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*Full Length Research Paper*

# Studies on antimicrobial activities of some selected ferns and lycophytes in Eastern India with special emphasis on ethno-medicinal uses

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**In this research, the occurrences of antimicrobial activity of some ferns were studied. The present paper dealt with the antimicrobial activities of some ethno-medicinally important ferns which were very common in West Mednapore District, as well as South West Bengal, against gram-positive and gram-negative bacterial pathogens. The plants were extracted with different solvents (70% ethanol, methanol and acetone) and controlled by deionized water (DW). Our experiment revealed significant results like *Dryopteris*, which showed remarkable antimicrobial activities among all the selected ferns.**

**Key words:** Antimicrobial activity, ferns, ethnomedicinal uses, Eastern India.

## INTRODUCTION

Although the medicinal value of the ferns have been known to man for more than 2000 years, they have been found with very little application in modern chemotherapy as compared to the angiosperms. However, researches on the antimicrobial activity of this plant group are still in their infancy.

The Greek botanist, Theophrastus (Ca. 372-287 B.C.), had referred to the medical value of ferns in one of his books. Although in India, total ferns' species were 1022, they have been recorded with respect to vast angiospermic diversity (15000 species), in that ferns' plants played a significant role on ethnomedicine. In ancient Indian medicine, several ferns were used, and in particular by Unani physicians in India and Western Asia. Reports showed that ferns were used by the people of India and in various other countries. Most of the diseases against which the lycophytes are said to have curative properties, are caused by bacteria (gram-positive, gram-negative or acid-fast). The plant extract preparations were used successfully in the treatment of such diseases and are expected to possess antimicrobial properties.

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A systematic survey of the antibiotic activity of the ferns, however, has been scarcely undertaken (Banerjee and Sen, 1980). In most cases, the antimicrobial activities of few ferns were being screened. Sen and Nandi (1951), however, restricted their studies to ferns. Of the 13 species examined by them, 10 species showed antibiotic activity; and the activity was particularly pronounced against the gram-positive bacteria. Maruzzella (1961) conducted the only extensive survey of antibiotic activity among the ferns. Thirty-three of the 34 ferns examined by him were found to be fairly active and half of them were active against both gram -positive and gram-negative bacteria, including plant pathogens. Mickell (1959) published a review on the antibiotic activity of vascular plants which included 26 ferns, of which 17 species were found to be antibiotically active. Horvath et al. (1967) and Creasey (1969) showed antitumoral activity in *Polypodium leucotomos* and *Cibotium schiedei,* respectively. Bhakuni et al. (1969) observed anticancerous activity in *Dicranopteris linearis* and *Selaginella plumosa.* Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem especially in tropical and subtropical countries.

The use of antibiotics and chemically synthesized medicines cures microbial infections very fast but they

**Table 1.** Antimicrobial activity of the leaf glands of *Lygodium altum.*

| Name of the test organism | Zones of inhibition (mm) | | | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Methanol | Acetone |
| *E. coli* | - | 6 | - | 4 |
| *B. cerus* | - | 10 | 4 | 5 |
| *V. cholarae* | - | - | - | - |
| *K. pneumoniae* | - | 6 | 7 | 4 |

may also disturb the natural immunity of the body and cause variety of side effects. This has aroused interest in plant products and these products are certainly an answer, which may partially support or substitute synthetic drugs. Thus, keeping this view in mind, medical communities are now trying to resolve the aforesaid problems from plant-based medicines in allopathy. In this context, the rich diversity of Indian medicinal plants has been evaluated for their antimicrobial properties, and this may have proved beneficial for mankind (Mandal and Mondal, 2008; Manickam et al., 2005; Davvamani et al., 2005).

The ferns which have ethnomedicinal importance are found and are used by the local and tribal people. Ferns show various economic values towards food and fodder indicators, biofertilizers, insect repellents, medicine and folk medicines (Ghosh et al., 2004).

In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous medical systems for their therapeutic utility (Britto et al., 2001).

Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry. They may provide natural source of anti-microbial drugs that will provide novel compounds that may be employed in controlling some infection globally. The global market of trade estimated medicinal plants at around US $ 60 billion per year and growing at the rate of 7% annually with varying shares of developed and developing countries (Dev, 1999). A study revealed that about 42% of the best selling pharmaceutical products in 1997 were biological or natural products or entities derived from natural resources worth US $ 17.5 billion (Larid and Kate, 2002). Another criterion was the study of plants used for cosmetic or alimentary purposes, especially as a preservative or the study of species to justify their use as antimicrobial agents.

## MATERIALS AND METHODS

The plants were collected from different parts of Paschim Medinipore District, West Bengal. Collections of five plants were made mostly in rainy season (July and August). After the collection of selected plants, extracts were prepared from fresh leaves' parts. The extracted solvents were deionized in water by ethanol (70%), methanol (70%) and acetone (70%). The final extracts were prepared from crude extract by the centrifugal process at 5000 rpm.

Antimicrobial activity of the extracts was assayed by the conventional agar cup method. The inhibitory effects of leaf glands was noted against each test organism after incubation for 24 h at 36.5 ± 1°C. Tests were conducted against 4 different bacteria (3 gram-negative and 1 gram-positive bacteria), that is, *Bacillus cerus, Escherichia coli, Klebsiella pneumoniae* and *Vibrio cholaerae.*

### Collection areas of the selected specimens

1. *Lygodium altum*: Chilkigarh, Bhadutala, Moupal forest, Kankrajhore, Jhargram.
2. *Salvinia molesta*: Kharagpur, Midnapur, Chowrangi, Temathani, Shyamchawk.
3. *Salvinia cuculata*: Kharagpur, Dujipur, Temathani, Chowrangi.
4. *Helminthostachys zeylanica*: Chilkigarh, Amlachoti, Moupal forest.
5. *Dryopteris filix-mas*: Jhargram, Midnapur, Chilkigarh, Kankrajhore.

An extensive survey (about different seasons) was done in the different collection areas and specimens were collected and kept at the Department of Botany and Forestry, Vidyasagar University, Paschim Medinipur. However, bacterial strains were supplied from Microbiology Department, Vidyasagar University, Midnapore, India.

## RESULTS AND DISCUSSION

The antimicrobial activities of crude extracts of the five selected medicinal ferns are shown in Tables 1 to 5. The results showed that all the plant extracts and their solvents gave better inhibition at high concentration of the extract. The ethanolic and acetone extract of *Lygodium altum* showed good inhibition against *B. cerus, K. pneumoniae* and *E. coli* bacteria (Table 1 and Figure 1). Moreover, only the methanolic extracts of *Salvinia molesta* showed good inhibition against *B. cerus* (Table 2 and Figure 2). The solvents' extracts of two plants like *Salvinia cuculata* and *Helminthostachys zeylanica* have no such activity against the selected bacteria (Tables 3 and 4, and Figures 3 and 4), but *Dryopteris filix-mas* has the most remarkable antimicrobial activity and its antimicrobial spectrum covers gram- positive and gram-negative bacteria (Table 5 and Figure 5).

The result showed that antibiotic activity was fairly well distributed in ferns and lycophytes. The five species of

**Table 2.** Antimicrobial activity of the leaf glands of *Salvinia molesta.*

| Name of the test organism | Zones of inhibition (mm) | | | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Methanol | Acetone |
| *E. coli* | - | - | - | - |
| *B. cerus* | - | - | 5 | - |
| *V. cholarae* | - | - | - | - |
| *K. pneumoniae* | - | - | - | - |

**Table 3.** Antimicrobial activity of the leaf glands of *Salvinia cuculata.*

| Name of the test organism | Zones of inhibition (mm) | | | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Methanol | Acetone |
| *E. coli* | - | - | - | - |
| *B. cerus* | - | - | - | - |
| *V. cholarae* | - | - | - | - |
| *K. pneumoniae* | - | - | - | - |

**Table 4.** Antimicrobial activity of the leaf glands of *Helminthostachys zeylanica.*

| Name of the test organism | Zones of inhibition (mm) | | | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Methanol | Acetone |
| *E. coli* | - | - | - | - |
| *B. cerus* | - | - | - | - |
| *V. cholarae* | - | - | - | - |
| *K. pneumoniae* | - | - | - | - |

