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PHYTO-CHEMICAL AND PHARMACOLOGICAL EVALUATION OF ETHNO-MEDICINAL PLANT DRUGS (EMP) AND TRIBAL MEDICINE FORMULATION (TMF) USED BY TRIBAL PRACTITIONERS FOR WOUND THERAPEUTICS IN THE REGION OF BILIGIRIRANGANA HILLS, KARNATAKA

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ABSTRACT

In the present investigation, an attempt has been made to appraise enthno-pharmacology of medicinal plants of B.R. Hill tracts of Chamarajanagara district of Karnataka. The ethno-medicinal plant materials were taxonomically identified and authenticated with standard flora. The selected ethno-medicinal plant drugs, *Andrographis serphyllifolia, Discorea hispida, Glycosmis mauritiana, Nothapodytes nimmoniana* and *Rauvolfia densiflora* were subjected for physico-chemical and preliminary phyto-chemical analysis. The physico-chemical analysis and other characteristics features of ethno-medicinal plants indicated the active status of pharmacognostic and efficiency of the plant material. In the study, active phyto-constituents of the five selected ethno-medicinal plant drugs (EMP) of different families and the tribal medicinal formulations (TMF) were recognized and their presence was correlated with the bioactivities of the plants. The phyto-chemicals have two categories *i.e.,* primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids, alkaloids, flavonoids, saponins, tannins, glycosides, fixed oils, fats, phyto-sterols and phenolic compounds. The phyto-chemical parameters were analyzed evidently for their active presence in the aqueous extracts followed by organic solvents like ethanol, petroleum ether and methanol extracts. The presence of active secondary metabolites in the extracts of ethno-medicinal plants may have profound activity and justifies the status for preparation of crude potential drug by the tribal people. The phyto-chemical screening of the ethno-medicinal plants showed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds and reducing sugars. *A. serphyllifolia* and *D. hispida* did not contain cardiac glycosides and coumarins while, *G. mauritiana*, *N. nimmoniana* and *R. densiflora* showed the presence of glycosides, tannins and alkaloids. The findings provided evidence that, crude aqueous and organic solvent extracts of ethno-medicinal plant drugs and TMF contain medicinally important bioactive compounds and it justifies their use in the Tribal Medicinal System (TMS) for the treatment of different relentless ailments apart from wound therapeutics.

KEYWORDS: Phyto-Chemicals and Pharmacology, Ethno-Medicinal Plant Drugs (EMP), Tribal Medicine Formulation (TMF), Tribal/Traditional Practitioners, B.R. Hills, Karnataka

INTRODUCTION

The nature has provided an absolute resource of remedies to cure the several ailments of mankind and is best friend of pharmacy. The herbal drugs are most effective in action without any side effects. The drugs obtained from plant source constitute a major part of therapeutics in the traditional systems of medicine. The shortcomings of the drugs available today, propel the discovery of new pharmaco-therapeutic agents in medicinal plants (Craig and David, 2001; Deepak and Anshu, 2008). The potentialities of herbal medicines are most essential to intensify the pharmacological study of ethno-medicinal plants that find place in folklore and also to promote the use of herbal medicine.

Ethno-pharmacology' is an interdisciplinary area of research that deals with the identification, description, observation and investigation of ingredients used in various recipes of traditional medicine and their effect on animal models. It is also the study of the relevant forms of knowledge, practice and cultures implementing them. 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 ailments. Therefore, standardization of plant material is the need of the day (Cordell, 1995). The increasing demand for herbal medicines both in the developing and developed countries inevitably led to maintaining the quality and purity of the herbal raw materials and finished products (Ajay *et al*., 2009; Reuben *et al.*,2008; Arunkumar and Muthuselva, 2011; Amjad *et al.,* 2013; Chitrashree *et al.,* 2014).

Plants are utilized extensively as raw drugs for many formulations in traditional systems of medicine. To check the genuineness of the raw drugs and to detect adulteration of these materials, an authentic pharmacognostic study is needed for each raw drug. Usually, the drugs are collected by traditional practitioners who have inherited Ayurvedic or other herbal practices. Their identification is mostly based on the morphological features or other traditionally known characteristics. In such cases, there is a chance of selecting incorrect raw drugs/adulterants. Our country has a long tradition of using herbal products for healthcare. There is an increasing awareness of the significance of ethnic and traditional knowledge in the development of therapeutics. In the current scenario of globalization, information technology and knowledge system on traditional medicine have significant importance (Edeoga, 2005; Kala, 2006; Good man and Gillman, 2008; Everaldo *et al.*, 2001).

Mankind has been continuously using the medicinal plants in one or the other way in the treatment of various ailments. In India, the sacred Vedas dating back between 3500 B.C and 800 B.C give many references of ethno-medicinal plants. One of the outermost works in traditional herbal medicine is "Vrikshayurveda", compiled even before the beginning of Christian era and formed the basis of medicinal studies in ancient India. The Rig Veda, dating between 3500 B.C. to 1800 B.C. seems to be the earliest record available on medicinal plants. Herbs seem to be very important component of medicine in other cultures too; Greek, African and Chinese medicines to mention a few. Nearly 80% of the world population depends upon traditional system of health care. Allopathic drugs have brought a revolution throughout the world, but the plant base medicines have its own status. The recent surveys had revealed that, 50% of the top prescription drugs in the USA are based on natural products and the raw materials are locked up in the tropical world–interiors of Africa, Asia and Latin America. The local uses of plants as a cure are common particularly in those areas, which have little or no access to modern health services such as the innumerable villages and hamlets in India (Fabricant and Fransworth, 2001; Parekh and Chandra, 2008; Francois *et al*., 2009).

The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been

transmitted orally for centuries is fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture. The collection of information about natural flora, classification, management and use of plants by the people holds importance among the ethno botanists. The local people and researchers face the challenging task of not only documenting knowledge on plants, but also applying the results of their studies to biodiversity conservation and community development. The vast diversity of flora with a deep concern and reverence which our country enjoys and with a sense of realization about the invaluable therapeutic properties of ethno-medicinal plants (Tripathi, 2003; Usman *et al.*,2007; Revathi *et al*., 2012; Sharma *et al*., 2012).

The current research is undertaken to carry-out qualitative and quantitative phyto-chemical analysis in five ethno-medicinal plants *viz.*, *Andrographis serphyllifolia* Vahl. *(Acanthaceae), Discorea hispida* Dennst. *(Dioscoreceae); Glycosmis mauritiana* Tanaka. *(Rutaceae); Nothapodytes nimmoniana* Blume. *(Icacinaceae) and Rauvolfia densiflora* (Wall.) Benth & Hook *(Apocynaceae)*. These plants used by the tribal communities that show significant medicinal potential are being investigated further for their biological activity using bio-assay guided fractionation. Besides, particular attention is placed on plants traditionally used to treat. This work mainly concentrate on the studies pertaining to evaluation of ethno-medicinal plant drugs and herbal formulation practiced by the tribal medicine men' of the area surveyed.

MATERIALS AND METHODS

The study area considered in and around of Biligirirangana hill tracts which come under reserved forest type located at the border region of Karnataka state during the period of January, 2011 to November, 2013. The Biligirirangana Hills (commonly called B.R. Hills, that is a hill range situated in south-eastern Karnataka, at its border with Tamil Nadu in South India). The area is also called Biligiriranga Swamy Temple Wildlife Sanctuary or simply BRT Wildlife Sanctuary (Figure.1). It is a protected reserve forest under the Wildlife Protection Act, 1973. The sanctuary is home to eco-systems that are unique to both the mountain ranges being located at the confluence of the Western Ghats and the Eastern Ghats. The hill tracts are distributed extensively in the Yelandur and Kollegal taluks in Chamarajanagar District of Karnataka. The hill tracts are contiguous with the Satyamangalam range southwards, in the Erode District of Tamil Nadu. The hills that give the range its name are situated 90km from Mysore and 220km from Bangalore. The hills may be reached either from Yelandur or via Chamarajanagar. The hills are located at the eastern most edge of the Western Ghats and support diverse flora and fauna in view of the various habitat types supported.

Biogeographically, the sanctuary is unique. It is located between 11° and 12° N and the ridges of the hills run in the north-south direction. It is a projection of the Western Ghats in a north-east direction and meets the splintered hills of the Eastern Ghats at 78° E. This unique extension of Western Ghats constitutes a live bridge between the Eastern and Western Ghats with the sanctuary located almost in the middle of this bridge (Ganeshaiah *et al.,* 1998; Ravishnkar and Panduranga Murthy, 2009).

