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# Antibacterial Activity of Five Selected Species of Pteridophytes

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***Abstract:***
*In the present study, the human pathogens such as Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Bacillus cereus were isolated and the effects of various fractions of Ceratopteris thalictroides, Christella dentata, Lygodium flexuosum, Pteris vittata and Salvinia molesta on these organisms were tested. By column chromatography the extracts of various solvents are made and they are petroleum ether, chloroform, acetone and methanol extracts. Using these extracts antibacterial activity of Ceratopteris thalictroides Christella dentata, Lygodium flexuosum. Pteris vittata and Salvinia molesta were tested. From the present investigation. It become clear that the five selected pteridophytes such as Ceratopteris thalictroides Christella dentata, Lygodium flexuosum, Pteris vittata and Salvinia molesta possess antibacterial activity.*
*The activity may be due to the presence of some phytochemicals, which can be later identified. The most effective result of these study point that the Petroleum ether extract of Salvinia molesta against E. coli shows 88 % of inhibition against E. coli. The Petroleum ether extract of Salvinia molesta shows the 104% of inhibitory effect against Klebsiella pneumoniae. The Petroleum ether extract of all five plants show 82% inhibition against Staphylococcus aureus and Petroleum ether extract of Lygodium flexousum show 77% of inhibitory effect against Bacillus cereus. These results show the petroleum ether extract of plants are most useful and most effective drug against the selected microbes. Especially the Petroleum ether extract of Salvinia molesta shows the 104 % of inhibitory effect against Klebsiella pneumoniae. So the inhibitory compounds are dissolved in the Petroleum ether extracts. The Petroleum ether extracts of plants will be effective against gram negative bacteria. The pure compound extraction and application in animals is necessary for the future study.*

***Keywords:*** *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus cereus, antibacterial activity, extraction.*

**1. Introduction**

Pteridophytes make as important contribution to the earth's plant diversity. Being the second largest group of vascular plants, they form a significant, dominant component of many plant communities (Benjamin & Manickam, 2000). Pteridophytes have been evaluated for their antimicrobial activity. A number of active constituents responsible for the medicinal action have been isolated and are being characterized. All over the world, scientific research is getting momentum to evaluate the effects, side effects and therapeutic uses in various acute and chronic pathological conditions. In this investigation, an attempt has been made to test in vitro antibacterial activity of *Ceratopteris thalictroides* (family: Parkeriaceae), *Christella dentate* (family: Thelypteridaceae), *Lygodium flexuosum* (family: Shizaeceae), *Pteris vittata* (family: Pteridaceae), *Salvinia molesta* (family; Salviniaceae) found in kerala against some human pathogenic bacteria like *Escherichia coli, Klebsiella pneumoniae, Bacillus cereus* and *Staphylococus aureus*. Some of these plants are ethnobotanically very important and used by tribal people to curing various diseases like asthma, dyspepsia, bronchitis, phthisis etc.Though recent ethnobotanical, phytochemical and pharmacological studies have reported the medicinal and pharmaceutical values of many species of Pteridophytes, still some species of Pteridophytes, used by the tribal are yet to be evaluated for their pharmaceutical value and to isolate the active principle. The main objectives of this project are Isolation and characterization of human pathogenic bacteria and the effect of various fractions of *Ceratopteris thalictroides, Christella dentata, Lygodium flexuosum, Pteris vittata*, and *Salvinia molesta* human pathogenic bacteria under in vitro conditions.

**2. Review of Literature**

Pteridophytes have been successfully used in the different systems of medicines like Ayurveda, Unani and Homeopathy of medicines (May, 1978). The antimicrobial activity of phenolic acids in *Pteridium aquilinum* (Michael and Cooper-Driver,1984). (Kumar and Kaushik1999) have conducted a study on antibacterial effect of *Adiantum capillus-veneris*. ( Lopez et al., 2001) have studied the antiviral and antimicrobial activities of *Adiantum latifolium.* Perumal (2010) have published a paper on comparison to higher plants pteridophytes have found little applications in medicine.

