Background to Work.
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The work embodied in this thesis started with the demonstration, in our laboratory, of the extraordinary ability of leaf extracts of *Tridax procumbens* to heal wounds in rats. When the juice obtained from the leaves of the plant were applied to deep cuts and badly bleeding wounds, it healed them quickly without leaving any scar. A search of literature also revealed that others have also observed the same type of effect using *Tridax procumbens* leaf extract but the mechanism of action is not known (152, 153).

*Tridax procumbens*, a perennial plant belonging to the Asteraceae family, grows in many parts of India, Africa and South America (fig.1). Various parts of this plant have been used in traditional system of medicine, not only for wound healing purposes but also, for the treatment of diseases like dysentery, mucosal infections and gastrointestinal disorders (154).

Wound healing is a complex physiological process involving inflammatory response, migration of different types of cells to the wounded site, matrix synthesis, deposition of collagen and re-epithelization (155). Inflammatory response occurs in the first 24 hour when platelets form a plug by adhering to the collagen exposed by damage to blood vessels. Neutrophils are the first leukocytes to arrive at the wounded site in numbers, due to their higher density in the bloodstream. The neutrophils are important for controlling local infection of bacteria through endocytosis as well as the release of lysosomal enzymes. This causes additional tissue death at the wound site, initiating the debridement process to remove dead tissue so that
the remaining living tissue can adequately heal (156). Neutrophils are attracted to the wound site by the action of several chemotactic substances. Fibroblasts and macrophages then infiltrate the wound to initiate reconstruction. Fibroblasts produce collagen that increases the wound strength, new epithelial cells cover the wound area, and capillaries join to form new blood vessels (157). Even though under normal conditions all these processes occur naturally and the wound gets healed, under certain abnormal situations like diabetes or when the wounded animal is immuno-compromised or the wound gets infected as in the case of burns or deep wounds, the process of healing gets very much delayed (158).

Amongst many proteins that may be playing important role in wound healing process, the growth factors, cytokines and cell adhesion molecules are the most important ones. It has been shown that platelets derived growth factor (PDGF), transforming growth factor beta (TGF-β) and fibroblast growth factor (FGF) play important role in the wound healing process (159).

Cytokines play an important role in regulating cell functions such as proliferation, migration and matrix synthesis. The main cytokines involved in inflammation are tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) and IL-8. IL-1 and IL-2 stimulate monocytes and T lymphocytes respectively. They have been evaluated for their effect on wound healing. IL-1 has been clearly detected in melanocytes and keratinocytes during wound healing process (160).

The cell adhesion molecules which are involved in cell to cell or cell to matrix interactions regulate a variety of functions, including, signal transduction, cell growth, differentiation, site specific gene
expression, morphogenesis, immunologic function, cell mortality, wound healing and inflammation. Vascular cell adhesion molecule (VCAM) and E selectin or endothelial leukocyte adhesion molecule (ELAM) are molecules that play a role in the recruitment of leukocytes into the site of inflammation. ELAM 1 is specifically expressed on cytokine activated endothelial cells. Inter cellular adhesion molecule (ICAM) is reported to be expressed on epithelial cells to a very high level coinciding with the onset of healing process. Specific signals produced in response to creation of wound control the expression and activation of certain of these adhesion molecules. TNF-α induces the activation of endothelial cell, which express several inducible cell adhesion molecules such as ICAM and VCAM (161).

Amongst many enzymes, which are involved in wound healing process, lysyl oxidase is very important. It is a Cu dependent amino-oxidase that plays a critical role in the biogenesis of connective tissue matrix by cross linking the extracellular matrix proteins such as collagen and elastin (162).

*Tridax procumbens* plant extracts have been reported to be employed in treatment of bronchial catarrh, dysentery, diarrhea and restoring the hair, anemia, cold and inflammation (163, 164). Studies using leaf extracts of *Tridax procumbens* in tissue of rats have shown increased lysyl oxidase activity, protein content and breaking strength of collagen in extract-treated animals as compared to controls. A fall in the lysyl oxidase activity was observed in extract-treated animals after day 8. The extract may be having a dual role: one a stimulatory (direct) effect and the other a depressant (indirect) effect in the later
stage (165, 166). The Juice from the leaves of *Tridax procumbens* significantly (P \( \leq 0.001 \)) counteracted the effects of dexamethasone on tensile strength and epithelization. It caused increase in adrenal weight and decrease in thymus weight. These observations suggest that the juice from the leaves of *Tridax procumbens* exerts a direct pro-healing effect along with an indirect anti-healing influence probably mediated through release of adrenal steroids (167-169). *Tridax procumbens* extracts have shown significant anti-inflammatory activity influencing exudates, leucocytes migration, rat paw oedema and granuloma formation (170). The essential oils of *Tridax procumbens* have been reported to possess insecticidal and insect repellent activities (171). The plant extracts have been reported to have anti-juvenile hormone like activity in *Culex quinquefaciatus* mosquitoes (172).

