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Research Article

Antimicrobial and Antioxidant Activities of Tribal Medicine formulation (TMF) accomplished for Wound related remedies in Biligirirangana Hill area of Chamarajanagara district, Karnataka (India)

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Abstract

The present investigation looking at some less known medicinal uses of ethno-medicinal 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 ethno-medicinal plants for other related ailments. The contents and amount of EMP present in the formulations were validated scientifically. The previous studies of these ethno-medicinal plants confirm that, the physico-chemical analysis and the active status of pharmacognostic parameters which evidently indicates the efficiency of these plant drugs. To ascertain these findings, a study on biological activities in the vein of Antimicrobial and Antioxidant activities were attempted. The antimicrobial activity was tested against standard strains such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Kliebsiella pneumonia and Pseudomonas aerugenosa* using disc diffusion method. The significant zone of inhibition was observed in all the ethno-medicinal plant drugs followed by TMF and the result was found to be superior over standard antibiotics. The antioxidant activity has 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 EMF individually. A superior ABTS radical scavenging (ranging between12.06-15.40 EC_{50}µg/ml), Frap assay (ranging between 8.25-21.26µmol Fe^{2+}/mg) and significantly higher total reduction capacity (1.28%) were realized in ethanolic extract of TMF. As a result, the EMF and TMF drugs can be used as functional foods to increase shelf-life of food items for human consumption and neutraceuticals to dissuade deleterious free radical-induced life threatening diseases.

Keywords: Antioxidant activity, Antimicrobial activities, Tribal Medicine Formulation (TMF), Tribal / Traditional Medicine Men (TMM), B.R. Hills, Karnataka.

1. Introduction

Plant-derived substances have recently become of great interest owing to their versatile applications in the 'Health care system'. Ethno-medicinal plants are the main source of large amount of herbal drugs comprising to different groups such as antimicrobials, anti-inflammatory, anti-cancer, anti-viral, anti-venom activities etc. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Globally, a large number of the ethno-medicinal plants are claimed to possess the antibiotic properties in the traditional system and are used extensively by the tribal/ folk medicinal community. It is now believed that

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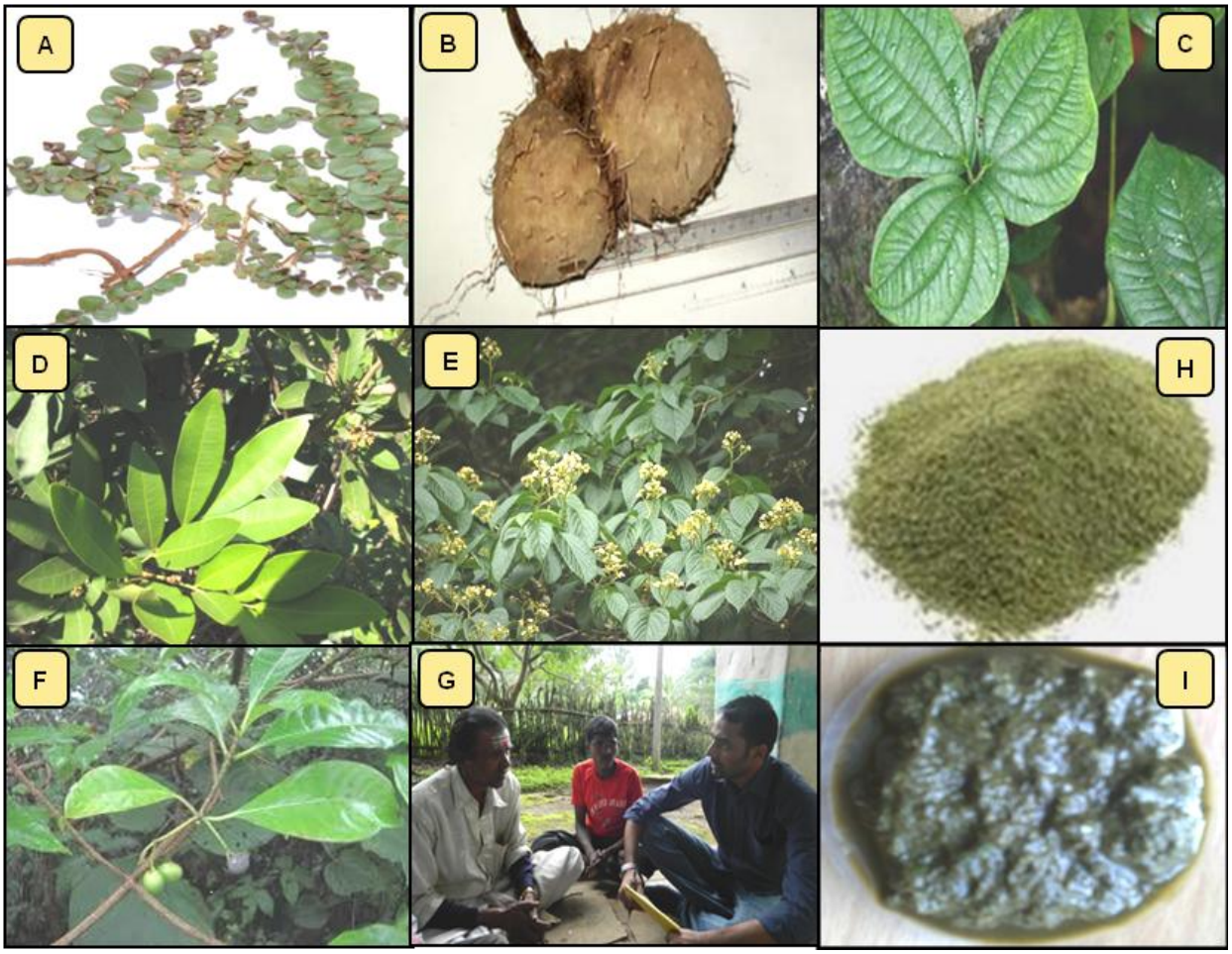
nature has given the cure of every disease in one way or another. Plants have been known to relieve various diseases in Ayurveda [2-5].

In India, almost 95% of the prescriptions were plant based in the traditional health care systems like, Ayurveda, Unani, Homeopathy and Siddha [6]. Therefore, the researchers today are emphasizing on evaluation, characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. The exploration of the bioactive constituents in the plant drugs has always been a challenging task for the researchers. The role of natural products, herbal medicine, tribal and traditional medicines is being increasingly appreciated in recent years for the prevention and cure of several human serious ailments. Therefore, standardization of plant drug formulation is the order of the day [7-12]. The increasing demand for herbal drugs both in the developing and developed countries inevitably led to maintaining the quality, purity of the herbal raw materials and finished products [5]. The study of ethno-medicinal plants continues principally for the discovery of novel secondary metabolites.

The present research study is undertaken to carry-out antimicrobial and antioxidant activities in the selected ethno-medicinal plants *viz., Andrographis serphyllifolia* Vahl., *Dioscorea hispida* Dennst., *Glycosmis mauritiana* Tanaka., *Nothapodytes nimmoniana* Blume. And *Rauvolfia densiflora* (Wall.) Benth & Hook and Tribal Medicine formulation. These plant drugs and their formulations used by the tribal practitioners that could be an evidence for getting into the further investigation on these ethno-medicinal plant drugs for the biological activity and isolation of active constituents using bio-assay guided fractionation.

