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Original Article

Physicochemical and phytochemical investigation on medicinal plants used by ethnic tribes of Nilgiri Mountains, South India

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ABSTRACT

Medicinal plants *Mahonia leschenaultii, Berberis tinctoria, Vaccinium leschenaultii, Rubus ellipticus* and *Passiflora mollissima* used to treat various ailments by ethnic tribes of Nilgiris was subjected to physiochemical, phytochemical screening and class of compounds quantification. Methanol followed by ethanol proved to be highly effective for sequential extractive value for all the plants. The fluorescence analysis of the powder showed the presence of fluorescent compounds when examined under day light and UV light. Ash values of total ash, acid-insoluble ash and water-soluble ash were observed and determined. Phytochemical screening of thirty six extracts of various solvent showed the existence of bioactive compounds such as alkaloids, tannins, phenols, quinones, saponins, flavonoids, flavones, glycosides, carbohydrates, terpines, triterpens and proteins. Further quantification of alkaloid, phenol, flavonoid, tannin and saponin content was determined. Versatile data produced by this investigation may be useful in probing of biochemistry and bioactive principles and subsequently may lead to the drug discovery and development of these plants in the future.

Keywords: Ethnomedicinal plants; physiochemical analysis; ash value; phytocompounds; fluorescence analysis

INTRODUCTION

De Materia Medica, a medicinal plant catalog written by Dioscorides in the first century A.D, became the prototype for modern pharmacopoeias. In the late fifth century B.C, Hippocrates mentioned 300 to 400 medicinal plants [1]. It is clear that finding healing powers in plants is an ancient idea. Herbal medicine or phytotherapy is the science of using herbal remedies to treat the diseases. The active ingredients in medicinal plants are defined as chemical compounds that act directly or indirectly prevent or treat disease. Secondary metabolites derived out of plants become greater importance because of their versatile applications, especially in medicinal field. Medicinal plants are the renewable richest living resource of phytochemical drugs of traditional systems of medicine, folk medicines, nutraceuticals, modern medicines, pharmaceutical intermediates, cosmetics, food supplements and chemical entities for synthetic drugs [2]. So far 12,000 secondary metabolites have been isolated, a number estimated to be less than 10% of the total. The phytochemical and pharmacognostical parameters are chief trustworthy and low-cost criteria for confirmation of the crude drugs. Recently many people show interest in embracing complementary herbal medicine instead of modern drugs due to their side effects. Screening for biologically active constituents is a crucial factor for success of the investigation while selecting plant species for study. The selection should be based on the therapeutic use of plant species used by a specific ethnic group. Any part of the plant may contain active components. Hence, we selected medicinal plant species used by ethnic group called Todas at Shola forest in Utagamund in Western Ghats, South India. The investigation was aimed to evaluate physiochemical characters, phytochemical constituents and to quantify the class of compounds from medicinal plants such as *Mahonia leschenaultii, Berberis tinctoria, Vaccinium leschenaultii, Rubus ellipticus* and *Passiflora mollissima* used to treat various ailments by ethnic tribes of Nilgiris.

MATERIALS AND METHODS

Ethnobotanical survey and selection of plant material: Plants were selected for this study based on tribals medicinal use. Fresh plant parts were

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collected from the tribal areas (Toda and Kattunayyaka tribe) in Western Ghats Nilgiris, Tamil Nadu, India during January – April 201.3 Figure 1 shows the plants selected for the study. The tribal villages were approximately lie at 11.411842°N longitudes and 76.6959°E latitude. The ethnobotanical data such as local name, mode of preparation and medicinal uses were collected through questionnaire, interviews and discussions among the tribal practitioners in their local language (Tamil). The voucher specimens in duplicate were identified by Dr.G. Jayajothi Taxonomist

Department of Plant Biology and Biotechnology, Loyola College, Chennai and deposited in the Loyola College, Loyola College, Chennai for future reference.

