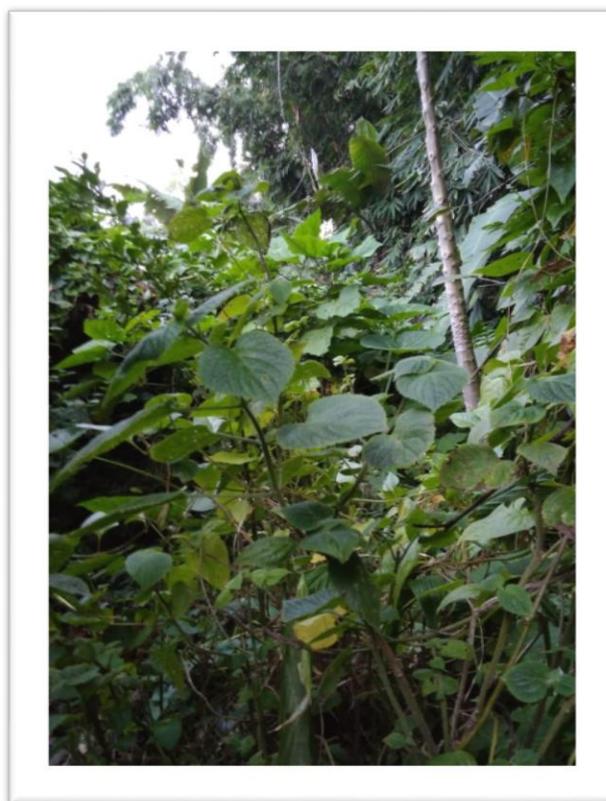


Project Report

STUDIES ON ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF HERBAL HAND WASH AND BATH SOAP USING MEDICINAL PLANTS OF MANIPUR

DST Letter No. 19/13/2017(R&D-Biotech)/DST/282 dated the 23rd October, 2017



Submitted by

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Submitted to

**DEPARTMENT OF SCIENCE & TECHNOLOGY
GOVERNMENT OF MANIPUR**

Completed Project

A. First Page

1. Project Title : **Studies on antibacterial and antifungal properties of herbal hand wash and bath soap using medicinal plants of Manipur**

2. Name & Designation of Principal Investigator & Co-Investigator:
 - (a) Dr. Ningthoujam Sanjoy Singh, Associate Professor, Department of Botany, Ghanapriya Women's College, Imphal – Principal Investigator

 - (b) Dr. Leimapokpam Geetabali Devi, Assistant Professor, Department of Botany, Ghanapriya Women's College, Imphal – Co-Investigator

3. DST Sanction Order No. and date : 19/13/2017(R&D-Biotech)/DST/282 dated the 23rd October, 2017

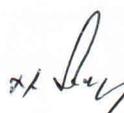
4. Project Serial Number (as per DST Sanction Order) :

5. Sanctioned Project cost and duration: Rs. 95,000/- (Rupees ninety-five thousand) only; one year

6. Actual Project cost and duration: Rs. 95,000/- (rupees ninety-five thousand) only; One year

7. Date of Project start and completion: 1st November, 2017 and 30th November, 2018

8. Signature of the Investigators:


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PROJECT REPORTS

Deviations made from original objectives, if any, while implementing the project, and reasons thereof.

There is no deviations made from original objectives.

Details of the Project work

Introduction :

Origin of the Proposal

Maintenance of physical cleanliness and use of deodorants are essentials of healthy living. Out of several practices for maintaining cleanliness, hand hygiene is one of the important simple and least expensive means of preventing health care associated infections. However, improper hand-hygiene practices are still practiced in many countries, some of them due to unavailability of hand-hygiene facilities (Larson, 1999) thereby increasing the chance of antimicrobial infections and other ramifications. Most of the skin infections on human beings are caused by *Staphylococcus aureus* and *Streptococcus species* (Millogo-Kone et al., 2008). Bacterial and fungal infections are usually treated with antibiotics and antifungal pharmaceutical preparations respectively (Kareru et al., 2010). Apart from these pharmaceuticals, there are different preparations of hand sanitizer like gel, foam, liquid solution etc. to protect the skin from harmful microorganisms. Most of the chemical antiseptics available in the market are alcohol based sanitizers, chlorohexidine products etc however they have adverse effects and other limitations (Joshi et al., 2008b; Winnefeld et al., 2000). Continuous use of these products can lead to skin irritation and development resistance among pathogens (Winnefeld et al., 2000). In the last few decades, attentions have been drawn to herbal formulations for skin care and individual hygiene for their eco-friendly and easily affordable conditions. From time immemorial, herbal extracts have been used in many countries as traditional medicine, functional food, natural eyes, cosmetics and detergents (Raskin et al., 2002). Manipur, with its biodiversity and different communities have a long heritage of using herbal products for various skin care and sanitation purposes. Documentation of the traditional technology for individual hygiene would increase our understanding of the underlying phytochemical properties of the medicinal plants. Scientific investigation into their efficacies and phytochemical profiling of the medicinal plants can help in development of eco-friendly broad-spectrum or target-specific herbal hygiene products.

Definition of the project

The present project is to develop an optimised protocol for production of herbal hand wash and bath soap using medicinal plants found in Manipur. Antibacterial and antifungal properties of these herbal extracts and their combined effects in herbal hand wash and bath soap were studied. The project is

defined as: **Studies on antibacterial and antifungal properties of herbal hand wash and bath soap using medicinal plants of Manipur**

Objectives of the project

Objectives of the project are to

- (a) Survey and document the medicinal plants and methods traditionally used in cosmetic purposes
- (b) Phytochemical Screening of the different plant parts of selected plants with various chemical extracts such as petroleum ether, benzene, ethanol and water
- (c) Study antibacterial and antifungal properties of different fractions of medicinal plants
- (d) Educate and disseminate knowledge about the bioactive properties of medicinal plants of Manipur

Science Technology content of the proposal

The proposed project was based on the previous knowledge of the biochemical properties of the medicinal plants. Plants reported to be used as traditional cosmetic purposes were scientifically validated for their biochemical properties. Data mining and wet lab techniques were applied to investigate the biochemical profile of the selected medicinal plants. Different extraction methods were tested to determine optimised technology for development of effective herbal hygienic products.

Importance of the proposal with reference to Manipur.

Manipur has rich biodiversity and has a long tradition of usage of medicinal plants for therapeutic uses. However, there is gradual lost in traditional knowledge and biodiversity in this state located in Indo-Burma hotspot region (Myers et al., 2000). Documentation and scientific validation of the biochemical properties of these medicinal plants in Manipur are the need of the hour. Moreover, both rural and urban folks in Manipur are facing many skin-related problems from various factors. Development of herbal hand wash and soap from locally available resources and imparting the knowledge to the interested individuals would contribute to the overall development of the state.

Review status of the subject:

International status

Potentials of herbal products in various skin care treatments have been studied in various countries. Topical applications of herbal extracts have been studied to establish their antimicrobial properties (Holetz et al., 2002; McCarrell et al., 2008; Millogo-Kone et al., 2008; Wagate et al., 2010). Gels, creams and soap formulations developed from variety of plant extracts have been assessed for their efficacies

against skin diseases caused by microbial infections (Azubuike, 2012; Barak-Shinar et al., 2017; Kareru et al., 2010; Semkina, 2005). Diverse statistical experiments have been applied to assess the formulation variables on pharmaceutical preparations for skin care treatments (Ajala et al., 2017). There are other attempts to introduce easily available plant materials as source of fatty materials in toilet soaps (Asthana et al., 2017).

National status

In India, there is also rich tradition of using Ayurvedic products for skin care and hygienic treatment (Vyas, 2011). Apart from concerted effort to rejuvenate the rich tradition, many scientific investigations are also done to study the antimicrobial potentials of medicinal plants through herbal hand wash and soap. Patel et al. developed and evaluated herbal hand wash containing ethanolic extract of liquorice root for antibacterial properties (Patel et al., 2017). There is another attempt to study antibacterial and antifungal properties of herbal bath soap formulation derived from *Vitex negundo* leaf extract (Ruckmani et al., 2014).

