CHAPTER 6

Discussion
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The natural occurrence of active phyto-chemicals in the traditional medicines are playing vigorous role in managing human health by treating majority of the ailments. In addition, the traditional therapeutic sources and their modes are the effectual basis for treating some deadly diseases which are unable to handle by the modern system of medicines. A large number of ethno-medicinal plants are used as herbal ointment in the traditional medicine system for the treatment of cuts, wounds and burns. Various research data revealed that plants may worked as healing and regeneration of the tissue by multiple mechanisms. There are several reports stating that the extracts of several plants, used for wound healing properties. Most of the recent reports suggest that, the active fractions of plants will act on targeted region, where, down regulation of the reactive oxygen species will be attempted specifically thereby, the system will be protected from the possible non-significant changes. This is because of the presence of wide range of medicinal properties in the plant drug components which can exhibit promising pharmacological activities. Moreover, the diversified class of phyto-chemicals from many plant drugs are still unexplored and also their actual mechanism not yet known to the system. Hence, the present research has been visualized on the phyto-chemical and pharmacological activities of traditional medicine formula practiced for wound healing and associated problems.

Screening of selected ethno-medicinal plant drugs and Tribal medicinal formulation clearly indicate the presence of maximum classes of active phyto-constituents is present in the extracts of both EMP and TMF drugs respectively. The status of crude drug along with efficacy will be resolute based on its physico-chemical characteristic features of the drug thereby; the crude drug will be standardized for its therapeutic application against ailments and diseases. The therapeutic approaches of herbal drug formula are conceivably based on the outstanding active metabolites present in the drug. Although, the absence of certain phyto-chemicals in one sample and its presence in the other can be safely attributed to the various physiological and biosynthetic reactions taking place inside the plant, the effect of the environment should not be neglected, as the environment always modify the things. The energetic fractions of the TMF drug showed the active metabolites such as, alkaloids, flavonoides, glycosides, terpenoides, tannins, saponins, phenols etc. These active constituents can
have profound effect on both physiological and biochemical changes which brings positively the normal functional status of the system\textsuperscript{234, 496, 497}. The significant contents of phenolic compounds indicated that, these compounds contribute to the antioxidant activity. The formulation comprising of all plant ingredients can be regarded as promising herbal drug explored from natural sources for antioxidant activity with high potential value for drug accomplishment. However, the presence of different horizons of phenols in the plant system is stabilizing the other associated group of active metabolites which is in the vein of a central dogma of the plants\textsuperscript{498-500}.

These phenolic derivatives in alliance with other active phyto-chemicals in the TMF drug indicates, the efficacious properties against some inflammatory diseases, carcinogenesis, oxidative stress apart from their defensive activity against other common ailments\textsuperscript{501-505}. Therefore, the plant phenols with their diverse analogs are playing crucial role in upholding the level of natural antioxidants which ultimately forms the herbal drug for its total biological activities\textsuperscript{24, 506-508}. The ability of active metabolites are channelized to synthesize the proteins to get amalgamate with other active constituents which breaks the complexity of extracellular nature of microorganisms belongs to diverse range of classes\textsuperscript{487, 488, 490-492}. Apart from the antimicrobial potentialities, the association of these active constituents can have strong antioxidant and anti-cancerous actions\textsuperscript{506, 507, 509}.

The confirmation of active metabolites in the TMF drug has exhibited better antimicrobial activities not only by smashing the complexity of the microbial cell wall but also complete degrading the cells matrix of pathogenic microorganisms\textsuperscript{510, 511}. The plant based drugs are the rich in source of active lead constituents which balances the status of human health by providing necessary elements as nutraceuticals. The intake of plants as medicines will have total benefit apart from its action on the ailments is concerned. The cells ultimately protected by oxidative damage in the system thus, the cells will be devoid of toxicity through the appropriate actions of the drug\textsuperscript{512-514}.

