Indian J. Applied & Pure Bio. Vol. 38(1), 324-338 (2023). **ISSN: 0970-2091**
A web of Science Journal

Phytochemical screening and TLC profiling of selected ethnomedicinal plants used by the Khasi Tribals of Meghalaya

Zoliansanga and R. Lalfakzuala

Microbiology Laboratory, Department of Botany, Mizoram University, Aizawl -796004 (India)
Address for Correspondence: Zoliansanga, Microbiology Laboratory, Department of Botany, Mizoram University, Aizawl -796004 (India)
E-mail: zlspautu@gmail.com

Abstract

In this investigation, collection and identification of Khasi ethnomedicinal plants growing in various sites of East Khasi Hill district of Meghalaya and its adjoining areas were carried out. Randomized approach was followed for the selection and collection of plant species. The phytochemical composition of the selected fifteen (15) ethno medicinal plants were analyse by phytochemical screening and TLC study. Phytochemical screening revealed variations and diversity in the content of phytochemical compounds, qualitatively, for the 15 selected medicinal plants investigated. The variations and diversity was observed not only between the family and genus, but also between the species. TLC profiling of all 15 extracts gives an impressive result that direct towards the presence of number of phytochemicals.

Key words : Phytochemical, TLC fingerprint, ethnomedicinal plants, Soxhlet apparatus, Assam Herbarium.

The use of medicinal plants for the treatment, prevention and cure of diseases accompanies man since the earliest civilizations[20]. The presence of significant secondary metabolites and the identification of biologically active compounds[9,16], makes the herbal alternative often effective for developing new therapeutic strategies for the treatment of infectious diseases[9,48]. This indicates that phytochemicals based approach on ethnopharmacological information is one of the important paradigms for new drug development and combinatorial chemistry[17]. There is no denying the fact that 70% of the modern medicines had been derived from the plants/ natural products or had their origin in them. Between 1981 and 2002, of the 877 new molecules introduced into the pharmaceutical market, 49% were substances isolated from natural products[13]. In 2010, herbal medicines

accounted for approximately 15% of the capital of the world pharmaceutical industry[40] .

Ethnomedicines and medicinal plants used by the Khasi tribe in Meghalaya have attracted attention of various scientists and researchers. However, most of these studies are restricted to documentation of uses of plants. Scientific analysis and evaluation like phytochemical screening, cytotoxicity activities and antimicrobial activities is the need of the hour but research work in this context is very scarce and limited. Hence, efforts are made in this direction in the form of phytochemical screening and TLC analysis of bio-active compounds.

Sample collection and identification of medicinal plants :

For the selection of the plant species to study, randomized approach suggested by Albuquerque and Hanazaki[2] was followed.

Fifteen (15) ethnomedicinal plants viz., *Achyranthes aspera* L., *Acmella paniculata* (Wall.ex DC) R.K. Jansen, *Ageratum conzyoides* (L) L., *Bidens pilosa* L., *Centella asiatica* (L.) Urb., *Garcinia pedunculata* Roxb.ex Buch-Ham, *Gaultheria fragrantissima* Wall, *Hibiscus sabdariffa* L., *Houttuynia cordata* Thunb, *Lantana camara* L., *Piper attenuatum* Buch-Ham ex Miq, *Potentilla lineata* Trevir., *Prunella vulgaris* L., *Sonchus oleraceus* (L) L., *Sonchus palustris* L. were selected and collected from various sites in East Khasi Hill district of Meghalaya and its adjoining areas. All plants were identified and authenticated by the Botanical Survey of India, Shillong.

Preparation of plant extracts :

The non-infected mature leaves (of *Achyranthes aspera* L., *Ageratum conzyoides* (L) L., *Bidens pilosa* L., *Centella asiatica* (L.) Urb.,*Gaultheria fragrantissima* Wall, *Hibiscus sabdariffa* L., *Houttuynia cordata* Thunb, *Lantana camara* L., *Prunella vulgaris* L., *Sonchus oleraceus* (L) L., *Sonchus palustris* L.), flower of *Acmella paniculata* (Wall.ex DC) RKJansen, fruit of *Garcinia pedunculata* Roxb.ex Buch-Ham, and root stock of *Potentilla lineata* Trevir. were collected, air-dried in shade and grinded. The powders were individually extracted in methanol by using Soxhlet apparatus. The individual extracts were filtered using a Whatmann filter paper No. 42 (125 mm) and concentrated using a rotary evaporator.

Phytochemical screening :

The presence of various phytochemicals in mature leaves (of *Achyranthes aspera* L., *Ageratum conzyoides* (L) L., *Bidens pilosa* L., *Centella asiatica* (L.) Urb.,*Gaultheria fragrantissima* Wall, *Hibiscus sabdariffa* L., *Houttuynia cordata* Thunb, *Lantana camara* L., *Prunella vulgaris* L., *Sonchus oleraceus* (L) L., *Sonchus palustris* L.), flower of *Acmella paniculata* (Wall.ex DC) RKJansen, fruit of *Garcinia pedunculata* Roxb.ex Buch-Ham, and root stock of *Potentilla lineata* Trevir. were determined as per standard methods described by Brain and Turner[8] and Evans[19] .