*2.1. The Plants under Study Are*

*Ceratopteris thalictroides* (L.)Brong., *Christella dentata* Forrsk., *Lygodium flexuosum* (L.) SW., *Pteris vittata* L. and *Salvinia molesta* D.S Mitch

**3. Materials and Methods**

*3.1. Materials*

Petridishes, conicalflasks, test tubes, beakers, glass rods, column apparatus, micropipettes etc were used for the study. All of these were thoroughly washed, dried and sterilized. The instruments like autoclave, laminar air flow chamber, hot air oven, electronic balance, burner, inoculation loop, swabs, and incubation chamber were unavoidable. In addition to these muslin cloth, filter paper discs (Whatmann's filter paper No.1), distilled water, forceps, needle, measuring jar, chemicals like chloroform, acetone, petroleum ether and methanol, pure extract of plant parts, antibiotic discs and bacterial culture. A nutrient agar medium prepared for growing microorganisms in a laboratory is called as culture medium.

*3.2. Methods*

3.2.1. Plant Collection

The plants were collected from local area during the period of 2012 – 2013 and their identity was confirmed at Dept. of Botany, St. Thomas' College, Thrissur.

3.2.2. Plant Extract Preparation

Fresh pinnae of the *Ceratopteris thalictroides, Christella dentata, Lygodium flexuosum, Pteris vittata* and *Salvinia molesta* were collected, washed thoroughly, under running tap water and wiped. Fresh pinnae (25g) were chopped and ground well. The homogenate obtained was filtered using a sterile cloth and poured into a test tube.The plant parts are subjected to extraction procedures using various solvents in the order of their polarity. The screening for antibacterial activity was carried on after evaporating the solvents completely. The homogenate of each fern were extracted with 95% methanol (25ml) overnight at room temperature. The extract was filtered and the pooled extracts were then evaporated under reduced pressure to free alcohol to a known volume (25ml). This is referred to as the crude extract. The concentrated extract (5ml) was then applied on a silica gel column (50 cm x 3 cm) equilibrated with petroleum ether (25ml). The column was eluted using the same solvent followed by chloroform (25ml), acetone (25ml), methanol (25ml), distilled water (25ml) successively. The fractions obtained were evaporated to yield solid mass free from solvents. The solid mass was again dissolved in 1 ml methanol to evaluate antibacterial efficiency.

3.2.3. Sterilization

Sterilization of plugged glass wares, petridishes, swabs and discs must be done to destroy all living organism adhering to the inner surfaces. Likewise the culture media must be sterilized prior to use to destroy all contaminating organisms present. The usual application for most media is steam under pressure in an autoclave at a temperature of $121^0$C and a pressure of 15 lbs/square inch for 15 minutes. After autoclaving, allowed the medium to remain as such for 10-15 minutes. Then the sterilized medium can be transferred into sterile petridishes. This is done under sterile conditions.

Antibiotic discs (Gentamicin-10 mcg- positive control) and distilled $H_2O$ (Negative control) were used for sensitivity testing. The streak – plate method is employed for getting a pure culture. The disc diffusion method was used to evaluate the antibacterial activity (Murray *et al.,* 1995). The nutrient agar media (25 ml) was poured into sterilized petriplates and left it to gel at room temperature. The culture suspensions from the pure culture after streak-plate method were swabbed on the medium. Whatmann's No.1 filter paper discs were loaded with the fractions (0.06 µml) obtained through chromatography. Distilled $H_2o$ alone serves as negative control and readymade antibiotic disc (Gentamicin) was used as positive control. Plates were incubated at $37^0$C for 24 hours. Three plates were employed per treatment and the average zone of inhibition was recorded.

Statistical analysis were made using ANOVA .Standard deviation of data were found out, significant levels were compared with one way ANOVA test using graph pad InStat software and p value $<0.05$ were considered as significant.