The results for Brine Shrimp Test reveals, five EMP drugs exhibited significant activity. These were \textit{A. serphyllifolia, D. hispida, G. mauritiana, N. nimmoniana} and \textit{R. densiflora}. In all, the LC\textsubscript{50} values were found to be below 0.01 \(\mu\)g/ml. The reason may be due to the low concentration or possible antagonistic activity between the phyto-chemicals from the different classes. It is evident through the individual plants
medicine and TMF drugs employed in the study with various parameters. Further, the aqueous and ethanol extracts, were exhibited considerable effects on brine shrimps as opposed to the ethanol extract reported in the previous study\textsuperscript{515}. The TMF drug have known to be very effective on biological parameters which could be attributed with a range of ingredients apart from other factors like, type of plant drugs, components, vigorous lead constituents, mechanism of action on affected area to normalize the biochemical and physiological functions. The most active fractions of both EMP and TMF were compared with the standard antibiotics, Pencillin, Streptomycin and Ampicillin 100mg/disc). The efficacy of the plant drugs was found to be promising and which can be a most candidate drugs used in treating infectious diseases caused by these pathogenic organisms \textsuperscript{516-519}.

In all, the TMF drug was shown to be effective antimicrobial agent with respect to increased dosages which was due to a driving force of active secondary metabolites like, tannins, flavonoids and glycosides in the plant drug. Additionally, the TMF drug which is formulated by the healers in association with the concoction of active ingredients and the results gives the impression about its valid traditional importance in the wound and related therapeutics. The antimicrobial activity is logically coupled with wound and its affected area either due to direct microbial infections or exposure of wound to the open environment\textsuperscript{520}. However, the development of potential antimicrobial drug is based on the effectiveness of the least measured quantity of the active fractions which annihilate the possibility of drug resistance microorganisms. Clinically, the minimum inhibitory concentrations (MIC) of the TMF drug will help to design an active principle with appropriate concentration which can kill the wide range of pathogenic organisms under irrespective of any diverse conditions\textsuperscript{521, 522,496}.

Further, the active principle of the TMF drug established a legitimate control over pro-oxidants by means of donating electrons which facilitate the effective free radical scavenging activity. This was also due to the probable contributions of ethno-medicines along with its efficiency in various therapeutic applications possessing majority of phyto-chemical classes of compounds and these uphold the phenomenon that is to say, ethno-medicines are the real sources of modern medicinal system\textsuperscript{506-508,510}. The diverse class of ingredients derived from poly herbal components which leads to synergistic actions of the TMF drug could possibly contributed in the total formulation as drug for multiple ailments\textsuperscript{523}. 


The bioactive analysis of the crude preparations for its standardization to know-how the occurrence of a wide range of active phyto-chemicals by the pharmaceutical industry will substantiate the use of the crude drug for wound and associated ailments in the traditional system of medicines. Later, the analysis on chromatogram gave significant approach in the TMF drug extract which confirms the presence of flavonoids, saponins in the qualitative tests together with TLC chromatogram which demonstrated a green fluorescence band at Rf 0.76 with NP/PEG spray. In addition, the TMF drug extract was premeditated to expose the appropriate mobile phases to obtain specificity in TLC chromatogram.

Besides, it is apparent that, the ploy herbal ingredients of these EMP drugs were of ethnic use, because, there possess some specific active chemical lead constituents in the EMP drugs namely, *A. serphyllifolia* possess, flavones like, Apigenin, Serpyllin and 5-hydroxy-7, 8, 2′, 3′, 4′-pentamethoxy flavones which validates the biological activities attempted in the study. Similarly, in *D. hispida*, an alkaloid called Dioscorine, *C_{13}H_{19}O_{2}N*, is present along with some steroidal sapogenin and suggested that, the alkaloid belonging to the tropane group and projected with these possible biological activities.

Later, one of the chief ingredients, *G. mauritiana* was known to have active metabolites like flavonoid and sugar moiety which confirms that, the lead compound might be a flavanoid glycoside. The presence of these active constituents may lead a key role in all the biological activities analyzed individually. Similarly, a potent alkaloid, namely Camptothecin present in *N. nimmoniana* which acts as compelling drug for cancerous ailments. The lead constituent Camptothecin known to have anti-inflammatory and antimicrobial properties out of apart from wide range of biological activities. Currently, the synthesis of Camptothecin is promising only from natural sources which have been possible from some specific class of ethno-medicinal plants.