**Table 5.** Antimicrobial activity of the leaf glands of *Dryopteris filix-mas.*

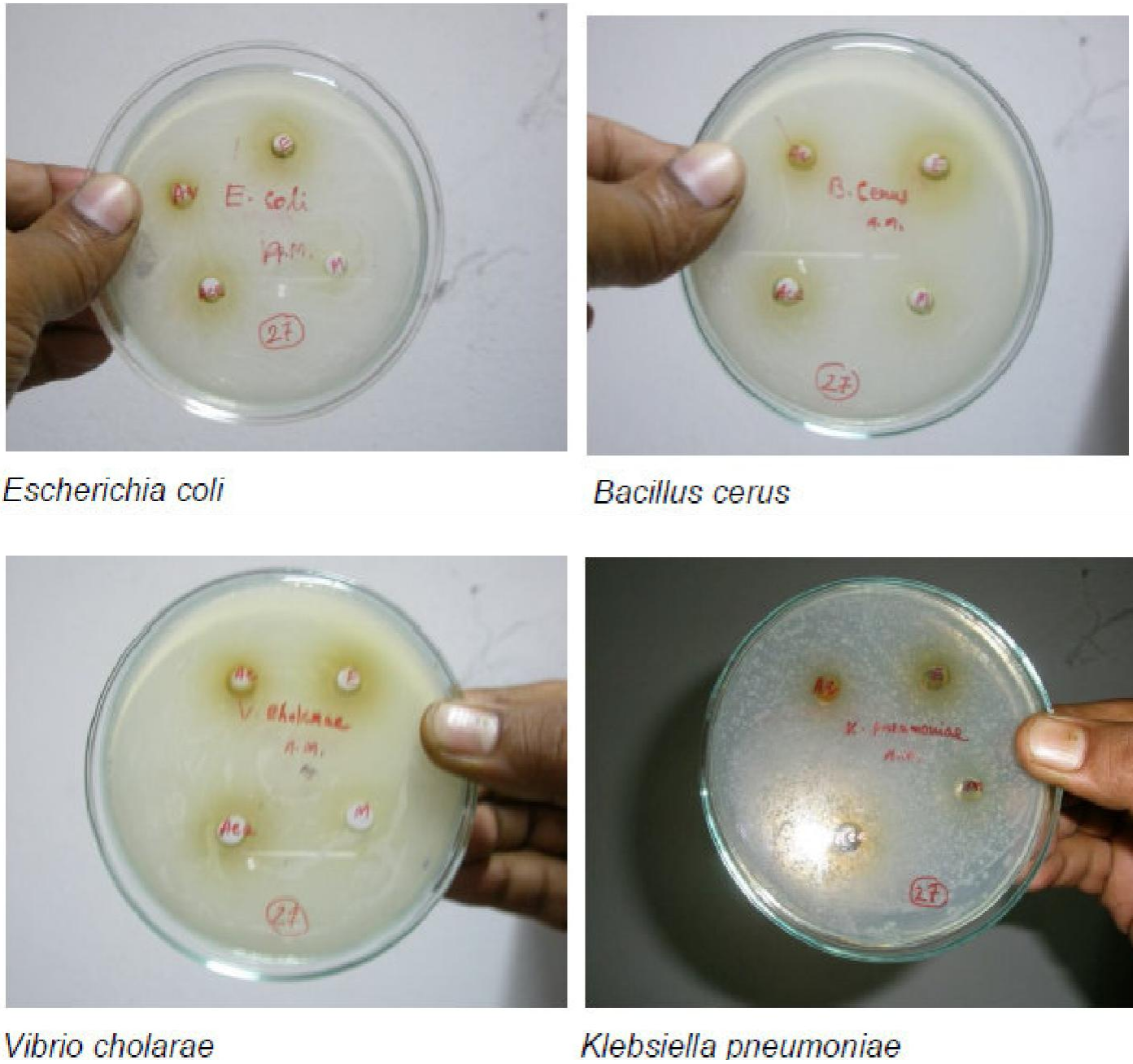
| Name of the test organism | Zones of inhibition (mm) | | | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Methanol | Acetone |
| *E. coli* | - | 4 | 4 | - |
| *B. cerus* | - | 4 | - | 7 |
| *V. cholarae* | - | 3 | 3 | 6 |
| *K. pneumoniae* | - | 4 | 4 | 5 |