Ethnobotanical data of studied plants and their medicinal uses

***Mahonia leschenaultii* takeda (LCH-301):** The ethnic tribe Toda use leaf extract of *Mahonia leschenaultii* takeda species of Berberidaceae family, in post-natal treatment in women and for checking fever, cold and jaundice. The bark juice is externally applied to treat dental ailments. The root of this plant is rich in alkaloids [3,4]. It is locally called Thovari, mullukadambu in Tamil. Description**:** Small tree, branches with persistent leaf-bases. Leaves even pinnate, leaflets 6 pairs, opposite oblong, coriaceous, glabrous, glossy above, margin serrately-spiny. Racemes terminal. Berry globose to 8mm, glaucous, 1-seeded**.**

***Berberis tinctoria* Lesch (LCH-302)**: It is commonly called as Nilgiri barberry, a common allied species of *B. aristata*. Toda call this plant by Thikmui, locally named by Oosikala, is an endemic plant to high hills of Nilgiris. The leaf extracts *B. tinctoria* species of Berberidaceae is orally given to relieve fever and gastric discomfort. The decoction of leaves is used to cure ulcer and sores above all, it is used in India traditional systems of medicine by the name of Daruharidra for skin disease, jaundice and rheumatism. The plant is reported to contain alkaloids, like berberine, berbamine, jatrorrhizine and palmatine [5]. Description: Shrub, long stem with branches bearing numerous slender leafy twigs, wood is tough yellow in colour. Leaves are ovate with triple spines in the axils of with three tuffs of leaves. Racemes of flower drooping. Fruit: berry sausage shaped and stigma attached.

***Vaccinium leschenaultii* Wight (LCH-303):** The plant belongs to Ericaceae family and sub-family is Vaccinaceae. *V.leschenaultii* (Bilberry), a member of the Ericaceous family, found in the mountains and forests of Western Ghats in high altitude. They are small trees. Bilberry is a relative of the blueberry and the leaf is used for diabetes. The fruits are eaten by local dwellers. Fruit or leaf extracts of *Vaccinium* species were found to induce apoptosis in cancer cells and to inhibit human leukemia [6, 7], breast [8], colon[9], lung and prostate [10] cancer cells *in vitro*. Description: Small tree with brownish bark. Leaves simple, alternate, spiral; elliptic or elliptic-obovate, apex acute or acuminate, margin serrate, glabrous, flowers solitary or in raceme, bellshaped, pink colour. Fruit is fleshy berry.

***Rubus ellipticus* Sm. (LCH-305):** The *Rubus ellipticus* species belongs to Rosaceae which is commonly called as weedy rasberry, a stout evergreen shrub grows abundantly in the forests at high altitudes like Himalaya and Nilgiris region. It is native to tropical and sub-tropical India. The leaf extracts of *R.ellipticus* is orally taken to cure fever and stomach upset while raw fruits are used to cure throat and mouth ulcer and root is used to cure stroke. Traditionally it is used for dysentery, antifertility, antimicrobial, analgesic, antiepileptic gastralgia, wound healing [11]. Description: Stout climbing shrubs stems often 30-40 cm long covered with spreading prickles. Leaves are palmately compound thick leaflets 3, obcordate. Flowers are in terminal panicles, tomentose. Fruit is yellow depressed, hemispherical, glabrous.

***Passiflora mollissima* Baily. (LCH-304):** *Passiflora mollissima* is also called by banana passion fruit. The local healers use the leaves of *P. mollissima* as an antidiabetic drug. The plant is used as antifungal, antibacterial and antifeedant [12]. Alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds are known in the genus [13]. Description: Lianas, Leaf with blades 6-16 cm long, 7-20 cm wide, deeply 3-lobed. Flower is pendent, salverform, 6-9 cm in diameter, peduncles solitary, 3.8-10 cm long. Fruit is yellow at maturity, pericarp softly coriaceous, obovate to oblong, 6-8 cm long, pubescent, aril orange [14]. Herbal extracts of *Passiflora* sp also used in traditional medicines to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases and in Ayurveda for several remedies such as sedative, anxiety and hypertension.