**4. Result and Discussion**

In the present study, the table 1 shows the petroleum ether extracts of *Salvinia molesta* exhibit the maximum 88 % of inhibitory effect (21/24) when compared to that of standard value. Both extracts of chloroform (12/22), acetone (19/27) *Ceratopteris thalictroides* show maximum inhibitory effect against E. coli. Methanol extract of *Pteris vittata* (14/27) shows the 52 % inhibitory effect when compared to that of standard value. The table 2 shows the petroleum ether extracts of *Salvinia molesta* against *Klebsiella pneumoniae*

shows 104 % inhibitory effect. So these extract is much more effective than the standard control measure. This extract will provide most powerful constituents of drug against these bacteria. Acetone extracts of *Lygodium flexuosum* (21/24) 88 % of inhibitory effect towards the *Klebsiella pneumoniae*. The chloroform extract of *Ceratopteris thalictroides* (19/23) shows 83 % of inhibitory effect against *Klebsiella pneumoniae* and methanol extract of Christelladentata (17/22) shows the 77 % inhibitory effect against *Klebsiella pneumoniae* when compared to that of standard inhibitory value. The table 3 shows the petroleum ether extracts of all five plants shows (18/23) almost 82 % of inhibitory effects against Staphylococcus aureus when compared to that of standard gentamicin inhibitory value. Acetone extract of all plant (12/26) except *Pteris vittata* (11/20) shows the 46 % inhibitory effects against Staphylococcus aureus,when compared to that of standard gentamicin inhibitory value. Chloroform and methanol extract of Pterisvittata (16/26), (16/22) shows the maximum 62 % and 73 % of inhibition against Staphylococcus aureus when compared to that of standard gentamicin inhibitory value. The petroleum ether extracts (82 %) of all five plants are more effective against Staphylococcus aureus than the other three extracts of studied plants. The table 4 shows the petroleum ether extracts of Lygodium flexuosum (17/22) shows maximum 77 % inhibitory effect against Bacillus cereus than that of standard gentamicin inhibitory value. The chloroform extract of *Pteris vittata* (14/21) shows maximum 67 % of inhibitory effect against Bacillus cereus than that of standard gentamicin inhibitory value. The acetone extract of *Ceratopteris thalictroides* (14/26) shows maximum 54 % of inhibition effect against Bacillus cereus than that of standard gentamicin inhibitory value. The methanol extract of *Ceratopteris thalictroides* (15/23) shows maximum 62 % of inhibitory effect against Bacillus cereus than that of standard gentamicin inhibitory value. The petroleum ether extracts of *Lygodium flexuosum* (17/22) is the most effective inhibitory drug among the four extract of five plants.

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## Annexure

**1. Activity against *Escherichia coli***

| Sample | Petroleum ether(A) | | Acetone (B) | | Chloroform (C) | | Methanol (D) | |
|---|---|---|---|---|---|---|---|---|
| | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value |
| *Ceratopteris thalictroides-A* | 18.33 | <6.9 | **19** | <3.3 | **12.33** | <2.3 | 9.33 | <1.2 |
| *Christella dentata – B* | 20 | <6.9 | 17.66 | <3.3 | 9 | <2.3 | 8 | <1.2 |
| *Lygodium flexuosum – C* | 16 | <6.9 | 14.66 | <3.3 | 11 | <2.3 | 11 | <1.2 |
| *Pteris vittata – D* | 20.33 | <6.9 | 13 | <3.3 | 10.66 | <2.3 | **14.66** | <1.2 |
| *Salvinia molesta–E* | **21.33** | <6.9 | 12.66 | <3.3 | 10.33 | <2.3 | 10.66 | <1.2 |
| Gentamicin | 24.33 | <6.9 | 27 | <3.3 | 22.33 | <2.3 | 27 | <1.2 |
| Distilled $H_2O$ | Nil | - | Nil | - | Nil | - | Nil | - |

*Table 1: Inhibitory effects of petroleum ether (A), Acetone (B), Chloroform (C) and Methanol (D) fractions of five selected plants against E.coli .*