In Guatemala, *Tridax procumbens* is used for the treatment of vaginitis, gastrointestinal disorders, stomach pain, diarrhearrhea, mucosal infections and skin infections (173-176). Extracts prepared from *Tridax procumbens* are used for the treatment of protozoal infections. Activity against bacteria and fungi, demonstrated by dilution procedures, showed its efficacy against *Trypanosoma cruzi* epimastigotes and promastigotes *in vitro* and against trypomastigotes *in vivo*. This plant extract had shown activity (\( \leq 2 \) mg/ml) against bacteria, (\( \leq 3 \) mg/ml) against yeasts, (\( \leq 5 \) mg/ml) against *Microsporum gypseum* and (\( \leq 5 \) mg/ml) against *T. cruzi in vitro* as well as *in vivo* (177). The hexane extracts of *Tridax procumbens* have been found to result in a marked inhibition of growth of promastigotes.
in vitro (178). Ethnobotanical studies in India, Nepal and Nigeria have shown that leaves from this plant are employed in the treatment of inflammations (179, 180). It has also been used in the treatment of cataract and to promote hair growth (181, 182). The leaf juice is also reported to stop hemorrhage and help in healing of cuts, bruises and wounds (183, 184). The hexane extract of the flowers showed activity against *Escherichia coli*. The same extract of the whole aerial parts was active against *Mycobacterium smegmatis*, *Escherichia coli*, *Salmonella group C* and *Salmonella paratyphi*. The ethyl-acetate extract of the flowers was active against *Bacillus cereus* and *Klebsiella* sp. The aerial parts extract in ethyl acetate also showed activity against *Mycobacterium smegmatis* and *Staphylococcus aureus*, while the aqueous extract showed no antimicrobial activity (185-187).

The aqueous extract of leaves of *Tridax procumbens* was lyophilized and studied on the excision wound model; rat skin fibroblast and rat paw oedema. Wound contraction was comparable in the *Tridax* and ibuprofen treated groups. Epithelialization was significant in the *Tridax* group. The specific activity of the enzyme gamma glutamyl transpeptidase was comparable in the *Tridax*, ibuprofen and aspirin treated groups at 200 mg/kg (188).

In chemical studies involving isolation and characterization of secondary metabolites from *Tridax procumbens*, saturated as well as unsaturated fatty acids and sterols have been obtained from the petroleum ether extract of the plant (189, 190). Eight new lipid constituents have also been characterized (191). Luteulin and glucoluteulin have been isolated from the flowers of *Tridax*
The ethyl acetate soluble part of hexane extract of *Tridax procumbens* yielded a new bis-bithiophene named tridbisbithiophene along with four known terpenoids: taraxasteryl acetate, beta-amyrenone, lupeol and oleanolic acid. The structures of all the isolated constituents have been elucidated with the aid of NMR spectroscopy whereas, the structure of new constituent tridbisbithiophene was confirmed via COSY and HMBC analysis. A new flavonoid (procumbenetin), isolated from the aerial parts of *Tridax procumbens* has been characterized as 3,6-dimethoxy-5,7,2,3,4'-pentahydroxyflavone 7-O-β-D-glucopyranoside. A flavone glycoside, 5,7,4'-trihydroxy-6,3'-dimethoxy-flavone 5-O-alpha-L-rhamnopyranoside I, has also been identified from the leaves of this plant.

Two water-soluble polysaccharide fractions, WSTP-IA and WSTP-IB were purified from the leaves of *Tridax procumbens* Linn. WSTP-IA contained L-Ara f and D-Gal p in similar to 1:3 molar proportions, and WSTP-IB contained only D-Gal p as the major sugar component. Methylation linkage analysis, and H-1 and C-13 NMR studies on the native and modified polysaccharides, indicated that WSTP-IA is an L-arabino-D-galactan with a beta-(1 -> 6)-D-galactan main chain in which at least one in every two D-Gal p residues carries single residues of either L-Ara f (alpha-/beta-) or beta-D-Gal p end-group as substituents at O-3. WSTP-IB is a linear beta- (1 -> 6)-D-galactan. This is the first polysaccharide containing a beta- (1 -> 6)-D-galactan main chain isolated from plant sources. The n-hexane extracts from the flower and leaves contained a mixture of hydrocarbons and long chain fatty acids such as Neo phytadiene & hexadecanoic acid as...
the main components. The ethyl acetate extract of leaf juice contained a mixture of fatty acids, aromatic compounds, polyaromatic carboxylic acids, polysubstituted phenols and thiols (197). The claims of traditional uses of *Tridax procumbens* in folk medicine, and the published literature are not sufficient to validate the use of this plant for medicine purposes. Therefore, further studies on the isolation and characterization of active principles present in the extracts are necessary. On the basis of published literature we have selected leaf extracts of *Tridax procumbens* for further study on isolation and characterization of active constituents involved in immunomodulatory activities associated with wound healing. Therefore we have analyzed the wound healing effect of aqueous as well as organic extract of *Tridax procumbens* leaves and have tried to identify the molecules involved in the process. Since it was observed that extracts of freshly plucked leaves of *T. procumbens* are much more effective in healing wounds as compared to that obtained from the whole plant, we have focused our attention on identifying the nature of molecules which are present in the extract of leaves which help in wound healing activity. We decided to assay for wound healing properties of the *T. procumbens* leaf extracts by the following *in-vitro* assays which can be quantitated for comparison purposes

1. Induction of IL-8, TGF-β1 and TNF-α secretion by human PBMC and THP-1 cell line
2. Activation of neutrophils by the aqueous extract.