Alkaloid :

Alkaloids (Dragendorff Test) – About 2 ml methanol extract of plant material powder

was mix with 1% HCl and then keep in a boiling water bath for 2 minutes. Appearance of brownish-red precipitate/orange precipitate on addition of few drops of Dragendorff reagent respectively indicated the presence of alkaloids.

Flavonoid :

sFlavonoids (Shinoda Test) – To the ethanol extract (2ml) of plant material, conc. HCl was added and then followed by addition of magnesium ribbon. Formation of pink-red color indicated the presence of flavonoids.

Saponin :

Saponin (Foam Test) - Appearance of froth and its persistence on addition of 5 ml distilled water to 0.5 ml of water extract indicated presence of saponin.

Tannin :

Tannins (Ferric Chloride Test) - Formation of blue-black precipitate/brownish green on addition of 2 ml of 5% $FeCl_3$ to 2 ml of water plant extract indicated the presence of Tannins.

Steroid :

Steroids (Liebermann-Burchard reaction) - Appearance of blue-green ring on addition of 2 ml acetic anhydride and conc. H_2SO_4 to 2ml chloroform extract of plant material indicated the presence of terpenoids.

Cardiac glycoside :

Cardiac glycosides (Keller-Kiliani test) - Add 1 ml glacial acetic acid to 2 ml of methanol plant extract, followed by the addition of $FeCl_3$ and conc. H_2SO_4. Appearance of green-blue color indicated the presence of cardiac glycosides.

Thin layer chromatography :

Phytochemical analysis by TLC were carried out by following the method of Harborne[24]. TLC is a quick, sensitive, and inexpensive technique, which separates the number of components present in any non-volatile complex mixture or plant sample using a suitable solvent for separation of different components.

For the separation of different phytochemical compounds in the methanol extract of 15 ethno-medicinal plants, the extract was spotted manually using a capillary tube on pre-coated silica gel 60F254 TLC plates (15X5 cm with 3 mm thickness). The spotted plates were developed in the appropriate solvent system. Different compositions of the mobile phase were tried in order to separate and obtain better resolution of the different secondary metabolites and to detect the suitable mobile phase as per the method of Wagner *et. al.*[49].

The various combinations of mobile phase allowed the separation of different components of the plant extracts that had distinct Rf values and develop TLC fingerprint profile. For each extract, six different solvent systems were used as developing solvents. These were Chloroform : Methanol (4 : 2), Ethyl Acetate : Methanol : Water : Glacial Acetic Acid (1.35 : 0.5 : 0.5 : 0.5), Chloroform: Methanol (6 : 1), Methanol : Water (6 : 4), Hexane : Ethyl Acetate (4 :1) and Ethyl Acetate : Methanol : Water (8.1 : 1.1 : 0.8). After the separation of phytochemical constituents, the spraying reagents namely,

Table-1. Selected 15 (fifteen) Ethnomedicinal plants of Meghalaya

S. No.	Medicinal plants (Family)	Local name	Parts used	Indigeneous uses	BSI Assam Herbarium Accession No.
1	*Achyranthes aspera* L. (Amaranthaceae)	Sohberthid	Leaves	Piles, Diuretic, Boils, Abscess, Painful delivery, Antifertility, Rabies, Antidiabetic, Pneumonia, Menstrual disorders, Insect stings and Snakebite[6]	96684
2	*Acmella paniculata* (Wall. ex DC) R.K.Jansen (Asteraceae)	Jasat	Flowers	Brushing teeth with inflorescence relieves toothache. Crushed inflorescence is put into aching tooth to get relief from toothache[25]	96572
3	*Ageratum conzyoides* (L.) L.(Asteraceae)	Kynbat Myngai	Leaves	Leaf paste is applied on cuts and wounds[25] Paste of leaves and lime is applied to cuts acting as homeostatic[29]	96565
4	*Bidens pilosa* L. (Asteraceae)	Sohberthid	Leaves	Leaves are grind and the juice is taken against gastric disorders [25]Leafs of *Bidens pilosa* alongwith leaf of *Drymaria diandra* are used as antidote for snakebite[30]	96566
5	*Centella asiatica* (L.) Urb.(Apiaceae)	Khliangsyiar	Leaves	Leaves are taken as raw to cure blood deficiency and helps in purification of blood. Whole plant is also taken for blood dysentery[25] Whole plant is ground and the juice is squeezed out of it and is used to get relieve from both diarrhea and dysentery[31]	96567
6	*Garcinia pedunculata*	Sohdanei	Fruit	The fruit is finely powdered after sun dried and	96682
	Roxb.ex Buch-Ham (Clusiaceae)			used for dysentery[31]	
7	*Gaultheria fragrantissima* Wall (Ericaceae)	Lathynrait	Leaves	Paste made from leaves is applied to bone fractures and sprains[29] Leaf juice of *Gaultheria fragrantissima* Wall., *Clerodendron colebroo kianum* Walp. and *Eucalyptus iarrhea* Hook is massaged over the body of persons suffering from rheumatism and paralysis. In case of migraines and pneumoniathe juice is applied over the	96574

