# EVALUATION OF ANTIMICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT *SCHIMA WALLICHII* BARK

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**Summary**

*Schima wallichii* Choisy (Ternstroemiaceae) is a well known plant of Sikkim Himalayan region. The bark of this plant is traditionally used as antipyretic, antiseptic, anthelmintic, wound healing agent. Present investigation was undertaken to investigate antimicrobial activity of hydroalcoholic extract of *Schima wallichii* bark (HAE). HAE was examined against some selective Gram positive and Gram negative bacterial (20) and fungal (4) strains. Preliminary antimicrobial activity was evaluated by agar disc diffusion method. Minimum inhibitory concentration (MIC) was determined by tube dilution method whilst minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by agar diffusion method. HAE showed highest sensitivity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella* species while least activity was found against selected Gram positive strains namely *Sarcina luteus, Bacillus pumilus* and *Bacillus subtilis*. In antifungal assay, HAE exhibited highest sensitivity against *Candida albicans* and least with *Penicillium funiculosum.* This study confirms that HAE possesses significant antimicrobial activity and may prove to be a useful antimicrobial agent in future.

**Key words:** *Schima wallichii,* hydroalcoholic extract, antimicrobial activity, ciprofloxacin, griseofulvin.

In recent times, the rapid development of multi-resistant microbial strains of clinically important pathogens fetches the interest of scientists to develop newer broad spectrum antimicrobial agents (1). The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures (2 - 4) but yet to be scientifically explored. Now it is aimed to explore scientifically the antimicrobial potential of a traditional plant to substantiate the folklore claim.

*Schima wallichii* Choisy, (Ternstroemiaceae) is a large evergreen tree up to 30 meters in height and 3.5 meters in girth and found in the Himalayan region from Nepal eastward to Assam and Manipur at an elevation up to 2100 meter. It is well known as ‘Chilauni’ (Hindi), ‘Makrisal’ (Bengali), ‘Alue-chilauni’ (Nepali), ‘Sumbrang-kung’(Lepcha) in traditional medicine.

The barks are used as an antiseptic for cut and wound, vermicide, mechanical irritant and to cure gonorrhoea. The barks juice is given to animal infested with liver flukes. Decoction of barks is good for fever and said effective against head lice (5 - 7). Present investigation was undertaken to explore scientifically the antimicrobial activity of hydroalcoholic extract of *Schima wallichii* barks.

## Material and Methods

### Plant material

The barks of *Schima wallichii* were collected from Majhitar, East Sikkim, India, in the month of April, 2006. The plant was authenticated by Botanical survey of India, Shibpur, Howrah (West Bengal). The voucher specimen (PPRT/DP/PT/JU/03/06) has been preserved in our laboratory for future reference.

### Preparation of the extract

The barks were dried under shade, pulverized into coarse powder and extracted exhaustively by using 90 % ethanol as a solvent in a soxhlet extraction apparatus. The extract was evaporated under reduced pressure in a rotary vacuum evaporator (Buchi type, Mumbai, India) until all the solvent had been removed to give a semisolid extract and finally lyophilized to ensure complete removal of solvent (Yield = 3 % w/w).

### Preliminary phytochemical screening

Preliminary phytochemical screening (8, 9) of the extract revealed the presence of tannins, saponins, steroids and triterpenoids.

### Preparation of sample

In the study of antimicrobial activity, the extract was dissolved in Dimethyl sulphoxide (DMSO). The corresponding concentration was expressed in term of µg of extract per ml of solvent (µg/ml).

### Chemicals

All chemicals and solvents used in this experiment were of analytical grade obtained from BDH, Poole, UK.

### Microorganisms

Twenty different bacterial strains namely *Staphylococcus aureus* 29737, *Staphylococcus aureus* ML 267, *Sarcina luteus* 9341, *Bacillus pumilus* 8241, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 10536, *Escherichia coli* VC Sonawave 3:37 C, *Escherichia coli* CD/99/1, *Escherichia coli* $RP_4$, *Escherichia coli* 18/9, *Escherichia coli* K88, *Shigella dysenteriae* 1, *Shigella soneii* 1, *Shigella soneii* BCH 217, *Shigella flexneri* type 6, *Shigella boydii* 937, *Pseudomonas aeruginosa* ATCC 25619, *Vibrio cholerae* 2, *Vibrio cholerae* 785, *Vibrio cholerae* 1037 and four different fungal strains namely *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 6275, *Penicillium notatum* ATCC 11625, *Penicillium funiculosum* NCTC 287 were collected from institute of microbial technology, Chandigarh, India. The bacterial strains were grown in MacConkey agar plates at 37 $^0$ C and maintained on nutrient agar slants, while fungi were grown at 30 $^0$ C and maintained in Saboraud glucose agar slants.

