., So based on the constituent present in the *Polypodium decumanum* it can be concluded that the antibacterial activity of the plant is due to the presence of these chemical constituent in the plant. We can also say that ethanolic extract of *Polypodium decumanum* showed a significant antibacterial effect against all 3 gram positive bacteria and 3 gram negative bacteria based on the zone of inhibition it. When compared with the antibacterial effect of standard drug Ampicillin, the plant extract showed only 20% lesser effect.

**REFERENCES**


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