				forehead. Powdered leaf mixed with water is taken orally to treat diarrhoae[31]	
8	*Hibiscus sabdariffa* L. (Malvaceae)	Jajew	Leaves	Leaf paste applied for boils[6]	96685
9	*Houttuynia cordata* Thunb. (Saururaceae)	Jamyrdoh	Leaves	Leaves are eaten raw for blood purification and also applied to treat sores and boils [29] Leaf juice is taken for cholera, dysentery, curing of blood deficiency and purification of blood[25] Roots and leaves are eaten raw to treat amoebic Dysentery[31] Stomachache, Cholera, Dysentery and Diuretic[6]	96568
10	*Lantana camara* L. (Verbenaceae)	Dieng Sohpang khlieh	Leaves	Used as remedy for whooping cough, sprain, chronic inflammable skin and treatment for fever and jaundice[37]	96569
11	*Piper attenuatum* Buch-Ham. ex Miq. (Piperaceae)	Sohmarit	Fruit	Anti-Blennorliagic, Stomachic, Dyspepsia, Malaria, Haemorrhoids, Delirium, Tremors and Migraine[6]	96681
12	*Potentilla lineata* Trevir. (Rosaceae)	Lynniang	Root	The rootstocks are believed to strengthen the gums and teeth and also reported to be used in diarrhea. Slices of the rootstock are chewed with betelnut, lime and betel leaf locally. It has been reported to be antidiabetic[15] Roots are edible and effective against high blood pressure[25]	96573
13	*Prunella vulgaris* L. (Lamiaceae)	Jahynwet	Leaves	Tender leaf paste is applied on cuts and wounds for quick healing[25]	96570
14	*Sonchus oleraceus* (L) L. (Asteraceae)	Jhurkthang	Leaves	Jaundice, Diuretic, Diaphoretic, Antiseptic, Coughs, Phthisis, Bronchitis, Asthma, Pertusis and Demulcent[6]	96571
15	*Sonchus palustris* L. (Asteraceae)	Jalynshir	Leaves	Mainly eaten as vegetable leaves but are claim to be useful for treatment of high blood pressure and diabetis[26]	96683

*Plants are arranged in alphabetical order of their names

Dragendroff reagent (for solvent system I), 5% Ferric Chloride solution (for solvent system II & IV), Vanillin Sulphuric Acid (for solvent system III & VI), Vanillin Phosphoric Acid (for solvent system V) were used to detect the bioactive compounds. After derivatization with proper spraying reagent, the bands/spots developed were noted and Rf values were calculated by using the following formula:

Retention time (Rf) = Distance travelled by the solute/Distance travelled by the solvent

The Rf value is a measure of the distance a compound travels. In each case the TLC spots were visualized under day light or UV light (254 nm or 356 nm).

Collection, identification and documentation of selected medicinal plants :

This present work is the result of investigations, carried out from September, 2016 to February, 2021 in the East Khasi Hills district and adjoining areas.

Fifteen (15) ethno medicinal plants were selected and collected for phytochemical and TLC analysis (Table-1). The voucher herbarium specimen were identified and authenticated by Botanical Survey of India, and deposited in the Assam Herbarium of BSI, Eastern Circle, Shillong, Meghalaya.

Qualitative phytochemical test :

The result of the phytochemical group tests for extract of 15 (fifteen) medicinal plants is given in Table 2.The result of the phytochemical group test revealed that there is great diversity in the phytochemical content of various species of plants.

Table-2. Preliminary phytochemical group tests of the crude extract of the fifteen (15) medicinal plants

S.N	Medicinal plants	Alkaloid	Flavonoid	Saponin	Tannin	Steroid	Cardiac glycoside
1	*Achyranthes aspera* (leaves)	+	-	+	+	+	+
2	*Acmella paniculata* (flowers)	+	-	-	-	+	-
3	*Ageratum conzyoides* (leaves)	+	-	-	-	+	+
4	*Bidens pilosa* (leaves)	-	+	+	-	+	-
5	*Centella asiatica* (leaves)	+	+	-	+	+	+
6	*Garcinia pedunculata* (fruit)	-	+	-	-	-	-
7	*Gaultheria fragrantissima* (leaves)	-	-	-	+	+	-
8	*Hibiscus sabdariffa* (leaves)	-	+	-	-	+	+
9	*Houttuynia cordata* (leaves)	+	+	-	-	+	-
10	*Lantana camara* (leaves)	+	+	+	+	+	+
11	*Piper attenuatum* (fruit)	+	-	-	-	+	-
12	*Potentilla lineata* (roots)	+	+	-	+	+	-
13	*Prunella vulgaris* (leaves)	+	+	+	+	+	-
14	*Sonchus oleraceus* (leaves)	+	+	+	+	+	-
15	*Sonchus palustris* (leaves)	+	+	-	+	+	-

Note: + (Positive) = present - (Negative) = absent.

Thin layer chromatography :

The present study was oriented towards the phytochemical screening of the 15 ethno-medicinal plants and development of TLC fingerprints. The results of Thin Layer Chromatography are presented in Table-3. Clear separated resolved bands/spots were observed in three solvent systems *i.e.*, Chloroform: Methanol (6:1), Hexane: Ethyl acetate (4:1) and Ethyl acetate: Methanol: Water: Glacial acetic Acid (1.35:0.5:0.5:0.5) after being sprayed with reagent. Therefore, they are recommendable as a solvent system for further analysis.