Chemicals and reagents: All solvents and chemicals used were of analytical grade and were procured from Merck (Mumbai, India).

Preparation of powder: The plant parts were subjected to shade dry and powdered. These powdered materials were used for further physiochemical and fluorescent analysis.

Physicochemical parameters of plant powder: The amount of active constituents extracted with solvent from given amount of medicinal plant material, determined by the extractive value. The ash remaining following ignition of medicinal plant materials was determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash. The total ash method is intended to measure the total amount of material remaining after ignition. This includes both physiological ash, which is derived from the plant tissue itself, and non-physiological ash, which is the residue of the extraneous matter such as sand and soil adhering to the plant surface. Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water [15, 16].

Determination of individual extractive values of cold extraction: The plant powder (1 kg) was extracted three times by cold percolation method with 3:l of hexane, ethyl acetate, methanol and ethanol at room temperature for 72 h. The filtrates were concentrated under reduced pressure at 40 ºC and stored in a refrigerator at 2-8 ºC for use in subsequent experiments. The maximum cold extractive value was noted in hexane, ethyl acetate, methanol and ethanol extract.

Fluorescence analysis: When the powder is exposed ultraviolet radiation, many drugs fluorescence. It is essential to examine all materials on reaction with different chemical reagents under U.V. light. The fluorescence characteristics of powdered drugs were studied under visible light and U.V. light after treating with different chemical reagents [17, 18]. The plant material was given treatment for 48 hours with various chemical and organic solvent like Conc. Hydrochloric acid, 1N Sodium hydroxide, Ammonia, Ferric chloride, Glacial acetic acid, Iodine, Chloroform, Hexane, Ethyl acetate, Methanol, Ethanol and Distilled water.

Loss on drying determination: 10 g of the sample was weighed in a tarred evaporating dish and dried at 105°C for 4 hours and weighed. The weighing and drying was continued at 1 hour interval until there was no difference between two successive weighing.

Total ash determination: 5g of the powered material was precisely weighed in an ignited and weighed silica crucible. On the bottom of the crucible, the powered material was spread as a fine layer. The crucible was incinerated at 450°C until free from carbon, was cooled and weighed. . The procedure was repeated until a constant weight was observed.

Acid insoluble ash determination

The ash obtained from the determination of total ash was boiled with 30 ml hydrochloric acid for six minutes. The insoluble ash was collected on filter paper and washed with hot water then transferred into weighed silica crucible. Now the acid insoluble ash percentage was calculated with reference to the air dried drug.

Water soluble ash determination: The ash obtained was boiled for five minutes in 30 ml of water then insoluble matter was collected on the filter paper and washed. The insoluble matter was transferred into a tarred a silica crucible and ignited for 17 minutes at not exceeding 450ºC. The experiment was repeated to get the constant weight. Now the weight of the insoluble matter was subtracted from the total weight of ash. The resulted weight is considered as water-soluble ash which was calculated with reference to the air-dried drug.

PHYTOCHEMICAL ANALYSIS

Phytochemical tests for the screening and detection of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by Evans [19], Harborne [20], Sofowara [21] and Trease and Evans [22].

Qualitative Analysis

Alkaloid detection

A. Wagner's Test: Filtrates were treated with Wagner's reagent. Brown or reddish precipitate formation indicates the presence of alkaloids.

B. Mayer's Test: Filtrates were treated with Mayer's reagent. Formation of a yellow colored precipitate indicates the presence of alkaloids.

C. Dragondroffs Test: Filtrates were treated with Dragondroffs reagent. Observation of red precipitate indicates the presence of alkaloids.

Tannins detection

A. Lead acetate test: The extract was dissolved in water and to that 10% lead acetate solution was added. The appearance of yellow precipitate confirms the tannins.

