ABSTRACT

The present investigation accentuate on some less known medicinal uses of ethnomedicinal plants drug formulation which is practiced by Tribal Medicine Men (TMM) at Biligirirangana Hill area of Chamarajanagara district, Karnataka, India. This was mainly based in the light of traditional practice of herbal drugs comprising of diverse ethnomedicinal plants for other related ailments. The contents and amount of EMP present in the formulations were validated scientifically. The ethnomedicinal plant materials of Tribal Medicine Formulation (TMF) were taxonomically identified and authenticated with standard flora and taxonomic experts. The authenticated ethnomedicinal plant drugs were of (EMP) viz, *Andrographis serphyllifolia* (Acanthaceae), *Dioscorea hispida* (Dioscoreaceae); *Glycosmis mauritiana* (Rutaceae); *Nothapodytes nimmoniana* (Icacinaceae) and *Rauwolfia densiflora* (Apocynaceae) and Tribal Medicine Formulation (TMF) drug were procured in its raw status. Further, the TMF was validated scientifically with authorized ayurvedic practitioner and was subjected for chemo-typing and characterization studies. Initially, the plant materials were subjected for physico-chemical parameters such as ash values, foreign organic matter, moisture contents and extractive values respectively. In addition, fluorescence analysis for the ethnomedicinal plants samples was carried out.

The proximate analysis reveals that, the value of acid insoluble ash and water soluble ash were found to be significantly lesser correspondingly, the solvent extraction rate was significantly higher as compared to the values of water soluble ratio. The physico-chemical analysis with distinctive characteristic features of ethnomedicinal plants pointed out that, the vigorous standing of pharmacognostic and efficiency of the plant drugs.

The phyto-chemical screening of aqueous extract and solvent extracts of both EMP and TMF demonstrated the presence of alkaloids, flavonoids, saponins, tannins, gums & mucilages, coumarins, terpenoids, steroids, glycosides, phyto-sterols, fixed oils and fats, anthraquinone and phycobalamin. The occurrence of active metabolites was also suggested that, the synergistic actions of these active metabolites exerted the significant pharmacological potentialities. The phyto-chemical tests were best answered by the aqueous extracts of TMF than that of solvent extracts of EMP. In addition, the amount of total phenolic compounds in all tested plant extracts was
higher than the TMF drug. The ranking order of five plant species from point of view of antioxidant (phenolic compounds) amounts was as follows: R. densiflora > N. nimmoniana > G. mauritiana > A. serphyllifolia > D. hispida.

Subsequently, in the cytotoxic assay, the brine shrimp mortality was found to be increased with increase in test concentrations. The noxious and momentous action was displayed to the nauplii of brine shrimp at active fractions of both EMP and TMF drugs.

Later, the antimicrobial evaluation was carried-out by employing both Gram-positive, Bacillus subtilis and Staphylococcus aureus; Gram-negative; Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia. As a whole, the active fractions of EMP and TMF showed perceptive affects against Gram positive bacteria, whereas, in case of Gram-negative organisms, they were considerably susceptible solvent extracts such as, Ethanol, Methanol, petroleum ether compared to aqueous extracts of both EMP and TMF. The zone of inhibition was found to be most effective at methanol and ethanolic extracts of EMP and TMF drug which was compared with standard antibiotics. The high degree of inhibition zone of antibacterial activity was noticed against E.coli by ethanolic extracts of EMP plant drugs. Similarly in TMF, the zone of inhibition was significantly superior to other solvent extracts. Correspondingly, in the antifungal activity, Aspergillus flavus showed efficient antifungal activity for ethanol extracts EMP drugs. On the contrary, the TMF Methanol drug extract showed proficient activity in Aspergillus niger, A. flavus, Fusarum oxysporum and Rhizopus stolonifer respectively. In the analysis of Minimum Inhibitory Concentration (MIC), the domino effect reveals, ethanolic extracts of TMF showed outstanding activities both in MIC (21.9 μg/ml) and MBC (58.7 μg/ml) in S. aureus followed other extracts against all the pathogenic organisms and were also significantly superior over all the EMP drugs. Similarly, the aqueous and ethanol extracts of EMP drugs (Glycosmis mauritiana) showed least MIC value 26.5μg/ml and 29.3 μg/ml against E. coli respectively. On the contrary, the highest MIC (56.2 μg/ml) and MFC (116.6 μg/ml) activity was observed in aqueous extract of A. serphyllifolia by A. flavus, like-wise, in petroleum ether extract of N. nimmoniana, MIC (57.4 μg/ml) and MFC (114.3 μg/ml) activity was recorded in A. niger. Whereas, petroleum ether extract of R. densiflora showed MIC (59.9 μg/ml) and MFC (119.8 μg/ml) in R. stolonifer. In all, the aqueous fractions of both EMP and TMF crude
fractions were exhibited maximum activity when compared with different solvent extracts on all the bacterial and fungal cultures tested.