Importance of the project in the context of current status

Many works have been attempted on the potential of herbal extracts for development of herbal hand wash and soap in other parts of India. However, there are limited attempt on the study of combinatorial effect of different herbal extracts on antimicrobial and antifungal properties. According to our knowledge, this is a pioneering attempt in Manipur on the development of herbal hand wash and soap using medicinal plants and study of their combinatorial antifungal and antibacterial properties.

Methodology adopted

The present project was based on the following components :

- (a) Ethnopharmacological field survey,
- (b) Phytochemical profiling using standard protocol
- (c) Antimicrobial studies using standard protocol.

Materials and Data collected

Study Site :

Ethnobotanical data collection was done in Tangjeng (Kakching district), Phoijing (Bishenpur district), Lamphel (Imphal West district) and Heingang and Andro (Imphal East district) respectively. Traditional knowledge holders in these villages were consulted. Because of the limitation of time and resources, only Meitei villages were selected for the study. Plant materials are also collected from these villages based on data mentioned in the ethnobotanical field surveys.

Interviews

Semi-structured questionnaires were administered to collect ethnobotanical data from the informants in Manipur. Informations were collected from 23 informants from the valley districts of Manipur distributed in 5 villages. All the informants belonged to Meitei community raised and brought up in their respective villages. Prior informed consent were taken before taking the data.

Specimen Collection

Plant samples were collected on the basis of information provided by the informants. Some specimens were collected from informant's homegardens while some are collected from the wild habitats. Collected plants were cross checked with the informants for their vernacular names. Plants were identified in the Department of Botany, Ghanapriya Women's College, Dhanamanjuri University. Their Scientific names were standardized according to interntional standards.

Quantitative Analysis

Ethnobotanical data were analysed as per quantitative methods. In the collected data, fidelity level index (Friedman et al., 1986), In this method, species are arranged in accordance with the percentage of informants suggesting the use for a given species as compared with the total number of informants reporting any sort of use for that plant. The obtained ratio was defined as the fidelity level (FL). The Relative frequency of citation (RFC) was also calculated on the collected ethnobotanical data (Tardío and Pardo-de-Santayana, 2008). The RFC is obtained by the equation $RFC = FC/N$ where FC is the frequency of citation i.e. the number of informants who mention the use of the species and N is the total number of informants interviewed in the survey. For selecting the most popular plant used in traditional hygiene and cosmetics, DCI was used with some modifications.

Ethnobotanical Data Collection

Ethnobotanical data collection was focused on plant and plant products traditionally used for cosmetic and hygienic products. At the foremost, knowledge on plant and plant products for cosmetic and hygienic products would be collected from ethnopharmacological field survey in Manipur. Priority plants would be selected through application of quantitative and statistical analyses such as FIC, Fidelity Level, RFC and DCI indices (Heinrich et al., 1998; Hoft et al., 1999; Phillips and Gentry, 1993; Trotter and Logan, 1986).

Informant Consensus Index was one of popular quantitative approaches for identifying the relative importance of medicinal plants with the ailment categories in a particular culture. The present index was initially developed by Trotter and Logan (Trotter and Logan, 1986) and lateron modified by Heinrich et al. (Heinrich et al., 1998). The present index calculated on the basis of the following equation-

$$FIC = \frac{N_{ur} - N_t}{N_{ur} - 1}$$

where Nur stands for the number of use reports for a particular use category and Nt stands for the number of taxa used for a particular ailment category by all informants. The indices could reflect the homogeneity in the use of plants in the ailment categories among the informants of the study area. As many species may be associated with the same disease, this factor becomes significant tool for determining the most used plant species for treating a particular ailment. A higher FIC value indicates the use of relatively few plants by the informants in the treatment of a particular ailment category whereas a lower FIC value indicates that there are disagreement among the informants with regard to use of a particular plant for treating a particular ailment category.

The RFC is obtained by the equation $RFC = FC/N$ where, FC is the frequency of citation i.e., the number of informants who mention the use of the species and N is the total number of informants interviewed in the survey (Tardío and Pardo-de-Santayana, 2008).

The Fidelity Level (FL) was initially proposed by Friedman et.al. (1986) to analyze plant use in Israel (Friedman et al., 1986). The index was defined as the ratio between the number of informants who independently suggested the use of a species for the same major purpose and the total number of informants who mentioned the plant for any use. The index is calculated according to following formula:

$$FL = \frac{N_p}{N \times 100}$$

Where, Np is the number of informants that reported a use of a plant species to treat a particular disease, and N is the number of informants that used the plants as a medicine to treat any given disease (Andrade-Cetto and Heinrich, 2011; Friedman et al., 1986).

Priority plants for selection of cosmetic and hygienic plants, modified Disease Consensus Index was adopted (Andrade-Cetto et al., 2006; Andrade-Cetto and Heinrich, 2011). During the survey, 24 informants were interviewed. In addition to the main questionnaire included in Annexure A, a separate questionnaire set with Yes/No response was used that consists of 10 specialized questions. This questionnaire was used for calculating DCI index. The following criteria were used to calculate the index:

- (i) Plant Names in Manipuri,
- (ii) General Plant Description in informant's own expression,
- (iii) Mode of preparation of the recipe,

- (iv) Method and way of administration of the herbal preparation for the usage,
- (v) Organoleptic properties, such as colour, taste, texture, smell etc.,
- (vi) Changes after using the preparation,
- (vii) Frequency of using the herbal preparation per day, month etc.,
- (viii) Impression of the user after taking the preparation,
- (ix) General knowledge about how the plant is collected, e.g. wild, cultivated, market etc. and
- (x) Whether the user has recommended the use to other people (it indicates confidence of the patient).

The analysis of the data consists of a binary evaluation – (1) or “yes”, which indicates the knowledge or (0) or “no” – a lack of such knowledge. In each case this refers to a single question, allowing a mathematical analysis of the results. The potential maximum value for one informant (about one plant, OP) is always 1. If all informants have knowledge about all aspects of this medicinal plant’s use and biology, a species can score a maximum of 1.

$$OP = \frac{\text{Answers (yes)} \times 100 / \text{Number of questions}}{100}$$

The DCI is a comparison based on Mathematical aspects (limit theory), the ideal answers of informants reports and the ideal answers for each species. It is calculated as follows:

$$DCI = \left(\sum_{i=1}^{\infty} \frac{V_{xi}}{Cc} mVx \right) Pm^{-0.1}$$

where, ‘x’ is any species; ‘ $\sum V_{xi}$ ’ is the sum of the individual values obtained for one species within the community which evaluates knowledge and mentions; ‘mVx’ is the statistical mean of the individual values, for one plant which evaluates knowledge; ‘Cc’ the maximal number of informants whom refer a plant. Which evaluates mentions; $Pm^{-0.1}$ is the compensation factor, and analyses the dispersion for one plant, considering the mode of preparation and parts used (Andrade-Cetto and Heinrich, 2011).

Phytochemical Analysis

Phytochemical analysis were conducted on selected plant materials used in traditional hygiene. The plant materials were washed properly and grind to powder after drying. Then the materials were stored in closed containers at room temperature until used. Plant extracts were prepared by soaking 5 g of the powdered materials in 50 ml each of different solvents, viz. chloroform, hexane, methanol, and water for 24 hr. The extracts were then filtered using Whatman Filter Paper and concentrated. Filtrates were centrifuged at 1000 rpm for 30 min, filtered and concentrated to dried residue.

Extracts of different solvents were analyzed for the detection of various constituents. Test for alkaloid was done by using Dragendroff and Wagner Reagent method (Sinha and Dogra, 1985). Phytosterol and terpenoids were evaluated by using Salkowski's Test (Auwal et al., 2014). Frothing Test was conducted for the presence of saponin (Auwal et al., 2014). Estimation were also done for the presence of glycosides, tannins and flavonoids (Auwal et al., 2014) as well as phenol (Sinha and Dogra, 1985). All the estimations were done in triplicates.

Antimicrobial Activities

The antibacterial activity of the plant extracts was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, gram positive *Bacillus subtilis* and gram negative *Escherichia coli*. The strains were provided by the Life Sciences Department, Manipur University and were maintained in the Mueller Hinton agar medium at 4°C. Inoculums were prepared by growing cells in Mueller Hinton broth (MHB) for 24 hours at 37°C.