B. Ferric chloride test: The extract was dissolved in water. The solution was purified by filtration; 10% ferric chloride solution was added to the filtrate. This was observed for a change in color to bluish black.

C. Potassium Dichromate Test: The extract was dissolved in water to that added strong potassium dichromate solution, a yellow colour precipitate indicates the presence of tannins and phenolic compounds.

Test for Phenols: Phenols are chemical compound characterized by at least one aromatic ring bearing one or more hydroxyl groups. To1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Appearance of blackish green, red, purple and blue color indicated the presence of phenols.

Test for Quinones: Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. Formation of red colour indicated the presence of quinines when 1 ml of concentrated sulphuric acid was added to 1 ml of extract.

Saponins detection: Saponins are glycosidic triterpenoids widely found in the plant kingdom. They are soluble in water and give stable foam.
Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Persistent foam was formed. This indicates the presence of saponins**.**

Detection of Flavonoids: Flavonoids are hydroxylated phenolic substances but occur as a C6-C3unit linked to an aromatic ring.
A. Shinoda's test: Few pieces of magnesium turnings were added to a 3 ml of the alcoholic extract and followed by 3 drops of concentrated hydrochloric acid. Effervescence and appearance of dark brown solution which gradually changes to a deep red solution slowly turning to a deep red solution or pink colouration indicates the presence of flavonoids [19].
B. Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
C. NaOH Test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, on addition of dilute acid, which becomes colorless, indicates the presence of flavonoids.

Detection of Flavones: Flavones are phenolic structures containing one carbonyl group. Few ml of extract was treated with 2 ml of fifty percent methanol solution, and then warmed. With this solution metal magnesium was added and orange color indicates presence of flavones.
Detection of Glycosides: To 2ml of extract, 3ml of chloroform and 10% ammonia solution was added. Pink color formation indicated the presence of glycosides.

Detection of carbohydrates:
A. Molisch's Test**:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Violet ring formation at the junction indicates the presence of Carbohydrates.
B. Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Observation of red precipitate indicates the presence of reducing sugars.

Terpenoids: To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown color formation at the interface indicated the presence of terpenoids.

Triterpenoids: It consists of six isoprene units e.g. squalene found in wheat germ, and olives.
Liebermann Burchard's test: The Extract was treated with chloroform and filtered. A few drops of acetic anhydride was added to the filtrate, boiled and cooled. Then Conc. sulphuric acid was added. Formation of deep red colour indicates the presence of triterpenoids.

Detection of Proteins: Xanthoproteic Test: Few drops of conc. Nitric acid were added to the extracts. Observation of yellow colour indicates the presence of proteins.

Quantitative determination of the chemical constituency: On the basis of qualitative analysis only selected parts of plant powder was subjected to quantitative analysis**.**

Preparation of fat free sample: 2g of the sample were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 h.

Determination of alkaloid: Alkaloid quantity of crude was determined by using Harborne method [20]. 5 g of the defatted sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hour. This was filtered and the filtrate was concentrated on a water bath to one-fourth of the original volume. Con. ammonium hydroxide was added drop by drop to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.
Determination of total phenols: The fat free sample was boiled with 50ml of ether for the

extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50ml flask, then 10ml of distilled water was added. To this solution, 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour progress. This was measured at 505nm [23, 20].

Flavanoid determination: Flavanoid was determined by using Bohm and Kocipai- Abyazan [24] method. 10g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Total tannin determination: Tannin determination was followed by Van-Burden and Robinson [25] method. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Saponin determination: Total saponin was determined according to Obadoni and Ochuko [23] method. About 20g of plant sample was taken in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90ºC. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of normal butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven into a constant weight. The saponin content was calculated in percentage.

RESULTS AND DISCUSSION

Extractive value: Comparative account of various solvent sequential extractive value percentage of medicinal plant parts are presented in Table1 and Figure2. Methanol followed by ethanol proved to be highly effective for sequential extractive value for the plants *M. leschenaultii, B. tinctoria* and *R.ellipticus* and *P. mollisima* where as ethyl acetate followed by methanol extracts of *V.leschenaultii* showed highest percentage of extractive value. Among all the selected plants, *M. leschenaultii* proved to be high extractive value in methanol.