Tribes and Culture

The tribal-soligas lives in the tropical evergreen forests of South India are the major indigenous tribes of B.R Hills situated in Chamarajanagar district of Karnataka state which belongs to southern India. Soliga means 'people of the bamboo', a name based on their belief that their ancestors originated from the bamboo. It also reflects the Soliga's close

association with nature, referring to the dense thickets they inhabit. Since time immemorial, Soliga's have led a semi-nomadic life and were engaged in shifting cultivation. The tribals (Soliga) are involved in collecting of non-timber forest products (NTFPs) like honey, lichens, soap nut, roots of Magali (*Decalapis hamiltonii*), fruits of Amla (*Emblica officinalis*), Chilla (*Strychnous patatorum*) and Alale (*Terminalia chebula*) is another important, but relatively recent occupation.

The Soligas inhabiting this range were nature worshippers originally and venerate a large Champaka tree (*Michelia champaca*), called 'Dodda Sampige' in the local language. The Soligas have a rich, deep traditional and indigenous knowledge of ethno-medicinal plants which is passed on from one generation to the next (Figure.3A-C). The tribals share their knowledge about different aspects of practicing of tribal medicines, drug formulation, mode and duration of treatment against different ailments/diseases starting form cold, cough, fever etc. and to serious diseases like cancer, wound healing, snake bite and respiratory disorders, respectively (Somasundaram and Kibe, 1990).

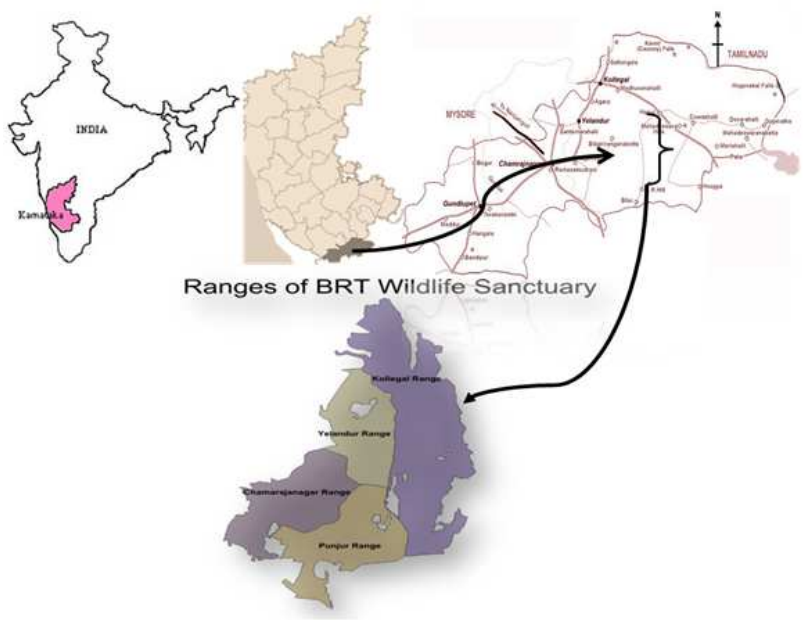

Figure 1: Geo-Graphical Location Showing the Study Area Biligirirangana Hills (B.R. Hills), Chamarajanagara District of Karnataka, India

Base-Line Survey and Collection of Ethno-Medicinal Plants

A survey was conducted at different podus of Biligirirangana Hills, Karnataka between 2011-2013 in order to collect the first-hand informations relating to ethno-medicinal plant drugs and their formulations practiced by Tribal community (Figure.1 and Figure. 3A-F).

Interaction with Tribal Healers

The indigenous information of the community herbalists, tribal practitioners, other rural traditional healers and the ethno-medicinal plant drugs (EMP) practiced for medicinal utility were collected through extensive base-line survey with Tribal Medicine Men (TMM) followed by personal interview and semi-structured questionnaire prepared for documentation of traditional knowledge. The exploration revealed some unknown and less known medicinal uses of ethno-

medicinal plants and formulations. In the exploration five important ethno-medicinal plant drugs were identified and the formulation was collected from TMM (Figure.3A-C). The scientific name, family, vernacular name, part used, mode of drug preparation, dosage and duration were systematically reported and traditional usage of these ethno-medicinal plant drugs are discussed here for the treatment of wound therapeutics, other related ailments(Figure.3D-F). For distribution, the locations for selected ethno-medicinal plant were recorded by using the Global Positioning System (GPS).

Collection of Ethno-Medicinal Plant Drugs

The Voucher specimens of Ethno-medicinal plants, *A. serphyllifolia*, *D. hispida*, *G. mauritiana*, *N. nimmoniana* and *R.densiflora* were collected and identified appropriately by consulting a floral expert. The species identified and the same have been deposited in the Bhoomigeetha Institute of Research & Development (BIRD), Tumkur in collaboration with Deptartment of Engineering Chemistry, Akshaya Institute of Technology, Tumkur, Karnataka respectively (Figure.2A-H).

Identification and Authentication of Ethno-Medicinal Plant Drugs

The different parts of five ethno-medicinal plant drugs, *A. serphyllifolia* Vahl. (leaves), *D.hispida* Dennst. (tubers)*; G.mauritiana* Tanaka. (Leaves)*; N.nimmoniana* Blume. (leaves) and *R.densiflora* (Wall.) Benth & Hook (*whole plant*) were collected from different tracts/regions of B.R. Hills of Chamarajanagara districts of Karnataka (Figure. 2A-H). During collection, any type of adulteration was strictly prohibited. The plants were mounted on paper and the sample was taxonomically identified with the help of Standard Flora of Mysore district. Then, the plant drug materials were taxonomically acknowledged and authenticated as per the procedures (Figure.2A-H). The baseline informations of selected ethno-medicinal plants are represented systematically (Table 1).

The experiment was conducted in the laboratory of Applied chemistry discipline, Akshaya Institute of Technology, Tumkur in association with Bhoomigeetha Institute of Research & Development (BIRD), Tumkur. Additional support was also taken from ‘Azyme Technologies’, Bengaluru for completion of technical analysis. The plant materials were then subjected for shade drying till plant materials competent for grinding. After drying, the plant materials were ground well using mechanical blender into fine powder and transferred into airtight containers with proper grouping for future use.

Validation of Ethno-Medicinal Plant Materials and Tribal Medicine Formulation

Ethno-medicinal plant materials and Tribal Medicine Formulations (TMF) were obtained from the Traditional Healers (Figure. 3A-J) during interactions 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, Karnataka state (India). The standard protocols were identified and the methodology was employed in the present study based on the descriptions of Chaithra (2013).

Preparation and Processing of Ethno-Medicinal Plant Drug Materials (EMP)

The collected plant materials were subjected for separating different desirable parts like leaves, stem, root/ tubers from the main plants or whole plant parts. The selected ethno-medicinal plant drug materials such as leaves, stem,

root/ tubers were dried under shade for 20 days to ensure the active constituents were free from decomposition and also care was taken to avoid any photochemical degradation. The selected plant parts were powdered using suitable electric blender. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced following the standard procedures (Anonymous, 2007).

Extraction of Ethno-Medicinal Plant Drug Materials (EMP) Via Successive Solvent Extraction

The powdered sample (170g) was extracted using soxhlet apparatus with different solvents from polar to less polar such as water, Ethanol, Petroleum ether and Methanol. The macerated extract was then 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 as described by Raman, 2007. The extracts were then placed in shaker incubator for 24 hours and later subjected to filtration using Whatmann No1. 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 and 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).

Water Extraction of Crude EMP Samples

Crude plant extract was prepared by Soxhlet extraction method. About 20g of 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 same was kept in refrigerator at 4ºC for their future use for phyto-chemical analysis.

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 various amounts and pasting it using cold or warm water. It can be directly applied on to the exterior parts of the body or given for the intake depending on the abnormality being treated. These formulations are the indigenous knowledge of Tribal people in India (Figure. 3B-F and Table 2).

Preparation and Processing of Tribal Medicine Formulation (TMF)

The biochemical activity of Tribal Medicine Formulation (TMF) will not be known to the tribals, but their action will be known because of the practice, since many years. The components react with each other and show the suitable activity on the patient. The different constituents in TMF was subjected for devastating to small pieces using pestle, mortar and then powdered in an electric grinder for further analysis.

Extraction of Tribal Medicine Formulation (TMF)

The powder of the Tribal Medicine Formulation (TMF) was subjected (50g) to successive extraction with different solvents in increasing order of polarity from petroleum ether to ethanol and methanol finally, to crude extraction with water. The organic solvent was specified based on the dissolving efficiency and 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 the dried extracts were subjected to various chemical tests in order to detect the presence of different phyto-constituents.