The evaluations of various plants extract showed presence of different bioactive compounds as indicated by varying number of spots on a TLC plate and different Rf values (Table-3). TLC fingerprint profiles of methanol extract of 15 ethno-medicinal plants in solvent systems III and V are shown in Figure 1 and 2, respectively.

Table-3. Thin Layer Chromatographic study of methanol extract of the fifteen (15) medicinal plants

SN	Medicinal Plants	Rf value in different Developing Solvent Systems					
		$CHCl_3$: MeOH 3:2 (Solvent systemI)	EA : MeOH : H_2O : GAA 1.35:0.5:0.5:0.5 (Solvent system II)	$CHCl_3$: MeOH 6:1 (Solvent system III)	MeOH: H_2O 6:4 (Solvent system IV)	Hx:EA 4:1 (Solvent system V)	EA : MeOH : Hx 8.1:1.1:0.8 (Solvent system VI)
1	*Achyranthes aspera* (leaves)	No spot	0.99,0.76 0.60	0.88	0.84	0.95,0.84 0.09	0.87 0.83
2	*Acmella paniculata* (flowers)	No spot	0.85	0.62,0.54	No spot	No spot	No spot
3	*Ageratum conzyoides* (leaves)	0.64	0.75	0.63,0.54 0.50,0.44	0.92	0.96,0.64	0.93
4	*Bidens pilosa* (leaves)	0.56	0.72	0.63	0.92	0.87,0.71	0.93
5	*Centella asiatica* (leaves)	0.11	0.65	0.84,0.67 0.57,0.46 0.41,0.15	0.83	0.95,0.48	No spot
6	*Garcinia pedunculata* (fruit)	No spot	0.64	0.18,0.20	0.51	0.95,0.90 0.48	No spot
7	*Gaultheria fragrantissima* (leaves)	No spot	0.95	0.82,0.67	0.84	0.96,0.17	0.60
8	*Hibiscus sabdariffa* (leaves)	0.67,0.52 0.49	0.91	0.60,0.52	0.68,0.64	0.98,0.94 0.75,0.64 0.59,0.53 0.48,0.35	0.90 0.77
9	*Houttuynia cordata* (leaves)	0.14	0.65,0.75 0.85	0.80,0.41 0.14	0.86,0.74 0.51	No spot	0.87,0.35 0.51
10	*Lantana camara* (leaves)	0.61,0.57 0.55,0.53 0.51	0.91	0.59,0.520 .47,0.350. 29,0.270.22	No spot	0.98,0.94 0.88,0.75 0.60,0.50	No spot

					0.43,0.35 0.28,0.19 0.10	
11 *Piper attenuatum* (fruit)	0.75	0.96	0.92	No spot	0.96,0.88 0.77,0.56 0.44,0.32 0.09	0.77 0.54
12 *Potentilla lineata* (root)	No spot	0.82	0.62	No spot	0.98,0.94 0.50,0.34 0.28,0.13	No spot
13 *Prunella vulgaris* (leaves)	0.57,0.54 0.50	0.61	0.66,0.51 0.50,0.43	0.90	0.93,0.13	0.93 0.49 0.20
14 *Sonchus oleraceus* (leaves)	No spot	0.41	0.70,0.66 0.62,0.57	No spot	No spot	No spot
15 *Sonchus palustris* (leaves)	0.08	0.68	0.87,0.73 0.66,0.50 0.14	0.86	0.98,0.94 0.65,0.55 0.48,0.33 0.31,0.25	0.89 0.81 0.72 0.61

Note : Hexane = Hx, ethyl acetate = EtOAc, chloroform = $CHCl_3$, methanol = MeOH, water = H_2O, and glacial acetic acid = GAA.

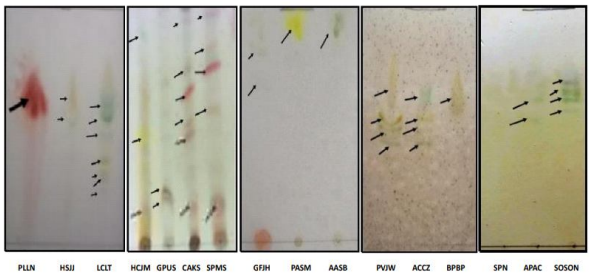

Figure 1. TLC fingerprint / profile of methanol extract of 15 ethnomedicinal plants in Solvent Systems - III.

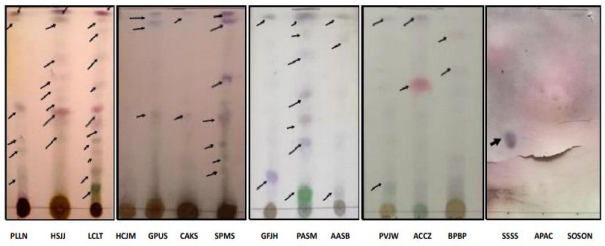

Figure 2. TLC fingerprint / profile of methanol extract of 15 ethnomedicinal plants in Solvent Systems - V.

