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# ANTIBACTERIAL POTENTIAL AND PHYTOCHEMICAL ANALYSIS OF FOUR WESTERN HIMALAYAN PLANTS

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## ABSTRACT

The antibacterial activities and phytochemical analysis of crude extracts of four indigenous plants (*Acorus calamus, Euphorbia hirta, Solanum surattense* and *Taxus baccata*) grown in Garhwal Hills region of western Himalaya, India were tested against pathogenic *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumoniae, Staphylococcus epidermidis,* and *Bacillus cereus*. Solvent extraction of investigated plants parts was performed using ethanol, methanol, ethyl acetate, hexane, aqueous and chloroform. Determination of the antibacterial activities revealed that aqueous, methanol and ethanol extracts of *Acorus calamus* and *Euphorbia hirta* significantly inhibited most of the pathogens while, in case of *Taxus baccata* and *Solanum surattense* solvent extracts moderately suppressed the pathogens. Minimum inhibitory concentration (MIC) values calculated for *Solanum* ranged 80 mg/ml–100 mg/ml. *Taxus* leaves MIC range were fell between 20 mg/ml – 60 mg/ml. *A. calamus* gave lowest and most promising MIC value of 6 mg/ml against *S. auerus* and 8 mg/ml against both *K. pneumoniae* and *E. coli*, however, the zones of inhibitions were also on lower side and *Euphorbia hirta* measured MIC range of 25 mg/ml – 100 mg/ml. Phytochemical analysis of these plants recorded presence of almost all major phytonutrients. The research outcome leads to conclusion that these four plants extracts possess antimicrobial activity against human pathogenic bacterial strains.

**KEYWORDS: Antimicrobial activity, Minimum inhibitory concentration, Zone of inhibition, Western Himalayan Garhwal hil**ls

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# INTRODUCTION

Epidemics of bacterial infectious diseases have been documented throughout the history. Microbial infections are considered second largest cause of deaths world-wide. Today, infectious diseases account for one-third of all deaths in the world[1]. Emerging and re-emerging zoonotic diseases, food-borne and waterborne diseases and diseases caused by multi-drug resistant organisms constitute the major threats in India[2].Today, the evolution of antibiotic resistance by important human pathogens has rendered these original antibiotics and most of their successors largely ineffective, and if replacements are not found, the golden age of antibiotics will soon come to an end[3]. The increase of multiple drug resistant microbes' brigade has slowed down the development of new antimicrobial drugs in the market and made scientific community brood over the alarming challenges coming year by year. Also, high cost and adverse side effects (such as hypersensitivity, allergic reactions, immunosuppressant etc.) are commonly associated with popular synthetic antibiotics and are major burning global issues in treating infectious diseases[4].Although hundreds of plant species have been tested for antimicrobial properties, vast majority have not yet been adequately evaluated[5]. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[6].India is considered a vast repository of medicinal plants those are used in traditional medical treatments[7]. Historically, all medicinal preparations were derived from plants, whether in simple form of plant parts or in more complex form of crude extracts, mixtures. Today, a substantial number of drugs are developed from plants[8].The Himalayas have a great wealth of medicinal plants and traditional medicinal knowledge. The pharmaceutical sector is using 280 medicinal plant species, out of which 175 are from the Indian Himalayan Region[9].The plants discussed in the present study; *Solanun surattense* (leaves), *Taxus baccata* (leaves), Acorus calamus (rhizome) and *E. hirta* (leaves) were evaluated for their phytochemical constituents and antibacterial potential against 5 bacterial pathogens using 6 different solvents.

# MATERIALS AND METHODS

The 4 study plants were collected from the different locations of Mussoorie–Dehradun region with an altitude ranging from 1676 m to 2286 m. The taxonomic identities of these plants were confirmed at Forest Research Institute, Dehradun. The various plant parts used in the study were detached, washed in clean tap water. Materials were shade-dried, homogenized to fine powder and stored in airtight bottles.

### *Solvent Extraction*

Fine powder of the plants materials was extracted. Extraction was done by gradient extraction in the order of increasing polarity (hexane < chloroform < ethylacetate < methanol < ethanol < water). Extraction process was carried out by soxhlet extractor. After the process solvent was removed with the help of rotary evaporator, weight of the dried material was measured. The dried extract was stored in sterile bottles at room temperature.

### *Microorganisms Used in the Study*

Six laboratory bacterial pathogens namely; *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Escherichia coli, Bacillus cereus and Streptococcus mutants* were obtained from Microbial Type Culture Collection (MTCC), IMTECH Chandigarh and maintained on nutrients agar slants at 4° C and sub-cultured 24 hours before use.