**2. Activity against *Klebsiella pneumoniae***

| Sample | Petroleum ether(A) | | Acetone (B) | | Chloroform (C) | | Methanol (D) | |
|---|---|---|---|---|---|---|---|---|
| | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value |
| *Ceratopteris thalictroides-A* | 17.66 | <4.4 | 8.66 | <3.2 | **19** | <2.5 | 10 | <3.5 |
| *Christella dentata – B* | 19.66 | <4.4 | 11 | <3.2 | 13.33 | <2.5 | **17.33** | <3.5 |
| *Lygodium flexuosum – C* | 16 | <4.4 | **21.33** | <3.2 | 12.66 | <2.5 | 15 | <3.5 |
| *Pteris vittata – D* | 19 | <4.4 | 9.33 | <3.2 | 9 | <2.5 | 8.66 | <3.5 |
| *Salvinia molesta–E* | **23** | <4.4 | 13.66 | <3.2 | 10.33 | <2.5 | 10.33 | <3.5 |
| Gentamicin | 22.33 | <4.4 | 24.33 | <3.2 | 23.33 | <2.5 | 21..66 | <3.5 |
| Distilled $H_2O$ | Nil | - | Nil | - | Nil | - | Nil | - |

*Table 2: Inhibitory effects of petroleum ether (A), Acetone (B), Chloroform (C) and Methanol (D) fractions of five selected plants against Klebsiella pneumonia*