Fluorescence analysis: The fluorescence analysis of the powder showed the presence of fluorescent compounds in all the plant parts [26] powder is examined as such under visible light, leaf powder of all the selected plants appears green color, then stem powder appears light green and light yellow whereas root powder was yellow of *M. leschenaultii* and *B. tinctoria* respectively. Besides bark of *V.leschenaultii* looked light brown in color. When treated with different chemicals and reagents various colors appeared when examined under visible light and UV light (254nm&366nm). The Table 2 and 2a enumerates a detailed fluorescence behavior of crude powder. Pharmacognostical parameters and standards must be established before any crude drug can be included in herbal pharmacopoeia since the evaluation of a crude drug is an integral part of establishing its correct identity [27].

Analysis of ash content: The ash value of any organic material is composed of their non volatile inorganic components. Extractive values are representing the presence of polar or non polar compounds and ash values that are representing the purity of drug, loss on drying value indicates that drug is safe regarding any contamination of growth of bacteria, fungi and yeast. Determination of percentage on loss on drying, total ash, acid insoluble ash and water soluble ash was evaluated with the crude drug powder. Table 3 exhibits the results on these physiochemical parameters. Loss on dry percentage of *B. tinctoria* stem was high and *M. leschenaultii* stem was less. Total ash content was high in the root of *B. tinctoria, M. leschenaultii* and *V.leschenaultii* bark. Likewise water soluble ash was relatively more in *P. mollissima* leaf, while *M. leschenaultii* leaf exhibited less percentage. Acid insoluble ash was less in *M. leschenaultii* and *P. mollissima*. A high value is an indicative of adulterations, substitution, contamination or carelessness in preparing the crude drug for marketing. The percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality of drugs.

Phytochemical investigation

Qualitative analysis: The present study revealed the presence of active class of phytoconstituents. The active compounds were qualitatively analyzed in leaf, stem, roots and bark separately and the results are presented in Table 4. In the screening

process alkaloids, tannins, phenols, quinones, saponins, flavonoids, flavones, glycosides, carbohydrates, terpines, triterpens and proteins showed different types of results in various solvents Figure 3, 3a. The results reveal that the methanol and ethanol extract of all the plants part showed the presence of all important compounds except quinones which was present in *V. leschenaultii*. Saponin was completely absent in *M. leschenaultii* whereas alkaloids was abundantly present in the plants *M. leschenaultii* and *B.tinctoria, V. leschenaultii* and *P.mollissima* but absent in *R,ellipticus.* Besides, saponin was more in ethyl acetate and methanol extract of *V. leschenaultii.* Phytochemicals are mostly absent in all the hexane extract, moderately present in ethyl acetate and abundant in methanol and ethanol extract. Medicinal plants contain plenty of secondary metabolites are responsible for several biological activities, which may help in protection against various chronic diseases in human beings and animals. The major phytochemical substances found in the surveys are alkaloids, phenols, steroids and saponins, flavonoids, tannins, unsaturated steroids, triterpenoids and essential oils [28]. Saponins protect against hypercholesterolemia and also have antibiotic properties. Alkaloids protect against chronic diseases. Steroids and triterpenoids show the analgesic properties whereas steroids and saponins were responsible for central nervous system activities. The pharmacological activities of the drugs are contributed by the presence of secondary metabolites.