Note: PLLN-*Potentilla lineata*; HSJJ-*Hibiscus sabdariffa*; LCLT-*Lantana camara*; HCJM-*Houttuynia cordata*; GPUS-*Garcinia pedunculata*; CAKS-*Centella asiatica*; SPMS-*Sonchus palustris*; GFJH-*Gaultheria fragrantissima*; PASM-*Piper attenuatum*; AASB-*Achyranthes aspera*; PVJW-*Prunella vulgaris*; ACCZ-*Ageratum conzyoides*; BPBP-*Bidens pilosa*; APAC-*Acmella paniculata*; SOSON-*Sonchus oleraceus;* CFCF-*Caffeine;* QCT-*Quercetin;* SPN-*Saponin;* TATA-*Tannic acid;* SSSS- *Stigmasterol;* DGDG-*Digoxin.*

In the investigation of medicinal plants, a relevant moment, which set the course of the work and its impact on all points of view, was the criterion used for the selection of the plant species to study. In the present study, random approach was followed in the selection of 15 medicinal plants. The randomized investigation involves random selection and collection of plant species for study, according to the plant availability. When carried out in regions with high diversity and endemism, the probability of finding novel substances, bioactive or not, is certainly higher in this type of selection[41] It is an indispensable approach, once it can demonstrate the potential of different plant species that had never been investigated. According to Souza Brito[46], this type of selection provides an endless source of new structures, since nature is a vast chemical laboratory.

Phytochemical screening revealed variations and diversity in the content of phytochemical compounds, qualitatively, for the 15 selected medicinal plants investigated. The variations and diversity was observed not only between the family and genus, but also between the species.

Alkaloids were found to be present in ten (10) of the medicinal plants (*Achyranthes aspera, Acmella paniculata, Ageratum conzyoides, Centella asiatica, Houttuynia cordata, Lantana camara, Piper attenuatum, Potentilla lineata, Prunella vulgaris* and *Sonchus oleraceus*). Alkaloid have a wide range of pharmacological activities such as antiasthma, antimalarial, anticancer, cholino-mimetic, vasodilatory, antiamyhyrithic, analgesic, antibacterial and anti-hyperglycemic activities[14]. Some alkaloids have been known to possess psychotropic and stimulant activities and have been used as recreational drugs and entheogenic rituals[23]. Alkaloids have great antimicrobial activity against bacterial pathogens such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*[35].

Some of the alkaloids such as morphine and cordine have been found to be active not only against bacterial and fungal pathogens but also trypanosomes and plasmodia[42]. Some of the Alkaloids found in dietary food materials have also been found to contain microbiocidal and antidiarrheal effect in the small intestines where they show the ability to intercalate with the microbial genetic material[43]. Other studies carried out on alkaloids extracted from a variety of medicinal plants in Nigeria showed a great antimicrobial activity against both Gram-negative and Gram-negative bacteria and also showed great antifungal activity[22].

Flavonoid were detected in nine (9) of the medicinal plants (*Bidens pilosa, Centella asiatica, Garcinia pedunculata, Hibiscus sabdariffa, Houttuynia cordata, Lantana camara, Potentilla lineata, Prunella vulgaris* and *Sonchus oleraceus*). In plants, flavonoid are responsible for floral pigmentation, ultraviolet ray's filtration in higher plants and symbiotic nitrogen fixation. They are also known to have inhibitory activities against organisms that cause plant diseases for example *Fusarium oxysporum*[21]. Flavonoids have been known to possess antimicrobial activity against bacterial, fungal and viral

microorganisms[12]. They are usually known for their antimicrobial activity of inhibiting the synthesis of the nucleic acids, tampering with the integrity of the cytoplasmic membrane function and the energy metabolism process. Flavonoids from some medicinal plants have been found to inhibit the synthesis of the nucleic acids, cause permeability of the inner bacterial membrane and a dissipation of the membrane potential of Gram negative and Gram positive bacteria[14]. Some of the bioactive components that have been isolated from flavonoids have been found to contain antifungal, antibacterial and insecticidal activities[1]. Previous studies carried out have shown that when mixed with antibiotics they have synergistic activity and suppress many pathogenic microorganisms in numerous in vitro and in vivo studies[50]. Additional in vivo studies have shown that flavonoids can be used as pharmaceutical drugs for bacterial infections or through the dietary intake to offer protection against infection[51].

In this research, saponin was found to be present in five (5) of the medicinal plants (*Achyranthes aspera, Bidens pilosa, Lantana camara, Prunella vulgaris* and *Sonchus oleraceus*). Saponins are also considered as one of the natural antimicrobial products that make up the defense system of the plants and some can be beneficial rather than harmful to animals[18]. There has been evidence of the presence of saponins in traditional medicine preparations where the administration is through oral means that is expected to lead to the hydrolysis of glycosides from terpenoids[4]. Studies carried out have shown medicinal plant extracts fractions rich in saponins are effective against microorganisms such as *Escherichia coli, Salmonella typhi, Aeromonas hydrophilia* and other fungal pathogens such as *Candida albicans*[15]. Saponins antimicrobial activity is attributed mainly to its capability of lysing microorganism's membranes rather than the surface tension of the extracellular medium[4]. Apart from antimicrobial activity, saponins have shown other biological properties with its cytotoxic activity on cancer or tumor cells being considered the most important one[50] Other plants are known to produce steroidal saponins for example cholestane glycosides which are known to have a broad spectrum of biological activity such as cytotoxic activity, antifungal, antibacterial and in vivo antitumor activities[33].