Physico-Chemical Analysis of EMP and TMF

The different extracts of the Ethno-medicinal plant drugs and tribal medicinal formulation were subjected to analyze various physico-chemical characteristic features based on WHO guidelines (Anonymous, 2007).

The extracts of ethno-medicinal plant (EMP) drugs and Tribal Medicine Formulation (TMF) were analyzed qualitatively for the detection of Ash value, Acid insoluble, Water soluble ash, Foreign organic matter, Moisture content etc (Wiart and Kumar, 2001; Khandelwal, 2010).

Total Ash Value

For its detection, 2g of powdered material of each EMP drugs were placed separately in a suitable tarred crucible of silica previously ignited and weighed. The powdered drugs were spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon and cooled in desiccators. The sample was weighed and the percentage ash was calculated by taking in account the difference of empty weight of crucible and that of crucible with total ash.

Determination of Total Ash Value

2g of the sample was weighed and heated in a china dish at 100^0C till black ash was obtained in the dish. It was cooled and weighed. The total ash was calculated by employing the following formula.

$[\%Ash = (M_{ash} / M_{dry}) \times 100]$

Where M_{ash} is mass of the ash sample and M_{dry} is dry weight of the sample

Acid Insoluble Ash Value

It was carried out as per the steps mentioned in the procedures for determination of total ash value. Further, 25ml of dilute hydrochloric acid was taken to wash the ash from the dish used for total ash in to a100 ml beaker. Wire gauze was placed over a Bunsen burner and boiled for five minutes and filtered through an “ash less” filter paper, then, the residue was washed twice with hot water. Crucible was ignited in the flame, cooled and weighed. Filter paper and residue was kept together into the crucible; heated gently until vapours cease to be evolved and then more strongly until all carbon has been removed. The sample was allowed to cool in desiccators. Finally, the residue was weighed and acid-insoluble ash was calculated of five EMP drugs with reference to the air dried sample of the same.

Determination of Acid Insoluble Ash

To the ash, 25 ml 0.5% diluted HCl was added and was boiled gently for 5 min. with the watch glass covering the dish. Rinse the contents present on the under-surface of the closed watch glass with 5ml of hot water. Filter the contents collect the insoluble ash matter from the top of the filter paper and transfer it to the china dish and allow to cool again and weighed. The Acid insoluble wash was determined using the formula described elsewhere.

Water Soluble Ash Value

This was determined in a similar way to acid insoluble ash, using 25 ml of water, in place of dilute hydrochloric acid.

Determination of Water Soluble Ash

To the total ash sample, 25 ml of water was added and boiled gently for 5 min. then; 5 ml of boiled water was added to it along the underside of the watch glass. The contents were then filtered using filter paper and transferred the same to the china dish and the contents were heated again. The final sample was allowed to cool and weighed. The water soluble ash was calculated using the method as described by (Khandelwal, 1999).

PROXIMATE ANALYSIS

Foreign Organic Matter

10g of the dried plant sample was taken in an appropriate container and the crude foreign matters were removed present in the sample (by hand picking). Then, the collected organic matter was subjected for weighing and the same was calculated as foreign organic matter (%) based on the following formula.

[% Foreign Organic matter = (Organic matter weight/Total weight) x 100]

Moisture Content Determination

The moisture content in the sample was subjected for weighing by adding water to the sample accurately and designated as A. Then, the sample was dried to a constant weight at a temperature not exceeding 115^0C. Further, the sample was allowed to cool, weighed again and then the dry weight of the sample was recorded, designated as B. The moisture content in the sample was reported to the nearest tenth of 1%. The calculation was attended based on the following formula.

[%W = [(A-B)/B] x 100]

Where, %W - % of moisture in the sample; A – Weight of wet sample (g) and B – Weight of dry sample (g)

Phyto-Chemical Analysis of EMP and TMF

The extracts from ethno-medicinal plant (EMP) drugs and Tribal Medicine Formulation (TMF) were used for the Phyto-chemical analysis, qualitatively for the detection of carbohydrates, proteins, also for the secondary metabolites like alkaloids, flavonoids, terpenoids, steroids, tannins, saponins and total phenols etc. The aqueous extracts of the plant was subjected to qualitative chemical screening for the identification of the alkaloids, flavonoids and tannins using standard procedures.

Test for Alkaloids

This was analyzed by following the procedures described by Trease and Evans, 1996. Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2 % hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. Turbidity or yellow precipitation in the samples indicates the presence of alkaloids in the extracts.

Test for Tannins

Tanins were analyzed by following the procedures depicted by Iyengar *et al.*, 1995. The dried powdered samples (0.5g) of EMP was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate. Development of brownish-green or blue-black color indicated the presence of tannins.

Test for Flavonoids

A portion of the aqueous extract (2 mL) was heated, with 10 ml of methanol over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids. The yellow colouration was disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. Alkaline reagent test- An aqueous solution of extract is treated with 10% Ammonium hydroxide solution. Yellow colour indicated the presence of flavonoids which was based on the methods described by Edeoga *et al.*, 2005.

Test for Steroids

1 ml of extract was dissolved in 10 ml of the chloroform and equal volume of concentrated H_2SO_4 is carefully added by sides of the test tubes. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Siddiqui and Ali, 1997).

Test for Saponins

Saponins were analyzed as per the standard protocol (Kalita *et al.*, 2011). 1 g of extract was boiled with 5 mL of distilled water and filtered. 3ml of distilled water was added to the filtrate, and the mixture was shaken vigorously for about 5 minutes. Frothing that persisted upon warming was taken as evidence of the presence of saponins; or the extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken vigorously in a graduated cylinder for 15 minutes; formation of emulsion indicates the presence of saponin or layer of foam indicated the presence of saponins.

Test for Glycosides

As per the protocols given by Mondal *et al.*, 2013, glycosides were analyzed. A small amount of an alcoholic extract of the fresh or dried plant material was placed in 1ml of water. A few drops of aqueous sodium hydroxide were added. The presence of yellow colour was considered as an indication for the presence of glycosides.

Borntrager's test- To 2ml of filtered hydrolysate, 3 ml of chloroform was added and shaken. Chloroform layer is separated and 10% Ammonia solution is added. Pink colour indicated the presence of glycosides.

Test for Gums

This was analyzed as per the protocol given by Mondal *et al.*, 2013. 5ml solution of the extract was taken and then Molish reagent and sulphuric acid were added to the container. Red violet ring produced at the junction of two liquids indicated the presence of gums.

Detection of Carbohydrates

Molish's test- 2ml of filtrate, 2 drops of alcoholic solution of α- naphthol were added. The mixture was shaken well and 1 ml conc. Sulphuric acid was added slowly along the sides of the test tube and allowed to stand. The formation a violet ring indicated the presence of carbohydrates and this was analyzed as per the procedure of Ramakrishnan *et al.*, 1994.

Detection of Proteins and Amino Acids

Millon's test-To 2ml of filtrate, few drops of Millon's reagents were added. The formation of a white precipitation indicated the presence of proteins (Ruthmann, 1970). Millon's reagent- Mercury (1 g) is dissolved in 9 ml of fuming nitric acid. When the reaction is completed, equal volume of distilled water was added. This was evaluated based on the method described by Rasch and Swift, 1960.

Biuret Test

An aliquot of 2ml of filtrate was treated with 1 drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. Formation of Pink colour in the ethanolic layer indicated the presence of proteins (Gahan, 1984).

Ninhydrin Test

2 drops of Ninhydrin solution (10mg of Ninhydrin in 200ml of acetone) was added to 2 ml of aqueous filtrate. A characteristic purple colour was formed and indicated the presence of amino acids (Yasuma and Inchikawa, 1953).

Detection of Phytosterols

Libermann – Buchard's test- The extract (50mg) was dissolved in 2 ml of acetic anhydride. To this, 1 or 2 drops of concentrated Sulphuric acid was added along the sides of the test tube. An array of colour changes confirmed the presence of Phytosterols (Finar, 1986).

Detection of Fixed Oils and Fat

Spot test- A known quantity of extract was pressed between 2 filter papers. Oil stain on the paper was formed and indicated the presence of fixed oil (Kokate, 1999).

Detection of Phenolic Compounds

Ferric Chloride test - The extract (5omg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green colour was formed and indicated the presence of phenolic compounds (Evans, 2009).