The antibacterial activity was evaluated in the methanolic extracts by the agar well diffusion method using Muller-Hinton agar plates (Karthikeyan et al., 2009). The agar plates were swabbed with bacterial strains using sterile cotton swab and wells of 6 mm diameter were punched in each plate using a sterile cork borer. Preparation of stock solution was done by dissolving 0.3 g of dried methanolic extract in 1.5 ml of DMSO (dimethyl sulfoxide). Different concentrations of 20, 40, 60, 80, 100 and 120 µl of the extracts were put in the wells with different concentrations and incubated at 37°C for 24 h. After incubation, the diameter of the zone of growth of inhibition of the plant extracts were compared with that of DMSO as negative control (Mandal et al., 2013).

Herbal Soap Preparation

Herbal soap preparation begins with the preparation of basic soap. Ten grams of coconut oil was taken in a beaker and serve as neutral fat. In another beaker, 12.5 ml of ethanol and equal amount of water were mixed with 3.5 g of Sodium hydroxide pellets. This preparation is stirred until completely dissolved. The solution was then added to the beaker containing oil. Stirring was continued on a hot plate, until the smell of fat or oil disappears and oil dissolves forming a homogenous solution. The mixture was allowed to cool and soap was filtered using Buchner funnel and Whatmann No. 1 filter paper. About 300 ml of saturated sodium chloride solution was allowed to pass through the funnel for salt washing to remove any impurities. Acid washing was done by passing 5 ml of 0.1N of dilute hydrochloric acid. Soap was solidified for an hour to get the consistency.

The two solidified basic soaps were taken and broken down to small pieces. These pieces were melted in a water bath. The two preparations were used for different concentrations of methanolic *Vitex negundo*. One was used for high concentration of Vitex extract (0.5 g) while another was used for lower

concentration (0.25 g) of *Vitex*. These extracts were added to the respective melted soaps along with 5 ml of ethanol. To the mixture, 0.033 g of stearic acid was added in hot water, stirred and added to the melted soap. Then, 0.033 g of TiO₂ was added to the melted soap and stirred and 2 ml of lemongrass oil was added. After that 1 g of sodium lauryl sulphate was added in 5 ml of distilled water and added to the mixture in drops. Respective soaps were gently mixed for about 30 minutes and moulded into different shapes. The soaps were allowed to solidify to room temperature and kept for physical observation.

Chemical characterizations:

The herbal bath soap with lower concentration was evaluated and characterized for pH, Total Fatty Matter and moisture content. The pH of the soap was determined by two methods – (i) using the pH strip and (ii) using the pH meter after dissolving 1 gram of soap in 10 ml of water.

Total Fatty Matter was estimated after reacting the soap with acid in the presence of hot water (Awang et al., 2001). About 10 g of the soap was added to 150 ml distilled water and heated. Then the mixture was dissolved in 20 ml of 15% H₂SO₄ and heated until a clear solution was obtained. Fatty acids appeared on the surface of the solution. Fatty acids were solidified by adding 7 g of wax and heating. Then, the mixture was allowed to cool down to form cake. After properly cooled down, cake was removed and blotted to dry. Weight of the cake was measured to obtain the TFM by using the formula,

$$\%TFM = \frac{(Weight\ of\ the\ oil - Weight\ of\ the\ wax)\ in\ g}{Weight\ of\ the\ soap\ in\ g} \times 100$$

Determination of the percentage of water in the soap was estimated by drying the soap to a constant weight. The water content is expressed as the percentage, by weight, of the dry sample. The soap was weighed and recorded as 'wet weight of sample' and was dried from 100 to 115°C using a dryer. The sample was cooled down. Weight of the sample was noted to find the 'dry weight of sample'. The moisture content was determined using the formula,

$$\%Moisture\ Content = \frac{(Weight\ of\ the\ wet\ sample - Weight\ of\ the\ dry\ sample)\ in\ g}{Weight\ of\ the\ dry\ sample\ in\ g} \times 100$$

Stability Studies

Stability studies of hand wash are analyzed for change in colour and phase separation.

Viscosity and pH are determined by using digital viscometer and digital pH meter respectively.

Antimicrobial activity would be studied by using Zone of Inhibition technique (Isenberg, 1998) by using agar dilution method. For the antibacterial studies, *Escherichia coli*, *Pseudomonas aeruginosa*,

Staphylococcus aureus and *Bacillus subtilis* were used. For the antifungal studies, *Candida sp.* was used.

Preparation of Herbal Hand Wash

Herbal hand wash was prepared by adding methanolic extracts of plant material (Borgatta et al., 1989). Extracts are mixed with glycerin and distilled water. This preparation is again mixed with sodium lauryl sulphate methyl paraben and some drops of lemon grass. The solution was made homogenous using homogenizer under room temperature. Stability studies were carried out by storing the preparation at different temperatures for one week (Joshi et al., 2008a). Changes in colour and phase separation were observed in the formulated hand wash. The pH was also determined with pH meter. The viscosity of the formulated hand wash was determined by using digital Viscometer.

Statistical Analysis

Estimates were done in triplicates. Statistical analyses were done using R software. Results are expressed as the mean \pm SD (standard deviation). Significance difference between the different means at $p=0.05$ were determined by using ANOVA tests.

Data collection format/questionnaire

Data collection format is enclosed separately as Annexure A

Outcome of the Project

Analysis of Results

Ethnobotanical Survey

In the ethnobotanical survey, 30 medicinal plants are cited as having used in cosmetic and hygienic uses among the Manipuri community. Some of the medicinal plants have multiple usage ranging from indigestion to injuries, in addition to use in hygienic products. Distribution of plant parts used in hygienic products are shown in the following figures.

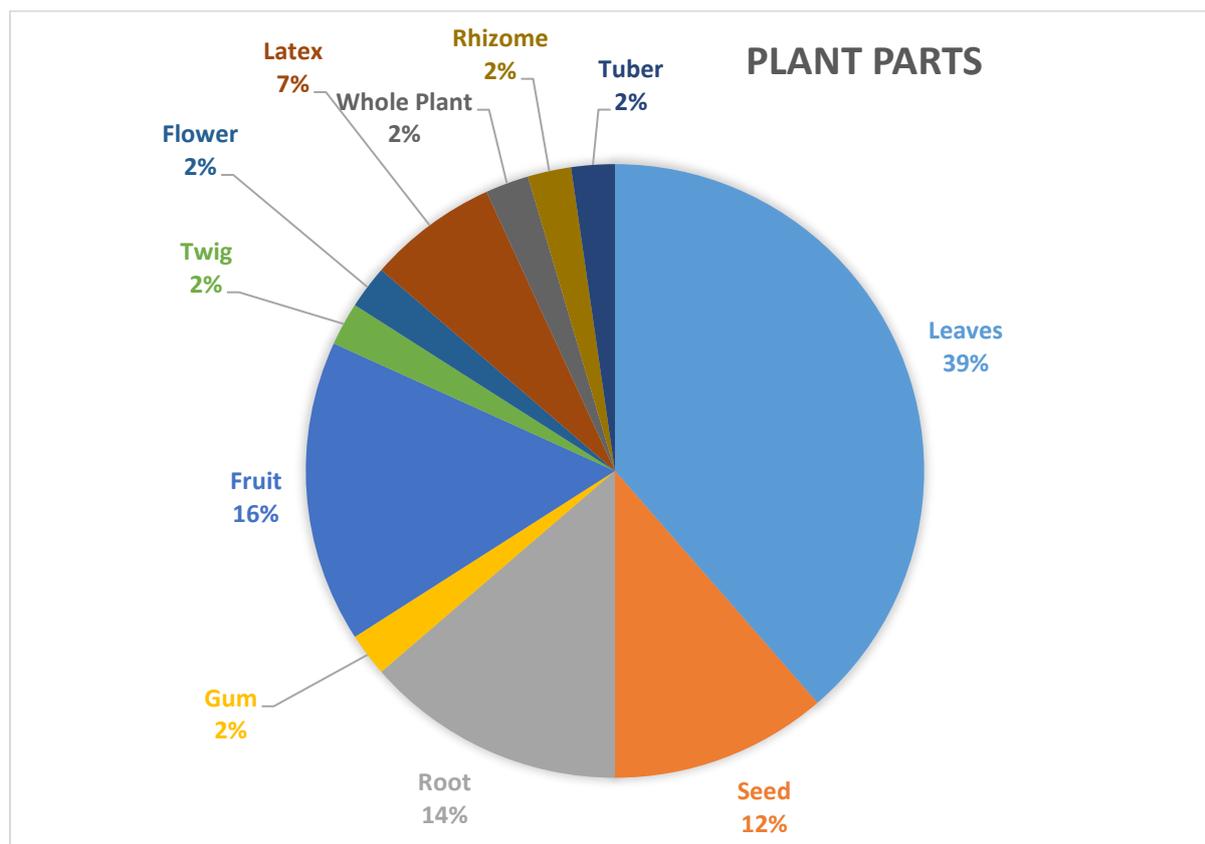


Figure 1: Plant parts used in hygienic and cosmetic purposes by the Manipuri community