Tannin was detected in eight (8) of the medicinal plants (*Achyranthes aspera, Centella asiatica, Gaultheria fragrantissima, Lantana camara, Potentilla lineata, Prunella vulgaris, Sonchus oleraceus* and *Sonchus palustris*). Thus, from the result of this research, the leaves of *Achyranthes aspera, Centella asiatica, Gaultheria fragrantissima, Lantana camara, Prunella vulgaris, Sonchus oleraceus* and *Sonchus palustris* and root of *P. lineata* may be an ideal sources for tannin extraction. Tannins are generally found in plants and they are thought to function as chemical defenses against pathogens and herbivores[23]. They have been commercially used primarily in the preservation of leather, making glue stains and mordant[28]. It has also been used in the vegetable industry in different concentration in pickling process to provide protection against bacteria, mold, and yeasts[3]. Antimicrobial activity of tannins has been tested in various fields of medicine providing positive results such as antioxidant activities, anticarcinogenic activities and antimutagenic properties[34]. Tannins have been used in

inhibiting the growth of many fungi, yeasts, bacteria and viruses[10]. Some of the bioactive compounds of tannins such as catechin and pyrogallol found in vegetable tannins have been found to be toxic to microorganisms[12]. Tannins have been found not only effective against pathogenic microbes but also have a significant value as a cytotoxic and an antitumor agent[27].

Out of the fifteen medicinal plants, fourteen (14) of them (*Achyranthes aspera, Acmella paniculata, Ageratum conzyoides, Bidens pilosa, Centella asiatica, Gaultheria fragrantissima, Hibiscus sabdariffa, Houttuynia cordata, Lantana camara, Piper attenuatum, Potentilla lineata, Prunella vulgaris, Sonchus oleraceus* and *Sonchus palustris*) have steroid as their phytochemical constituents. Steroids are mainly used to treat reproductive complications such as treatment of venereal diseases, used during pregnancy to ensure an easy delivery, as well as to promote fertility in women and libido in men. They also act as sex hormones derivatives, (for example, they can be metabolized to either androgen or estrogen-like substances) and hence they are potential source of contraceptives[44]. They are also anti-microbial, analgesic, anti-inflammatory, and of use in treating stomach ailments and in decreasing serum cholesterol levels[39]. They have also been indicated as potent inhibitors of macrophage activation, blocking the production of pro-inflammatory cytokines and LPS-induced lethality and therefore they have potential use as immunosuppressive agents especially the physalins[45].

Cardiac glycoside were detected in five (5) of the medicinal plants (*Achyranthes aspera, Ageratum conzyoides, Centella asiatica, Hibiscus sabdariffa* and *Lantana camara*). Therefore, they could be exploited for their medicinal properties. Cardiac glycosides (also called cardenoloids) are used in treatment of congestive heart failure, whereby they inhibit Na+/K+-AT Pase pump that causes positive ionotropic effects and electrophysiological changes. This strengthens heart muscle and the power of systolic concentration against congestive heart failure[39]. They are also used in treatment of atrial fibrillation, flatter, and they acts as emetics and as diuretics[5].

The non-detection of certain phytochemicals in the medicinal plants does not absolutely rule out their presence in the plants. The phytochemicals in the plant material could be below the limit of detection, or they are insoluble in the solvent used for the extraction. Another important factor to be considered is the part of the plant used for the extraction of the phytochemicals since secondary may not be equally distributed in the plant organs. The solvent of plant extraction has an important effect on biologically active content but there are contradicting findings. For instance, while Cheung *et al.*,[10] reported that methanol can extract the higher amount of phenolic compounds than aqueous extract, Mohaddese *et al*.[38] showed that the highest amount of phenolic is present in aqueous extract but the total flavonoid content is higher in the methanol extract. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. This therefore underscores the need to try as much solvents as possible in

screening plant parts for phytochemicals. Diversity and difference in the amount of phytochemicals content can be attributed to many factors like growth conditions (solar radiation, temperature, precipitation and relative humidity), genetic inbuilt, *etc*.[24].

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. The quantitative amount varies from plant to plant depending upon the variety, processing, cooking and growing conditions[11].

Thin layer chromatography (TLC) is a fast, efficient and inexpensive technique used to determine the number of components in a mixture, verify the identity and purity of a compound, monitor the progress of a reaction, determine the solvent composition for preparative separations, and analyze the fractions obtained from column chromatography. TLC results are reliable and reproducible[32].

TLC profiling of all 15 extracts gives an impressive result that direct towards the presence of number of phytochemicals. Various phytochemicals gives different Rf values in different solvent system. Compound showing high Rf value in less polar solvent system have low polarity and with less Rf value have high polarity. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by Column Chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the Rf values of compounds in different solvent system[47].

The plants synthesize several chemicals for various purposes and these chemicals have medicinal and healing effects in humans. Analysis of chemical components of plants is useful in new drug development and also synthesis of some complex chemicals for medical use. The phytochemical analysis of these plants indicates that the traditional healers though may be ignorant about their phytochemical constituents, certainly knew their beneficial effects in the human healthcare.