Gelatin test-The extract (50mg) was dissolved in 5ml of distilled water and 2ml of 1% solution of gelatine containing 10% sodium chloride was added to it. White precipitation was formed and indicated the presence of phenolic compounds.

Lead acetate test- The extract (50mg) was dissolved in distilled water and to this, 3ml of 10% lead acetate solution is added. A bulky white precipitate was formed and indicated the presence of phenolic compounds.

Detection of Gums and Mucilages

This was analyzed on the basis of protocol described by Whistler and Bemiller, 1993. The extract (100 mg) was dissolved in 10ml of distilled water and to this; 25ml of absolute alcohol was added with constant stirring. White or cloudy precipitate was formed and indicated the presence of gums and mucilage.

Quantitative Total Phenolic Content

The amount of phenol in the aqueous extract was determined by Folin-Ciocalteu (FC) reagent method with some modifications (Kujala *et al.,* 2000). 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 2% solution of Na2CO3 were added to 1ml of plant extract. The resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

Anthraquinones

The crude powder (0.5gm) was shaken with 10 ml of benzene and was filtered. 0.5 ml of 10 % ammonia was added to the filtrate and was shaken well and the development of a pink, red or violet color in the ammonical (lower) phase indicated the presence of the anthraquinones which was confirmed following the procedure of Sodipo *et al* (1990).

Phlobatannins

Deposition of red precipitate when an aqueous extract of plant sample was boiled with 10 % aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins (Ajayi *et al.,* 2011).

Assay for Cytotoxicity of TMF Extract

Assay for Brine Shrimp Lethality

The eggs of brine shrimp (Artemia salina Leach) were collected and hatched in a tank at a temperature around 37°C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). 4ml of seawater was given to each of the vials. Specific volumes of sample were transferred from the stock solution to the vials to get final sample concentrations of 0.1, 0.5, 1, 10, 20, 40, 60, 80 and 100 µg/mL. In the control vials same volumes of DMSO (as in the sample vials) were taken. With a pasteur pipette, 10 living nauplii were put into each of the vials. After 24 h, the vials were observed and the number of nauplii that survived in each vial was counted. From this, the percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extract.

In a set of 12-well plates, each well contained 10 nauplii, 1ml sea water and 1 ml of extract diluted to final concentrations of 1%, 0.1%, 0.01%, 0.001% and 0.0001% respectively. The tests were set out in triplicate so that a total of fifteen wells per extract were used. Numbers of living nauplii were counted after 24 hours. The LC_{50} values and 95 % confidence intervals were determined in µg/ml, using the Finney probit analysis computer program. A median lethal concentration (LC_{50}) smaller than 1000 µg/ml indicates pharmacological activity (Alkofahi *et al.,* 1997).

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.

RESULTS

The studies on Phyto-chemical and Pharmacological screening for Ethno-medicinal Plant drugs (EMP) and Tribal Medicine Formulation (TMF) is of paramount importance in identifying new sources of therapeutically and industrially valuable compounds having tremendous medicinal significance to make the best and judicious use of available natural ethno-medicinal wealth. In the present study, five EMP and TMF comprising mixture of ethno-medicinal plants have been chemically evaluated respectively.

The results generated from the present study on the selected ethno-medicinal plant drugs (EMP) viz, *A.serphyllifolia, D.hispida, G.mauritiana, N.nimmoniana* and *R.densiflora* and TMF are represented in the tables, figures and graphs, respectively. 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 represented in the Table 1. Similarly, the details on medicinal formulation were obtained from tribal medicine men and the collected tribal medicine formulation (TMF) was validated with an authorized Ayurvedic Medical Practitioner (Table 2).

Physico-Chemical Analysis

The physico-chemical analysis was carried out for EMP viz., *A.serphyllifolia*, *D.hispida*, *G.mauritiana*, *N.nimmoniana* and *R.densiflora*. Of these, *G.mauritiana* was added as the major component in the TMF. Whereas, the other EMP such as *A.serphyllifolia, D.hispida, N.nimmoniana* and *R.densiflora* were added as the trivial component in the descending manner with respect to quantity. The Fluorescence analysis was carried out and the calculations of the Ash value and proximate analyses are tabulated (Table 3).

The amount of total ash present in the samples of *A.serphyllifolia* was 55.25%, the acid insoluble ash present in the leaves was 60.15%, the water soluble ash extractive value was 78.40%, the foreign organic matter present in leaves was 24.50% and moisture content present in the sample was 45.01%.

Similarly, the amount of total ash present in the samples of *D.hispida* was 56.75%, the acid insoluble ash present in the leaves was 44.16%, the water soluble ash extractive value was 63%, the foreign organic matter present in leaves was 58.40% and moisture content present in the sample was 52.52%.

In the sample, *G.mauritiana,* the total ash (44.55%), acid insoluble ash (82.26%), water soluble ash (98.77%), foreign organic matter (40.20%), moisture content (42.20%). For *N.nimmoniana;* the total ash (52.26%), acid insoluble ash (65.16), water soluble ash (70.66%), foreign organic matter (39.10%), moisture content (35.10%) and *R.densiflora showed* the total ash (40.20),acid insoluble ash (55.70%), water soluble ash (72.50%), foreign organic matter (60.30%), moisture content (22.22%) were represented (Table 3).

Fluorescence Analysis of the EMP Sample

Fluorescence analysis was done for the extracts of all ethno-medicinal plant drugs (EMP) according the standard procedure. The results shows potential setting of the EMP samples indicating their effectiveness in the subsequent study (Table 4 to 13).

Phyto-Chemical Analysis

The phyto-chemical screening of aqueous extract and solvent extracts of EMP and TMF demonstrated the presence of alkaloids, flavonoids, saponins, tannins, gums and mucilages, coumarins, terpenoids, steroids, glycosides, phyto sterols, fixed oils and fats, anthraquinone and phycobalamin (Table 14), which are suggested to act synergistically to exert the observed pharmacological activity (37, 38). The fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound, is always overlooked. Moreover, this may help to nullify the toxic effects (if any) of individual constituents.

In the phyto-chemical screening; the ethno-medicinal plants; *A. serphyllifolia* and *D. hispida* did not contain cardiac glycosides and coumarins while, *G. mauritiana*, *N. nimmoniana* and *R. densiflora* showed the presence of glycosides, tannins and alkaloids. Besides, important medicinal phytochemicals such as terpenoids, reducing sugar, flavonoids, alkaloids and phlobatannins were present in all most all the samples used in the analysis (Table 14). The phyto-chemical tests were best answered by the aqueous extracts of TMF than that of solvent extracts of EMP. The presence of most primary and secondary metabolites was also found in aqueous extracts of TMF (Table 14).

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 14). The amount of total phenolic compounds in all tested plant extracts was higher than the TMF drug. The ranking order of five EMP species from point of view of phenolic compounds (antioxidants) amounts was as follows: *R. densiflora* > *N. nimoniana* > *G. mauritiana* > *A. serphyllifolia* > *D. hispida*.

Cytotoxic Activity

In brine shrimp lethality bioassay, test sample showed different mortality rate at different concentrations (Table 15). The mortality rate of brine shrimp was found to increase with the increase in concentration of the different sample and the extracts of both EMP and TMF drugs showed significant (p=0.001) toxicity to the brine shrimp nauplii. The concentrations of aqueous extract for 50% mortality (LC_{50}); 18.78 µg/ml and 90% mortality (LC_{90}); 41.16 µg/ml were recorded.

DISCUSSIONS

The presence of important phyto-chemicals make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. The quantitative determination of pharmacognostic parameters will help

for setting standards for crude drugs. The 'total ash' is particularly important in evaluating the purity of drugs. The pharmacognostic constants for the different samples of EMP, the diagnostic microscopic features and the numerical standards are reported, which is useful for the compilation of a suitable monograph for its proper identification. Microscopic and morphological characters were examined by pharmacognostic evaluation, which also includes the determination of leaf content, ash value, powder analysis and extractive values. The Phytochemical screenings including qualitative chemical examination were also observed (Anthereden, 1969; 1996; Parek and Chandra, 2007 & 2008; Goodman and Gilman, 2008; Murthy *et al.*, 2011a).

The curative properties of medicinal plants are perhaps due to the presence of various primary and secondary metabolites. 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 (Alali, 2002; Mukesh and Smitha, 2010; Pulak *et al.*,2013).

The phyto-chemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993). Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids (Mojab *et al*., 2003; Sazada *et al*., 2009; Mahammad *et al*., 2010).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.*, 2007). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.*, 2007).Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown *et al.*, 1998; Krings and Berger, 2001; Sudawadee and baker, 2009; Nazeerullah *et al.*, 2013).