**Preliminary screening for antimicrobial activity**

The test was performed by disc diffusion assay as per NCCLS, 1993 (10). The nutrient agar plates containing an inoculum size of $10^6$ cfu / ml for bacteria and Saboraud glucose agar plates containing $2 \times 10^5$ spores for fungi were used (11). Previously prepared extract impregnated disc (6 mm in diameter) at the concentrations of 200 µg/ml for bacterial and 2000 µg/ml for fungal strains were placed aseptically on sensitivity plates with appropriate controls. Ciprofloxacin (200 µg/ml) and griseofulvin (2000 µg/ml) were used as standard antibacterial and antifungal antibiotics respectively. Plates were incubated at 37 $^0$C for 24 hours for bacteria and 30 $^0$C for 3 days for fungal spores (12). Sensitivity was recorded by measuring the clean zone of inhibition on agar surface around the disc.

**Determination of Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) and Minimum fungicidal Concentration (MFC)**

MIC was determined by tube dilution method for each of the test organism in triplicates (13). To 0.5 ml of varying concentrations of the extracts (0 – 200 µg/ml for bacterial strains and 0 - 2000 µg/ml for fungal strains), 2ml of nutrient broth was added and then a loopful of test organism previously diluted to 0.5 McFarland turbidity standard for (Bacterial isolates) and $10^6$ cfu/ml (for fungal strains) was introduced to the tubes. The procedures were repeated on the test organisms using standard antibiotics ciprofloxacin (for bacteria) and griseofulvin (for fungi). A tube containing nutrient broth only seeded with the test organisms was served as control. Tubes containing bacterial cultures were then incubated at 37 $^0$C for 24 hours for bacteria and 30 $^0$C for 3 days for fungal spores. After incubation the tubes were examined for microbial growth by observing the turbidity.

To determine the MBC and MFC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and Saboraud glucose agar (for fungi) by streaking. Plates inoculated with bacteria and fungi were then incubated at 37 $^0$C for 24 hours and 30 $^0$C for 3 days respectively. After incubation the concentration at which no visible growth was seen was noted as MBC (for bacteria) and MFC (For fungi).

## Results

The preliminary antimicrobial activity of HAE was shown in table 1. The extract has shown maximum activity against *Escherichia coli* and *Pseudomonas aeruginosa* strains in term of zone of inhibition at the concentration of 200 µg/ml whilst no distinct zone was observed at the same concentration against selected Gram positive bacteria namely *Sarcina luteus*, *Bacillus pumilus* and *Bacillus subtilis* as. In preliminary antifungal assay HAE showed maximum zone of inhibition against *Candida albicans* at the concentration of 2000 µg/ml. HAE was found least effective against *Penicillium funiculosum* in term of zone diameter. In this preliminary antimicrobial assay ciprofloxacin (200 µg/ml), griseofulvin (2000 µg/ml) were taken as standard antibacterial and antifungal agents. The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were shown in Table 2. The results showed that HAE is highly sensitive against *Escherichia coli* and *Pseudomonas aeruginosa* strains in term of MIC and MBC, moderately sensitive to *Shigella* species, *Vibrio cholerae* and *Staphylococcus aureus* strains and less sensitive to Gram positive bacteria namely *Sarcina luteus* and *Bacillus* species. Results indicated that the antifungal activity of HAE exists at a concentration range of 800 – 1200 µg/ml. The extract was found most effective against *Candida albicans* and least effective against *Penicillium funiculosum* in term of MIC and MFC.

**Table-1. Results of preliminary antimicrobial activity of HAE**

| **Name of Bacteria** | *Zone diameters in mm* | |
|---|---|---|
| | **HAE (200 µg/ml)** | **Ciprofloxacin (200 µg/ml)** |
| **Gram positive bacteria** | | |
| *Staphylococcus aureus* 29737 | 8.63 ± 0.07 | 14.13 ± 0.07 |
| *Staphylococcus aureus* ML 267 | 8.67 ± 0.03 | 13.53 ± 0.67 |
| *Sarcina luteus* 9341 | - | 12.63 ± 0.12 |
| *Bacillus pumilus* 8241 | - | 13.03 ± 0.12 |
| *Bacillus subtilis* ATCC 6633 | - | 13.60 ± 0.10 |
| **Gram negative bacteria** | | |
| *Escherichia coli* ATCC 10536 | 11.23 ± 0.12 | 13.50 ± 0.10 |
| *Escherichia coli* VC Sonawave3:37 C | 11.67 ± 0.03 | 13.00 ± 0.10 |
| *Escherichia coli* CD/99/1 | 12.07 ± 0.13 | 12.63 ± 0.70 |
| *Escherichia coli* $RP_4$ | 11.60 ± 0.10 | 12.13 ± 0.07 |
| *Escherichia coli* 18/9 | 12.53 ± 0.07 | 13.00 ± 0.12 |
| *Escherichia coli* K88 | 12.53 ± 0.12 | 14.06 ± 0.09 |
| *Shigella dysenteriae* 1 | 9.53 ± 0.13 | 15.63 ± 0.07 |
| *Shigella soneii* 1 | 11.13 ± 0.03 | 15.07 ± 0.13 |
| *Shigella soneii* BCH 217 | 10.10 ± 0.10 | 15.57 ± 0.09 |
| *Shigella flexneri* type 6 | 9.03 ± 0.17 | 15.07 ± 0.12 |
| *Shigella boydii* 937 | 8.97 ± 0.09 | 14.43 ± 0.13 |
| *Pseudomonas aeruginosa* ATCC 25619 | 12.03 ± 0.13 | 16.07 ± 0.13 |
| *Vibrio cholerae* 2 | 9.63 ± 0.03 | 14.03 ± 0.13 |
| *Vibrio cholerae* 785 | 10.00 ± 0.20 | 14.60 ± 0.06 |
| *Vibrio cholerae* 1037 | 10.03 ± 0.17 | 14.07 ± 0.13 |
| **Fungal strains** | **HAE (2000 µg/ml)** | **Griseofulvin (2000 µg/ml)** |
| *Candida albicans* ATCC 10231 | 17.13 ± 0.07 | 18.2 ± 0.20 |
| *Aspergillus niger* ATCC 6275 | 13.67 ± 0.07 | 14.03 ± 0.09 |
| *Penicillium notatum* ATCC 11625 | 10.13 ± 0.17 | 11.10 ± 0.10 |
| *Penicillium funiculosum* NCTC 287 | 8.13 ± 0.03 | 12.06 ± 0.06 |