Further, the antioxidant activity was been evaluated by DPPH radical scavenging and ABTS methods, respectively. All the EMP drugs registered significantly higher in phenolic content, individually (107.67mg GAE/g) than that of TMF (102.45 mg GAE/g). Similarly, antioxidant potential was found to be noteworthy as determined by DPPH (12.24 EC_{50} μg/ml) radical scavenging activity which was registered in the EMP individually followed by TMF. An appreciable ABTS radical scavenging (12 -15EC_{50} μg/ml) activity, Frap assay (8-21μmol Fe^{2+}/mg) were realized in ethanolic extract of TMF and were found to be considerably superior. In the total reduction capability, the IC_{50} values of reducing activities of aqueous fractions EMP revealed in the order of activity as: A. serphyllifolia > D.hispida > G. mauritiana > N. nimmoniana > R. densiflora. The bioactive compounds in TLC chromatograms were measured by calculating retention factor. There were two to five bands of whole TMF drug extract at Rf values of 0.32 and 0.44 in A. serphyllifolia; 0.52 and 0.26 in D. hispida; 0.59 and 0.23 in G. mauritiana; 0.34, 0.14, 0.16, 0.62 and 0.95 in N. nimmoniana similarly, in case of R. densiflora 0.76 and 0.85 were present.

The effect of active fractions of Tribal Medicine Formulation (TMF) was employed to analyze the wound healing potentialities via excision and incision models. The assessment on wound healing tests was performed for drug formulation using the simple ointment base (B.P) as control along with reference hypothesis, Nitrofurazone ointment 0.2% w/w by way of excision wound model. The momentous effect from the topical therapy at the dosage of 10 and 15% w/w ointment of EMP and TMF drugs exhibited superior action on wound contraction as compared to the control treatment administered to the groups of animals of excision wound model. Correspondingly, the striking effect of the active fractions of the drug on tensile strength in the incised wound after 10th day of wound healing process was also found to be extremely significant. In the incision wound model, increase in tensile strength was found to be noteworthy after 10 days of wound due to treatment with test formulation. The dose dependent effect was noticed by the TMF drug on wound recovery followed by ethanolic fractions emphasizing on tensile strength after the termination of the wound healing phases. Subsequently, the ethanolic fraction of TMF drug over dead space wound model exhibited enhanced effect on granuloma weight followed by breaking
strength which was correlated to level of hydroxyproline. In the histological studies, the animal group treated with TMF drug elicited the formation of collagen which facilitates wound contraction. Later, the defence related enzymes like, superoxide dismutase and catalase were showed significant activities which might have contributed to the existing antioxidant potentials in the system. Thus, the re-epithelialisation duration was considerably reduced during the phases of wound contraction and the treatment with TMF drug was found to be significantly superior over all other treatments including control. It was evident that, the crude fractions of TMF drug were considerable promising at both the wound models which substantiate the practice of the drug by local traditional healers.

The formulated Tribal Medicine extracts was tested for anti-proliferative activity against HeLa cancer cell lines. The inhibition concentration as observed in the 24h duration of trypan blue assay. The cells show positive ‘Hoechst assay’ as the DNA has acquired the Hoechst stain and the same is seen under the fluorescence microscope. The results analysis for MTT assay indicated that the cell viability decreases with increase in concentration of the drug. The inhibition concentration value (IC_{50}) for the MTT assay at 24h shows to be 5.1\mu g/ml and for 48h at 5.4\mu g/ml. The outcome of mamarian cancer cell analysis showed that, the methanolic fractions of TMF drug exhibited profound activities over tested MCF-7cells. The Apoptotic assays showed that, TMF drug both ethanolic and crude fractions induced mitochondrial depolarization in MCF cells leads to 20-30\% followed 40-50\% Cell death by Trypan Blue Assay at lowest exposure periods of lowest concentrations.

The protein profile of both TMF drug and snake venom on SDS-PAGE was demonstrated in association with the standard markers, which were laden on the gel and performed the electrophoretic experiment by setting the standard parameters. The assessment showed that, the final impression was found to be low molecular weight proteins. Correspondingly, the partially purified bioactive principle of TMF drug neutralized the PLA_{2} and interaction of Proteins of both snake venom and TMF. This may be owing to occurrence of diverse range of vigorous phyto-chemicals in the TMF formula which played an imperative role in inhibition of PLA_{2} activity. In the Anti-hemorrhagic analysis, the mouse lung tissue and muscle tissue layer added with Saline buffer showed no cell death or even desertion in the tissue system was observed. The second layer added with Snake venom showed the cells started dying
by forming tumors whereas, in case of the third layer added with the Snake venom sample along with the active principle of TMF drug was found to be very effective in decreasing the activity of the enzymes which were present in the snake venom and this confirms the efficacy of TMF drug extract over venom in neutralizing the effect of the snake venom. However, this study may serve as a stepping stone for future research on the biological and pharmacological activities in the extracts of EMP and TMF drugs. In addition, the outcome of the study clearly confirms that, the practice of TMF drug in Tribal medicine system for wound healing has been substantiated. Hence, the pharmacologist can harness the potentials in the ethno-medicinal plants and in the TMF extracts of ointments for the treatment of contemporary diseases relating to wound and associated ailments such as skin cut, skin infection, tissue damage etc. The ethno-medicinal plants and formulation certainly which are the real gift from the nature having traditional knowledge, provides excellent novel raw material towards better management of wound and other multiple ailments. Further, this TMF drug formulation can perform as an alternative and efficient surrogate medicine that possibly will even barter the other type of modern wound healing medicines.