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 (Okwu, 2004). More than 4000 phenol compounds (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 Del rio *et al.*, 1997; Salah *et al.*, 1995;1998; Okwu, 2004).

Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Ali *et al.*, 2008).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 (Parek and Chandra, 2007 & 2008; Murthy *et al.*, 20011a & b).

They also are effective antioxidant and show strong anticancer activities (Salah *et al.*, 1995; Delrio *et al.*, 1997; Okwu, 2004). 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 (Sodipo *et al.,* 2000). Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds such as sex hormones.

Alkaloids have been associated with ethno-medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids (Siddiqui and Ali, 1997; Stray, 1998; Okwu and Okwu, 2004). Glycosides are known to lower the blood pressure according to many reports. 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 (Javed *et al.,* 2010 and 2011).

The results for Brine Shrimp Test reveals, five EMP drugs exhibited significant activity. These were *A. serphyllifolia, D. hispida, G. mauritiana, N. nimoniana* and *R. densiflora.* In all, the LC_{50} values were found to be below 0.01 µg/ml. The reason may be due to the low concentration or possible antagonistic activity between the phytochemicals from the different classes. It is evident through phyto-chemical and pharmacological evaluation of Ethno-medicinal plants and Tribal medicinal formulation drugs employed in the study with various parameters (Meyer *et al.,* 1982; Mc.Laughlin 1991 and Jimenez *et al,* 2007).. Further, the aqueous and ethanol extracts, were exhibited considerable effects on brine shrimps as opposed to the ethanol extract reported in the study by Erdogan (2009).

Pharmacological evaluation of extracts of both EMP and TMF reveals some interesting activities like biological activity of active Phyto-chemicls, Antibacterial activity, Antioxidant activities and Cytotoxicity of all the plant drugs respectively. From these, it can assume that, different active secondary metabolites are present in its extracts and perhaps some of these compounds may function in a synergistic manner. The screening of five selected ethno-medicinal plant drugs and Tribal medicinal formulation clearly reveals that, the maximum classes of phyto-constituents are present in the extracts of both EMP and TMF as compared to the previous reports which are correlated in the study. Hence, the active principles of above plant extracts and the formulation could be explored for their highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs towards wound and related ailments.

Further, the ethno-medicinal plant drugs studied are found to be of great importance due to the presence of most of the tested major active phyto-constituents. Since, these plant drugs have also been used by tribal practitioners for the treatment of supplementary ailments in association with other additional plant drugs, the medicinal roles of these plants could be related to such identified bioactive compounds. The quantitative analyses of these phyto-compounds will be an interesting area for further study. The efforts should be geared up to exploit the biomedical applications of these screened plant drugs due to the presence of certain class of energetic phyto-compounds for their full utilization. Besides, Alkaloids are one of the characteristic secondary metabolites in EMP drugs. Flavonoids are known to be synthesized by plants in response to microbial infections. Tannins (commonly referred to as tannic acid) are known as antimicrobial agents, which are water-soluble poly-phenols and precipitated proteins present in many plant foods. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein. Now a day the standardization of crude

drugs has become very important for identification and authentication of drug.

However, this report may serve as a stepping stone for future research on the biological activities and action of chief constituents present 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 these 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. If these effects are confirmed via lead molecules in the subsequent studies, it should be possible to make rationale research focused on development of new functional food and nutraceuticals or new secondary metabolites as bioactive compounds.

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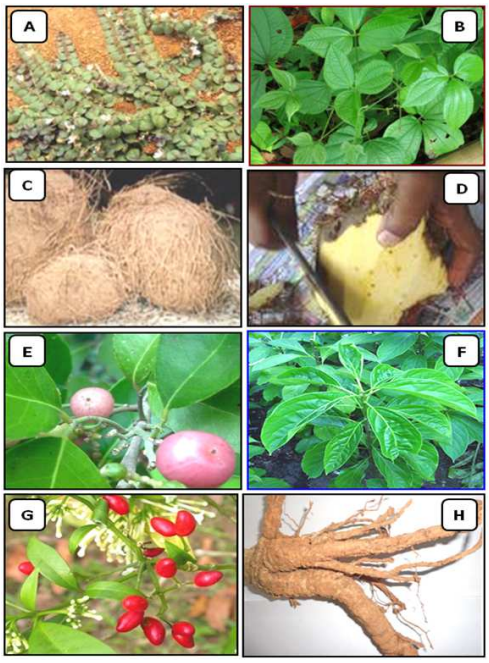

Figure.2A-H: Ethno-Medicinal Plant Drugs (Emp) and Parts of Tribal Medicinal Formulation (Tmf)

A: *Andrographis serphyllifolia*, **B:** *Dioscorea hispida*, **C & D:** Tubers of *Dioscorea hispida;*

And processing of Tubers, **E:** *Glycosmis mauritiana*, **F:** *Nothopodytes nimoniana*,

G & H: Habit and Root samples of *Rauwolfia densiflora*

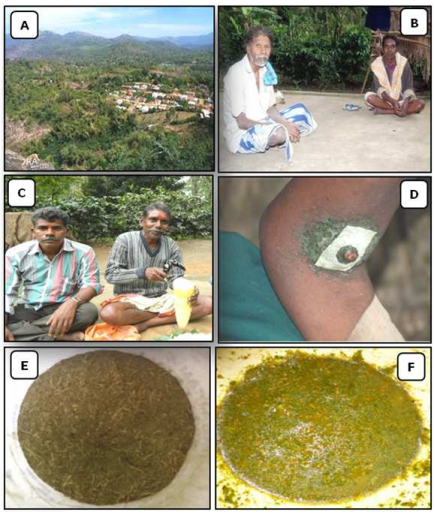

Figure 3A-J: Tribal Healers and Components of Tribal Medicine Formulation (Tmf)

A: A view of Tribal podus at B.R. Hill Tract, **B**: Tribal Healers at B.R. Hills, **C:** Groundwork for TMF,

D: Mode of Wound healing Treatment, **E:** Formulation of TMF Drug (Powder) and **F:** TMF Drug (Paste)

Table 1: List of Ethno-Medicinal Plant Drugs Practiced By Tribal Healers for Wound Related Therapeutics at Biligirirangana Hill Tracts, Karnataka state, India

Sl. No.	Ethno-Medicinal Plant With Botanical Name and Vernacular Name In Kannada	Family	Plant Parts Used	Ethno-Medicinal Value	Formulation & Mode of Treatment Against Ailments/Diseases	Dosage and Duration of the Treatment
1.	*Andrographis serphyllifolia*, Vahl. Vr. Name: Kasinasara	Acanthaceae	Stem, leaf	Gangrene, skin infection by microbes, wound treatment etc	Paste of leaves is applied on affected part in snake bite and Scorpion bite. Decoction of the leaves is used to cure fever and cough.	Paste application three/four times/week Tonic One tsp three times a day for a week.
2.	*Dioscorea hispida*, Dennst. Vr. Name: Noolana hambu	Dioscoreaceae	Tuber, leaf, seeds	Wound healing, excess bleeding, Pharynx inflammation, disturbances in Gastro-intestinal tract, Anti-allergic and inflammatory bowel disease etc.	Decoction of tuber, stem & leaf Tonic form Oral administration & Paste with lime juice for external applications for wounds and infected area	Decoction/ Tonic 1tsp three times a day for a week Paste application four times/week
3.	*Glycosmis mauritiana* (Lam), Tanaka. Vr. Name: Orrange	Rutaceae	Root, stem, leaf	Wound healing, Healing of Cancer tumour, Antimicrobial and	Paste with water And apply externally Crushed with warm water and swallowed	Apply paste at wound area & cover with a thin

	berry			Antigangrene etc		cloth 3times/week Tonic One tsp three times a day for a week
4.	Nothapodytes nimoniana, Blume. Vr. Name: *Durvasane mara*	Icacinaceae	Leaf stem	Wound healing, Anticancer activity, Microbial infection etc	Paste with warm water Decoction with warm water/ goat milk	Tablets/ Decoction One tab three times a day for a week. Decoction- two times/week
5.	*Rauwolfia densiflora* Benth & Hook. Vr. Name: Snake root	Apocynaceae	Leaf, stem & root	Decoction for reduce Blood pressure, Snake bite, Skin infection, treating insomnia etc.	Ground & juice boiled with warm water & swallowed. Paste with honey drops/warm water and apply	Tonic One tsp two times a day for 8 days Paste for external wound application

Table 2: Validated Tribal Medicine Formulation (TMF)* Practiced for Wound Healing and Related Ailments at Biligirirangana Hill Tracts, Karnataka