'-' no measurable zone. Values are mean ± S.E.M. of 3 replications. HAE – hydroalcoholic extract of *Schima wallichii* bark.

## Discussion

The antimicrobial activities of various plants have been reported by many researchers (14, 15). As the plants produce secondary metabolites in order to protect themselves from microorganisms, herbivores and insects thus antimicrobial effect is somehow expected form plants. Phytoconstituents present in plants namely flavonoids, alkaloids and triterpenoids are producing exhilarating opportunity for the expansion of modern chemotherapies against wide range of microorganisms (16, 17).

**Table-2. Results of Minimum inhibitory concentration (MIC) of HAE**

| **Name of the organisms** | *HAE (µg/ml)* | |
|---|---|---|
| | **MIC** | **MBC** |
| **Gram positive bacteria** | | |
| *Staphylococcus aureus 29737* | 100 | 100 |
| *Staphylococcus aureus* ML 267 | 100 | 100 |
| *Sarcina luteus* 9341 | 200 | > 200 |
| *Bacillus pumilus* 8241 | 200 | > 200 |
| *Bacillus subtilis* ATCC 6633 | 200 | > 200 |
| **Gram negative bacteria** | | |
| *Escherichia coli* ATCC 10536 | 25 | 25 |
| *Escherichia coli* VC Sonawave 3:37 C | 25 | 50 |
| *Escherichia coli* CD/99/1 | 25 | 25 |
| *Escherichia coli* $RP_4$ | 25 | 50 |
| *Escherichia coli 18/9* | 50 | 50 |
| *Escherichiacoli* K88 | 50 | 50 |
| *Shigella dysenteriae* 1 | 50 | 100 |
| *Shigella soneii* 1 | 50 | 75 |
| *Shigella soneii* BCH 217 | 50 | 75 |
| *Shigella flexneri type* 6 | 50 | 100 |
| *Shigella boydii* 937 | 50 | 50 |
| *Pseudomonas aeruginosa* ATCC 25619 | 25 | 50 |
| *Vibrio cholerae* 2 | 100 | 150 |
| *Vibrio cholerae* 785 | 100 | 150 |
| *Vibrio cholerae* 1037 | 50 | 100 |
| **Fungal strains** | | **MFC** |
| *Candida albicans* ATCC 10231 | 800 | 1000 |
| *Aspergillus niger* ATCC 6275 | 1000 | 1200 |
| *Penicillium notatum* ATCC 11625 | 1000 | 1200 |
| *Penicillium funiculosum* NCTC 287 | 1000 | 1200 |

MIC – Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, MFC – Minimum fungicidal Concentration, HAE – hydroalcoholic extract of *Schima wallichii* bark.

In present study a variety of Gram positive, Gram negative bacteria and fungal stains were selected for the screening of antimicrobial effect of hydroalcoholic extract of *Schima wallichii* barks to perceive the antimicrobial spectrum as well to authenticate ethnomedicinal claims. The results of this study showed that the extract exhibited varied antimicrobial activities against the tested organisms including both Gram positive and Gram negative bacterial and fungal strains, which may be indicative of the presence of broad spectrum antibiotic compounds in this extract. This may be an immense advantage in fighting the hazard of antibiotic refractive pathogens in recent times.

The broad spectrum antimicrobial activity of said extract may not be for a single phytomolecule but may be due to the presence of a number of bioactive metabolites. Thus our motto is not to trace the responsible molecules but to develop standardized extract to combat against the multifactorial pathogenesis of microorganisms by multimodal therapeutic approaches of multi-molecules present in developed extract.

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