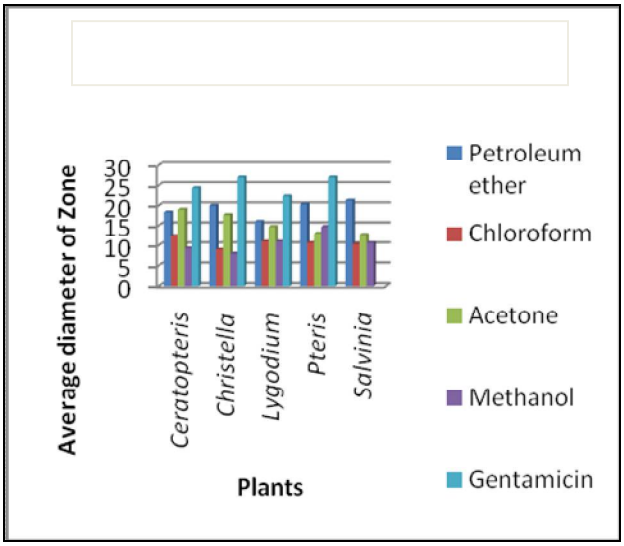


*Figure 1: Activity against Escherichia coli*

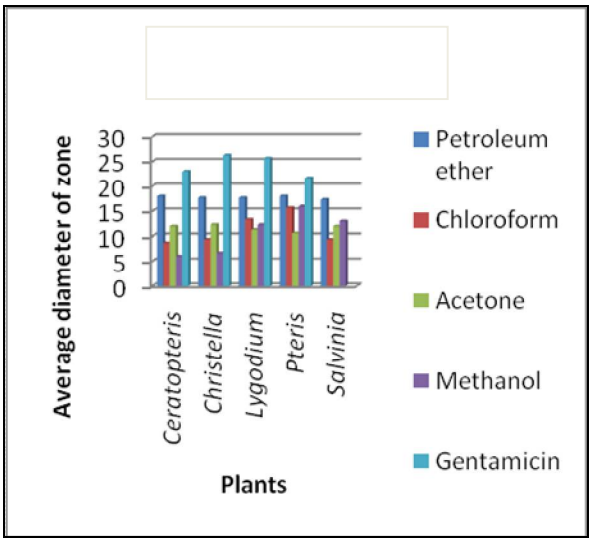


*Figure 2: Activity against Staphylococcus aureus*

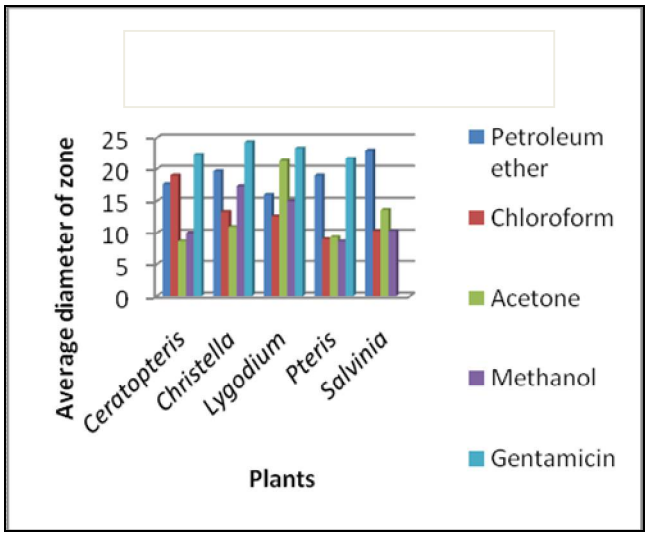


*Figure 3: Activity against Klebsiella pneumonia*

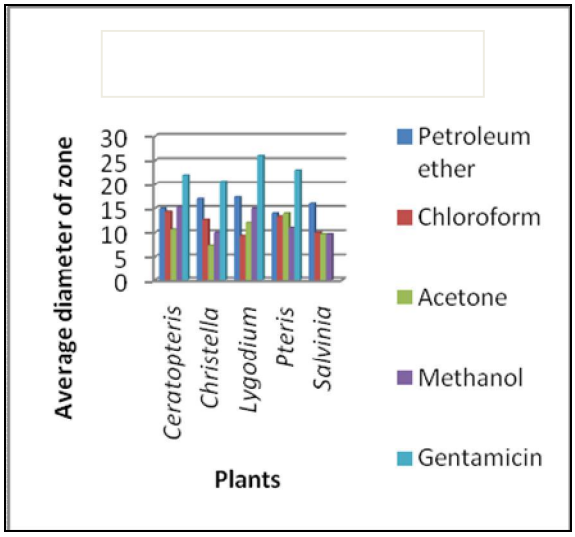


*Figure 4: Activity against Bacillus cereus*

**3. Activity against *Staphylococcus aureus***

| Sample | Petroleum ether(A) | | Acetone (B) | | Chloroform (C) | | Methanol (D) | |
|---|---|---|---|---|---|---|---|---|
| | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value |
| *Ceratopteris thalictroides-A* | **18** | <4.2 | 12 | <1.5 | 8.66 | <3.67 | 6 | <2.78 |
| *Christella dentata – B* | 17.66 | <4.2 | **12.33** | <1.5 | 9.33 | <3.67 | 6.66 | <2.78 |
| *Lygodium flexuosum – C* | 17.66 | <4.2 | 11.33 | <1.5 | 13.33 | <3.67 | **12.33** | <2.78 |
| *Pteris vittata – D* | 18 | <4.2 | 10.66 | <1.5 | **15.66** | <3.67 | 16 | <2.78 |
| *Salvinia molesta–E* | 17.33 | <4.2 | 12 | <1.5 | 9.33 | <3.67 | 13 | <2.78 |
| Gentamicin | 23 | <4.2 | 26.33 | <1.5 | 26.33 | <3.67 | 21.66 | <2.78 |
| Distilled $H_2O$ | Nil | - | Nil | - | Nil | - | Nil | - |

*Table 3: Inhibitory effects of petroleum ether (A), Acetone (B), Chloroform (C) and Methanol (D) fractions.*

**4. Activity against *Bacillus cereus***

| Sample | Petroleum ether(A) | | Acetone (B) | | Chloroform (C) | | Methanol (D) | |
|---|---|---|---|---|---|---|---|---|
| | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value | Average Zone in mm | P value |
| *Ceratopteris thalictroides-A* | 15 | <3.49 | 10.66 | <1.32 | **14.33** | <1.34 | **15.33** | <2.75 |
| *Christella dentata – B* | 17 | <3.49 | 7.33 | <1.32 | 12.66 | <1.34 | 10 | <2.75 |
| *Lygodium flexuosum – C* | **17.33** | <3.49 | 12 | <1.32 | 9.33 | <1.34 | 15 | <2.75 |
| *Pteris vittata – D* | 14 | <3.49 | **14** | <1.32 | 13.33 | <1.34 | 11 | <2.75 |
| *Salvinia molesta–E* | 16 | <3.49 | 9.66 | <1.32 | 10 | <1.34 | 9.66 | <2.75 |
| Gentamicin | 22 | <3.49 | 20.66 | <1.32 | 26 | <1.34 | 23 | <2.75 |
| Distilled $H_2O$ | Nil | - | Nil | - | Nil | - | Nil | - |

*Table 4: Inhibitory effects of petroleum ether (A), Acetone (B), Chloroform (C) and Methanol (D) fractions of five selected plants against Bacillus cereus*