### *Antibiotic Sensitivity Testing*

Antibiotic sensitivity of bacterial strains was determined by standard disc diffusion method against a number of antibiotics such as amoxicillin, chloramphenicol, cephotaxime, gentamicine, and kanamycin.

### *Antibacterial Activity Assay*

The antibacterial activities were carried out using agar disc diffusion assay[10] using 6 various concentrations of the different plants parts. Six

different pathogenic strains of microorganisms were inoculated into flask containing nutrient broth and incubated for 37° C for 24 hours. 20 ml Muller-Hinton agar poured on Petri dishes, 0.2 ml of bacterial suspension prepared with turbidity equivalent to 0.5M McFarland solution aseptically introduced and evenly spread using sterile glass rod. Six wells were punched in Muller-Hinton-Agar plate with sterile cork borer of 6 mm diameter. 100 mg/ml solutions of the methanol, ethanol, and aqueous extracts of plants were prepared in 1% DMSO. However, the hexane, chloroform and ethyl acetate extracts were not directly soluble in 1% DMSO. Thus, 100 mg/ml solution of these extract were made in 30% solution of their respective solvents in 1% DMSO. The wells were then filled with 20 µl of test extract solution and incubated at 37° C for 24 hours to 48 hours. After incubation, plates were observed for antibacterial activity by measuring zone of inhibition.

***Minimum Inhibitory Concentration***

All the extracts exhibiting antibacterial activity were further analysed for Minimum inhibitory concentration by micro-dilution method. Different concentrations of the extract solution were prepared viz. 100 mg/ml, 70 mg/ml, 30 mg/ml, 10 mg/ml, 1 mg/ml and 0.5 mg/ml in 1% DMSO. Bacterial suspensions of 24 hours old culture of the different test organisms were prepared with turbidity equivalent to 0.5M McFarland solution. 5 µl of the suspension was added to each micro liter well with 95 µl of Muller-Hinton broth. 100 µl of all different concentrations of the extracts solution were added to respectively mark well for different isolates. The microliter plates were incubated at 37° C for 24 hours. After incubation, 5 µl of the broth culture from each well was spot inoculated on Muller-Hinton agar plates.

***Qualitative Phytochemical Analysis***

Qualitative phytochemical analysis was performed to detect various phyto-constituents in different plants material[11].

***1. Detection of Alkaloids***

50 mg of solvent free extract was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested with alkaloid reagents as follows:

***Mayer's Test***

To a few ml of the filtrate, one or two drop(s) of Mayer's reagent was added by the side of test tube / bottle. Mayer's reagent: Mercuric chloride (1.358 g) was dissolved in 60 ml of water and potassium iodide (5.0 g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water. Development of creamish / brownish red / orange precipitate indicated the presence of alkaloids.

***2. Detection of Cardiac glycosides***

100 mg of extract was dissolved in 5 ml of water and filtered. 2 ml filtrate was dissolved in 1 ml of glacial acetic acid, .5 ml $FeCl_3$ and .5 ml $H_2SO_4$. Development of green blue colour indicated the presence of cardiacglucosides.

***3. Detection of Saponins – Frothing Test***

50 mg of extract was diluted with distilled water and made up to 20 ml finally. The suspension was shaken in a graduated cylinder for 15 minutes. Development of foams concluded the presence of saponins.

***4. Detection of Tannins: Ferric Chloride Test***

50 mg of extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. Blue black precipitate observed indicated positive test for tannins.

***5. Detection of Flavonoids***

20 mg of plant material was dissolved in 10 ml of ethanol and filtered. 2 ml filtrate, few drops of HCL was added by the side of test tube / bottle and trace of mg were added to this solution. Ribbon pink/ tomato red colour indicates the presence of favonoids.

***6. Detection of Steroids***

200 mg plant material added on 10 ml chloroform, 1 ml acidic anhydride added on bottle having filtrate and concentrated $H_2SO_4$ drop wise added on side of the bottle. Development of blue green ring indicated presence of steroids.