Figure 1A-I: Ethno-medicinal Plant components in Tribal Medicinal Formulation (TMF)



A: Leaves of *Andrographis serphyllifolia***, B:** Tubers of *Dioscorea hispida,* **C:** Leaves of *Dioscorea hispida*
D: Leaves of *Glycosmis mauritiana,* **E:** Leaves of *Nothopodytes nimoniana* Blume**.,**
F: Leaves of *Rauwolfia densiflora*, **G:** Interaction with Tribal Healers,
H: Powder of Tribal Medicine Formulation, **I:** Paste/slurry of Tribal Medicine Formulation

2. Materials and Methods

2.1 Base-line survey and collection of Ethno-medicinal plants

The data on ethno-medicinal plants, *Andrographis serphyllifolia*, Vahl (leaves), *Dioscorea hispida*, Dennst, (tubers); *Glycosmis mauritiana* Tanaka, (leaves); *Nothapodytes nimmoniana* Blume (leaves) *and Rauvolfia densiflora* (Wall.) Benth & Hook (whole plant) were collected during the survey conducted between 2012 and 2014, at Biligirirangana Hill area, Karnataka. The species identified appropriately and authenticated by consulting a flora of Mysore and the same have been deposited in the Centre for Shridevi Research Foundation, SIET in collaboration with Bhoomigeetha Institute of Research & Development (Tumkur), Karnataka. The baseline informations of selected ethno-medicinal plants are represented in the Table 1.

Table 1: Validated Tribal Medicine formulation (TMF)* and its components practiced for wound healing and related ailments at Biligirirangana Hill Tracts, Karnataka.

Sl. No.	Ethno-medicinal plants with Vernacular Name.	Family	Plant parts used	Quantity (powder) (g/kg)	Validated Quantity of TMF (g)*
1	*Andrographis serphyllifolia* **(A)** Vr. Name: Kasinasara	Acanthaceae	Whole plant	20	**(A)** 20+ **(D)**15+ **(G)** 25+ **(N)** 25+ **(R)** 15+ **TMF ADGNR = 100**
2	*Dioscorea hispida* **(D)** Vr. Name: Noolana hambu	Dioscoreaceae	Tubers	15	
3	*Glycosmis mauritiana* **(G)** Vr. Name: Orrange berry	Rutaceae	leaves	25	
4	*Nothapodytes nimoniana* **(N)** Vr. Name: Durvasane mara	Icacinaceae	Leaves	25	
5	*Rauwolfia densiflora* (**R**) Vr. Name: Snake root	Apocynaceae	Leaves	15	

DOSAGE, DURATION AND MODE OF TREATMENTS OF TRIBAL MEDICINE FORMULATION

Paste of TMF	It is applied on affected part of wound followed by snake bite and Scorpion bite region with few drops of Honey and Lime juice for wounds and infected area.	**Duration:** Apply paste at wound area & cover with a thin cloth 3times/week
Decoction of TMF	Ground & juice boiled with warm water & swallowed for internal problems Decoction with warm water/ goat milk for. inflammation and related ailments	**Duration:** One tsp two times a day for 8 days.

*****TMF obtained from TMM was validated by Authorized Ayurvedic Practitioner

2.2 Validation of Tribal Medicine Formulation (TMF)

Interactions were conducted with Tribal Medicine Men with a semi-structured questionnaire and the data on Medicine formulation responsible plant components were obtained. Further, the Tribal Medicine Formulations (TMF) and Ethno-medicinal plant materials were obtained from the Tribal Medicine Men (Fig. 3A-J) and then the samples were scientifically validated based on its physical characteristics in association with an authorized Ayurvedic practitioner, Nisarga Ayurveda Research Foundation, Sakaleshpur, Hassan district, India. The standard protocols were identified and the methodology was employed in the present study, based on the description of Authorized Herbal Practitioner [13].

2.3 Preparation and Processing of TMF

The collected Ethno-medicinal plant materials were subjected for separating different desirable parts like leaves, stem, root/ tubers from the main plants or whole plant parts were shade dried for 20 days to ensure the active constituents free from decomposition and also to avoid any photo-chemical degradation. The different parts of plant materials were powdered using suitable electric blender. The powdered samples of both EMP and TMF were stored in airtight containers and kept in a cool, dark, dry place until the further analysis is commenced following the standard procedures [6].

2.4 Successive solvent extraction

2.4.1 Tribal Medicine Formulation: Medicine Formulation is a mixture of the tribal medicinal components of various parts of plants that are used to treat various abnormalities. The parts used for the mixture can be leaves, roots, stem, tubers, twigs, fruits, seeds, flowers and whole plants. The formulation is usually prepared by mixing the components in variable amounts and pasting it using cold or warm water. It can be directly applied on to the exterior parts of the body with honey drops or given for the intake depending on the abnormality being treated through warm

water or goat milk. These formulations are based on the indigenous knowledge of Tribal people in India (Fig. 3A-J and Table 2).

The bio-chemical activity of Tribal Medicine Formulation (TMF) will not be known to the tribal healers but their action will be known, because the TMF is practiced by them, since many years. The components react with each other and show the suitable activity on the patient. The TMF constituent was subjected for devastating into small pieces using pestle and mortar and then powdered in an electric grinder for further analysis.

50g powder of the Tribal Medicine Formulation (TMF) was subjected to successive solvent extraction with different solvents in increasing order of polarity from petroleum ether to ethanol and methanol finally to the level of crude extraction with water. The organic solvent was made particular based on the dissolving efficiency and total recovery of the ethno-medicinal plant drugs amongst the organic solvents used in the study, Meanwhile, the extracts were kept for evaporation to dryness and set aside for further bio-physical analysis.

2.4.2 Ethno-medicinal plants: The powdered sample (170g) was extracted by maceration method using soxhlet apparatus with different solvents from polar to less polar solvents such as Water, Ethanol, Methanol and Petroleum ether. The macerated extract was centrifuged at 5000rpm for 15 min and the supernatant was taken for the excess solvent evaporation. After evaporation of the excess solvent, the crude extract was taken for further analysis. The macerated extracts were then placed in shaker incubator for 24 hours and later subjected to filtration using Whatman No. 1 filter paper.

The test solvents were confirmed based on the yield and feasibility of the solvents during extraction processes and then, organic solvents were removed, firstly by means of a water bath, then in an oven, yielding the extracted compound. The concentrate was designated as crude ethanolic extract of EMP. These extracts were used to conduct the phyto-chemical and pharmacological evaluation of ethno-medicinal plant drugs (EMP).

2.5 Water extraction of Crude EMP Samples

Crude plant extract was prepared by Soxhlet extraction method. The powdered plant material was uniformly packed into a thimble for extraction. The mixture was heated on a hot plate with continuous stirring at 30-40ºC for 20 minutes. Then, the water extract was filtered through Whatmann No. 1 filter paper. The filtrate was subjected to dryness and the dried extract was kept in refrigerator at 4ºC for their future use for biological activities and bio-chemical analysis.

2.6 Antimicrobial activity

2.6.1 Test Microorganisms: Test organisms were used in the study, gram-positive bacterium: *Staphylococcus aureus, Bacillus subtilis* and gram-negative bacteria: *Escherichia coli, Kliebsiella pneumoniae* and *Pseudomonas* sp. were collected from the infected individuals at Siddhartha Medical College, Tumkur. The Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All the cultures were checked for viability, purity by regular plating and biochemical tests. Test cultures were prepared by transferring a loop full of bacteria from stock culture to nutrient broth in the flasks and incubated at 37°C for 24 h.

2.6.2 Preparation of Inocula: Stock cultures were maintained at 4°C on slopes of nutrient agar. The active cultures for experiments were prepared by transferring a loop-full of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) and were incubated as still cultures for 24h at 37°C and were used for the determination of Antimicrobial activity.

2.6.3 Disc diffusion method: *In vitro* antimicrobial activity was screened through disc diffusion method as proposed by Kirby-Bauer using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. the discs were formed at appropriate distance in the petriplates and loaded with the Control (solvent), Standard Antibiotic (Chloramphenicol - 0.025%) and Plant extracts (concentration- 100 mg/ml) in polar, non-polar and not-so-polar solvents [14]. The plates were incubated at 37^0C for 24 h. at the end of incubation; the inhibition zones formed around the disc were measured with transparent ruler and recorded.