ferns and lycophytes examined exhibited antimicrobial properties against the test organism used. This compares very favourably with the antibiotic activity of any other group of the plant kingdom. Our observations are in good agreement with the findings of Sen and Nandi (1951), Mickell (1959) and Maruzzella (1961). These are, however, in sharp contradiction with the findings of Dhar et al. (1968), Bhakuni et al. (1969, 1971) and Dhawan et al. (1977), who did not find any antimicrobial activity in the 42 species of pteridophytes examined by them. They used only one solvent (50% ethanol) and the dilution at which the antibiotic activity was tested was rather high.

Three species of the ferns were found to be capable of elaborating antimicrobial substances. This is in agreement with the common usage of the ferns in folk medicine for staphylococcal and streptococcal infections, such as infections of the throat, carbuncles, boils, ulcers and in wound healing (Banerjee and Sen, 1980).

*H. zeylanica* and *S. cuculata* which are used in folk medicine, however, could not be confirmed in the antimicrobial activities.

Extracts of 2 species were inhibitory to both




*Escherichia coli* *Bacillus cerus*

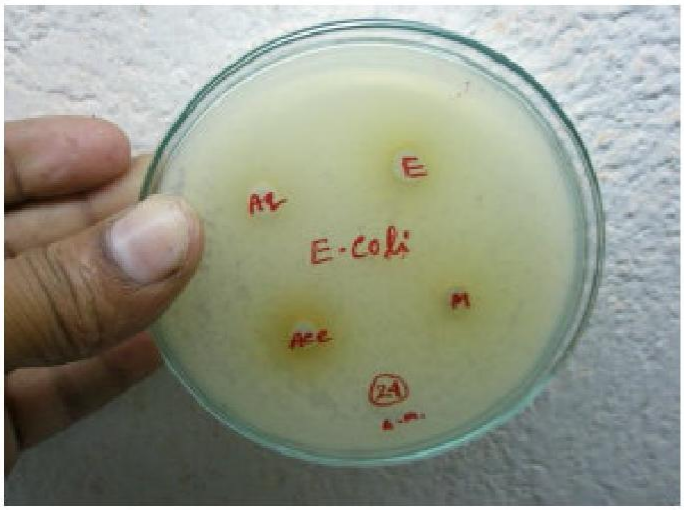
*Vibrio cholarae* *Klebsiella pneumoniae*

**Figure 1.** Activity of *Lygodium altum* leaf glands extract against four bacteria by disk diffusion method (A: deionized water, B: ethanol, C: methanol, and D: acetone).

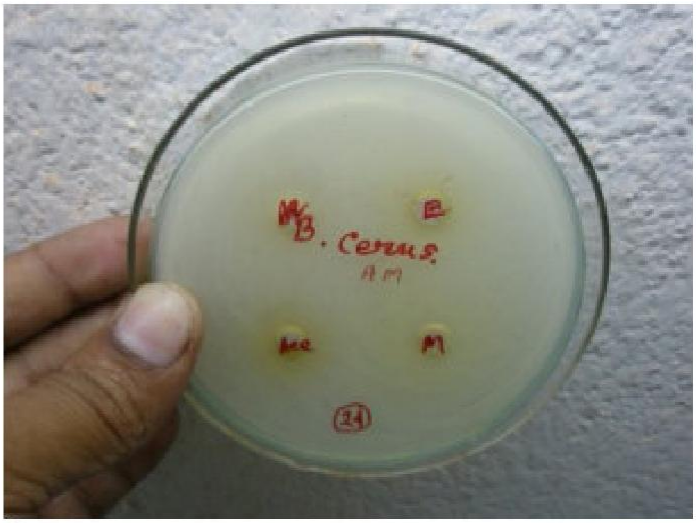
gram-positive and gram-negative bacteria. The antibiotic spectra of 2 species covered gram-positive and gram-negative bacteria. Most of the active principles of ferns are soluble in water, particularly at high temperature. About one-fourth of them are soluble in methanol and ethanol and about one-tenth in ether or acetone. These observations along with the differences in antimicrobial spectra suggest that the ferns produce a variety of antibiotic substances.