Quantitative determination: Quantitative determination of alkaloid, phenol, tannin and saponin of selected plants was analyzed and tabulated (Table 4a). Alkaloid content was high in *M. leschenaultii* and *B.tinctoria* while flavonoids and tannin were high in *V. leschenaultii, P.mollissima* and *R.ellipticus* likewise saponin content was more in *V. leschenaultii.* The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by [29, 30]. Reports of alkaloids in 12 leafy vegetables studied recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension [31]. *Rubus* species are used in folk medicine such as diabetes mellitus, inflammatory disorders and ulcers. The literature survey revealed the presence of triterpenes in various *Rubus* species [32]. The presence of anti-inflammatory activity in triterpenes has remarkably less side effects. The percentage of alkaloid content of the *M. leschenaultii* and *B.tinctoria* leaf, stem and root was found to be 3.58, 5.67, 6.94 and 3.29, 4.49 and 5.86 respectively. Whereas *P.mollissima* leaf was 5.83% and remaining plants showed moderate amount of alkaloid content. The alkaloids represent a very extensive group of secondary metabolites with diverse structures, distribution in nature, and biological effects. They are often biologically active in humans meaning they participate in our biochemistry actively. The results revealed that percentage crude yield of phenol of *M.leschenaultii* root, *B.tinctoria* root, *P.mollissima* leaf, *V. leschenaultii* leaf, bark and *R.ellipticus* leaf was 18 .07, 19.21, 24.09, 9.09, 11.23 and 14.73 respectively. Phenols are associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis; structural components and allelopathy [33]. Phenolics show an array of health promoting benefits in human health. They are of current interest due to their important biological and pharmacological properties, especially the anti-inflammatory [34], antioxidant, antimutagenic and anticarcinogenic activities[35]. Phenolic compounds are a class of antioxidant compounds and act as free radical terminators. Phenolic compounds, flavanoids and tannins are the major group of compounds acting as primary antioxidants or free radical scavengers. The percentage crude yield of flavonoid content was found to be high in *V. leschenaultii* leaf, bark, *R.ellipticus* and *P.mollissima* that was 9.62, 3.22, 3.56 and 2.97 respectively. Flavonoids show antioxidant activity and their mechanism of actions through scavenging or chelating process and act as free radical terminators [36]. Flavonoids functions as stress protectants in plant calls by scavenging reactive oxygen species produced by the photosynthetic electron transport system [37]. The percentage crude yield of tannin content was more in *V. leschenaultii* leaf, bark, *R.ellipticus* and *P.mollissima* that found to be16.24, 18.12, 15.15 and 25.17 respectively, whereas remaining plants showed less amount. Tannins are known to possess general antimicrobial and antioxidant activities. Recent reports show that tannins may have the potential value as cytotoxic and antineoplastic agents [38]. Leaves of *Rubus* species contain tannins [39]. Derivatives of kaempferol and quercetin, phenolic acids, triterpenes, mineral salts as well as vitamin C are reported in *Rubus* species [40, 41]. Tannin is a group of polymeric phenolic substances and found in almost every plant part [42]. The percentage crude yield of saponin content was significantly high in *V. leschenaultii* leaf and bark while showed 6.05 and 8.21 respectively. Other plants showed moderate amount but berberidaceae plants showed very less. Saponin is a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used as hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti inflammatory and for weight loss,

etc. It is also known to have antifungal properties. Saponins have been implicated as bioactive antimicrobial agents of plants [43]. Plant saponins boost the effectiveness of certain vaccines and knock out some kinds of tumor cells, particularly lung and blood cancers [44]. They also lower blood cholesterol there by reducing heart diseases. Medicinal plants are the immense source for varied phytoconstituents exhibiting diverse pharmacological property. The curative properties of plants are perhaps due to the presence of various secondary metabolites. Identifying potential medicinal plants with high amount of bioactive compounds is of significance in medicine, since it is indispensable to evaluate the pharmacognostic characteristic of the plant before its use in the field of pharmaceutical formulation and also in research.