2.7 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC), MBC and MFCs were performed by a serial dilution technique using 96-well microtiter plates as described by [15]. The different plant extracts *viz*., Methanol, Ethanol, Petroleum Ether, Aqueous were taken (1 mg/ml) and serial dilution of the extract with Luria broth for bacterial culture and potato dextrose broth medium for fungi with respective inoculums were used. The extracts were serially

diluted (two-fold) to a working concentration ranging from 400mg/ml to 12.5mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms namely *Escherichia coli*, *Bacillis subtilis*, *Staphylococcus aureus*, *Kliebsiella pneumoniae* and *Pseudomonas* sp and the microplates were incubated for 72 hours at 28^0C, respectively. After incubation period, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value. The lowest concentrations without visible growth (under binocular microscope) were defined as MICs and the calculation was done as per the following formula.

MIC=Minimum Inhibitory Concentration, C=Concentration of Antibiotic in mg/ml in total volume,

X=Length of Microbial growth in cm. and Y=Total length of possible growth in cm.

2.8 Determination of Minimum Bacterial Concentration (MBC)

The MBCs were determined by serial sub-culturing of 2 µl into microtitre plates containing 100µl of broth per well and further incubation for 72 hours. The lowest concentration with no visible growth was defined as the MBC, indicating assassination (99.5%) of the original inoculum. The optical density of each well was measured at a wavelength of 655nm by Microplate reader (Perlong, ENM8602) and compared with the standard Ampicillin for Bacteria (Hi-media lab, India) as the positive control. All the experiments were performed in duplicate and repeated three times.

2.9 Determination of MFC

The fungicidal concentrations (MFCs) were determined by serial sub-culturing of a 2 µl into microtiter plates containing 100µl of broth per well and further incubation for 72 hours at 28^oC. The lowest concentration with no visible growth was defined as MFC indicated 99.5% killing of the original inoculums. The commercial standard, Flucanazole (Sigma), was used as positive controls (1–3000 µg/ml) for fungi. All experiments were performed in duplicate and repeated three times.

2.10 Antioxidant Activity

2.10.1 DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity: The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β- picryl hydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour. The free radical scavenging capacity of the methanol extracts was determined using DPPH [16,17].

The DPPH solution (0.006% w/v) was prepared in 95% methanol. The different concentrations of the test sample which is to be examined for antioxidant activity is prepared (50-300µg/ml). 3 ml of different concentration of test samples of EMP and TMF extracts were mixed with 1 ml of DPPH solution in dark. Ascorbic acid which is strong anti-oxidizing agent is taken as standard. The different concentrations (3 ml) of standard solution of ascorbic acid were mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and plant extracts samples was incubated for half an hour and then the absorbance was taken with the help of U.V. Spectrophotometer at 517nm. The concerned extract or solvent served as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD} \times 100}{\text{Control OD}}$$

2.10.2 ABTS (2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity: ABTS assay was performed according to the standard protocol; the stock solution was prepared by mixing equal volumes of 7mM ABTS solution and 2.45mM potassium per-sulfate solution followed by incubation for 12h at room temperature in the dark to yield a dark-colored solution containing ABTS radicals. The working solution was prepared freshly before each assay by diluting the stock solution by mixing of stock solution to 50% methanol for an initial absorbance of about 0.700 (± 0.02) at 745 nm, with temperature control set at 30°C. The varying concentrations (50-3000µg/ml) of the plant extracts of EMP and TMF were allowed to react with 3 ml of the ABTS solution and the absorbance readings were recorded at 734 nm. Ascorbic acid was used as positive controls. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula as described by Lie-fan *et al.*, (2011).

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD} \times 100}{\text{Control OD}}$$

2.11 FRAP Assay and Determination of Total Phenolic Content

The FRAP assay was done for the extracts of EMP drugs as per the standard protocol [17] and total phenolic content was determined based on the standard formula [18].

1.12. Assay for Total reducing ability

The total reduction capabilities of the aqueous extract of TMF were estimated by using the standard method [19]. The absorbance of the reaction mixture after incubation was measured at 700nm using a spectrophotometer (UV-Cintra Spectrophotometer). Higher absorbance of the reaction mixture indicated greater reducing power.

2.13 Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained from all the test samples of EMP and TMF were compared with each other. The p values <0.05 were considered to be statistically significant. The concentration producing 50% of the maximum response (LC_{50} or IC_{50}) was obtained by the best visual fit from the plot of the individual experiments.

3. Results

The data on ethno-medicinal plant drugs comprising of family, vernacular name, parts used, medicinal value, formulation, treatment of ailment, dosage and duration of the treatment are well represented (Table 1). Similarly, the details on medicinal formulation were obtained from the tribal medicine men. Further, the collected tribal medicine formulation (TMF) was validated with an authorized Ayurvedic Medical Practitioner (Table 2).

3.1 Antimicrobial activity

In the study, Gram-positive: *S. aureus; B. subtilis* and Gram-negative; *E. coli, P. aeruginosa, K. pneumonia* were used for evaluation. In all, two Gram-positive bacteria were found to be sensitive to the extracts of EMP and TMF, whereas in case of Gram-negative organisms, they were considerably susceptible to solvent extracts such as Ethanol, Methanol, Petroleum ether compared to aqueous extracts of both EMP and TMF (Table 2). The antimicrobial potential of both the EMP and TMF plant drugs was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standards *viz.*, Ampicillin (1.0 mg/disc), Flucanazole (1.0 mg/disc). The results revealed that, all the extracts are potent antimicrobials against all the microorganisms studied. Among the different solvent extracts studied, methanol and ethanol showed high degree of inhibition followed by aqueous and Petroleum ether extracts. For all the tested microorganisms, Ethanol and Methanol showed maximum antibacterial activity in EMP. In model organism, *E.coli*, the Ethanol extract showed maximum inhibition zone diameter with *A. serphyllifolia* (14±0.2), *D. hispida* (14±0.6), *G. mauritiana* (16±3.5) *N. nimmoniana* (14±1.5) and *R. densiflora* (16±1.0). Similarly in TMF, the zone of inhibition was (19±0.2) significantly superior than other solvent extracts. For *S. aureus*, the ethanolic extract exhibited radical increase in zone of inhibition at *A. serphyllifolia* (13±0.3), *D. hispida* (16±2.5), *G. mauritiana* (19±1.5) *N. nimmoniana* (15±1.0) and *R. densiflora* (21±3.0) and the maximum inhibition (23±2.0) was recorded. Correspondingly, the Methanol extract showed maximum inhibition zone with diameter of 24±2.5 mm in *S. aureus* and similar values (20±1.6) were observed in *B. subtilis* and *Pseudomonas* sp. Further, the Petroleum Ether (10-20 mm) and aqueous extract (12-19 mm) showed poised status and the activity was notable. More specifically, aqueous extract was found to be equally effective and represented trivial susceptibility to all bacterial strains as compared to other tested organisms (Table 2).

In the antifungal activity, ethanol extracts of EMP drugs showed efficient antifungal activity against *A. flavus viz., A. serphyllifolia* (19.6±0.6), *D. hispida* (16.9±0.5), *G. mauritiana* (17±0.4) *N. nimmoniana* (16±0.3) and *R. densiflora* (15.5±0.4). Consequently, the TMF Methanol drug extract alone showed proficient activity in *A. niger* (21±0.3), *A. flavus* (22.1±0.6), *F. oxysporum* (24.8±0.11) and *R. stolonifer* (26.1±0.5) respectively. In contrast, Aqueous and Petroleum Ether extracts showed lowest inhibition zone with diameter ranging between 11-18 mm and 14-21 mm against all the pathogenic fungal strains, respectively (Table 3).