Ethnobotanical Data and Relative Frequency of Citation

Altogether 30 medicinal plants were reported in the present study. Similar use reports are also observed in other parts of the world. Ethnobotanical reports are presented in the following data (Table 1) along with the Relative Frequency of Citation mentioned by the informants. Relative frequency of citation (RFC) of each species has been evaluated from the available information. Highest RFC was observed in *Phyllanthus emblica* and *Bauhinia purpurea* (1.250) followed by *Pogostemon parviflorus* (1.208), *Azadirachta indica* (1.083), *Vitex negundo* and *Citrus aurantifolia* (1.000) respectively.

Table 1 : Ethnobotanical plant along with the Relative Frequency of Citation

SI No	Scientific Name	Local Name	parts used	Usage	RFC
1	<i>Ageratum conyzoides</i> (L.) L.	Khongjai Napi	leaves	Hair care, injuries	0.917
2	<i>Aloe vera</i> (L.) Burm.f.	Ghritakumari	leaves	skin care, headache	0.750
3	<i>Andrographis paniculata</i> (Burm.f.) Nees	Bhubati	Leaves, seed	small pox, purgative, fever, stomach pain	0.625
4	<i>Argemone mexicana</i> L.	Yenkhumit	Leaves, Seed, Root	Pimples, skin care, jaundice, laxative	0.292
5	<i>Artemisia vulgaris</i> L.	Laibakngou	leaves, roots	Hair care, fever, antiseptic	0.542
6	<i>Azadirachta indica</i> A.Juss.	Neem	leaves, gum, fruits, twig	Insecticide, leprosy, contraceptive	1.083
7	<i>Bauhinia purpurea</i> L.	Chingthrao Angangba	Bark, Leaves, Flowers	Dysentery, Leprosy, Small pox, Asthma	0.250
8	<i>Calotropis gigantea</i> (L.) Dryand.	Angkot	Latex	Eczema, ringworm, Leprosy, leucoderma	0.333
9	<i>Cassia fistula</i> L.	Chahui	Root, Fruit	Leprosy, Jaundice, Fever, Dysentery	0.292
10	<i>Chenopodium album</i> L.	Monsaobi	Whole Plant	Leucoderma, indigestion	0.333
11	<i>Citrus aurantiifolia</i> (Christm.) Swingle	Champra	fruit	Hair care, hand wash, pimples, indigestion	1.000
12	<i>Coix lacryma-job15i</i> L.	Chaning	Seed	Small pox, diarrhoea, fever	0.250
13	<i>Curcuma longa</i> L.	Yaingang	Rhizome	Skin care, spice, pimples, pigmentation, injuries	0.292
14	<i>Dichrocephala integrifolia</i> (L.f.) Kuntze	Lallukok	leaves	Hair care, injuries	0.375
15	<i>Dioscorea bulbifera</i> L.	Lam Haa	Leaves, tuber	Eczema, dysentery, goiter, pharyngitis	0.417
16	<i>Drymaria cordata</i> (L.) Willd. ex Schult.	Tandan pambi	Leaves	Scabies, Jaundice, Asthma, Fever	0.417

SI No	Scientific Name	Local Name	parts used	Usage	RFC
17	<i>Euphorbia antiquorum</i> L.	Tengnou	Latex	skin disease	0.250
18	<i>Garcinia pedunculata</i> Roxb. Ex Buch-Ham.	Heibung	Fruit	Skin care, indigestion, dysentery	0.375
19	<i>Holmskioldia sanguinea</i> Retz.	Kharam Leithong	leaves	Hair care, headache	0.292
20	<i>Jatropha curcas</i> L.	Awa Kege	Latex, seeds, roots	Eczema, purgative, wounds	0.583
21	<i>Melia azedarach</i> L.	Seizrak	leaves	skin disease, insect repellent	0.292
22	<i>Meyna spinosa</i> Roxb. ex Link	Heibi	Fruit, leaves	skin care, hair care	0.375
23	<i>Mussaenda glabra</i> Vahl	Hanurei	leaves	Hair care	0.333
24	<i>Ophiopogon intermedius</i> D.Don	Ching Charot	shoot, root	Hair care, aromatic item, sinusitis, wounds	0.625
25	<i>Oxalis debilis</i> Kunth	Yensil	leaves	Hair care, indigestion	0.583
26	<i>Phyllanthus emblica</i> L.	Heikru	fruit	Hair care, cold, indigestion	1.250
27	<i>Pogostemon parviflorus</i> Benth.	Sangbrei	leaves, roots	Hair care, injuries, haemorrhage	1.208
28	<i>Sapindus mukorossi</i> Gaertn.	Kekru	Fruit	Hand Wash, Epilepsy, Headache, Asthma	0.833
29	<i>Senna tora</i> (L.) Roxb.	Thaonam	Seed, Leaves	Eczema, ringworm	0.167
30	<i>Vitex negundo</i> L.	Urikshibi	leaves	smallpox, eczema, hair care	1.000

Factor for Informant Consensus (FIC)

Factor for Informant Consensus was used to determine plants of particular intercultural relevance and to agree on their use. High values correspond only when plant species are consensus in a particular disease category. From the data, it was observed that disease category of Skin and Subcutaneous Cellular

Tissue have high consensus (less than 1) on the use of plants among the informants. High value on Circulatory System Disorder, Genitourinary System and Sensory System Disorder are because of very few respondents.

Table 2: FIC of the plants reported in the survey

Disease Category	FIC
Digestive System	0.756
Respiratory System Disorder	0.556
Circulatory System Disorder	1.000
Nervous System Disorder	0.786
Infection	0.781
Injuries	0.722
Genitourinary System	1.00
Skin/Subcutaneous Cellular Tissue Disorder	0.884
Sensory System Disorder	1.00

Fidelity Level (FL)

The Fidelity Level was estimated to quantify the importance of the species for a given purpose (Table 2). In the present study, highest fidelity levels were observed in *Senna tora*, *Vitex negundo*, *Meyna spinosa*, *Melia azaderach* and *Euphrobia antiquorum* (100). Lowest fidelity level were observed in *Ophiopogon intermedius*, *Bauhinia purpurea* and *Coix lacryma-jobi* (33.333)

Table 3: Fidelity level of the medicinal plants used in cosmetic and hygienic purpose in Manipur

Sl No	Scientific Name	Digestive	Respiratory system disorder	Circulatory system disorder	Nervous System Disorders	Infection	Injuries	Genitourinary system disorder	Skin/ Subcutaneous Cellular Tissue Disorders	Total	Highest Report	FL
1	<i>Ageratum conyzoides</i> (L.) L.						3		19	22	19	86.364
2	<i>Aloe vera</i> (L.) Burm.f.				8				10	18	10	55.556
3	<i>Andrographis paniculata</i> (Burm.f.) Nees	3				10			2	15	10	66.667
4	<i>Argemone mexicana</i> L.	2							5	7	5	71.429
5	<i>Artemisia vulgaris</i> L.	2				4			7	13	7	53.846
6	<i>Azadirachta indica</i> A.Juss.				10			2	14	26	14	53.846
7	<i>Bauhinia purpurea</i> L.		2			2			2	6	2	33.333
8	<i>Calotropis gigantea</i> (L.) Dryand.								8	8	8	100
9	<i>Cassia fistula</i> L.	1				2			4	7	4	57.143
10	<i>Chenopodium album</i> L.	2							6	8	6	75
11	<i>Citrus aurantiifolia</i> (Christm.) Swingle	10							14	24	14	58.333