The reasons for this discrepancy are not known. This may be due to variations in extraction methods including the solvents used, ecological factors, genetic changes, plant parts examined, season of collection, state of maturity and the strains of the test microorganisms used.

It is necessary to keep in mind the various factors which determine the antibiotic activity of a fern before conclusions are made regarding its capacity to elaborate antibiotic substances. The discrepancy between the




*Escherichia coli*




*Bacillus cerus*


*Vibrio cholarae*


*Klebsiella pneumoniae*

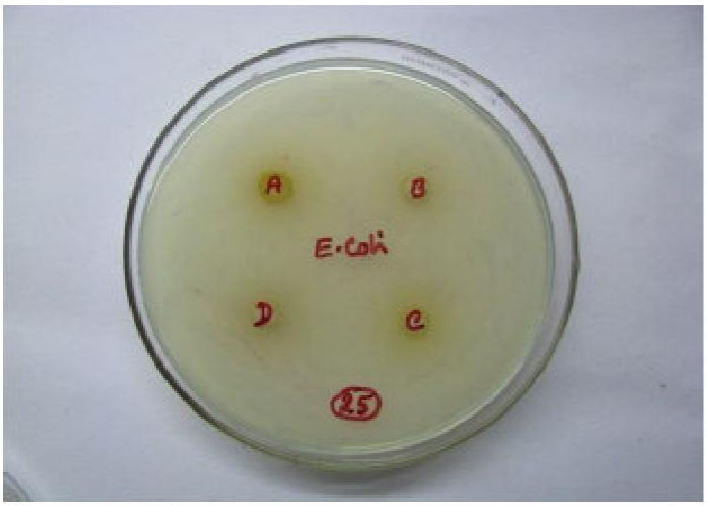
**Figure 2.** Activity of *Salvinia molesta* leaf glands' extract against four bacteria by disk diffusion method (A: deionized water, B: ethanol, C: methanol, and D: acetone).

laboratory results and those of folk medicine may be partly due to the aforementioned factors, in addition to incorrect identifications of the plants by those who use them in folk medicine.

There was considerable variation in the distribution of antibiotic substances within the plant. Apart from seasonal variations, ecological conditions may also vary in antibiotic activity. Although in many cases, it was observed that fern species collected from different parts of Paschim Medinipur District showed similar activity at a particular time of the year, considerable agreement between the observations was recorded by us regarding the antibiotic activity of the ferns and lycophytes and their uses in folk medicine. However, the ferns used for ethnomedicinal purposes are enumerated as follows:

1. *Lygodium altum* Clarke

i) It has expectorant properties.
ii) Fresh roots are used in external application for rheumatism, scabies, eczema and wound, and also in carbuncle.




*Escherichia coli*


*Bacillus cerus*


*Vibrio cholarae*


*Klebsiella pneumoniae*

**Figure 3.** Activity of *Salvinia cuculata* leaf glands' extract against four bacteria by disk diffusion method (A: deionized water, B: ethanol, C: methanol, and D: acetone).

2. *Salvinia molesta* Mitchell

i) Used as a therapeutic utility.
ii) Used as a biofertilizer

3. *Salvinia cuculata* Roxb.