CONCLUSIONS

The plants selected in our study are found to be well known for their ethnomedicinal and nutritional values in addition showed significant physiochemical characters. Further the successive extracts of various parts of the five plants have revealed the presence of class of compounds which are known to have activity against several pathogens and therefore could suggest their traditional use for the treatment of various ailments. The physicochemical and phytochemical parameters are major reliable and inexpensive criteria for confirmation of the crude drugs, which serve as standard data for quality control studies of pharmaceutical preparations moreover, useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Furthermore, this data may be versatile in probing of biochemistry of these plants in the future.

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Table: 1 Percentage of extractive value of selected medicinal plants

	M. leschenaultii			***B. tinctoria***			***V. Leschenaultii***		***R.ellipticus***	***P. mollissima***
Types of extract	**MLL**	**MST**	**MLRT**	**BTL**	**BTS**	**BTR**	**VLL**	**VLB**	**R.E**	**P.M**
Hexane	8.2	4.05	5.1	4.32	3.77	4.88	4.03	5.46	3.08	4.41
Ethyl acetate	5.65	4.33	6.3	3.02	2.18	3.49	13.82	12.09	7.58	5.37
Methanol	17.12	14.36	18.47	15.43	16.21	14.76	11.94	10.25	9.44	8.06
Ethanol	5.97	8.56	7.41	6.13	6.63	8.71				

M. leschenaultii **(MLL-leaf, MST-stem, MLRT-root)** ***B. tinctoria.*** **(BTL-leaf, BTS-stem, BTR-root)**
V. leschenaultii **(VLL-leaf, VLB-bark)** ***R.ellipticus*** **(REL-leaf)** ***P. mollissima*** **(PML-leaf)**

Table:2 Fluorescence Analysis of medicinal plants

	Light	*M. leschenaultii*			*B. tinctoria*		
Powder treatement	**Visiblelight /UV light**	**MLL**	**MST**	**MLRT**	**BTL**	**BTS**	**BTR**
As such	Visible	Light Green	Greenish yellow	Yellow	Green	L.Yellow	L.Yellow
	UV 254 nm	Brown	Yellow	Yellow	Brown	Yellow	Yellow
	UV 366 nm	Dark brown	Yellow	Yellow	Dark brown	Dark yellow	Dark yellow
Concent.Hcl	Visible	Dark green	Green	Green	Dark green	Dark green	Dark green
	UV 254 nm	Green	Dark green	Dark green	Dark green	Black	Black
	UV 366 nm	Dark green	Dark green	Dark green	Dark green	Dark green	Black
NaOH	Visible	Dark green	Light Green	Light Green	Greenish brown	Green	Dark brown
	UV t 254 nm	Dark green	Green	Green	Green	Yellow	Black
	UV 366 nm	Black	Dark green	Dark green	Dark green	Yellow	Black
Ammonia	Visible	Dark green	Brown	Brown	Brown	Dark green	Green
	UV 254 nm	Brown	Brown	Brown	Brown	Dark green	Yellow
	UV 366 nm	Black	Black	Black	Black	Yellow	Black
Ferric chloride	Visible	Dark green	Dark green	Dark green	Dark green	Dark green	Dark green
	UV 254 nm	Brown	Dark green	Dark green	Dark brown	Dark green	Dark green
	UV 366 nm	Black	Dark green	Dark green	Black	Yellow	Yellow
Glacial acetic acid	Visible	Green	Yellow	Yellow	Light Brown	Yellow	Yellow
	UV 254 nm	Light brown	Yellow	Yellow	Yellow	Yellow	Yellow
	UV 366 nm	Brown	Yellow	Yellow	Yellow	Yellow	Yellow
Iodine	Visible	Dark green	Light yellow	Light yellow	Light green	Light green	Light green
	UV 254 nm	Brown	Yellow	Yellow	Yellow	Yellow	Yellow
	UV 366 nm	Black	Yellow	Yellow	Yellow	Yellow	Yellow
Chloroform	Visible	Light green	Light yellow	Light yellow	Light yellow	Yellow	Yellow
	UV 254 nm	Light brown	Yellow	Yellow	Light yellow	Light yellow	Light brown
	UV 366 nm	Dark brown	Dark yellow	Dark yellow	Light brown	Dark yellow	Dark brown
Hexane	Visible	Dark green	Light yellow	Light yellow	Light yellow	Light yellow	Dark green
	UV 254 nm	Blackish brown	Yellow	Yellow	Light Brown	Dark yellow	Brown
	UV 366 nm	Light brown	Yellow	Yellow	Light brown	Dark yellow	Black
Ethyl acetate	Visible	Dark green	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
	UV t 254 nm	Brown	Yellow	Yellow	Light yellow	Light yellow	Light yellow
	UV 366 nm	Black	Yellowish green	Yellowish green	Dark brown	Dark yellow	Dark yellow
Methanol	Visible	Green	Light yellow	Light yellow	Light yellow	Light yellow	Light green
	UV 254 nm	Brown	Light yellow	Light yellow	Brown	Yellow	Green
	UV 366 nm	Dark brown	Light yellow	Light yellow	Dark brown	Yellow	Light yellow
Ethanol	Visible	Light green	Yellow	Yellow	Brown	Yellow	Yellow
	UV 254 nm	Green	Yellow	Yellow	Yellow	Yellow	Yellow
	UV 366 nm	Dark brown	Dark yellow	Dark yellow	Brown	Dark yellow	Dark yellow
Distilled water	Visible	Light green	Light yellow	Light yellow	Light yellow	Light yellow	Yellow
	UV 254 nm	Dark brown	Yellow	Yellow	Dark green	Yellow	Yellow
	UV 366 nm	Brown	Yellow	Yellow	Brown	Yellow	Yellow

