Chapter - VIII

Summary and Conclusion
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Wound is delineated as disruption of structural and physiological continuity of a skin tissue. It may be produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. Healing of wounds is one of the important areas of clinical medicines explained in many Traditional medicine and folklore systems.

Plants possess immense potential for managing and treating wounds. Tribals all over world use plants for treating wounds and burns. These natural agents induce healing and regeneration of the lost tissue through various mechanisms. These phytomedicine are not only cheap and affordable but are also safe. Presence of wide range of life-sustaining constituents in plants including wound healing molecules has prompted me to evaluate wound healing potential of a common plant around Tiruchirapalli, Tamilnadu and to contribute towards the development of an eco-friendly safe wound healer from natural sources.

In the present dissertation, based on the literature review, Centratherum punctatum Cass. (CP) belonging to the family Asteraceae was selected and its ethanolic and aqueous extracts were subjected to wound healing activity employing in-vivo methods. Besides, the selected plant was also studied from standardised point of view before conducting in-vivo experiments, which is necessary to maintain the consistency and reproducibility in the results.

Following studies were carried out to determine the standards

♣ Botanical studies.
♣ Chemical studies.
♣ Heavy metal analysis.
♣ Quantitative analysis of major chemical constituents.
♣ HPTLC finger printing.
♣ LCMSMS analysis.
In the present dissertation, standards to be determined are selected based on WHO guidelines. These standards could be used in identifying and authenticating this wound healing plant drug as well to check the quality and genuineness.

As there is no pharmacognostical work on record for this traditional wound healer botanical and chemical standardisation studies were carried out and reported for the first time. These quality standards determined could be used to check the genuineness of the drug and can contribute to the Indian Herbal Pharmacopeia.

8.1. BOTANICAL STUDIES

Distinguishing organoleptic, macroscopic and microscopic features of the plant drug (CP) were studied and botanical standards determined.

8.2. CHEMICAL STUDIES

The physicochemical constants and extractive values such as petroleum ether, chloroform, ethanol and water were determined for specific identification.

The behaviour of the leaf powder with different chemical reagents was also studied. The plant powder revealed various shades of green, yellow and red colour under visible light and green, black, brick red and brown colour under ultraviolet light after treatment with different chemical reagents.

Preliminary phytochemical screening revealed the presence of proteins, alkaloids, phenols, coumarins, sugars, and quinones in all the extracts of *C. punctatum*. Flavonoids and proteins were absent in petroleum ether extract and present in the other extracts. Flavonoids are proven antioxidants and free radical scavengers and can contribute in hastening the wound healing process. Thus, the enhanced wound healing efficacy observed in the selected drug may be attributed to this property of the flavonoid such as Kaempferol and Biochanin present in the plant. Thus Phytochemical screening provided supporting evidence for the scientific validation of the wound healing property of the plant.

*C. punctatum* is a source of biologically important elements, which might have played significant role in the observed wound healing efficacy of the plant. Essential elements such as zinc, vitamins C and E might have accelerate the process of wound repair, particularly auto debridement and keratinocyte migration.
It also provides resistance to epithelial apoptosis via cytoprotection, probably through antioxidant activity of the cysteine-rich metallothioneins, against reactive oxygen species and bacterial toxins. Vitamin C also acts as cofactors or coenzymes in a number of metabolic functions involved in wound healing.

HPTLC fingerprints of the methanol and chloroform extract of the plant drug were determined using single mobile phase Toluene:Ethyl acetate:Formic acid (5:4:1) was used as mobile phase and scanned at 254nm. The chloroform extract gave 7 peaks for 10µl and gave 4 peaks for 5µl. The methanol extract gave 9 peaks for 10µl and gave 6 peaks for 5µl. This HPTLC profile could serve as chemical standard.

To assess the wound healing potentials *in-vivo* wound healing activity experiments were conducted and supporting studies like analgesic and anti inflammatory activities were also carried out employing standard protocols.

**8.3. WOUND HEALING STUDIES**

Aqueous extract of *C.punctatum* possessed a definite pro-healing action. This is demonstrated by a significant increase in the rate of wound contraction and by enhanced epithelization. There was a significant decrease observed in the epithelization period. The drug extract also facilitated the rate of wound contraction significantly at the dose levels administered.

The promotion of wound healing activity is also well gazed by the tensile strength observed the incision wound. Significant increase was also observed in skin breaking strength and hydroxyproline content which was a reflection of increased collagen levels that was further supported by histopathological studied and gain in granuloma breaking strength.

Lipid peroxidation was found to be elevated in wounded animals and consequent generation of free radicals were reduced significantly in the experimental animals on administration of the plant extracts. The antioxidant property was found to be enhanced with the increased levels of antioxidants such as GSH and Catalase. Due to this antioxidant efficacy the oxidative stress caused by wound induction was effectively reduced.