SI No	Scientific Name	Digestive	Respiratory system disorder	Circulatory system disorder	Nervous System Disorders	Infection	Injuries	Genitourinary system disorder	Skin/ Subcutaneous Cellular Tissue Disorders	Total	Highest Report	FL
12	<i>Coix lacryma-job15i</i> L.	2				2			2	6	2	33.333
13	<i>Curcuma longa</i> L.						2		5	7	5	71.429
14	<i>Dichrocephala integrifolia</i> (L.f.) Kuntze						3		6	9	6	66.667
15	<i>Dioscorea bulbifera</i> L.		1		4	3			2	10	4	40
16	<i>Drymaria cordata</i> (L.) Willd. ex Schult.		2			4			4	10	4	40
17	<i>Euphorbia antiquorum</i> L.								6	6	6	100
18	<i>Garcinia pedunculata</i> Roxb. Ex Buch-Ham.	5			1				3	9	5	55.556
19	<i>Holmskioldia sanguinea</i> Retz.				2				5	7	5	71.429
20	<i>Jatropha curcas</i> L.	2					3		9	14	9	64.286
21	<i>Melia azedarach</i> L.								7	7	7	100
22	<i>Meyna spinosa</i> Roxb. ex Link								9	9	9	100

SI No	Scientific Name	Digestive	Respiratory system disorder	Circulatory system disorder	Nervous System Disorders	Infection	Injuries	Genitourinary system disorder	Skin/ Subcutaneous Cellular Tissue Disorders	Total	Highest Report	FL
23	<i>Mussaenda glabra</i> Vahl				2				6	8	6	75
24	<i>Ophiopogon intermedius</i> D.Don	5	2				4		4	15	5	33.333
25	<i>Oxalis debilis</i> Kunth	2							12	14	12	85.714
26	<i>Phyllanthus emblica</i> L.	10				6			14	30	14	46.667
27	<i>Pogostemon parviflorus</i> Benth.			1			4		22	29	22	75.862
28	<i>Sapindus mukorossi</i> Gaertn.		3		2				15	20	15	75
29	<i>Senna tora</i> (L.) Roxb.								4	4	4	100
30	<i>Vitex negundo</i> L.								24	24	24	100

Disease Consensus Index

When there is need for selecting species to treat a particular problem, Disease Consensus Index (DCI) can be applied. In the original work, the index was used to find priority species for a particular disease. However, in this survey, this index was used to determine the plants specifically used for hygienic purposes in Manipur. In the analysis, the highest DCI were observed in *Citrus aurantifolia* (1.192), *Vitex negundo* (0.729) and *Pogostemon parviflorus* (0.693) respectively.

Table 4: Modified DCI for selecting plants in hygienic purposes

Sl No	Local Name	Scientific Name	ΣVxi	#mentioned	Cc (only informant)	mVxi	#parts used	Pm ^{-0.1}	DCI
1	Khongjai Napi	<i>Ageratum conyzoides</i> (L.) L.	16.4	22	24	0.745	1	1	0.509
2	Ghritakumari	<i>Aloe vera</i> (L.) Burm.f.	7.3	18	24	0.406	1	1	0.123
3	Bhubati	<i>Andrographis paniculata</i> (Burm.f.) Nees	1.7	6	24	0.283	2	1.072	0.022
4	Yenkhumit	<i>Argemone mexicana</i> L.	1.8	7	24	0.257	3	1.116	0.022
5	Laibakngou	<i>Artemisia vulgaris</i> L.	8.4	13	24	0.646	2	1.072	0.242
6	Neem	<i>Azadirachta indica</i> A.Juss.	14	22	24	0.636	4	1.149	0.426
7	Chingthrao Angangba	<i>Bauhinia purpurea</i> L.	1.5	4	24	0.375	3	1.116	0.026
8	Angkot	<i>Calotropis gigantea</i> (L.) Dryand.	2.3	4	24	0.575	1	1	0.055
9	Chahui	<i>Cassia fistula</i> L.	1.1	3	24	0.367	2	1.072	0.018
10	Monsaobi	<i>Chenopodium album</i> L.	0.9	2	24	0.45	1	1	0.017
11	Champra	<i>Citrus aurantiifolia</i> (Christm.) Swingle	26.2	24	24	1.092	1	1	1.192
12	Chaning	<i>Coix lacryma-jobi</i> L.	0.4	2	24	0.2	1	1	0.003
13	Yaingang	<i>Curcuma longa</i> L.	3.3	8	24	0.413	1	1	0.057
14	Lallukok	<i>Dichrocephala integrifolia</i> (L.f.) Kuntze	2.4	6	24	0.4	1	1	0.04
15	Lam Haa	<i>Dioscorea bulbifera</i> L.	1.3	3	24	0.433	2	1.072	0.025
16	Tandan pambi	<i>Drymaria cordata</i> (L.) Willd. ex Schult.	1.2	3	24	0.4	1	1	0.02
17	Tengnou	<i>Euphorbia antiquorum</i> L.	1.7	5	24	0.34	1	1	0.024
18	Heibung	<i>Garcinia pedunculata</i> Roxb. Ex Buch-Ham.	7.1	9	24	0.789	1	1	0.233

SI No	Local Name	Scientific Name	ΣV_{xi}	#mentioned	Cc (only informant)	mVxi	#parts used	Pm ^{0.1}	DCI
19	Kharam Leithong	<i>Holmskioldia sanguinea</i> Retz.	1.7	5	24	0.34	1	1	0.024
20	Awa Kege	<i>Jatropha curcas</i> L.	3.4	9	24	0.378	3	1.116123	0.060
21	Seizrak	<i>Melia azedarach</i> L.	2.2	6	24	0.367	1	1	0.034
22	Heibi	<i>Meyna spinosa</i> Roxb. ex Link	3.4	9	24	0.378	2	1.072	0.058
23	Hanurei	<i>Mussaenda glabra</i> Vahl	3.5	8	24	0.438	1	1	0.064
24	Ching Charot	<i>Ophiopogon intermedius</i> D.Don	4.3	11	24	0.391	2	1.072	0.075
25	Yensil	<i>Oxalis debilis</i> Kunth	8	13	24	0.615	1	1	0.205
26	Heikru	<i>Phyllanthus emblica</i> L.	16.5	24	24	0.687	1	1	0.473
27	Sangbrei	<i>Pogostemon parviflorus</i> Benth.	19.3	24	24	0.804	2	1.072	0.693
28	Kekru	<i>Sapindus mukorossi</i> Gaertn.	13.1	17	24	0.770	1	1	0.420
29	Thaonam	<i>Senna tora</i> (L.) Roxb.	0.4	2	24	0.2	2	1.072	0.003
30	Urikshibi	<i>Vitex negundo</i> L.	20.5	24	24	0.854	1	1	0.729

Consensus from different Ethnobotanical Indices

Different ethnobotanical indices have their own criteria and methodologies and useful in their own context. Cultural significant plant can be estimated by taking consensus of different ethnobotanical indices.

Table 5: Comparason of different ethnobotanical indices for high ranking plants

Name of Plants	RFC	DCI	FL	Remarks
<i>Citrus aurantifolia</i>	1.000	1.192	58.33	Selected
<i>Vitex negundo</i>	1.000	0.729	100.00	Selected
<i>Pogostemon parviflorus</i>	1.208	0.693	75.862	Selected
<i>Senna tora</i>	0.167	0.003	100.00	
<i>Meyna spinosa</i>	0.375	0.058	100.00	
<i>Melia azaderach</i>	0.292	0.034	100.00	
<i>Euphrobia antiquorum</i>	0.250	0.024	100.00	
<i>Phyllanthus emblica</i>	1.250	0.473	46.67	

According to the consensus analysis, *Citrus aurantifolia*, *Vitex negundo* and *Pogostemon parviflorus* have comparative high values in most of the three ethnobotanical indices. Among these plants, *Citrus aurantifolia* is widely used in commercial soap and hand wash products in both domestic and international markets. It is also one of the widely studied plants in food, nutraceuticals and medicinal aspects. On the other remaining two plants of *Vitex negundo* and *Pogostemon parviflorus* are less used in commercial bath soap and herbal hand wash. Though there are some scientific literatures on the potential use of these two plants on bath soap and herbal hand wash, these two plants are selected for further phytochemical analyses. Prioritization of these two plants are because of limited resource and time constraints in the project.