Among all the extracts employed in the antimicrobial activity, the TMF drug and the EMP namely *G. mauritiana and R. densiflora* demonstrated relatively strong antibacterial activity with hops and presenting a highest activity in *Staphylococcus aureus* compared to model organism. Of these, ethanol extracts of

A.serphyllifolia, D.hispida, had the maximum activity against *Escherichia coli and Bacillus subtilis* followed by Petroleum ether. Similarly, ethanol extracts of *N. nimmoniana* also showed significant activity in *K. pneumoniae* and *Pseudomonas* species. Correspondingly, extracts of TMF and methanolic extracts of *A. serphyllifolia* and *G. mauritiana* exhibited most significant antifungal activity against all the fungal organisms (Table 2 and 3).

3.2 Determination of MIC, MBC and MFC values

Minimum Inhibitory Concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganisms to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MBC and MFC was determined by sub-culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the bacteria and fungi was taken as MBC and MFC, respectively. Moreover, it was noted that, most of the antimicrobial properties in different plant part extractions shows, MBC value that is almost two fold higher than there corresponding MICs.

The ethanolic extracts of TMF showed outstanding activities both at MIC (21.9 µg/ml) and MBC (58.7 µg/ml) in *S. aureous* 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 (Table 4).

The highest MIC (56.2 µg/ml) and MFC (116.6 µg/ml) activity was observed in aqueous extract of *A. serphyllifolia* at *A. flavus*, like-wise, in petroleum ether extract of *N, nimmoniana*, MIC (57.4 µg/ml) and MFC (114.3 µg/ml) activity at *A. niger* was recorded respectively. Whereas, petroleum ether extract of *R. densiflora* showed considerable values *i.e.*, MIC (59.9 µg/ml) and MFC (119.8 µg/ml) in *R. stolonifer*. The TMF was proved to have most significant activity in all the extracts against all the organisms (Table-5). In all, the aqueous fractions of both EMP and TMF crude extracts showed ceiling activity compared to other solvent extracts, respectively against all the bacterial and fungal cultures tested (Table 4 and 5).

3.3 Evaluation of Antioxidant activity by DPPH Radical Scavenging Assay

The free radical scavenging activity of extracts of EMP and TMF drugs was assessed by DPPH and ABTS assay. The result shows the significant decrease in the concentration of free radicals due to the scavenging ability of extracts of EMP drugs, respectively. The scavenging activity was compared with that of standard Ascorbic acid. The significant DPPH scavenging activity in methanolic extracts of EMP drugs, *A. serpyllifolia* (18.42±0.05 EC_{50}µg/ml), *D. hispida* (18.90±1.42 EC_{50}µg/ml), *G. mauritiana* (29.12±0.56 EC_{50}µg/ml), *N. nimmoniana* (16.66±0.85 EC_{50}µg/ml) *and R. densiflora* (31.55±0.65 EC_{50}µg/ml) respectively were recorded (Table 7 and Graph 1). Whereas, in case of TMF the % DPPH activity was found to be 90% at 150µl concentration and the activity was increased with increase in concentrations of TMF (Table 6 and 7).

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants. The extracts of EMP and TMF drugs efficiently scavenged ABTS radicals generated by the reaction between 2, 2-azinobis (3-ethylbenothiazoline-6-sulfonic acid) and ammonium persulfate (Table 7 and Graph 2). The activity was found to be increased in a dose dependent manner from 15- 64% at a concentration of 60-150 µg/ml.

The DPPH radical scavenging activity of organic fractions of ethno-medicinal plant (EMP) drugs was increased with increase in fraction concentration. The percent scavenging ability of TMF fractions at150µg/ml was higher (90.85%) was noticed. The results obtained in the study reveals that, the fractions of extracts of both EMP and TMF drugs are free radical scavenger and able to react with the DPPH radical, which might be attributed to their electron donating ability (Table 6 & 7 and Graph1).

The ABTS radical scavenging assay gave the measure of antioxidant activity of the ethanolic extract determined by the de-colorization of the $ABTS^{+}$, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734nm. The effects of ethanolic extracts of EMP on ABTS free radical scavenging activities was assayed at specific concentrations. Significant ABTS free radical scavenging activity was evident in all the extracts (Table 7 and Graph 2).

3.4 FRAP Assay

There is a consistency in the total polyphenol, DPPH and FRAP assays in that high values were obtained for the total polyphenols and FRAP assay and low values were obtained for the DPPH assay. The antioxidant activity of the standards was found to be much superior to all fractions from the leaf, shoot, root (whole plant) and

tuber samples. This is probably due to the low concentration of antioxidant compounds present in the fractions. In all the samples, a correlation between the total phenol assay and antioxidant activity (FRAP assay) was observed for the aqueous fractions of EMP drugs with the value of 18.57, 21.26, 8.25, 11.68 and 10.67 µmol/mg, respectively (Table 7). In all, the sample of *D. hispida* the ethanol fraction (31.22) and methanol fraction gave the highest value (29.70) when compared to all other extracts of EMP drugs, respectively and therefore, the ethanol and methanol fractions of the EMP drugs was analyzed for comparison in the study (Table 7).

3.5 Antioxidant activity in TMF drug

The ethanolic extract of tribal medicine formulation was used to evaluate Antioxidant activity using DPPH Radical scavenging assay. The absorbance value for TMF is represented in the Table 6. The value of % inhibition for TMF drug was noticed (Graph-3). The percentage of inhibition was found to be increasing with the increasing concentration of TMF extracts. The O.D values were found to be decreasing.

Another comparison was made with the Standard O.D values of Ascorbic acid, which is an antioxidant by itself. The O.D values of Ascorbic acid were found to be decreasing and so as the TMF drug values. This also demonstrated the presence of the antioxidant activity for the samples (Graph-3 and Table 6).

3.6 Evaluation of Total Phenolic content

The amount of total phenolics varied in different extracts of EMP drugs and the values of aqueous extracts of all the EMP drugs ranged from 56.24, 52.17, 72.46, 89.5 to 107.67 mg GAE/g of EMP. The highest total phenolic levels were detected in the extract of *R. densiflra* and the lowest in the extract of *D. hispida* (Table 7). The amount of total phenolic compounds in all tested plant extracts was higher than the TMF drug. The ranking order of eight plant species from point of view of antioxidant (phenolic compounds) amounts was as follows: *R. densiflora* > *N. nimoniana* > *G. mauritiana* > *A. serphyllifolia* > *D. hispida*.

3.7 Evaluation of Total Reduction capability

In the extracts of both EMP drugs, the IC_{50} values of reducing activities of aqueous fractions revealed the order of activity as: 0.36% in *Andrographis serphyllifolia*, 0.45% in *Dioscorea hispida*, 1.17% in *Glycosmis mauritiana*, 1.28% in *Nothapodytes nimoniana* and 1.12% in *Rauwolfia densiflora* were recorded. Similarly, the solvent fractions were also found to be significant as compared to aqueous crude extract. The reducing potentiality of EMP drugs is directly proportional to different extracts at variable concentrations (Table 7).