References :

1. Abdel Ghani, S.B., P.J. Mugisha, J.C. Wilcox, E.A. Gado, E.O. Medu, A.J. Lamb, and R.C. Brown, (2013). *Journal of Synthetic Communications*. *43*(11): 1549-1556. DOI:10.1080/00397911.2011.647222
2. Albuquerque, N.P., and N. Hanazaki, (2006). *Brazilian Journal of Pharmacognosy, 16* (Supl.): 678-689. DOI:10.1590/S0102-695X2006000500015
3. Andrade, R.G., L.T. Dalvi, J.M.C. Silva, G.K. Lopes, A. Alonso, and M. Hermes-Lima, (2005). *Archives of Biochemistry and Biophysics, 437*(1), 1-9. DOI:10.1016/j.abb.2005.02.016
4. Asl, M.N., and H. Hosseinzadeh, (2008). *Journal of Phytotherapy Research, 22*(6): 709-724. DOI: 10.1002/ptr.2362
5. Awoyinka O.A., I.O. Balogun, and A.A.

Ogunnowo (2007). *Journal of Medicinal Plants Research, 1:* 063-065.

6. Bidyasagar Singh, Tripathi, S.K, and B.P. Mishra, (2019) Traditional Knowledge on Medicinal Plants Used in Khasi Hills District of Meghalaya. Medicinal Plants of India: Conservation and sustainable use (Editors: S. K. Tripathi Kalidas Upadhyaya Nagaraj Hegde). 177-193
7. Blankenship, J.D., J. B. Houseknecht, S. Pal, L.P. Bush, R.B. Grossman, and C.L. Schardl, (2005). *Journal of Chemistry and Biochemistry. 6*(6): 1016-1022. DOI: 10.1002/cbic.200400327.
8. Brain, K.R., and T.D. Turner, (1975). The practical evaluation of phytopharmaceuticals, Wrightscience technical, 1st Ed. Bristol Britain.144.
9. Calixto, .J.B. (2005) *Brazilian Journal of Medical and Biological Research, 33*(2): 179-89.
10. Chung, K.T., T.Y. Wong, C.I. Wei, Y.W. Huang and Y. Lin (1998). *Critical Reviews in Food Science and Nutrition. 38*(6): 421-464. DOI:10.1080/10408699891274273
11. Costa M.A., Z.Q. Zia, L.B. Davin, and N.G. Lewis (1999). Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, ed. JT Romeo, New York, 67-87
12. Cowan, M.M. (1999). *Journal of Clinical Microbiology Reviews. 12*(4): 564-582. DOI: 10.1128/CMR.12.4.564
13. Cragg, G.M., and D.J. Newman, (2013) *Biochim Biophys Acta 1830:* 3670-3695. DOI: 10.1016/j.bbagen.2013.02.008
14. Cushnie, T.T., and A.J. Lamb, (2014). *International Journal of antimicrobial agents. 44*(5): 377-386. DOI: 10.1016/j.ijantimicag.2014.06.001.
15. Deshpande, S., S. Kewatkar, and V. Paithankar (2013). *International Current Pharmaceutical Journal. 2*(4): 85-87. DOI:10.3329/icpj.v2i4.14056
16. Duarte, M.C.T. (2004) *Revista Brasileira de Farmacognosia, 14*(1): 06-08.
17. Duraipandiyan, V., Ayyanar, M., and S. Ignacimuthu (2006) *BMC Complementary Alternative Medicine 6 :* 35. DOI: 10.1186/1472-6882-6-35
18. Edeoga, H.O., D.E. Okwu, and B.O. Mbaebie, (2005). *African Journal of Biotechnology. 4*(7): 685- 688. DOI: 10.5897/AJB2005.000-3127
19. Evans, W.C. (1966). Trease Evans Pharmacognosy. 14th Ed, London: WB Saunders Ltd, 119-159. DOI:10.1177/096032719701600311
20. Firmo, W.D.C.A. (2011) Historical background, popular usage and scientific conception on medicinal plants. Research Notebook, v.18.
21. Galeotti, F., E. Barile, P. Curir, M. Dolci, and V. Lanzotti, (2008). *Journal of Phytochemistry Letters. 1*(1): 44-48. DOI:10.1016/j.phytol.2007.10.001
22. Garba, S., and S.O. Okeniyi, (2012). *Journal of Microbiology and Antimicrobial Agents. 4*(3): 60-63. DOI:10.5897/JMA11.081
23. Gedir, J.V., P. Sporns, and R.J. Hudson, (2005). *Journal of Chemical Ecology. 31*(12): 2761-2773. DOI: 10.1007/s10886-005-8392-1
24. Harborne, J.B. (2005). Phytochemical methods – A guide to modern technique