Phytochemical Analysis

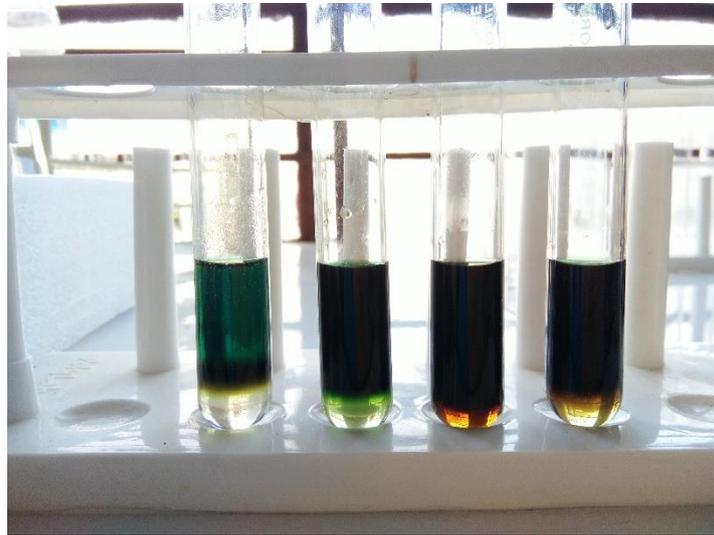
Phytochemical analyses were performed in the *Vitex negundo* and *Pogestemon parviflorus*. The methanolic extract of *Vitex trifolia* contains alkaloids, flavonoids, phenolic compounds, phytosterols, tannins and terpenoids. On the other hand, methanolic extract of *Pogestemon* contains alkaloids, flavonoids, phenolic compounds, phytosterols, saponin, annins, termpenoids and glycosides. The chloroform extract of *Vitex* contains only phytosterols, terpenoids and glycosides while that of *Pogestemon* contains phenolic compounds and glycosides only.



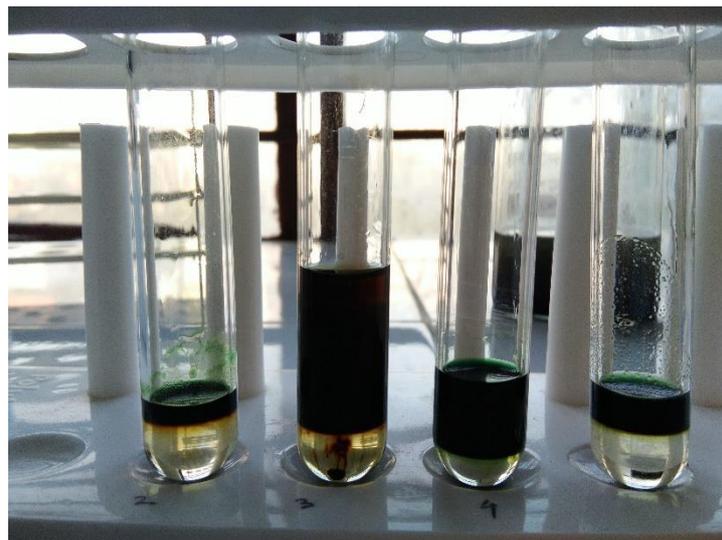
Powdered Plant parts



Plant Extract



Analysis in *Vitex negundo*



Analysis in *Pogostemon parviflorus*

Photographs showing the plant extracts and analyses.

Presence of different phytochemical compounds in *Vitex negundo* in different solvents were provided in following table (Table 4).

Table 6: Phytochemical groups present in different solvent extracts of *Vitex negundo*

Phytochemical	Tests	Chloroform	Hexane	Methanol	Water
Alkaloids	Dragendroff's	-	-	+	-
	Hager's	-	-	+	-
	Mayer's	-	-	+	-
	Wagner's	-	-	+	-
Flavonoids	Alkaline Reagent	-	-	+	-
	Ferric Chloride	-	-	+	+
	Lead Acetate	-	-	+	+
Phenolic Compounds	Ferric Chloride	-	-	+	+
	Lead Acetate	-	-	-	+
Phytosterols	Salkowski's	+	+	+	+
	Liebermann	-	+	-	+
Saponin	Froth	-	-	-	+
	Foam	-	-	-	+
Tannins	Ferric Chloride	-	-	+	+
Terpenids	Salkowski's	+	+	+	+
Glycosides	Keller Killani	+	+	-	+

Presence of different phytochemical compounds in *Pogostemon parviflorus* in different solvents were provided in Table 5.

Table 7 : Phytochemical groups present in different solvent extracts of *Pogostemon*

Phytochemical	Tests	Chloroform	Hexane	Methanol	Water
Alkaloids	Dragendroff's	-	-	+	-
	Hager's	-	-	+	-
	Mayer's	-	-	+	-

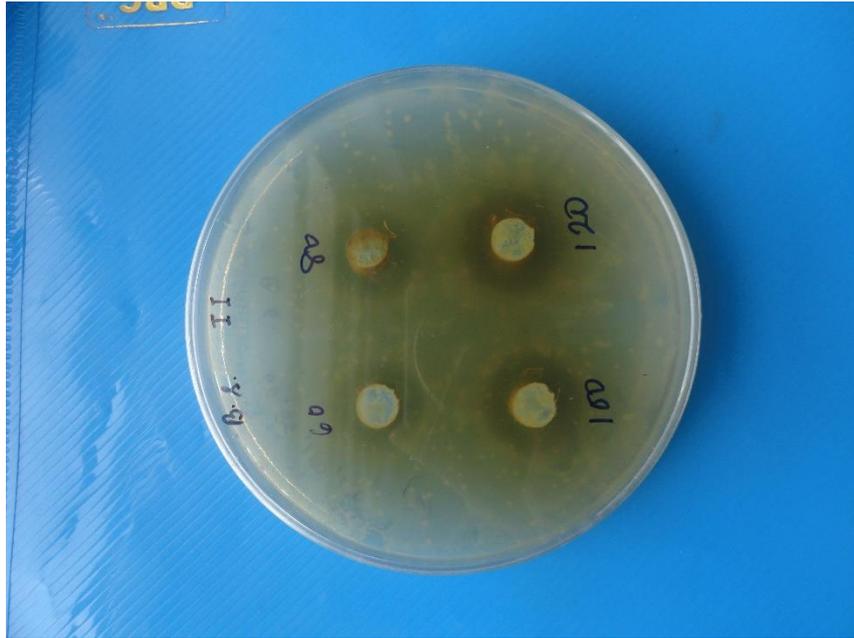
Phytochemical	Tests	Chloroform	Hexane	Methanol	Water
	Wagner's	-	-	+	-
Flavonoids	Alkaline Reagent	-	-	+	-
	Ferric Chloride	-	-	+	-
	Lead Acetate	-	-	+	+
Phenolic Compounds	Ferric Chloride	-	-	+	-
	Lead Acetate	+	-	+	+
Phytosterols	Salkowski's	-	+	+	+
	Libermann	-	+	-	+
Saponin	Froth	-	-	+	+
	Foam	-	-	+	+
Tannins	Ferric Chloride	-	-	+	-
Terpenids	Salkowski's	-	+	+	+
Glycosides	Keller Killani	+	+	+	+

