6. Summary and Conclusion

The reason for the selection of foothills of Assam-Arunachal Pradesh border area for
the present study was the unexplored nature of the area and abundance of unique and
diverse medicinal plants there. The information on the uses of some of these plants by
the local people for treatment of their ailments was the rationale behind the selection
of these plants for exploration of plants with antidermatophytic activity. So far, no
systematic study on medicinal value of the plants grown in this region has been
undertaken. The survey of different literature revealed that there was ample scope for
characterization of these plants and their active components for fighting microbial
skin diseases particularly dermatophytes infections.

The survey on medicinal plants in the foothills of Assam-Arunachal Pradesh border
and its surroundings revealed presence of enormous number of medicinal plants there.
Initial in vitro antidermatophytic screening revealed that methanol extracts obtained
from leaves of *P. longum, A. vera, L. inermis, O. sanctum, S. melongena, C. sinensis,*
*O. basillicum, O. gratissimum, P. betleoides, V. negundo, A. indica, C. sophera, C. ternatea* and *L. plukentii* exhibited activity against five dermatophyte species (MIC:
$1.25 \times 10^4$ - $10 \times 10^4 \mu g ml^{-1}$). *P. longum*, due its highest activity against test
dermatophytes, was selected for further study.

Methanol extracts from leaves of *A. cathartica, C. sinensis O. sanctum, O. gratissimum,* and *P. betle* were found very effective at $20 \times 10^4 \mu g ml^{-1}$ concentration in
inhibiting the growth of *Trichosporon beigeli*, an opportunistic fungus often
associated with dermatophytes infections.

The identity of the test dermatophytes was authenticated at MTCC and subsequently
MTCC numbers of the test dermatophytes were obtained by depositing the pure
dermatophyte culture at Microbial Type Culture Collection & Gene Bank, Chandigarh, India.

Sequential extraction of *P. longum* leaves with solvents of increasing polarity and subsequent bioassay revealed better efficacy of chloroform and methanol extracts against dermatophytes. Longevity of the chloroform and methanol extracts at 4°C and room temperature (25-34°C) was found to be 150 days and 90 days respectively. Except water, chloroform extract was found to be soluble in coconut oil, DMSO, methanol, ethyl acetate and hexane. The methanol extract was soluble in coconut oil, methanol and DMSO only. Phytochemical analysis of the extracts revealed presence of tannins and phenolic compounds and absence of alkaloids in both chloroform and methanol extracts of *P. longum* (leaf). Saponins were found only in the methanol extract.

Bioassay-guided fractionation of chloroform and methanol extracts on silica gel column chromatography resulted in the successful isolation of highly active antidermatophytic components. Isolation of active antidermatophytic components in the chloroform extract of *P. longum* leaves, by repeated column chromatography produced number of column fractions, of which four fractions i.e., fraction 1, 4, 5 and 7 were found to be active against *T. mentagrophytes* (Inhibition zone 25-32 mm at 0.5×10^4 μg ml⁻¹ concentration).

The first column fraction was yellowish semi solid, showing single spot on TLC plates. Further spectroscopic analysis detected different compounds in the fraction. Four major compounds identified by comparing spectroscopic data with NIST mass spectral library, in this fraction were 2,4-di-tert-butylphenol, methyl 14-methylpentadecanoate, methyl linolenate and octadecanoic acid, methyl ester. The
antidermatophytic activity of *P. longum* may be attributed to the activity of these compounds either singly or their combined effect on the test dermatophytes.

The repeated column chromatography of column fraction-4 yielded a deep orange coloured solid fraction. GC-MS analysis of this fraction detected 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester, 2,2-dimethoxybutane and β-myrcene from this column fraction.

From column fraction-5 by repeated column chromatography and recrystallization three antidermatophytic components (5a, 5b and 5c) were separated from three subfractions 10, 17 and 24 respectively. Component 5a was obtained from sub fraction-10 by recrystallization from methanol. Sub fraction 17 was eluted with 10% petroleum ether in ethyl acetate and obtained component- 5b. Component- 5c was separated from sub fraction-24 on recrystallization from petroleum ether. GC-MS spectra of these three components (5a, 5b and 5c) were compared with the data on mass spectra of those compounds available in the NIST libraries (NIST/EPA/NIH mass spectral library). However the GC-MS spectra of these components (5a, 5b and 5c) did not match any spectrum of known compound. The compounds represented by the GC-MS spectra of 5a, 5b and 5c probably have not been isolated yet. However more compound libraries will have to be consulted. Hence the samples were retained for future studies.

A needle-shaped crystal was obtained on recrystallization of the sub fraction-7 of column fraction-7. Analysis of this compound (crystal) using FT-IR revealed the presence of aromatic ring and primary amine as functional group in the component. \(^1\)H NMR and \(^{13}\)C NMR showed the presence of aromatic protons and carbons along with the presence of alkyl chain. Further, the component showed positive test for primary
aliphatic amine. Based on these spectroscopic data as well as the positive test for primary amine, the compound was identified as an aryl substituted aliphatic primary amine. The presence of amino group attached to aromatic ring may be attributed to the antidermatophytic activity of this compound.

The MIC values of these fractions / compounds (1, 4, 5a, 5b, 5c and 7) isolated from the chloroform extract of *P. longum* (leaf) against *T. mentagrophytes* and *T. rubrum* ranged between $0.625 \times 10^3$ and $2.5 \times 10^3 \mu g ml^{-1}$.

From methanol extract of *P. longum* leaf two active antidermatophytic column fractions were separated using hexane-chloroform (40:60) and hexane-ethyl acetate (70:30 – 60:40) as eluent respectively. The MIC values of these two fractions were $1.25 \times 10^3 \mu g ml^{-1}$ and $2.5 \times 10^3 \mu g ml^{-1}$ respectively, against *T. mentagrophytes* and *T. rubrum*.

DNA fingerprinting using RAPD technique showed high genetic variations among different *Piper* species. RAPD fragments, showing fixed frequencies in four samples of *P. longum*, generated with OPN-5 primer were observed that can be considered as species-specific marker.

**Conclusion**

The present investigation encompasses successful attempts in identification of some of the promising antidermatophytic plants inhabiting the foothills of Assam-Arunachal Pradesh border. On the basis of the results of this investigation *P. longum* was identified as the most potent plant. Subsequent isolation of the active antidermatophytic components from *P. longum* and their better activity profile highlighted the possibility of finding novel compounds, which would lead to the
development of an effective antidermatophytic agent. This study also validated the scientific rationale behind traditional uses of *P. longum* in folk medicines.

It would be of great interest to concentrate more on *P. longum* for further investigation as regards to the development of plant based efficient antidermatophytic agent. More information like safety studies and clinical trials would be needed to determine the viability of the isolated components for development into drugs for treatment of microbial skin diseases in general and dermatophytes infections in particular.

The RAPD data have ensured the correct identity of *P. longum* and the results would serve as baseline information. We believe that further investigation with inclusion of larger sample size and more primers is required to generate reproducible DNA marker, unique to *P. longum*. Further sequencing, of characterized amplified region as marker can be used as fingerprint to differentiate *P. longum* from other morphologically similar species. Such effort might prove useful in accurate authentication of the species as well as detection of adulterants to ensure the quality, efficacy and safety of plant material for future need.