Sl. No.	Ethno-Medicinal Plant 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	15	**(A)20+ (D)15+ (G)25+ (N)25+ (R)15+ ADGNR= 100**
2.	*Dioscorea hispida* **(D)** Vr. Name: *Noolana hambu*	Dioscoreaceae	Tubers	20	
3.	*Glycosmis mauritiana* **(G)** Vr. Name: *Orrange berry*	Rutaceae	leaves	25	
4.	Nothapodytes nimoniana **(N)** Vr. Name: *Durvasane mara*	Icacinaceae	Leaves	15	
5.	Rauwolfia densiflora **(R)** Vr. Name: *Snake root*	Apocynaceae	Leaves	15	

TMF obtained from TMM was validated by Authorized Ayurvedic Practitioner

Table 3: Physico-Chemical Characterizations of Selected Ethno-Medicinal Plant Drugs Collected at B.R. Hills

Sl. No.	Who Parameters					
	Ethno-Medicinal Plants	Total Ash (Value % W/W)	Acid Insoluble Ash (Value % W/W)	Water Soluble Ash (Value % W/W)	Foreign Organic Matter (%)	Moisture Content (%)
01	*Andrographis serphyllifolia*	55.25	60.15	76.40	24.50	45.00
02	*Dioscorea hispida*	56.75	44.16	63.00	58.40	52.52
03	*Glycosmis mauritiana*	44.55	82.26	98.77	40.20	42.20
04	*Nothapodytes nimoniana*	52.26	65.16	70.16	39.10	35.10

05	*Rauvolfia densiflora*	40.20	55.70	72.50	60.30	22.22
06	Tribal Medicine Formulation (TMF)	63.54	70.21	91.42	51.22	39.84

Table 4: Fluorescence Analysis of Extracts of Andrographis Serphyllifolia with Variable Components

Sl. No.	Components	Ordinary light	UV light
1.	Powder as such	Dark golden red	Dark green
2.	Powder + Chloroform	Olive	Medium sea green
3.	Powder + Methanol	Forest green	Dark green
4.	Powder + Water	Chocolate	Dark salmon
5.	Powder + Acetone	Dark green	Dark olive green
6.	Powder + 1N NaOH	Dark green	Lime green

Table 5: Fluorescence Analysis of Plant Powder of Andrographis Serphyllifolia with Variable Treatments

Sl. No	Powder With Treatment	Under Ordinary Light	Under UV Light
1	Powder as such	Apple Green	Dark Brown
2	Powder + H_2SO_4 (5%)	Brass	Dark Tan
3	Powder + NH_3 (25%)	Dark Green	Dark Green
4	Powder + 1N NaoH	Dark Olive Green	Black

Table 6: Fluorescence Analysis of Extracts of Dioscorea Hispida with Variable Components

Sl. No.	Components	Ordinary light	UV light
1.	Powder as such	Golden red	Dark green
2.	Powder + Chloroform	Olive	Medium lime green
3.	Powder + Methanol	Pale green	bright green
4.	Powder + Water	brown	Shady salmon
5.	Powder + Acetone	Sea green	Dark sea green
6.	Powder + 1N NaOH	Dark green	Lime green

Table 7: Fluorescence Analysis of Plant Powder of Dioscorea Hispida with Variable Treatments

Sl. No	**Powder with Treatment**	**Under Ordinary Light**	**Under UV Light**
1	Powder as such	Pale Green	Light Brown
2	Powder + MeOH (10%)	Sea blue	Dark Blue
3	Powder + NH_3 (25%)	Forest Green	Olive green
4	Powder + 1N NaOH	Lime Green	Blackish lime

Table 8: Fluorescence Analysis of Plant Extracts of Glycosmis Mauritiana with Variable Components

Sl. No	**Components**	**Under ordinary light**	**Under UV light**
1	Petroleum ether	Dark Olive Green	Black
2	Benzene	Dark Olive Green	Brown
3	Chloroform	Dark Olive Green	Dark Brown
4	Acetone	Dark Green	Black
5	Methanol	Dartmouth Green	Light Green
6	Ethanol	Cadmium Green	Light Green
7	Water	Apple Green	Light Blue

Table 9: Fluorescence Analysis of Plant Powder of Glycosmis Mauritiana with Variable Treatments

Sl. No	Powder with Treatment	Under ordinary light	Under UV light
1	Powder as such	Apple Green	Dark Brown
2	Powder + H_2SO_4 (5%)	Brass	Dark Tan
3	Powder + NH_3 (25%)	Dark Green	Dark Green
4	Powder + 1N NaoH	Dark Olive Green	Black

Table 10: Fluorescence Analysis of Extracts of Nothapodytes Nimoniana with Variable Components

Sl. No.	Components	Ordinary light	UV light
1.	Powder as such	Dark golden red	Dark green
2.	Powder + Chloroform	Olive	Medium sea green
3.	Powder + Methanol	Forest green	Dark green
4.	Powder + Water	Chocolate	Dark salmon
5.	Powder + Acetone	Dark green	Dark olive green
6.	Powder + 1N NaOH	Dark green	Lime green

Table 11: Fluorescence Analysis of Plant Powder of Nothapodytes Nimoniana with Variable Treatments

Sl. No	Powder with Treatment	Under Ordinary Light	Under UV Light
1	Powder as such	Apple Green	Dark Brown
2	Powder + H_2SO_4 (5%)	Brass (Yellowish)	Dark Tan
3	Powder + NH_3 (25%)	Dark Green	Dark Green
4	Powder + 1N NaoH	Dark Olive Green	Black

Table 12: Fluorescence Analysis of Extracts of Rauvolfia Densiflora with Variable Components

Sl. No.	Components	Ordinary Light	UV Light
1.	Powder	Dark golden rod	Dark green
2.	Powder + Chloroform	Olive	Medium sea green
3.	Powder + Methanol	Forest green	Dark green
4.	Powder + Water	Chocolate	Dark salmon
5.	Powder + Acetone	Dark green	Dark olive green
6.	Powder + 1N NaOH	Dark green	Lime green

Table 13: Fluorescence Analysis of Plant Powder of Rauvolfia Densiflora with Variable Treatments

Sl. No	Powder With Treatment	Under Ordinary Light	Under UV Light
1	Powder as such	Pale Green	Green (fluorescent)
2	Powder + Distilled Water	Bluish green	Bluish Green (fluorescent)
3	Powder + Alcohol (50%)	Green Olive	Pale Orange
4	Powder + NaoH (10%)	Light Brown	Bluish brown
5	Powder+HNO_3 (50%)	Yellowish Green	Blackish green (fluorescent)

Table 14: Analyses of Phyto-Chemicals (Qualitative)* of the Selected Ethno-Medicinal Plants in Different Extracts

Sl. No	Variable Compounds	Andrographis Serphyllifolia (Leaves)				Dioscorea Hispida, (Tubers)				Glycosmis Mauritiana (Leaves)				Nothapodytes Nimoniana, (Leaves)				Rauvolfia Densiflora (Whole Plant)				Tribal Medicine Formulation (Tmf)				
		A	E	P	M	A	E	P	M	A	E	P	M	A	E	P	M	A	E	P	M	A	E	P	M	
1.	Alkaloids	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	-	-	+	+	+	+	
2.	Flavonoids	+	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+	+	-	-	
3.	Tannins	+	-	-	+	-	-	-	-	+	+	-	+	+	+	-	+	+	-	+	-	+	-	-	+	
4.	Gums & Mucilages	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	-	
5.	Glycosides	-	+	-	-	-	+	-	-	+	+	+	+	+	+	-	-	+	-	-	-	+	+	-	+	
6.	Saponin	+	-	-	-	+	-	+	+	+	-	+	+	-	+	-	-	-	-	-	+	+	+	+	+	
7.	Steroids	-	+	-	-	+	-	+	+	-	+	-	-	-	+	+	+	+	+	-	-	-	+	+	+	
8.	Terpenoids	+	+	-	+	-	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	+	
9.	Coumarins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
10.	Phytosterols	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	
11.	Proteins & Amino acids	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	+	+	
12.	Carbohydrates	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	
13.	Phenolic compounds	+	+	+	-	-	+	+	+	-	+	-	+	+	+	-	-	+	+	+	-	+	+	+	+	
14.	Fixed oils & Fats	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	+	-	+	+	-	-	
15.	Anthraquinones	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	
16.	Phycobalamin	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	