Antimicrobial Studies

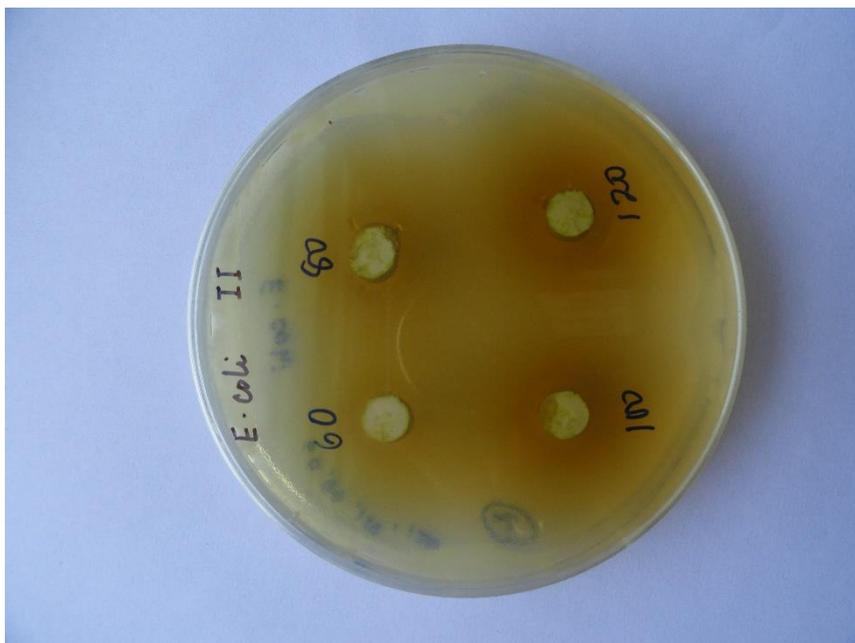
Methanolic extracts of *Vitex negundo* and *Pogestemon parviflorus* were tested for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *E. coli*. The results from the present study showed that the extracts have antibacterial activities against the tested organisms. However, the activities differed according to the tested organisms and the concentrations of extracts.

Table 8: Antibacterial activity of methanolic extract of *Vitex negundo* leaf (Zone of inhibition in mm)

Bacteria	Zones of Inhibition (mm) Methanolic extract <i>Vitex negundo</i> leaves			
	60 mg/ml	10 mg/ml	50 mg/ml	100 mg/ml
<i>Staphylococcus aureus</i>	12.2 ± 0.26	13.19 ± 0.18	13.43 ± 0.06	13.77 ± 0.15
<i>Escherichia coli</i>	10.37 ± 0.06	10.53 ± 0.058	10.93 ± 0.15	12.07 ± 0.15
<i>Pseudomonas aeruginosa</i>	17.06 ± 0.15	17.4 ± 0.2	18.6 ± 0.36	20.4 ± 0.7
<i>Bacillus subtilis</i>	10.76 ± 0.90	11.87 ± 0.45	12.27 ± 0.90	13.17 ± 0.65



Antibacterial study of *Vitex negundo* in *Bacillus subtilis* strain



Antibacterial study of *Vitex negundo* in *Escherichia coli* strain

Photograph of antibacterial study

Methanolic extracts of *Pogostemon parviflorus* were tested for antibacterial properties in different microbes. Antibacterial properties were shown in the following table 9. However, *Pogostemon parviflorus* did not exhibit antibacterial property against *Staphylococcus aureus* and *Escherichia coli* in the study.

Table 9: Antibacterial property of methanolic extract of *Pogostemon parviflorus* leaves

Bacteria	Zones of Inhibition (mm) Methanolic extract <i>Pogostemon parviflorus</i> leaves			
	60 mg/ml	10 mg/ml	50 mg/ml	100 mg/ml
<i>Staphylococcus aureus</i>	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-
<i>Pseudomonas aeruginosa</i>	6.3 + 0.17	6.57 + 0.21	6.63 + 0.23	6.83 + 0.12
<i>Bacillus subtilis</i>	3.83 + 0.35	4.23 + 10.15	4.77 + 0.12	5.07 + 0.15

Antifungal activities of both *Vitex negundo* and *Pogostemon parviflorus* were tested through their methanolic extract in *Candida* sp. The effective concentration in these two plants were different (Table 9 and 10)

Table 10: Antifungal activity of *Vitex negundo* methanolic extract

Fungus	Zone of Inhibition (mm) of <i>Vitex negundo</i> methanolic extract in <i>Candida</i>		
	10 mg/ml	20 mg/ml	30 mg/ml
<i>Candida</i> sp.	8.47 ± 0.31	10.03 ± 0.21	10.78 ± 0.10

Table 11: Antifungal activity of the *Pogostemon parviflorus* methanolic extract

Fungus	Zone of Inhibition (mm) of <i>Pogostemon parviflorus</i> methanolic extract		
	2.5 mg/ml	5 mg/ml	10 mg/ml
<i>Candida</i> sp.	8.63 ± 0.55	11.5 ± 0.5	17.17 ± 0.76

Apart from methanolic extracts of leaves, prepared herbal bath soaps were also checked for antibacterial property. In the analysis, *Vitex negundo* have more efficacy and spectrum in antibacterial property (Table 12)

Table 12: Antibacterial activity of herbal bath soap

Bacteria	Zones of Inhibition (mm) of formulated herbal bath soap (100 mg/ml)	
	<i>Vitex negundo</i>	<i>Pogostemon parviflorus</i>
<i>Staphylococcus aureus</i>	10.3 ± 0.1	-
<i>Escherichia coli</i>	9.3 ± 0.15	-
<i>Pseudomonas aeruginosa</i>	14.93 ± 0.38	4.97 ± 0.40
<i>Bacillus subtilis</i>	9.27 ± 0.5	2.64 ± 0.22

Antifungal properties of the herbal bath soap prepared from *Vitex negundo* and *Pogostemon parviflorus* were tested for analyzed by taking 100 mg/ml. Their activities are summarized in Table 13. *Pogostemon parviflorus* have comparatively more efficacy in anti-*Candida* properties.

Table 13: Antifungal activity (in mm) for bath soap

Fungus	Zones of Inhibition of bath soap prepared with <i>herbal</i> extract (100 mg/ml)	
	<i>Vitex negundo</i>	<i>Pogostemon parviflorus</i>
<i>Candida</i>	27.53 ± 0.59	38.2 ± 0.25

Antibacterial property of herbal hand wash

Antibacterial property of herbal hand wash prepared with *Vitex negundo* was tested with *Staphylococcus aureus* and *Bacillus subtilis* by dip well technique. The prepared hand wash have significant activity against selected species. *Staphylococcus aureus* and *Bacillus subtilis* have Zone of inhibitions of 8.13 ± 0.05 mm and 7.37 ± 0.12 mm respectively.

Stability Status

In the stability test, the prepared hand wash was found to be clear and homogeneous with pH value of 6.8 which is optimum condition for skin use. The stability study of formulaton was also performed and was found to be stable without phase separation. The pH of the soap was observed to range from 9.5 to 9.6. Total Fatty Matter was observed to be around 68%. The moisture content of the soap was found to be about 6.25%.

Conclusion, summarizing the achievements and indicating scope of future work.

The present study was focused on the probability and potential of plants traditionally used for hygienic purpose in the development of commercial soap and herbal hand wash. There are large resources of plants having various medicinal properties. Selection of plants from these wide resources are difficult.

To overcome that difficulty, an ethnobotanical survey was conducted to shortlist plants having aromatic, antibacterial and antifungal properties. Prioritization and selection were done by ethnobotanical survey, estimation of various ethnobotanical indices (RFC, FIC, FL and DCI). Consensus were taken from these ethnobotanical indices to target two plants – *Vitex negundo* and *Pogostemon parviflorus*. Phytochemical screenings were conducted on these two selected plants. There is difference in the presence of phytochemicals in these two plants. The methanolic extract of *Vitex trifolia* contains alkaloids, flavonoids, phenolic compounds, phytosterols, tannins and terpenoids. On the other hand, methanolic extract of *Pogostemon* contains alkaloids, flavonoids, phenolic compounds, phytosterols, saponin, tannins, terpenoids and glycosides. Antibacterial and antifungal properties were analyzed. There are differential antibacterial activities of these two plants. *Vitex negundo* have comparatively wider spectrum on the bacteria selected in the study. *Vitex negundo* have antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. On the other hand, *Pogostemon parviflorus* have antibacterial activity only against *Pseudomonas aeruginosa* and *Bacillus subtilis*. However, *Pogostemon parviflorus* have more antifungal activity in the study against *Candida* sp. In both herbal extracts and herbal soap preparations. With regard to the physical and organoleptic properties, *Pogostemon parviflorus* have more attractive odour and smell as compared to *Vitex negundo*. Soap prepared from these two plants have different activities giving rise to specialized uses. Soap based on *Vitex negundo* is preferred for antibacterial property while soap based on *Pogostemon parviflorus* is preferred for antifungal property.