Consequently, *R. densiflora* showed considerable pharmacological status which may be due to presence of medicinally important phyto-chemicals such as reserpine, densiflorine which are falling under the group of alkaloids. Apart from being used in the treatment of maternity complications, beriberi, syphilis, dysentery, diabetes, asthma, snake bite in the system of traditional medicines followed by the treatment of inflammatory diseases and wound related infections are critically evaluated and documented.
In the study, both models were used and the incision wound model has triggered the tensile strength on employing the TMF drug followed by ethanolic fractions treated group at variable concentrations. The recovery of wound has been stimulated by means of TMF drug and ethanol fraction followed by other treated groups. The outcome of the result was compared with control group and standard reference with Nitrofurazone (0.2%w/w). The contraction in wound was perhaps due to vigorous nature in the ingredients of total herbal formula which elicited the phases wound healing. The inflammatory phases of the wound healing have been resolved particularly by active metabolites present in the TMF drug which protects the cells from oxidative damage explicitly at affected area. In addition, it was observed that, all the extracts were compared with the standard. Thus it may be accomplished that TMF drug has the potential to satisfy all requirements of an ideal dressing material in that it provides an environment at the surface of the wound in which healing took place at the maximum rate consistent with the formation of granulation tissue. Further, the extracts TMF drug left no prominent disfigurement at the wound sites while reference Nitrofurazone ointment treated groups had considerable scars. Consequently, the natural anti-oxidants have known to be profound influence over wound contraction which is contemporaneous with all the remodeling phases. The TMF drug coupled with active ingredients derived from poly herbal combinations stimulated the stabilization in mass and breaking strength of granuloma followed by intensification of hydroxyproline content. Further, the formation of collagen was influenced after achieving all the progression stages which ultimately admits the wound contraction through re-epitelialization in the wound region.

The inflammation in the wound was thrived away with defense enzymes like, superoxide dismutase and catalase were alleviated which down regulate the free radicals thereby elicited the affected area with normal cells along with regular functional status through fitting remodeling stages. In the wound models, it was evident that, the TMF drug was known to have remarkable influence over wound contraction with reduced period of re-epithelialization which was perceptible when compared to the control group of treated animals. The TMF drug was activated by the active components of the poly herbal recipe which make possible in healing the wound at considerably a shorter duration. This was due to occurrence of
active phyto-chemicals in the different fractions of TMF drug which boosted the antioxidant activities in the granuloma tissues that in turn responsible for wound recovery through its energetic actions at the affected area\textsuperscript{543,544}. This gives a superlative model from traditional system of medicine explicitly for wound and all associated ailments\textsuperscript{545,546}.

The treated group of TMF drug was identified as remarkable wound therapeutics as it was achieved the complete proportion of wound contraction as compared to standard reference followed by control. The outcome of the study particularly at its crude fraction was significantly superior over other group of treatment. This was due to the effect of active ingredients with its natural conditions. Thus, the wound contraction was accomplished completely after 18\textsuperscript{th} day of treatment. However, the upshot provides a rationale for the use of TMF drug and its basis in the traditional system of medicine to promote wound healing. This effect may be explained by several mechanisms such as coating the wound, forming complexes with proteins of microorganism cell wall, chelating free radicals and reactive oxygen species, stimulating the contraction of the wound and increasing the formation of new capillaries and fibroblasts. Further, the TMF drug fractions did not produce any adverse effect and because of this; it is possible to justify the practice by Traditional healers and the same can be put forward its exercise in the treatment of wounds,\textsuperscript{539,490,491,547-549}.