Table 2: Antibacterial activity in the extracts of the selected Ethno-medicinal plants EMP and TMF against pathogenic organisms

SL. No	Bacterial species	Extracts	*Andrographis serphyllifolia* (Leaves)	*Dioscorea hispida* (Tubers)	*Glycosmis mauritiana* (Leaves)	*Nothapodytes nimoniana* (Leaves)	*Rauvolfia densiflora* (Whole plant)	Tribal Medicine Formulation (TMF)
1	*Escherichia coli*	A	12± 0.3	13±0.6	17±1.2	12±0.2	16±0.3	18±0.5
		E	14±0.2	14±0.6	16±3.5	14±1.5	16±1.0	19±0.2
		P	10±1.2	16±0.2	19±2.2	13±1.2	20±2.5	17±1.2
		M	13±0.6	15±0.5	17±1.6	15±2.2	18±1.5	18±1.5
2	*Bacillus subtilis*	A	11±0.5	13±1.2	11±0.3	14±0.4	15±0.0	16±1.0
		E	9±0.4	12±0.6	13±1.2	10±0.6	17±1.5	19±2.0
		P	10±2.2	14±1.0	14±0.5	11±0.0	15±2.0	16±2.2
		M	10±1.6	11±0.2	12±2.0	13±0.2	17±0.1	20±1.6
3	*Staphylococcus aureus*	A	15±1.5	14±1.6	17±2.6	13±2.6	18±0.5	19±1.2
		E	13±0.3	16±2.5	19±1.5	15±1.0	21±3.0	23±2.0
		P	18±1.6	18±3.0	16±2.5	14±0.5	19±1.5	20±1.6
		M	19±0.5	19±1.5	21±0.6	15±3.0	20±3.0	24±2.5
4	*Klibsiella pneumoniae*	A	10±0.6	10±2.0	13±0.0	13±1.5	14±2.0	17±0.6
		E	12±0.1	13±00	12±1.0	12±2.0	13±0.5	15±1.0
		P	10±0.2	16±1.5	13±0.6	12±1.5	11±0.2	18±2.5
		M	13±0.5	15±1.0	15±0.5	14±1.0	14±0.5	17±0.5
5	*Pseudomonas* sp.	A	07±0.3	13±3.0	11±0.2	10±1.0	14±1.2	15±1.5
		E	10±0.5	12±0.3	13±0.5	13±0.4	15±1.0	16±1.6
		P	13±0.3	10±0.5	10±0.0	14±0.0	17±3.0	18±1.2
		M	12±1.5	14±1.5	13±2.0	14±2.2	16±0.5	20±2.5

*Data are the Mean values of triplicate and expressed as M±SD (P<0.05).

Mean diameter of growth inhibition zones in mm

A: Aqueous extract, E: Ethanolic extract, P: Petroleum ether, M: Methanolic extract

Table 3: Anti-fungal activity in the extracts of the selected Ethno-medicinal plants *EMP and TMF against pathogenic organisms

SL. No.	Fungal species	Ext	*Andrographis serphyllifolia* (Leaves)	*Dioscorea hispida* Tubers)	*Glycosmis mauritiana* (Leaves)	*Nothapodytes nimoniana* (Leaves)	*Rauvolfia densiflora* (Whole plant)	Tribal Medicine Formulation (TMF)
1	*Aspergillus niger*	A	15±0.5	14±0.1	17±0.3	17±0.4	13±0.4	18±0.4
		E	20±0.6	17±0.5	17±0.5	16±0.3	16±0.4	19±0.4
		P	17±0.5	16±0.4	16±0.6	16±0.1	15±0.5	18±0.5
		M	20±0.5	19±0.7	20±0.4	21±0.5	18±0.3	21±0.3
2	*Aspergillus flavus*	A	14±0.9	12±0.8	16±0.5	17±0.5	14±0.7	17±0.4
		E	22±0.9	16±0.9	14±0.9	17±1.0	17±0.5	18±0.9
		P	18±0.6	16±0.5	15±0.9	15±0.9	17±0.5	19±0.9
		M	19±0.9	20±0.4	20±0.7	20±0.7	19±0.1	22±0.6
3	*Fusarium oxysporum*	A	11±0.1	16±0.3	17±0.5	17±0.5	14±0.4	18±0.5
		E	19±0.5	19±0.4	18±0.6	19±0.3	16±0.7	20±0.5
		P	17±0.4	18±0.6	18±0.2	18±0.4	15±0.3	21±0.6
		M	22±0.5	22±0.6	24±0.3	23±0.6	19±0.6	25±0.1
4	*Rhizopus stolonifer*	A	13±0.4	13±0.4	15±0.5	15±0.2	18±0.9	18±0.6
		E	20±0.9	19±0.6	16±0.68	16±0.9	19.4±0.6	22±0.2
		P	18±0.7	18±0.7	16±0.13	16±0.5	18.4±0.7	20±0.1
		M	22±0.3	23±0.9	22±0.40	20±0.4	21.4±0.5	26±0.5

* Values are mean of triplicate readings (Mean±SD);
Mean diameter of growth inhibition zones in mm
A: Aqueous extract, E: Ethanolic extract, P: Petroleum ether, M: Methanolic extract, Ext: Extracts

Table 4: Determination of MIC and MBC performance of different extracts of EMP and TMF against pathogenic organisms (µg/ml)

SL. No.	Bacterial Species	Ext	*Andrographis serphyllifolia* (Leaves)		*Dioscorea hispida,* (Tubers)		*Glycosmis mauritiana* (Leaves)		*Nothapodytes nimoniana,* (Leaves)		*Rauvolfia densiflora* (Whole plant)		Tribal Medicine Formulation (TMF)	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1.	*Escherichia coli*	A	38.4	86.2	34.2	78.1	26.5	69.3	40.2	87.2	37.6	80.2	26.1	69.6
		E	41.2	94.6	39.5	90.2	29.3	70.1	39.0	79.6	32.1	87.5	24.4	63.5
		P	36.7	74.5	35.5	79.6	32.4	74.3	42.5	85.1	35.3	90.0	25.3	66.1
		M	37.9	78.3	40.4	83.5	30.5	77.8	46.3	91.4	38.7	92.5	26.9	67.2
2.	*Bacillus subtilis*	A	33.7	67.5	36.3	71.7	29.6	80.1	42.5	82.2	41.1	101.5	23.1	66.5
		E	26.9	53.2	34.1	80.2	31.4	79.2	45.1	89.6	45.2	109.2	22.9	69.7
		P	31.4	65.7	35.5	68.1	35.8	82.5	44.8	91.5	48.1	114.1	24.5	70.1
		M	29.4	49.8	37.2	69.8	39.1	87.2	46.5	96.8	44.9	116.6	25.9	68.9
3.	*Staphylococcus aureus*	A	47.3	96.7	41.5	90.7	36.4	81.5	44.5	92.1	34.2	79.3	22.6	59.1
		E	36.7	81.5	36.1	86.6	34.9	77.4	42.1	90.4	36.8	78.2	21.9	58.7
		P	44.3	86.4	45.0	85.2	35.3	76.4	46.5	93.7	37.5	80.5	24.6	62.4
		M	42.4	85.9	41.5	87.4	36.1	75.8	43.3	89.5	39.6	87.6	26.5	66.18
4.	*Klibsiella pneumoniae*	A	46.4	97.8	39.5	84.1	38.9	86.9	37.9	76.5	43.1	89.2	31.4	69.5
		E	36.6	76.3	40.5	89.5	39.5	90.2	40.1	79.8	42.5	88.2	33.3	72.6
		P	41.3	94.9	42.5	94.0	44.2	95.1	43.5	84.1	45.2	94.5	39.2	75.4
		M	40.1	94.5	43.3	95.6	41.9	88.4	42.7	82.2	47.8	100.4	40.1	82.3
5.	*Pseudomonas* sp.	A	47.5	92.1	37.5	79.2	40.6	81.5	39.1	77.8	39.4	76.2	30.3	70.8
		E	40.8	81.3	39.9	83.5	37.1	76.5	40.4	78.9	37.6	73.1	29.5	69.5
		P	43.1	92.6	41.4	94.2	38.9	78.5	44.2	87.5	42.4	80.8	32.5	75.4
		M	38.9	79.9	44.5	92.3	40.1	80.4	46.5	91.2	43.1	82.6	31.5	74.6

*Values are mean of triplicate readings (Mean±SD);
Mean diameter of growth inhibition zones in mm
A: Aqueous extract, E: Ethanolic extract, P: Petroleum ether, M: Methanolic extract, Ext: Extracts, MIC: Minimum Inhibitory Concentrations, MBC: Minimum Bacterial Concentrations