Future Directions

1. Soap based on herbal mixture of *Vitex negundo* and *Pogostemon parviflorus* may be studied for antibacterial and antifungal properties to check whether there is supplementary and synergistic activity for both extracts.
2. Antibacterial and antifungal studies may be expanded by including more bacteria and fungi that cause dermatological diseases.
3. Stability, pH and viscosity of the soap and hand wash prepared from herbal mixture might be studied for production of more enhanced production.
4. Study may be conducted to test whether there is enhancement or reduction in the antibacterial and antifungal properties of the two plants when other plant parts or essential oils such as Lemongrass were added to the prepared mixture.
5. Antifungal and antibacterial properties on prepared soap may be extended to other remaining plants reported in the study.

Benefits accorded from the Project

Academic benefits:

- List of Research Publication- indicating Authors, Title of paper, Name of Journal, Volume, Page, Year.
 - Sanjoy Singh Ningthoujam, Geetabali Devi Leimapokpam, Sanjita Chanu Konsam, B. Koshoni Pekosii, Bidyapati Asem. Ethnomedicinal plant used in Manipur, North East India for cosmetic and skin diseases. - Communicated

- Scientists interacted with
 - Prof. Guruaribam Shantibala Devi, Department of Life Sciences, Manipur University
 - Prof. Potsangbam Kumar Singh, former Professor, Department of Life Sciences, Manipur University.

- Scientific manpower trained, indicating Names with qualifications and dates.
 - B. Kosoni Pekosii, M.Sc. Research Scholar, Manipur University
 - Asem Bidyapati Devi, M.Sc., Research Scholar, Manipur University
 - Sanjita Konsam, M.Sc., Research Scholar, Manipur University
 - Ashem Binarani Devi, Post graduate student, G.P. Women's College
 - Kh. Bidyarani, Post graduate student, G.P. Women's College
 - Hijam Soniya, Post graduate student, G.P. Women's College
 - Salome Thenneineng, Post graduate student, G.P. Women's College

- Non-Scientific manpower trained, indicating Names and dates.
 - K. Veteshore Sharma
 - S. Hemarjit Singh
 - W. Krishnamani Singh
 - M. Sandhyarani Devi

Contributions towards socio-economic development:

- Practical benefits resulting from the project outcome.
 - Documentation of the traditional handwash methods used in the state
 - Understand the antimicrobial properties of the selected plant extracts.
 - Understand the biochemical constituents of the selected plant extracts
 - Provide knowledge for developing skin-care products in non-hazardous manner.
New formulations and processing techniques evolved during the proposed project have a potential for production of eco-friendly, efficient and economical combination in the production of herbal skin care products.

- Specific benefits, if any, to the people of Manipur. Idea collected from the project have been utilised in the preparation of herbal-based hand-wash by the students for common usage. Though the original composition of the hand-wash have been maintained, such prepared hand wash have been supplemented with the herbal extracts.
- Patents taken/proposed, if any.
 - NO



Knowledge for preparing herbal hand wash acquired from the Project is shared with the students of the G.P. Women's College. Students are preparing their own hand wash using the herbal products



Knowledge acquired from the Project is shared with the non-technical personnel during the critical periods

Knowledge acquired from the Project are shared with technical and non-technical personnel after the completion of the Project.

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Document Reference No.

Date of Collection :

Data Acquisition Format for documenting Cosmetic and Hygienic Plants in Manipur.

Part I : Detail of Informant

Name :

Sex : Male/Female

Age : Years

Profession :

Knowledge on Plant : Healer/User/Other

Address :

- Village :
- Sub-division :
- District :

Level of education :

Training, if any :

Name of community :

Language :

Religion:

Acquisition of Knowledge :

Part II : Informant's Consent Agreement

I, do hereby agree to participate in this study with my full consent and conscience to disclose the details of Traditional Health and Hygiene Practices for documentation of plants. I declare to the best of my knowledge that the information provided here is true, accurate and complete.

Place :

Date :

Signature/Thumb Impression

Part III : Researcher's Declaration

The information collected will be used only for the research purpose and not in any undisclosed intention. We will not under any circumstances edit or tamper the information provided by the informant.

Signature of the Researcher

FORM GFR 19-A
(See Government of India's Decision (1) below Rule 150)
FORM OF UTILISATION CERTIFICATE

(to be submitted in duplicate to DST- Manipur)

Sl. No.	Letter No. & date	Amount (Rs.)	
	19/13/2017(R&D-Biotech)/DST/282 dated the 23rd October, 2017	Rs. 95,000/-	Certified that out of Rs. 95,000/- (Rupees ninetyfive thousand) of Grants-in-aid sanctioned during the year 2017 under the Ministry/Department letter given in the margin, and Rs. NIL on account of unspent balance of the previous year, a sum of Rs. 95,000/- (Rupees ninetyfive thousand) only has been utilised for the purpose for which it was sanctioned and that the balance of Rs. NIL remaining utilized at the end of the year has been surrendered to Government (vide No. dtd.....)/will be adjusted towards Grants-in-aid payable during the next year.
	Total :	Rs. 95,000/-	

2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly / are being fulfilled and that I have exercised the following check to see that the money was actually utilised for the purpose for which it was sanctioned.

Kinds of Checks exercised.

- 1 Acquisition Roll
- 2 Accounts & Bill
- 3 Field verification

Signature of Principal Investigator

Name : Dr. Ningthoujam Sanjoy Singh
Designation : Associate Professor
Date : 13 July 2020
Associate Professor
G.P. Women's College
Imphal

Signature of Accounts Officer

Name : Chingakham Sancho Devi
Designation : Accounts Officer
Date :
OFFICE OF THE PRINCIPAL
GOVERNMENT OF MANIPUR
G.P. WOMEN'S COLLEGE, IMPHAL

Counter Signature of Head of Institution

Name : Dr. Rajkumari Tamphasana
Designation : Principal, G.P. Women's College
Date :

Principal
G.P. Women's College, Imphal
Government of Manipur

STATEMENT OF EXPENDITURE

(As on 10 December, 2019)

(To be submitted in duplicate to DST, Manipur)

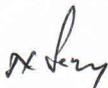
Name of the Project : **Studies on antibacterial and antifungal properties of herbal hand wash and bath soap using medicinal plants of Manipur**

Name of the Principal Investigator : **Dr. Ningthoujam Sanjoy Singh**

Name of the Institute : **Ghanapriya Women's College, Imphal**

DST Letter No. & date sanctioning the project : **19/13/2017(R&D-Biotech)/DST/282 dated the 23rd October, 2017**

Sl. No	Items	Amount sanctioned	Actual Expenditure	Variation (if any)	Remarks
1	Materials :	Rs. 25,000/-	Rs. 25,000/-	No	
2	Consumables :	Rs. 43,000/-	Rs. 43,000/-	No	
3	Project manpower :	Rs. 12,000/-	Rs. 12,000/-	No	
4.	Other Costs/contingencies	Rs. 15,000/-	Rs. 15,000/-	No	
	Total :	Rs. 95,000/-	Rs. 95,000/-		


Signature of Principal Investigator

Name : Dr. Ningthoujam Sanjoy Singh

Designation : Associate Professor

Date : 13 July 2020

Associate Professor
G.P. Women's College
Imphal

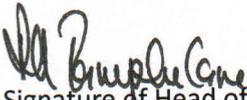

Signature of Accounts Officer

Name : Chingakham Sana Devi

Designation : Accounts Officer I/c

Date :




Counter Signature of Head of Institution

Name : Dr. Rajkumari Tamphasana

Designation : Principal, G.P. Women's College

Date :

Principal
G.P. Women's College, Imphal
Government of Manipur