of plant analysis 3rd edition, New Delhi: Springer Pvt Ltd

25. Hynniewta, S.R. and Yogedra Kumar, (2008). Herbal remedies among the Khasi traditional healers and village folks in Meghalaya. Indian Journal of Traditional Knowledge. pp 581-586.
26. Hynniewta, S. R. (2010). Ethnobotanical studies in Khasi hills, Meghalaya. Unpublished PhD Thesis.
27. Joshi, Nupur., Shashank Bhatt, S. Dhyani, and Joyti Nain, (2013). *International Journal of Current Pharmaceutical Research. 5*(2): 144-147.
28. Kanth, S.V., R. Venba, B. Madhan, N.K. Chandrababu, and S. Sadulla, (2009). *Journal of Cleaner Production. 17*(5): 507-515.
    DOI : 10.1016/j.jclepro.2008.08.021
29. Kayang, H., B. Kharbuli, B. Myrboh, and D. Syiem, (2005). Medicinal Plants of Khasi Hills of Meghalaya, India. Proc. WOCMAP, Vol. 1 : Bioprospecting & Ethnopharmacology (Eds. Bernath J, Nemeth E, Craker LE and Gardner ZE). DOI:10.17660/ActaHortic.2005.675.9
30. Kharkongor, P., and J. Joseph, (1981). Folklore medicobotany of rural Khasi and Jaintia tribes in Meghalaya. In: Glimpses of Indian Ethnobotany. (ed.Jain SK), Oxford and IBH New Delhi, 115.
31. Laloo, D., M. Kumar, S.K. Prasad, and S. Hemlatha, (2017). Gastroprotective activity of the alcoholic root extract of *Potentilla fulgens* Wall. DOI: https://doi.org/10.17816/RCF10275-76
32. Li Cai (2014). Current Protocols Essential Laboratory Techniques, Wiley Online Library. 6.3.1-6.3.18. DOI:10.1002/9780470089941.et0603s08
33. Li, R., M.Y. Wang, and X. B. Li, (2012). *Journal of Medicinal Plants Research. 6*(14): 2704-2713.
    DOI: 10.5897/JMPR11.1123
34. Lopes, G.K., H.M. Schulman, and M. Hermes-Lima, (1999). *Biochimica et Biophysica Acta (BBA)-General Subjects, 1472*(1): 142-152.
    DOI: 10.1016/s0304-4165(99)00117-8
35. Maatalah, M.B., N.K. Bouzidi, S. Bellahouel, B. Merah, Z. Fortas, R. Soulimani, and A. Derdour (2012). *Journal of Biotechnology Pharmaceutical Research. 3*(3): 54-57.
36. Manner, S., M. Skogman, D. Goeres, P. Vuorela, and A. Fallarero, (2013). *International Journal of Molecular Sciences. 14*(10): 19434-19451.
    DOI: 10.3390/ijms141019434
37. Medicinal Plants used by the people of Khasi and Jaintia Hills. Posters. Series 23. Meghalaya Biodiversity Board.
38. Mohaddese, Mahboubi, Atefeh Mahboubi, Nastaran Kazempour. (2015). *Heria Polonica. 61*(1): 31-37.
    DOI: 10.1515/hepo-2015-0008
39. Ngoci S.N., C.M. Mwendia, and C.G. Mwaniki, (2011). *Journal of Animal & Plant Sciences. 11*(1): 1364-1373.
40. Niero, R. (2010) Drugs, phytopharmaceuticals and phytotherapics: Economic and Market Approach. In: BRESOLIN, TM; CECHINEL-FILHO, V. (Org.). Drugs and Medications. a multidisciplinary approach . 1ed. São Paulo: Editora Santos. 65p.
41. Oliveira, D.R. (2011). *Rev. Bras. Farmacogn 21* (5): 793-806
    DOI:10.1590/S0102-695X2011005000084
42. Omulokoli, E., B. Khan and S.C. Chhabra, (1997). *Journal of Ethnopharmacology.*

56(2), 133-137.
DOI: 10.1016/s0378-8741(97)01521-3

43. Phillipson, J.D., and M.J. O Neill, (1989). *Acta Pharmaceutica Nordica. 1*(3): 131-144.
44. Rupasinghe, H.V., C.J.C. Jackson, V. Poysa, C. Di Berardo, J.D. Bewley, and J. Jenkinson, (2003). *Journal of Agricultural and Food Chemistry. 51*(20): 5888-5894.
45. Soares M.B., D. Brustolim, L.A. Santos, M.C. Bellintani, F.P. Paiva, Y.M. Ribeiro, T.C. Tossami, and R. Santos, (2005). *Journal of International Immunopharmacology 6:* 408-414.
DOI: 10.1016/j.intimp.2005.09.007
46. Souza Brito, A.R.M., and A.A. Souza Brito, (1996). Medicinal plant research in Brazil: Data from regional and national meetings. In: Medicinal Resources of the Tropical Forest - Biodiversity and its importance to human health (Cap. 28, 386-401). Ed. by M.J. Balick, E. Elisabetsky and S.A. Laird. Columbia University Press, 440.
47. Talukdar, A.D., M.D. Choudhury, M. Chakraborty, and B.K. Dutta (2010). *Assam University Journal of Science & Technology, Biological and Environmental Sciences, 5*(I): 70-74, 2010
48. Vargas, A.C. (2004) *Ciência Rural, 34*(1): p. 159-163.
49. Wagner, H., S. Baldt, and E.M. Zgainski, (1996). Plant drug analysis. New York, Berlin, Springer. 17.
DOI:10.1007/978-3-642-00574-9
50. Yokosuka, A., and Y. Mimaki, (2009). *Journal of Phytochemistry. 70*(6): 807-815.
DOI: 10.1016/j.phytochem.2009.02.013
51. Zamora-Ros, R., A. Agudo, L. Luján-Barroso, I. Romieu, P. Ferrari, V. Knaze, and E. Sánchez-Cantalejo, (2012). *The American Journal of Clinical Nutrition. 96*(6): 1398-1408.
DOI: 10.3945/ajcn.112.037358