Table 5: Determination of MIC and MFC performance of different extracts of EMP and TMF against pathogenic organisms (µg/ml)

SL. No.	Fungal species	Ext	*Andrographis serphyllifolia* (Leaves)		*Dioscorea hispida,* (Tubers)		*Glycosmis mauritiana* (Leaves)		*Nothapodytes nimoniana,* (Leaves)		*Rauvolfia densiflora* (Whole plant)		Tribal Medicine Formulation (TMF)	
			MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1.	*Aspergillus niger*	A	53.1	110.2	47.	101	41.5	97.5	55.1	110.2	47.1	99.2	34.1	72.4
		E	49.4	101.6	46.	104	39.5	95.1	52.4	106.1	50.2	105.2	36.3	76.5
		P	54.2	111.5	49.	108	42.6	99.1	57.4	114.3	54.1	109.1	40.5	79.6
		M	50.5	99.6	51.	105	49.5	101.2	54.3	109.3	56.5	116.4	43.2	82.5
2.	*Aspergillus flavus*	A	56.2	116.6	53.	112	42.6	101.9	51.5	102.4	53.3	111.5	37.2	77.6
		E	48.4	95.7	46.	97.	43.5	99.2	52.1	106.3	56.1	115.4	40.4	79.7
		P	56.8	113.9	52.	115	45.1	95.2	55.2	112.4	54.3	113.3	42.3	83.5
		M	51.2	102.8	54.	105	47.5	93.6	54.8	111.2	58.3	118.5	41.7	80.2
3.	*Fusarium oxysporum*	A	57.2	118.6	55.	106	44.1	97.9	53.5	103.5	49.1	100.3	40.2	79.4
		E	53.6	108.7	56.	115	46.4	98.2	48.3	100.2	52.5	103.5	41.4	82.8
		P	56.9	109.8	59.	116	45.4	101.5	53.5	104.1	54.3	107.6	39.2	78.5
		M	48.3	97.9	52.	100	48.5	102.6	56.2	110.5	53.3	105.7	43.2	85.2
4.	*Rhizopus stolonifer*	A	48.9	98.8	49.	101	49.2	98.4	45.5	92.1	50.3	101.6	38.1	77.7
		E	38.5	81.6	42.	90.	52.4	104.2	49.5	101.6	53.4	106.2	41.2	83.3
		P	46.4	97.8	49.	99.	57.1	107.2	44.3	95.6	59.9	119.8	46.4	90.2
		M	41.6	96.5	47.	102	52.3	102.4	42.5	92.7	55.5	114.4	40.6	89.9

* Values are mean of triplicate readings (Mean±SD); Mean diameter of growth inhibition zones in mm

A: Aqueous extract, E: Ethanolic extract, P: Petroleum ether, M: Methanolic extract, Ext: Extracts, MIC: Minimum Inhibitory Concentrations, MFC:Minimum Fungal Concentrations

Table 6: DPPH Radical scavenging assay for TMF practiced by Tribal Medicine Men at B.R. Hills, Karnataka.

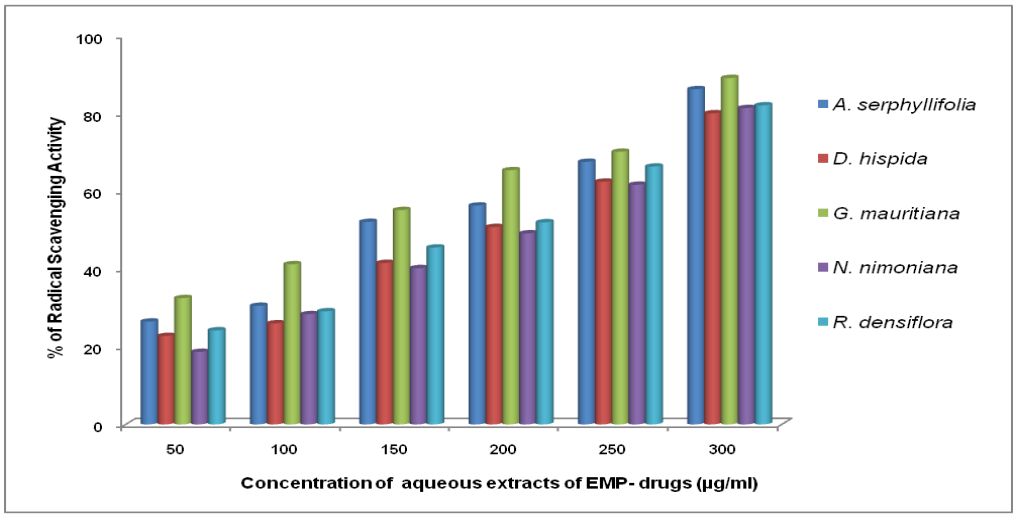
SL. No.	Concentration of Sample (TMF extract)	Absorbance of Sample	Control	DPPH radical scavenging activity (%)
1.	30µl	1.449		48.01
2.	60µl	1.334	2.788	52.13
3.	90µl	1.158		58.44
4.	120µl	1.058		62.03
5.	150µl	0.255		90.85

Table 7: Antioxidant activities and Total Phenolic content of Tribal medicine formulation practiced by Tribal Medicine Men at B.R. Hills, Karnataka

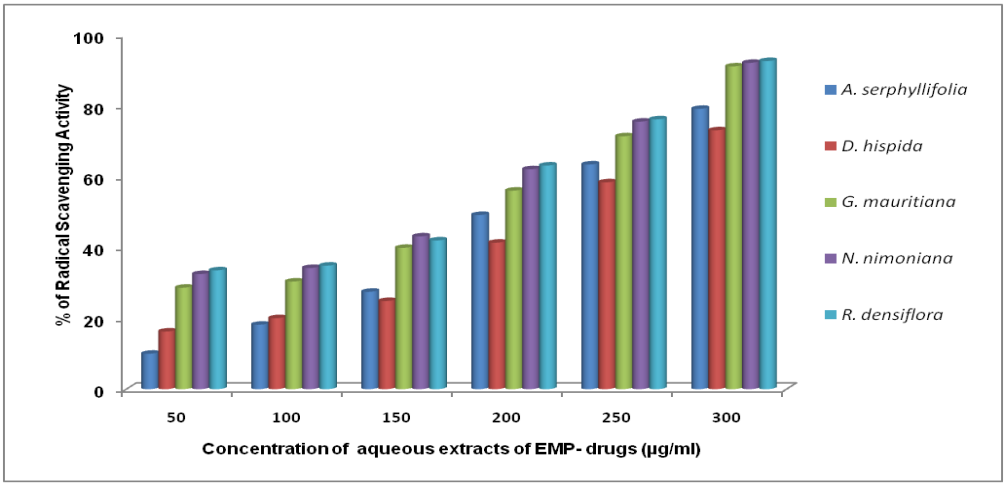
SL. No	Parameters*	Extracts	*Andrographis serphyllifolia* (Leaves)	*Dioscorea hispida* (Tubers)	*Glycosmis mauritiana* (Leaves)	*Nothapodytes nimoniana,* (Leaves)	*Rauvolfia Densiflora* (Whole plant)
1.	**DPPH Assay** (EC50µg/ml)	A	12.24±0.2	06.40±0.2	07.22±0.2	04.14±1.2	06.15±1.7
		E	16.14±1.0	17.10±0.2	26.45±1.2	07.14±0.6	11.14±1.3
		P	07.16±0.7	09.25±1.3	14.00±1.4	09.18±1.5	23.20±0.9
		M	18.42±0.1	18.90±1.4	29.12±0.6	16.66±0.9	31.55±0.7
2.	**FRAP Assay** (µmol $Fe2^{+}$ /mg)	A	18.57±2.7	21.26±1.6	08.25±1.2	11.68±0.5	10.67±0.3
		E	11.12±1.5	31.22±0.0	19.80±1.5	16.40±0.5	10.12±1.5
		P	13.65±0.1	24.32±1.5	17.85±0.1	13.33±0.1	15.42±1.5
		M	24.51±0.7	28.90±0.5	29.70±0.5	11.90±1.1	16.30±0.8
3.	**ABTS Radical Scavenging Activity** (%)	A	12.64±0.7	15.40±0.5	12.20±0.5	13.71±0.6	12.06±1.4
		E	15.45±1.5	19.00±1.6	13.32±0.0	16.32±1.5	19.10±0.0
		P	13.55±1.5	14.33±0.5	10.20±0.1	11.00±0.6	14.44±0.1
		M	13.89±0.5	18.74±0.5	15.80±0.1	17.30±0.9	22.53±0.1
4.	**Total Phenolic content** (mg of GAE/g)	A	56.24±0.1	52.17±1.0	72.46±0.7	89.65±0.8	107.67±1.7
		E	61.20±1.2	48.64±0.1	67.21±1.6	78.52±0.7	102.32±0.4
		P	57.60±0.3	59.80±1.0	53.63±1.0	23.12±0.0	83.55±0.9
		M	36.42±0.6	55.00±1.5	76.50±0.7	19.00±0.1	31.32±0.6
5.	**Total reduction capacity (%) (A700nm)**	A	00.36±0.0	0.45±0.02	01.17±0.0	01.28±0.0	01.12±0.5
		E	00.26±0.7	0.36±0.00	00.90±0.0	00.96±0.0	01.08±0.5
		P	00.94±0.1	00.43±0.0	00.74±0.0	00.88±0.0	00.96±1.5
		M	00.41±0.0	00.52±0.0	00.94±0.1	01.12±0.0	01.21±0.0