In the *in-silico* studies, the chemical constituents identified in the LCMS studies such as Kaempferol and Biochanin-1 were subjected to docking studies. The
study revealed that these compounds effectively docked with target protein Metalloproteinases 8 (MMP 8) suggesting that, the selected plant is an effective wound healer by preventing the degradation of collagen.

Histopathological study of granuloma tissues of animals treated with ethanol extract and aqueous extract revealed a high level of fibrosis as well as well-formed collagen fibres. This also further suggested nontoxic nature and wound healing efficacy of the selected drug.

8.4. SUPPORTING ACTIVITY STUDIES

8.4.1. Analgesic activity

Analgesic potential of the selected plant drug was determined employing both tail immersion test and writhing test methods. In the tail immersion test oral pretreatment with the ethanol and aqueous extract caused a moderate and dose related analgesia in the treated rats, whereas in the writhing test oral pretreatment with ethanol and aqueous extract caused a profound analgesic activity.

8.4.2. Anti-inflammatory activity

Anti-inflammatory efficacy of the selected plant extracts were evaluated employing carrageenin induced hind rat paw oedema and cotton pellet induced granuloma methods. The data of the results depicted that the both the tested extracts possess good anti-inflammatory activity but the activity was more in the cotton pellet induced granuloma method.

8.5. ANTIMICROBIAL ACTIVITY

The ethanol and aqueous extract of the selected plant were screened for their activity against wound pathogens such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Candida albicans* and *Aspergillus niger*. The tested extracts revealed pronounced activity. The data of the results obtained clearly depicted that both ethanol and aqueous extracts of the *Centratherum punctatum* Cass. were found to be having pronounced wound healing activity particularly at the dose levels of 300mg/kg.b.w and the results were comparable with standard drug, povidone iodine. Among the two extracts selected, aqueous extract showed better wound healing activity.
To sum up

In the present work following standards were determined for the selected plant powder of *Centratherum punctatum* Cass. a traditional wound healer.

**Organoleptic standards**
- ♣ Colour - Green.
- ♣ Odour - Strong Aromatic.
- ♣ Taste - Bitter.

**Macroscopic standards: green powder**

**Microscopic standards**
- ♣ Presence of thick walled cuticle cells.
- ♣ Stomata anisocytic.
- ♣ Uniseriate and glandular peltate trichome.
- ♣ Calcium oxalate crystals - prismatic and needle like.
- ♣ Cells containing oil globules, lignins, terpenoids and flavonoids are present.

**Tests for identity, purity and strength**

<table>
<thead>
<tr>
<th>Test</th>
<th>Limit</th>
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<tbody>
<tr>
<td>Foreign Matter</td>
<td>Not more than 1%</td>
</tr>
<tr>
<td>Loss on Drying</td>
<td>Not more than 6%</td>
</tr>
<tr>
<td>Total Ash</td>
<td>Not more than 10.5%</td>
</tr>
<tr>
<td>Acid insoluble Ash</td>
<td>Not more than 2%</td>
</tr>
<tr>
<td>Water Soluble Ash</td>
<td>Not more than 7%</td>
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**Extractive values**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Limit</th>
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</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Not less than 12%</td>
</tr>
<tr>
<td>Water</td>
<td>Not less than 23%</td>
</tr>
</tbody>
</table>

**Important chemical constituents**: Flavonoids, Alkaloids, Tannins and Lignins.

**Elemental composition**: Zinc, Iron and Magnesium.
Heavy metal content

- Lead: Below detectable limit
- Mercury: Below detectable limit
- Arsenic: Below detectable limit
- Cadmium: Nil

The data of the results obtained in the wound healing efficacy studies carried out in the present work suggested that the selected plant *Centratherum punctatum* Cass. possess promising wound healing efficacy. The probable mechanism of action might be due to its:

- Faster wound contraction potentials.
- Enhanced granulation tissue formation and epithelisation.
- Increased collagen content and tensile strength.
- Presence of enhanced supporting activities of wound healing process like analgesic, antiinflammatory and antibacterial efficacy.
- Ability to maintain antioxidant defence system.
- Presence of wound healing molecules such as Flavanoids such as Biochanin-1 and Kaempferol and Phenols.
- Presence of antioxidant element such as zinc.
- Presence of elements useful in collagen synthesis like iron.
- Presence of copper that can contribute in accelerating wound healing process along with other metabolites and minerals present in the selected drug.
- Effective docking of the selected ligands against collagen synthesis inhibitor Metallo proteinases 8 (MMP 8).