CHAPTER 6

SUMMARY & CONCLUSION
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Medicinal plants are an integral part of human health care system. India is one of twelve leading bio-diversity center with presence of over 45,000 different plant species. There has been resurgence in uses of herbal medicine in the past few years, not only among the traditional medicinal users but also among the modern consumers of herbal products. Herbal preparation called “Phytopharmaceuticals” or “phytomedicine” are preparation made from different parts of plants. Many countries have included herbal products in their National Health Programs and National Health Schemes as an important alternative for treatment of various ailments. As per the WHO definition of herbal medicines, these are formulations prepared as finished herbal products or mixture herbal products which may contain excipients in addition to the active herbal ingredients. The first chapter of the thesis elaborately deals with the general introduction, traditional systems of medicines, herbs as medicines, anatomy, physiology and diseases of skin, classification of dermatological formulations and merits and demerits of synthetic and natural/herbal topical formulations.

There are many potential plants with medicinal properties in the repertoire of traditional systems of Indian medicines such as Ayurveda, Unani and Siddha, Melaleuca leucadendron Linn and Pandanus tectorius soland ex Parkinson are two such plants that have been used traditional for the treatment of skin ailments. Scientific data was observed to be very empiric and hence there is a need for more extensive research on these indigenous plants and development of new herbal products using current methodologies and modern techniques.

In the present study novel herbal dermal formulation using essential oil obtained from Melaleuca leucadendron Linn leaves and extracts of Pandanus tectorius soland ex Parkinson leaves were developed so as to provide a natural herbal based antimicrobial activity over the skin.

The plan of work was divided into five parts initially an extensive literature was done details of which are given in chapter three.

The fourth chapter describes the material and methods used for the pharmacognostic studies of Melaleuca leucadendron and Pandanus tectorius leaves, extraction of essential oil from Melaleuca leucadendron leaves and successive solvent extraction of Pandanus tectorius leaves,
phytochemical screening using modern instrumental techniques, antimicrobial assay, experimental design, development and evaluation of formulations, stability and skin irritancy studies. The leaves of plants under consideration were initially authenticated and Pharmacognostic and phytochemical analysis were done.

Further phytochemical analysis of the essential oil extract (EO) from fresh leaves of *Melaleuca leucadendron* was done by Gas Chromatography Mass spectrometer (GC-MS) which revealed the presence of 1, 8-cineole, α-terpinene, and other terpenoids.

The dried powdered leaves of *Pandanus tectorius* were extracted successively with petroleum ether (PE), ethyl acetate (EA) and methanol (M) and also directly extracted with methanol to get total methanol extract (M1). Phytochemical analysis of dried extracts were done using Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC) Column Chromatography (CC), and Gas Chromatography with High Resolution Mass spectrometer (GC-HRMS), which confirmed the presence of squalene, stigmasterol, β-sitosterol, tirucallane triterpene and lupenone in PE, M and M1 extracts.

The essential oil EO and dried extracts PE, EA, M and M1 were screened for antimicrobial activity against *Staphylococcus aureus* NCIM-2079 (National Collection of Industrial Microorganisms), *Bacillus subtilis* NCIM 2063, *Pseudomonas aeruginosa* NCIM-2200, *Escherichia coli* NCIM-2065 and *Aspergillus flavus* NCIM-304 obtained from Indian type culture collection.

The essential oil of *Melaleuca leucadendron* showed good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Aspergillus flavus*.

The extracts PE, M and M1 of *Pandanus tectorius* also showed good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Aspergillus flavus* respectively.

To study the combined antimicrobial activity of the essential oil (EO) of *Melaleuca leucadendron* leaves along with the extracts PE and M of *Pandanus tectorius*, antimicrobial activity of EO:PE: M were studies in the ratio of 1:1:1, 1:2:2, 2:2:1 and 5:3:1 respectively.
EO: PE: M in the ratio 2:2:1 showed the best antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Aspergillus flavus* when compared to other combination as well as individual effect of extracts.

Semisolid topical applications using various ointment bases and gelling systems were prepared by incorporating EO: PE: M in the ratio 2:2:1 as the active medicament.

Preliminary studies were done using EVOP (Evolutionary Operation) approach. The response studied includes appearance, pH, viscosity, spreadability and extrudability.

The formulations containing the active medicament (EO:PE:M in ratio of 2:2:1) in various percent weight by weight were studied and examined for antimicrobial activity, the *Aloe vera* gel containing 1% active medicament had better result as compared to other gels and ointment and hence gel was selected for further studies.

The experimental design was done by using $3^2$ factorial, carbopol 940 and *Aloe vera* were selected as the independent variables and studied for experimental runs G1 to G9. The effect of the independent variable on the responses such as appearance, pH, spreadability, viscosity and extrudability were studied. G7 gave the better results.

To further confirm the appropriate ratio of EO: PE: M in the optimized gel (G7) a $3^3$ factorial design was applied to the G7 formulation by varying EO: PE: M at three levels the resultant 27 formulations were then studied for the physical parameters and antimicrobial activity. The gel formulation GE10 was found to be the best, with maximum antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Aspergillus flavus*.

The optimized GE 10 formulation showed no skin irritancy and was found to be stable after 6 months and 12 month period confirmed by physical parameters, antimicrobial activity and chromatographic techniques like TLC and HPTLC compared with marker compounds stigmasterol and squalene as per ICH guidelines. Further GE10 formulation was observed to have better ex-vivo antimicrobial activity when compared with other marketed herbal antimicrobial topical semisolids.
Thus novel herbal gel formulations containing 1% of combination of essential oil of *Melaleuca leucadendron* leaves and petroleum ether and methanol extract of *Pandanus tectorius* leaves in the ratio of 2:2:1 respectively was developed with good antimicrobial activity, stability and no skin irritancy.

**Future Prospects:**

The research work in the development of the novel herbal gel formulation studies need to be applied for patent and further studied clinically on humans, prior to approval by regulators for commercial adaptation by the pharmaceutical.