Data are the Mean values of triplicate and expressed as M±SD ($P<0.05$). A: Aqueous extract, E: Ethanolic extract & P: Petroleum ether & M: Methanolic extract.

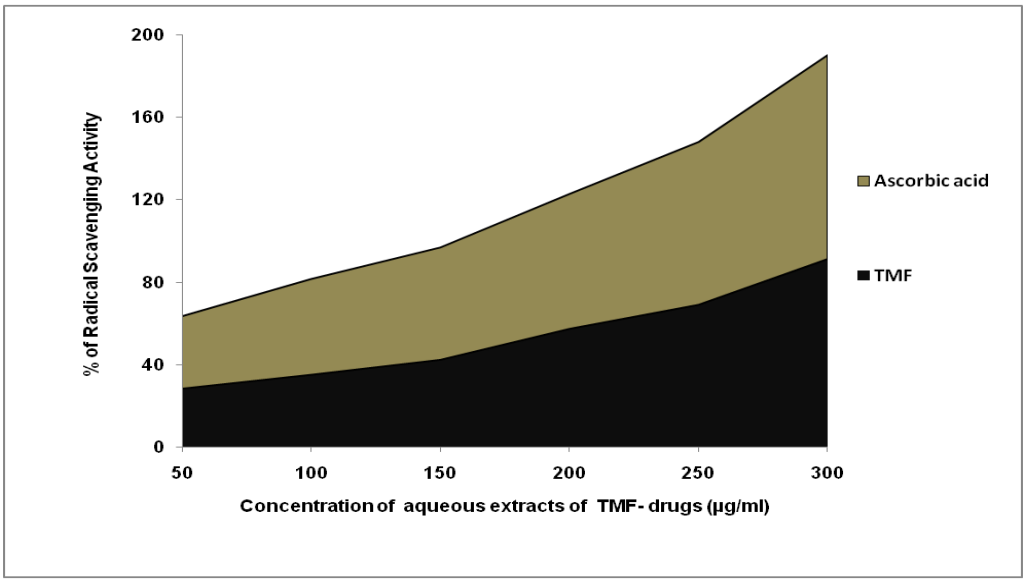
Graph 1: Radicals scavenging activity by DPPH method in aqueous extracts of EMP

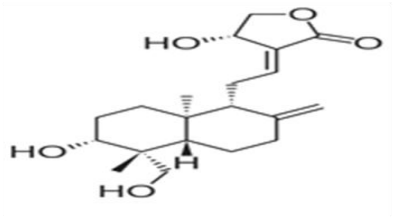


Graph 2: Radicals scavenging activity by ABTS method in aqueous extracts of EMP



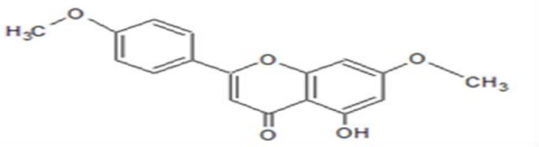
Graph 3: Radical scavenging activity by DPPH method in aqueous extracts of Tribal Medicine Formulation (TMF) and Ascorbic acid



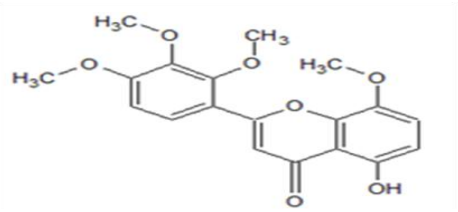


Andrographis serpyllifolia

A: Structure of Andrographidine-C (flavones)

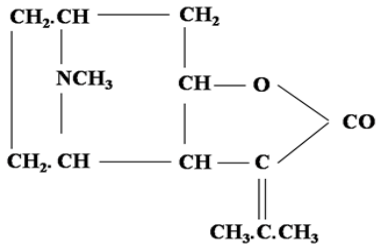


B: Structure of Apigenin 7, 4′-dimethyl ether



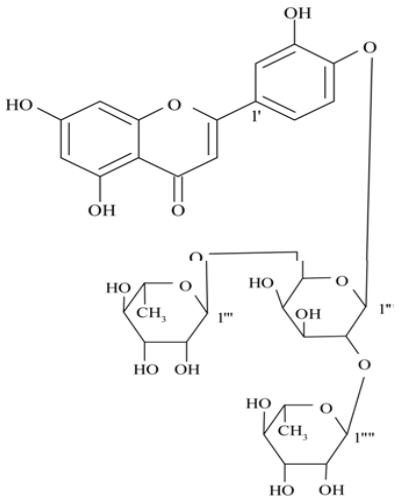
C: Serpyllin, IUPAC Name: 5-hydroxy-7, 8, 2′, 3′, 4′-pentamethoxyflavone ($C_{20}H_{20}O_8$)

Figure 2A-C: Bioactive constituents present in the *A. serphyllifolia*-a component of Tribal Medicinal Formulation (TMF) (Rao *et al*., 2014)



Dioscorea hispida

A: The Alkaloid, Dioscorine, $C_{13}H_{19}O_2N$



Glycosmis mauritiana

B: Luteolin-4′-O-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyra-nosyl-(1→6)}-β-D-glycopyranoside].