## RESULTS AND DISSCUSSION

The in-vitro antibacterial activity assay included total 24 extracts of the 4 western Himalayan hills plants. Extracts those possessed to have promising antibacterial activity were further evaluated for Minimum Inhibitory Concentration by micro-dilution methods. Results of assessment of antibacterial activity revealed variability in different extracts of different plants against the test bacterial pathogens (Table 2).In the present findings, aqueous extract of *Acorus calamus* (rhizome) found to be most active with zones of inhibition 10 mm to 20 mm against majority of pathogens including *S. aureus, E. coli, K. pneumoniae,* and *B. cereus* (Table 2). Present results get support from previous findings that reported activity of aqueous extract of *A. calamus* rhizome[12]. In another study it has been reported that methanol extract of plant rhizome exhibited a zone of inhibition of 25 mm against *S. aureus*[13], which is in the agreement of our present investigation that methanol and ethanol extracts of the plant rhizome were inhibitory to *S. aureus* (14 mm and 12 mm) respectively.The various extracts of this plant also found effective on yielding promising inhibitory concentrations range. In current investigation, it was interesting to note that ethyl acetate extract of *A. calamus* gave lowest and most promising MIC value of 6 mg/ml against *S. auerus* and 8 mg/ml against both *K. pneumoniae* and *E. coli* (Table 3). However, the zones of inhibitions were also on lower side. Aqueous extract of the plant exhibited 10 mg/ml MIC value against all the 6 tested pathogens. There are various reports suggesting and supporting the present study having effective MICs of different extracts of the *A. calamus*[14, 15].Aqueous extracts of *Euphorbia hirta* exhibited promising activity against *S. aureus, E. coli,* and *K. pneumoniae* with maximum inhibition measured 17 mm to 20 mm respectively, followed, by ethanol and ethanol extracts which also proved out to be active against *K. pneumoniae*, *S. aureus, S. epidermidis* and *E. coli.* Our study gets strong support that *E. hirta* (aqueous, methanol, and hexane) extracts promisingly inhibited *E. coli* with 18 mm, 13 mm, and 11 mm zones while, *K. pneumoniae* was inhibited with 18 mm, 16 mm and 11 mm zones[16]. Many previous research endorsed the antimicrobial activities of the methanol extracts of *E. hirta* leaves, flowers, stems and roots against some medically important bacteria and yeast[17, 18, 19].A previous study on *Solanum surattense* revealed that ethanol extract of the plant inhibited *S. aureus, B. cereus, E. coli* and *K. pneumoniae* with zones inhibition of 16 mm, 13 mm, 19 mm and 17 mm[20]. Our results are consistent with the above mentioned findings that ethanol extract inhibited the growth of *S. aureus, S. epidermidis* and *E. coli.* Study carried out on *Taxus baccata* revealed that hexane, ethanol and aqueous leaves extracts were active against *E. coli* and *K. pneumonia*[21]. Present study gives credence to this in results that *T. baccata* leaf aqueous extracts suppressed growth of most of the pathogens. Ethanol extract of the plant recorded 14 mm zones inhibition against both *S. aureus* and *E. coli.* While, methanol extract maximally inhibited *E. coli* (12 mm) and *S. aureus* (10 mm).

**Table I**
***Phytochemical analysis of different plants used in the study***

| S. No. | Plant/ Part | | Phytochemicals | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Tannins | Alkaloids | Flavonoids | Saponins | Cardic Glucosides | Steroids |
| 1. | *Acorus calamus* | Tuber | - | + | + | + | - | + |
| 2. | *Euphorbia hirta* | Leaf | + | + | + | + | + | + |
| 3. | *Taxus baccata* | Leaf | ++ | ++ | + | + | + | - |
| 4. | *Solanum surattense* | Leaf | + | + | ++ | + | - | + |

***+ positive, ++ strongly positive, - negative***

**Table II**
***Antibacterial activity of solvents extracts of different plants against different bacterial pathogens***

| | Plant Extracts | | Zones of inhibitions (mm) against different pathogens | | | | |
|---|---|---|---|---|---|---|---|
| | | | Sa | Se | Kp | Ec | Bc |
| 1. | *Acorus calamus* (rhizoms) | Et | 14 | 9 | 8 | 12 | - |
| | | Mt | 12 | 8 | 11 | 14 | - |
| | | Eta | 6 | - | 8 | 8 | - |
| | | Hx | 6 | - | 6 | 10 | - |
| | | Chl | 10 | 8 | 8 | 14 | - |
| | | Wat | 20 | 12 | 14 | 18 | 10.5 |
| 2. | *Euphorbia hirta* (leaves) | Et | 12 | 12 | 14 | 12 | - |
| | | Mt | 13 | 12 | 16 | 12 | - |
| | | Eta | - | - | - | - | - |
| | | Hx | - | - | 9 | 11 | - |
| | | Chl | - | - | - | - | - |
| | | Wat | 20 | 17 | 17 | 18 | - |
| 3. | *Solanum surattense* (leaves) | Et | 10.0 | 7.0 | - | 8.0 | |
| | | Mt | 9.0 | 7.0 | 6.0 | 7.0 | - |
| | | Eta | - | - | - | - | - |
| | | Hx | - | - | - | - | - |
| | | Chl | - | - | - | - | - |
| | | Wat | 8.0 | - | - | 10 | - |
| 4. | *Taxus baccata* (leaves) | Et | 14 | 12.5 | 10.0 | 14.0 | - |
| | | Mt | 11.5 | 10.0 | 9 | 12 | - |
| | | Eta | - | - | - | - | - |
| | | Hx | - | - | - | - | - |
| | | Chl | - | - | - | - | - |
| | | Wat | 11.0 | 10.5 | 10.0 | 8.0 | 12.0 |