The anti-proliferative activity in the methanolic fractions of TMF drug was analyzed against MCF-7 cells and the noxious effect was observed with the considerable apoptotic behavior which was superior with respect to variable concentrations in the drug. The ingredients derived from multi-herbal combinations of the TMF drug formula have known to exhibit the significant dogmatic effect over MCF-7 cells, which can be optional as chemo-preventive herbal drug that is extremely evident through its practice in traditional therapeutics. The lead constituents present in the TMF drug may have justified that, the possible risk of breast cancer can be prevail over by down regulating the oxidative stress and associated ailments. The practice of different ethno-medicinal plants are of potential value for identifying anti-proliferative agents and determination of the anti-inflammatory and related ailments, which was apparent through repression of breast cancer cells thereby apoptotic behavior of cells was regulated\textsuperscript{550}. Elsewhere, the active metabolites derived from
ingredients of TMF drug was known to have cyto-toxic effects against both MCF-7 and HeLa cancer cells which further indicates that, the efficacy on \textit{in vitro} modulation of the immune system by attempting deterioration of tumour cells under diverse situation\textsuperscript{561}.

Some studies indicated that, the active ingredients of the herbal formula can produce reflective vigorous health benefits by scavenging the free radicals by natural antioxidants which is further coupled with detoxification in the human system. The TMF drug possesses polyphenolic compounds which is a derivative of natural antioxidant agents perhaps accountable for defensive actions against the degenerative ailments occur due to oxidative damages\textsuperscript{552, 553, 554}. The active solvent fractions of TMF drug encompass with concoction of wide range of multifarious composites apart from other active metabolites which contributes in the form of hydrogen atoms to affect complete radical scavenging activities and can further enumerate this drug as potent anti-proliferative remedial tool\textsuperscript{555}. Many researchers have also opined that, the herbal drugs are explicitly with the association of phenolic substances can manipulate the total antioxidant activity\textsuperscript{556, 177, 557}. The ability of quenching a wide range of free radicals is due to presence of phenolic compounds in association with other active constituents of the plant drugs.

The outcome clearly indicates that, the mixture of multi-herbal ingredients known to have significant cytotoxic effects over breast cancer cells and the increased concentrations of the active principle of TMF drug have exhibited superior antioxidant activities which collectively down regulate the proliferation of cancer cells in the system. All together, the individual components of EMP and TMF drug in the study put forwarded the hypothesis that, the vigorous ingredients of the purified drug could be most effective in decelerating the ailments and disorders induced by oxidative trauma such as mammalian cancer by inhibiting the proliferation of MCF cells. The measured apoptosis was due to suppression of malignant cells by the active principle of the TMF drug which is dominated with phenolic substances as one of the chief sources. Finally, since it is the focal report on tribal medicine formulation, the characteristic mechanism of actions on fate of cell cycle along with proliferation of cancerous cells at the purified fractions of the plant drug needs to be furnished.
The venom isolated from the snake *Naja naja* was evaluated for neutralization with the protein of TMF drug. This reveals that the significant and effective neutralization was noticed between both proteins isolated from biological resources. Initially, the interaction between two proteins was found to be most efficient, however the interaction was found to be differing with two prominent peaks from proteins of both plant drug and snake venom and interestingly, at the end again these two proteins were found two interact significantly. As a result, the inhibition of enzyme, TMF-VPLA$_2$ at the peak two was appraised by crude fraction of TMF cold extract.

The extremely reactive lipid peroxides was formed due to oxidative free radicals which are responsible for tissue destruction through inflammation caused by venom PLA$_2$. It was apparent from the similar approaches suggesting that, the active fractions of herbal formula which can regulate the lethality of snake venom thereby restraining the possibility of tissue damage$^{432, 433}$. Correspondingly, the polyphenols from the aqueous extracts of the TMF drug formula completely inhibited the lethality of *Naja naja* venom in which the efficacy of plants to neutralize snake venom may be due to certain active chemical lead constituents possessing antilethal effects$^{391}$. Among them *Terpenoids, Flavonoids, Polyphenols* have contributed significantly as antivenom potential. Therefore multi-functionality of TMF drug has built an illustration in the capacity of such chemical substances to stick to proteins. Hence, obstruct the functions of many macromolecules. However it can be suggested that, these substances are responsible for observed protection, but the time has come to validate pharmacologically traditional evidences for this ethno-medicinal plant as antidote for Snake victims.