i) Used as a biofertilizer

4. *Helminthostachys zeylanica* (L.) Hook

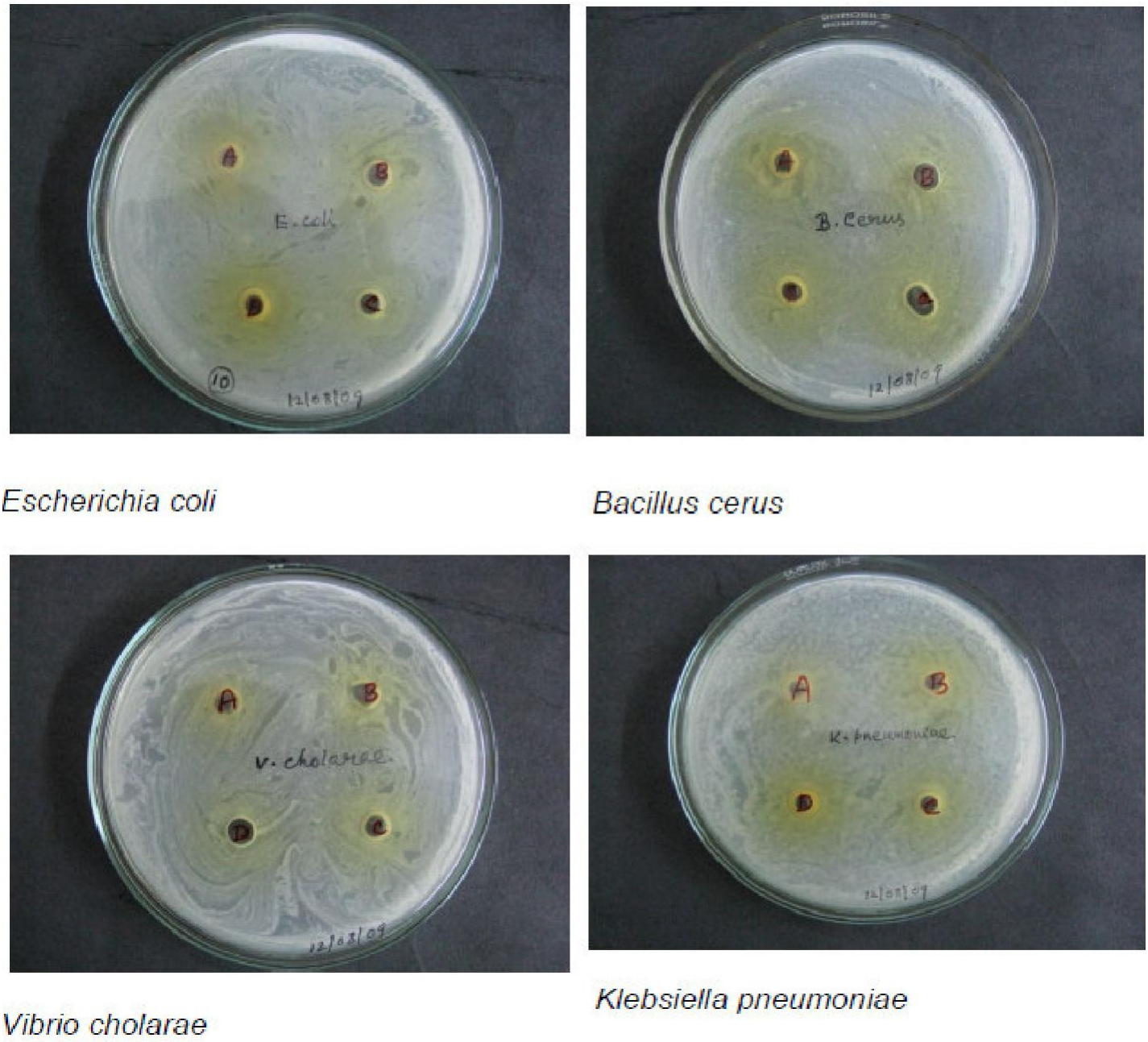
i) Its rhizomes are used in sciatica and are also considered as aperients (Mitra, 2006).
ii) Its rhizome is used for whooping cough and also for dysentry, cataract and early stages of phthises.

5. *Dryopteris filix-mas* (L.) Schott

i) It is one of the oldest anthelmintic drug known and has been used since ancient times for expelling worms from the intestines of man and animals.
ii) At present, it is one of the best taeniacidal drugs available and is administered in the form of a liquid extract of *D. filix-mas.*

**Conclusion**

We have recorded 29 species from the study area, but




*Escherichia coli*

*Bacillus cerus*

*Vibrio cholarae*

*Klebsiella pneumoniae*

**Figure 4.** Activity of *Helminthostachys zeylanica* leaf glands' extract against four bacteria by disk diffusion method (A: deionized water, B: ethanol, C: methanol, and D: acetone).

initially, only 5 plants were selected in this presentation. It is a preliminary work, but it shows significant results. Thus, in future studies, all the species used for this particular study will be included.