Figure 3A-B: Bioactive constituents present in the *D.hispida*- and *G.mauritiana*-components of Tribal Medicinal Formulation (TMF) (Javed *et al*., 2011; Pinder, 1951)

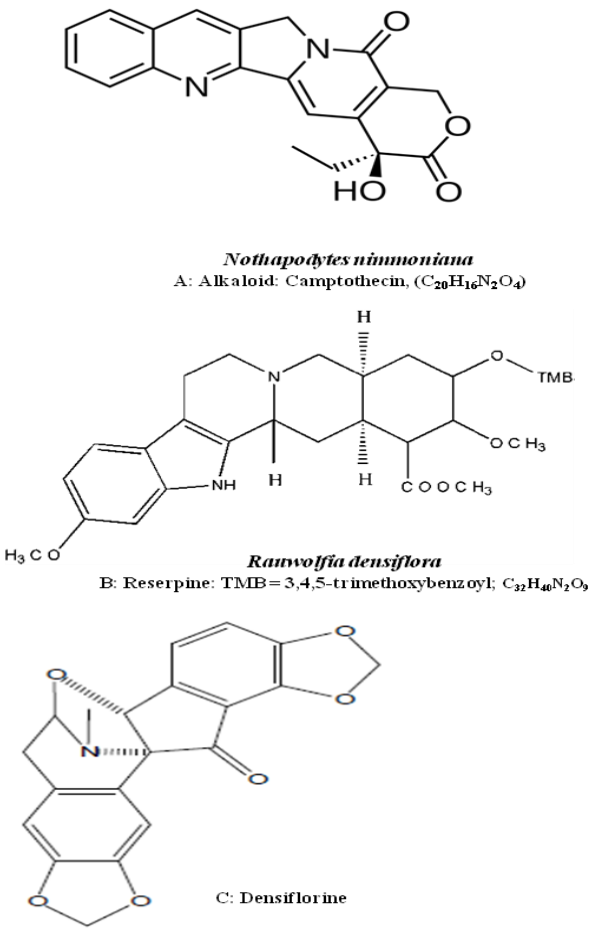


Figure 4A-C: Bioactive constituents present in the *N. nimmoniana*- and *R. densiflora*-components of Tribal Medicinal Formulation (TMF) (Khan, *et al.*, 2013; Amjad *et al*., 2013)

4. Discussion

The evaluation of pharmacognostic parameters will help for setting standards for crude herbal drugs. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites.

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [20-22]. Many factors could contribute to this variation, such as the plant variety, growing condition, maturity, season, geographic location, soil type, storage conditions and amount of sunlight received. Other contributing factor for this difference may be also due to sample preparation and analytical procedures [23].

The results obtained in this study thus suggest the identified phyto-chemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit [24,25]. The most active extracts of both EMP and TMF were compared with the standard antibiotics, pencillin, Streptomycin and Ampicillin 100mg/disc). The results obtained in the present study suggest that, all the EMP and TMF drugs could be used in treating diseases caused by these pathogenic organisms [2, 8, 15, 26- 32].

The observed antimicrobial activity against the tested organisms could be due to the presence of tannins and cyano-genetic glycosides in the extracts of EMP as these have previously been reported to possess antimicrobial activities. These could explain the rationale for the use of the plant in the treatment of the various conditions in traditional medical practice. The results seem to justify their continued use in the treatment of Wound related ailments and microbial infections [33,34,].

Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism [35]. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of

antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents.

The ethno-medicinal plants contain generally different class of phenolic compounds with antioxidant activity at significant level and the result reveals, the fractions of extracts of ethno-medicinal plants and formulations are free radical scavenger and able to react with the DPPH radical, which might be attributed to their electron donating ability. The free radical scavenging (antioxidant) activities of these ethno-medicines probably contribute to the effectiveness in various therapeutic applications possessing majority of phyto-chemical classes of compounds and these uphold the phenomenon that is to say, 'natural products are the source of synthetic and traditional herbal medicine' [36]. More than 4000 phenol com-pounds (flavonoids monophenols and polyphenols) are found in vascular plants. Phenolic compounds, such as querecetin, rutin, narigin, catechine, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents. This is in accordance with the reports of [23,30,37- 40].

DPPH is a kind of stable free radical and accepts an electron (or) hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. In the DPPH radical scavenging assay, antioxidants react with DPPH and exist naturally in deep violet colour to turn into a yellow coloured diphenyl-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant [41- 43].

Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. [4,44,45]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [46-48].

They also are effective antioxidant and show strong anticancer activities [38,37,23, 28, 44, 49]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [50,40]. Steroids have been reported to have antibacterial properties [28] and they are very important compounds especially due to their relationship with compounds such as sex hormones [23,51,52].

From the literature survey, it is evident that, all these EMP drugs were of ethnic use, because, there are some specific active chemical constituents present in the EMP drugs namely, *A. serpyllifolia* possess, flavones like, Apigenin, Serpyllin and 5-hydroxy-7, 8, 2′, 3′, 4′-pentamethoxy flavone [53] which validates the biological activities attempted in the study (Fig. 2A-C).

In *D. hispida*, an alkaloid called Dioscorine, $C_{13}H_{19}O_2N$, is present along with some steroidal sapogenin and suggested that, the alkaloid belonging to the tropane group [40] and projected with these possible biological activities (Fig. 3A).

In the study, *G. mauritiana* showed positive test for sugar and flavonoid moiety suggested that, the compound might be a flavanoid glycoside. Besides, among the other bioactive compounds, Luteolin-4'-O-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→6)}-β-D-glycopyranoside is new for this plant (Fig. 3B). The presence of these active constituents may lead a key role in all the biological activities analyzed individually [54].
Similarly, a potent alkaloid, namely Camptothecin present in *N. nimmoniana* of a wide spectrum of pharmacological activities like anti-cancer, anti-HIV, anti-malarial, antibacterial, anti-oxidant, anti-inflammatory, anti-fungal and also applied in the treatment of anemia (Fig. 4A). The Camptothecin is still not synthesized; therefore, its production entirely depends on natural sources [55].

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 (Fig. 4B and C). Apart from being used in the treatment of maternity complications, beri beri, syphilis, dysentery, diabetes, asthma, snake bite in the traditional system of medicine, the treatment of skin diseases and wound related infections etc. is of great interest which is critically evaluated and documented [56].

So far, little work has been done to bridge up the vast ethno-medicinal utilization of these EMP drugs and their active principles related to treatment of wound related ailments. It has been confirmed that, the tribal use of these EMP drugs in the form of TMF against Wound related ailments apart from other diseases. Further, the

mechanism of action needs to be stabilized through complete purification by employing specific biophysical techniques, which can be recommended for development of an appropriate drug specifically for Wound related ailments.

In conclusion, the evaluation of extracts of both TMF and EMP reveals some interesting activities like Phyto-chemicals, Antibacterial activity, Antioxidant activities of all the plant drugs respectively. From these we can assume that different active secondary metabolites are present in its extracts and perhaps some of these compounds may function in a synergistic manner. 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. Efforts should be geared up to exploit the biomedical applications of these screened plants due to the presence of certain class of phyto-compounds for their full utilization. Now a day the standardization of crude drugs has become very important for identification and authentication of drug. For this reason, the above plant extract could be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs towards wound related ailments.

The ethno-medicinal plants studied are of great importance due to the presence of both antimicrobials and antioxidant constituents. Since these plants have also been used by tribal practitioners for the treatment of supplementary ailments in association with additional plant drugs, the medicinal roles of these plants could be related to such identified bioactive compounds. The identification of these active phyto-compounds would be an interesting area for further study.

The observed antimicrobial activity against the tested organisms could be due to the presence of tannins and glycosides in the extracts of EMP as these have previously been reported to possess antimicrobial activities. These could explain the rationale for the use of the plant in the treatment of the various conditions in traditional medical practice. The results seem to justify their continued use in the treatment of 'Wound related' ailments and microbial infections.

However, this report 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, many evidences gathered in earlier studies which confirmed the identified phyto-chemicals to bioactive. Several earlier studies confirmed that, the presence of some active phyto-chemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from EMP and TMF plant drugs could be seen as a good source for useful drugs. The traditional medicine practice is strongly recommended for these plants as well as it is suggested that further work should be carried out to purify and characterize the chief active constituents responsible for the activity of the extracts of EMP and TMF. Besides, extension investigation is encouraged to elucidate the possible mechanism of action lying with effects of these extracts against the ailments to develop a novel drug with the status of functional food and nutraceuticals.

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