Overlapping of both proteins indicates that there might be presence of effective and interactive mechanism between these two proteins and also the neutralizing the effect of the enzymes present in the venom. Thus, the Anti-PLA$_2$ and Anti-Protease activity was effectively carried out in neutralizing the effect of the enzymes like PLA$_2$ and Protease present in the venom. Thus this Ethno-medicinal plant can be used as a life saving drug by conducting the further studies $^{437, 558}$.

The traditional medicines are the most promising way of treating the snake bite victims in rural India where the primary health care is considerably inadequate. This is because of the authenticity in the traditional therapeutic claims along with a hypothesis relating to the active principle on neutralizing the snake venom. Therefore,
effective new fangled anti-snake venom drug has been possible in the present study by means of herbal drug formula comprising of ploy herbal concoction with diversified class of lead constituents. This could be a most potential approach from TMF drug for its efficacy in effective management of snake envenomation. However, the observed result were of central importance which was evident from the aqueous crude TMF drug for its total significant biological activities explicitly supports the practice of TMF drug by Traditional Medicine Men. The presence of the bioactive compounds and phenolic content demonstrated the presence of the antimicrobial activity against the human pathogenic microorganisms followed by antioxidant activity. The TMF drug with both ethanolic and cold water fractions administered to the mice after they received snake venom of Naja naja were found to be significantly increased with respect to mean survival time and protection fold but could not protect mice from death when used alone and the results were found to be better when the cold water extract was used at higher dose. This might be possible in making precipitation of snake venom by the vigorous temperament of cold water extract of TMF drug. The multi-faceted ingredients derived from ploy herbal mixture is necessary to down-regulate the complexity of snake venom lethality through neutralizing the effect of anti-inflammatory enzymes. This has been possible by traditional system of medicines through scientific validation of the effectiveness of the herbal drug formula.

Moreover, considering the fact that, plant derived secondary metabolites may not necessarily be benign molecules but, the appraisal on the efficiency of the herbal drug coupled with all necessary bio-safety standards are very much requisite to ascertain the therapeutic potentials of the herbal drug formulations towards quantifiable management of snake envenomation. Further studies should also involve region-specific extensive ethno-botanical/ethno-pharmacological survey of our natural resources and creation of database of plants used as pharmaceuticals, nutraceuticals, anticancer drug, anti-dote for snakebite in ethno-medicinal practice. It is quite obvious that the high market demand of such plant derived medicines will build a pressure on our natural resources that might lead to the habitat loss, habitat degradation and over harvesting and exploitation of these medicinal plants resulting in a major threat to our biodiversity. Hence, a well thought road map should be designed for the proper consumption as well as maintenance of our valuable natural resources. This in turn,
will not only provide affordable and effective plant based curative molecule(s) for treatment of various ailments but will preserve our indigenous knowledge as well.

However, the discussion of the present study may serve as a stepping stone for future research on the biological and pharmacological activities in the extracts of EMP and TMF drugs. The study thus demonstrated the wound healing activity in the fractions of TMF drug which substantiate the practice of this drug formula (with slight amendment via scientific validation) positively. The efficacy of the TMF drug found to be most effective in the functional recovery of the wound healing by dose dependent manner. The result may also be attributed to the phytoconstituents present in it which may be both due to their individual and cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal practice and futures of TMF drug accomplishments. In addition, the reported experimental results can provide a concrete and legitimate platform for designing newfangled herbal drug comprising of multi-herbal concoction exhibit most prospective biological activities. The TMF principle has been confirmed for its efficacy and emerged as most useful drug against multiple ailments and degenerative disorders such as wound healing, antioxidant properties, antimicrobial, anti-proliferative and anti-snake venom respectively which led us further, to know how the molecular mechanism of the proposed drug apart from its role as functional food and nutraceuticals in the biological system.