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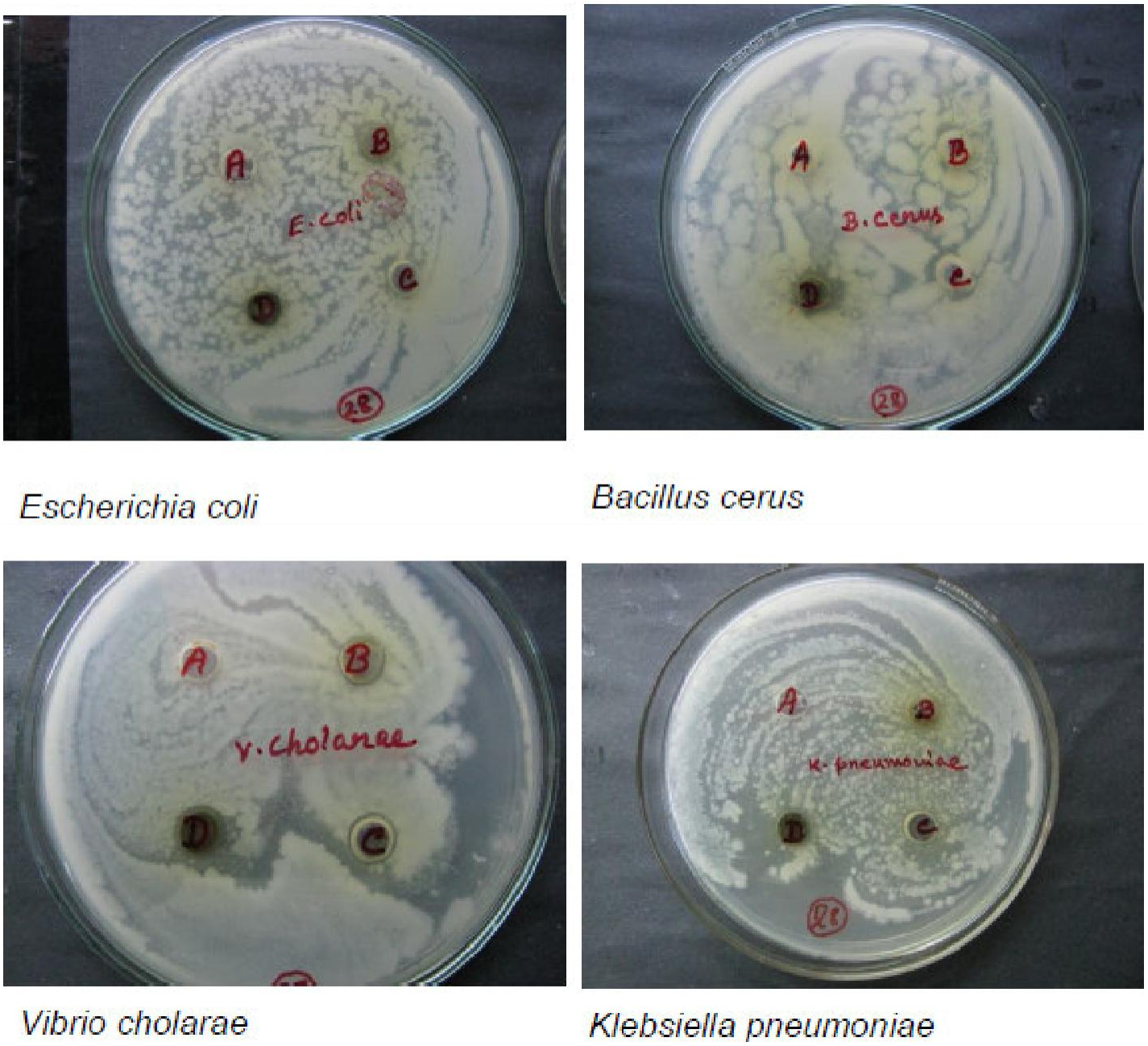


**Figure 5.** Activity of *Dryopteris filix-mas* leaf glands' extract against four bacteria by disk diffusion method (A: deionized water, B: ethanol, C: methanol, and D: acetone).

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