***** **+** = present, **-** = absent; A: Aqueous extract, **E:** Ethanolic extract & **P:** Petroleum ether & **M:** Methanolic extract. **EMP:** Ethno-medicinal Plant drugs, **TMF:** Tribal medicine Formulation

Table 15: Brine Shrimp Lethality Bioassay* in Extract of TMF Drug Practiced by Tribal Practitioners at B.R.Hills Area Karnataka

Sl. No.	Extract Concentration	Log Concentration	% of Mortality	Lc_{50} (Mg/Ml)	Lc_{90} (Mg/Ml)
1	100	2	100	18.78	41.16
2	80	1.8641	100		
3	60	1.7944	100		
4	40	1.62242	100		
5	20	1.41302	60		
6	10	1.4	30		
7	1	0	20		
8	0.1	-0.42031	0		
9	0.5	-1.3	0		

* LC_{50} and LC_{90} were determined from 24h counts using Probit analysis method described by FINNEY Computer program

Graph 1: Patterns in treatment of Ethno-medicinal plant drugs for Wound related therapeutics practiced by Tribal Medicine Men at B.R. Hills of Karnataka

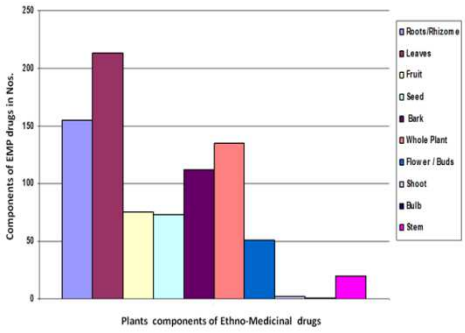

Figure 1

REFERENCES

1. Ahmad, I., Mehamood, Z and Mohammed, F.(1998). Screening of some Indian medicinal plants for their antimicrobial properties, *Journal of Etnopharmacology*; 62 (2), 183-193.
2. Ajay K Meena, Rao, M M., Ajit Kandale, R Sannd, Kiran, U Niranjan 2 and Yadav, A K.(2010), *Journal of Drug Invention Today* (2010),Volume- 2,Issu-2, pages182-186.
3. Ajay kumar meena, Kandale Ajith, Rao, M.M , Panda, P and Reddy Govind, (2011).Review on Citron-Pharmocognosy, Phyto-chemistry and medicinal uses. *International Research Journal of Pharmacy*.
4. Alali, FQ.(2002). Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an *ICBG* project.
5. Amjad Ali M. Iqbal, Imtiyaz Ansari, Mohib Khan. (2014). Comparative Pharmacognostical Evaluation of Roots of Four Rauwolfia Species, *International Journal of Pharmaceutical and Phytopharmacological Research*; 3 (4): 289-295.
6. Amjad Ali M. Iqbal1, Firoz A. Kalam Khan, Imtiyaz Ansari, Altamash Quraishi, Mohib Khan. (2013). Ethno-Phyto-Pharmacological Overview on Rauwolfia densiflora (Wall) Benth.ex Hook.f; *International Journal of Pharmaceutical and Phytopharmacological Research*, 2(5): 372-376.
7. Anonymous. (2007). Wealth of India. First supplementary Series, Vol-3, (D-I), Raw materials. Niscom. 130.
8. Antherden, L.M. (1969). Textbook Of Pharmaceutical Chemistry, 8th edn., *Oxford University Press*, London, pp. 813-814.
9. Arunkumar, G.S., Seema Kumara,B., Chandrasekhar raju, B and Ramaro, M. (2011). Biological activity of Mathanolic and Aqueous extract of *Glycosmis mauritiana* and *Streblus asper*; *International Research Journal of Pharmacy*, 2(12):267-269.
10. Arunkumar, S., Muthuselvam . (2009). Analysis of phyto-chemical constituents and antimicrobial activities of aloevera L. against clinical pathogens. *World J. Agril. Sc*., 5(5): 572-576.
11. Ch. V. Rao, Lubna azmi, Shyam Sundar Gupta. (2014). Phyto-chemical and Pharmacological review on *Andrographis serpyll*ifolia: *Potential herbal cure-all*; Vol 3 (3) Pp-1.13.
12. Chaithra, D (Registered Ayurvedic Practitioner and consultanat of traditional herbal drugs), Nisaraga Ayurvedic

Research Foundation, Sakaleshpur, Hassan district (India): Validated Tribal Medicine formulation (TMF): Ref. No.176/2013.

13. Chandra Prakash Kala. (2006). Developing the medicinal plants sector in northern India. *Journal of Ethno-biology and Etnomedicine*.75:16-24.
14. Chithrashree, Narasimha Murthy. K and Srinivas. C. (2014). Phyto-chemical screening and In vitro assessment of antimicrobial and antioxidant potential of *Andrographis serpyllifolia* - An endemic medicinal plant from South India. *International Journal of Advanced Research*, Volume 2, Issue 2, 917-928.
15. Cordell GA. (1995). Changing strategies in natural products chemistry. *Phytochemistry 40*: 1585-1612.
16. Criagg, G.M and David, J.N. (2001). Natural product drug discovery in the next millennium. *J. Pharm. Biol.*, 39: 8-17.
17. Deepak, A and Anshu, S. (2008). Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices, *Aavishkar Publishers Distributor*, Jaipur- India, P.440.
18. Del-Rio, A., Obdululio, B.G., Casfillo, J., Main, F.G., Ortuno, A. (1997). Uses and properties of citrus flavonoids. *J. Agric. Food Chem*., 45: 4505-4515.
19. Dewick, P.M. 1996. Tumor inhibition from plants: Tease and Evans.
20. Dubois, M., Gilles, KA., Hamilton, JK., Rebers, PA and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, Volume 28, 350-356.
21. Edoga, H.O., Okwu, D.E., Mbaebie, B.O. (2005). Phytochemicals constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*., 4(7): 685-688.
22. Erdoğan, T.F. (2009). Brine Shrimp Lethality Bioassay of Fumaria densiflora Dc. and Fumaria officinalis L. Extracts. Hacettepe University, *Journal of the Faculty of Pharmacy*, Vol. 28, No. 2, pp. 125-132.
23. Evans, W.C. (2009). *Trease and Evans' Pharmacognosy* (16th Edition), Saunders, ISBN 978-0- 7020-2933-2, London.
24. Evans WC. (1997). Pharmacology. Harcourt Brace and Company. Asia, Singapore. 226
25. Everaldo Attard and Pierpaolo Pacioni (2012). The Phyto-chemical and *In Vitro* Pharmacological Testing of Maltese Medicinal Plants, Bioactive Compounds in Phyto-medicine, Prof. Iraj Rasooli (Ed.), ISBN: 978-953-307-805-2.
26. Fabricant DS and Farnsworth NR. The value of plants used in traditional medicine for drug discovery. PMID, (2001), p.69–75..
27. Finar G (1986). Plants of economic importance. Medicinal Plants and Medicine in Africa. Spectrum Books Ltd. Ibadan. 78: 150-153.
28. Francois Muanda, Donatien Kone, Amadou Dicko, Rachid Soulimani and Chafique Younos (2009).

Phytochemical Composition and Antioxidant Capacity of Threev Malian Medicinal Plant Parts., *eCAM* ., 10:1-8.