***Abr: Sa: Staphylococcus aureus, Se: Streptococcus epidermidis, , Bc: Bcillus cereus, Kp: Klebsiella pneumoniae, Ec: Escherichia coli, Hx: Hexane, Chl: Chloroform, Eta: Ethyl acetate, Mt: Methanol, Et: Ethanol, Wt: Water, - No Activity.***

**Table III**
***Evaluation of Minimum inhibitory concentration (mg/ml) of different extracts against bacterial pathogens***

| S. No. | Plants | Solvents | Sa | Se | Kp | Ec | Bc |
|---|---|---|---|---|---|---|---|
| 1 | *Acorus calamus* (rhizome) | Et | 20 | 20 | 20 | 20 | - |
| | | Mt | 10 | 10 | 20 | 20 | - |
| | | Eta | 6 | - | 8 | 8 | - |
| | | Hx | 20 | - | 20 | 20 | - |
| | | Chl | 10 | 10 | 20 | 20 | - |
| | | Wat | 10 | 10 | 10 | 10 | 10 |
| 2 | *Euphorbia hirta* (leaves) | Et | 50 | 50 | 100 | 100 | - |
| | | Mt | 50 | 80 | 100 | 100 | - |
| | | Eta | - | - | - | - | - |
| | | Hx | - | - | 25 | 25 | - |
| | | Chl | - | - | - | - | - |
| | | Wat | 25 | 25 | 50 | 40 | - |
| 3 | *Solanum surattense* (leaves) | Et | 40 | 80 | 100 | 60 | 30 |
| | | Mt | 40 | 90 | 60 | 80 | - |
| | | Eta | - | - | - | - | - |
| | | Hx | - | - | - | - | - |
| | | Chl | - | - | - | - | - |
| | | Wat | 50 | 80 | - | 20 | - |
| 4 | *Taxus baccata* (leaves) | Et | 50 | 40 | - | 40 | - |
| | | Mt | 50 | 70 | - | 60 | - |
| | | Eta | - | - | - | | - |
| | | Hx | - | - | - | - | - |
| | | Chl | - | - | - | - | - |
| | | Wat | 20 | 40 | - | 20 | 20 |

***Abr: Sa: Staphylococcus aureus, Se: Streptococcus epidermidis, Bc: Bcillus cereus, Kp: Klebsiella pneumoniae, Ec: Escherichia coli, Hx: Hexane, Chl: Chloroform, Eta: Ethyl acetate, Mt: Methanol, Et: Ethanol, Wt: Water, - No Inhibition Recorded.***

The phytochemical screening of these four plants found to be rich in most of secondary metabolites (Table 1). In *Solanum* leaves, all major phytonutrients were present, only cardiac glucosides remains absent. *Taxus* leaves also exhibited all major phytochemicals tested except of steroids. For *Acorus calamus,* tannins and cardiac glucosides found negative while, alkaloids, flavonoids, saponins and steroids were present and *Euphorbia hirta* leaves recorded all the 6 phyto-nutrients. Different plants possess different constituents and in different concentrations, which accounts for differential antimicrobial effects, as also suggested earlier[22]. Screening of medicinal plants for antimicrobial agents has gained much importance because lately, World Health Organization (WHO) is keenly interested in the development and utilization of medicinal plant resources in traditional system of medicine in developing countries so as to extend health care to maximum number of population in these countries[23]. Present investigation has provided preliminary evidences of antibacterial properties of tested plants parts and observed significant effects on some pathogenic bacterial species. Aqueous extract of *A. calamus* and *E. hirta* exhibited remarkable activity against *S. aureus, E. coli* and *K. pneumonia*. Ethanol extract of *T. baccata* leaves showed good activity against *S.*

*aureus, S. epidermidis, and E. coli*. Interestingly, aqueous leaves extract of *Taxus* also exhibited activity against *B. cereus* unlike all other tested extracts. However, *S. surattense* moderately suppressed growth of the pathogens. The results lend credence to the folkloric use of these western Himalayan hills plants in treating microbial infections and could be exploited for antimicrobial agents.

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**Conflict of Interest**

Conflict of interest declared none.

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