29. Gahan PB (1984). Plant Histochemistry and cytochemistry: An Introduction. Academic Press, Florida, USA., pp. 1-123.
30. Ganeshaiah K. N; R. Uma Shaanker and K. S. Bawa. (*Biligiri Rangaswamy Temple Wildlife Sanctuary: Natural history, biodiversity and conservation*. ATREE and VGKK, Bangalore, (1998),p.432
31. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 10th ed. (2008):p.123.
32. Han, X., Shen, T., Lou, H. 2007. Dietry poly-phenols and their biological significance. *Int. J. Mol. Sci.*, : 950-988.
33. Harborne, J.B. (1973). Phyto-chemicals Methods. Chapman and Hall Ltd., London, pp. 49-188.
34. Harish K Handral, Prashanth Kumar Jha, Shruthi SD. (2010). Pharmacognostic and phyto-chemical studies on the leaves of Murraya koenigii L Spreng. *Pharmacophore*, 1 3 :231-238.
35. Javed Intekhab, Mohammad Aslam, Hira Khalid. (2011). Phytochemical Study of *Glycosmis Mauritiana*, *American Journal of Plant Sciences*, 2, 657-659.
36. Javed Intekhab, Mohammad Aslam. (2010). Isolation of a flavone glucoside from *Glycosmis mauritiana* (Rutaceae); *Arabian Journal of Chemistry*: 4, 79–81.
37. Jimenez PC, Wilke DV, Takeara R, Lotufo TMC, Pessoa C, Moraes MO. (2007). Cytotoxic activity of a dichloromethane extract and fractions obtained from *Eudistoma vannamei (Tunicata Ascidiacea)*. *Comp Biochem Physio*; p1-8.
38. Kavitha R, Premalakshmi V. (2013)Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. *Int J Pharm Bio.Sci*; 4:236-242.
39. Khandelwal K R, Practical Pharmacognosy, Techniques and Experiments, 20th Edn. *Nirali Prakashan*, Pune, (2010):23.8-23.10.
40. Kokate CK, Purohit AP and Gokhale SB, Practical Pharmacognosy; 2nd Ed. Vallabh Prakashan, Delhi. (2004).
41. Krishnaswamy NR. Chemistry of Natural products, A laboratory hand book, 1st edition, Universities Press India (pvt.) Ltd, Hyderabad, (2003), p 15, 26-30, 70-73, 87-88.
42. Kujala TS, Lopnen JM, Klika KD, Pihlaja K (2000): Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on content of total phenolics and 3 individual compounds. *J.Agric, Food Chem*; 50:6490-6496.
43. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. & Mc.laughlin, J. L. (1982). Brine shrimp: A Convenient general Bioassay for active plant constituents, *Planta Medica*, 45: 31–34.
44. Mohamed Sham Shihabudeen. H., Hansi Priscilla. D and Kavitha Thirumurugan. (2010). Antimicrobial activity and phyto-chemical analysis of selected Indian folk medicinal plants., *International Journal of Pharma Sciences and Research (IJPSR)*., 1(10):430-434.
45. Mojab, F., Kamalinejad, M., Ghaderi, N., Vanidipour, H.R. (2003). Phyto-chemicals screening of some species of

Iranian plants. *Iran. J. Pharm. Res*., 3: 77-82.

46. Mondal S, Marouthu I, Pushyami P , Suresh P. (2013). Toxicity studies of ethanol extract from *Ixora pavetta* andrews leaf. *World J of Pharm and Pharma Sci*. 3(1):350-360.
47. Mukesh Chandra Sharma and Smita Sharma. (2010). Phyto-chemical and Pharmacological Screening of Combined *Mimosa pudica* Linn and *Tridax procumbens* for In vitro Antimicrobial Activity, *International Journal of Microbiological Research*; 1 (3): 171-174.
48. Nazeerullah khan, Ennus Tajuddin Tamboli, V.K. (2013). Sharma, Sunil kumar. Phyto-chemical and pharmacological aspects of *Nothapodytes nimmoniana*. An overview, *Herba Rolonica*; Vol-59 (1):53-66.
49. Okwu, D.E. (2004). Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *J.Sustain. Agric. Environ*., 6(1): 30-37.
50. Panduranga Murthy,G., Mamatharani, D.R., Tejas, T.S and Niranjan M. Suralikerimath. (2011). Phyto-chemical analysis, invitro antibacterial and antioxidant activities of wild onion sps. *International Journal of Pharma and Biosciences*; Vol: 2(3): Pp-230-237.
51. Panduranga Murthy,G., Mokshith, M.C., Ravishankar, H.G. (2011). Isolation, partial purification of protein and detection of Antibacterial acivity in leaf extracts of *Tephrosia cinerea* (L.) Pers.- An Ethno-medicinal plant practiced by Tribal community at Biligirirangana Hills of Karnataka, India, *International Journal of Pharma & Biosciences*; 2(3):513-519.
52. Parekh, J., Chanda, S. (2007). Antibacterial and phyto-chemical studies on twelve species of Indian medicinal plants. *Afr. J. Biomed. Res*., 10: 175-181.
53. Parekh, J., Chanda, S. (2008). Phyto-chemicals screening of some plants from western region of India. *Plant Arch*., 8: 657- 662.
54. Pulak Majumder and Paridhavi, M. (2013). An Ethno-Phyto-chemical and Pharmacological review on Novel Indian Medicinal Plants used in Herbal Formulations, *International Journal of Pharmacy and Pharmaceutical Sciences*; Vol 5 (4):74-83.
55. Rasch E, Swift H (1960). Microphotometric analysis of the cytochemical Millon action. J. Histochem. Cytochem., 8: 4-17.
56. Ravishankar, H.G and Panduranga Murthy, G. (2009). Ethno-medicinal wealth of Biligirirangana Hills (B.R. Hills), Karnataka, India. *M.Phil thesis*: Annamalai University, Tamilnadu (India); EMP-Data Base: 1-415.
57. Reuben, K.D., F. I. Abdulrahman., J.C.Akan., H. Usman., O.A. Sodipo., G.O. Egwu. (2008). Phytochemical Screening and In Vitro Antimicrobial Investigation of the Methanolic Extract of Croton Zambesicus Muell ARG. Stem Bark. *European Journal of Scientific Research*; 23(1):134-140.
58. Revathi S.L., Dr. Suresh Kumar P, Sudarshana Deepa V, Nadana Rajavadivu G. (2012). Phytochemical analysis of Andrograpis serpyllifollia (Rohl.ex.Vahl) Wright; *International Journal of Pharmacutical Science and Health*

Care: 2, (1) Pp-1-16.

59. Salah, N., Miller, N.J., Pagange, G., Tijburg, L., Bolwell, G.P, Rice, E., Evans, C. (1995). Polyphenolic flavonoids as scavenger of aqueous phase radicals as chai breaking antioxidant. *Arc. Biochem. Broph*., 2: 339-346.
60. Sharma, A., Ajay, G. Namdeo, Kakasaheb, R and Mahadik. (2009). Pharmacognostic studies on *Nothapodytes nimmoniana* (J. Graham) Mabberly; *International Journal of Pharmaceutical Research and Development*, Vol-1 (9): 1-10.
61. Sharma, S., Ajay kumar, Ajay, G and Namdeo. (2012). Pharmacognostical and Phyto-chemical analysis of *Nothapodytes nimmoniana* stem; *International Journal of Pharmacy and Pharmaceutical Sciences*; Vol 4(4):455-459.
62. Siddiqui,S Arti verma, Ayaz Ahmad Rather, Faraha Jabeen and Mukesh K.Meghvansi (2009). Preliminary Phytochemicals Analysis of some Important Medicinal and Aromatic Plants. *Advances in Biological Resesrch*. 3(5-6):188-195.
63. Sodipo, O.A., Akiniyi, J.A., Ogunbamosu, J.U. (2000). Studies on certain on certain characteristics of extracts of bark of Pansinystalia macruceras (K schemp) picrre Exbeille. *Global J. Pure Appl. Sci*., 6: 83-87.
64. Sofowra, A. (1993). Medicinal Plants And traditional Medicine In Africa. Spectrum Books Ltd., *Ibadan*, Nigeria, pp. 191-289.
65. Somasundaram, H. N. and Kibe, R. V. Soliga – The Tribe and its Stride, Vivekananda Girijana Kalyana Kendra, BR Hills. (1990), pp.154
66. Stray, F. (1998). The Natural Guide to Medicinal herbs And Plants. *Tiger Books International*, London, pp. 12-16.
67. Sudawadee Theerasin and A.T. Baker. (2009). Analysis and identification of Phenolic compounds in *Dioscorea hispida* Dennst; *Asian Journal of Food and Agro-Industry*, 2(04), 547- 560.
68. Trease, G. E. & Evans, W. C. (1996). Pharmacognosy, pp. 89–122 (London: *Bailliere Tindall*).
69. Tripathi KD. *Essentials of medical pharmacology*. 5th ed. New Delhi (Ind) 2003.p232.
70. Usman, H., F.I. Abdulrahman and A.H. Ladan. (2007). Phytochemical and Antimicrobial Evaluation of *Tribulus terrestris* L. (*Zygophylaceae*). Growing in Nigeria. *Res. J. Bio. Sci. Medwell Journals*, 2007 2(3): 244-247.
71. WHO, (2000). General guidelines for methodologies on research and evaluation of traditional medicine. *World Health Organization*, Geneva.
72. Whistler, R.L and Bemiller, J.N. (Eds) (1993). Industrial Gums. Academic Press; San Diego. CA
73. Wiart, C., Kumar, A. (2001). Practical Handbook of Pharmacognosy. Malaysia: *Pearson Education* Malaysia Sdn Bhd.
74. Yasuma A, Ichikawa T (1953). Ninhydrin-schiff and alloxan- Schiff staining. A new histochemical staining method for proteins. J. Lab. Clin. Med., 41: 296-299.