M. leschenaultii **(MLL-leaf, MST-stem, MLRT-root)** ***B. tinctoria.*** **(BTL-Leaf, BTS-stem, BTR-root)**
L.green-Light green, L.yellow-Light yellow, G.brown- Greenish brown, Bl.brown- Blackish brown, Y.green-Yellowish green.

Table: 2a Fluorescence Analysis of medicinal plants

	Light	*V. Leschenaultii*	*R.ellipticus*	*P. mollissima*	*V. Leschenaultii*
Powder treatement	**Visible light/UV light**	**VLL**	**VLB**	**REL**	**PML**
As such	Visible	Green	Brown	Green	Green
	UV 254 nm	Brown	Light brown	Light brown	Brown
	UV 366 nm	Dark brown	Black	Blackish brown	Dark brown
Concent.Hcl	Visible	Black	Black	Dark green	Dark green
	UV 254 nm	Black	Black	Black	Black
	UV 366 nm	Black	Black	Black	Black
NaOH	Visible	Dark brown	Dark brown	Brown	Dark green
	UV t 254 nm	Black	Black	Black	Black
	UV 366 nm	Black	Black	Black	Black
Ammonia	Visible	Dark brown	Dark brown	Dark green	Green
	UV 254 nm	Black	Black	Black	Light brown
	UV 366 nm	Black	Black	Black	Brown
Ferric chloride	Visible	Brown	Brown	Black	Black
	UV 254 nm	Dark brown	Dark brown	Black	Black
	UV 366 nm	Black	Black	Black	Black
Glacial acetic acid	Visible	Light Brown	Dark Brown	Green	Dark green
	UV 254 nm	Brown	Brown	Dark green	Dark green
	UV 366 nm	Black	Black	Dark Brown	Brown
Iodine	Visible	Light green	Brown	Light green	Light green
	UV 254 nm	Dark green	Dark brown	Dark brown	Dark brown
	UV 366 nm	Black	Black	Black	Black
Chloroform	Visible	Light Brown	Brown	Light green	Green
	UV 254 nm	Light Brown	Light Brown	Light green	Green
	UV 366 nm	Dark Brown	Black	Green	Brown
Hexane	Visible	Dark green	Brown	Light green	Dark green
	UV 254 nm	Brown	Light Brown	Light Brown	Light Brown
	UV 366 nm	Black	Black	Dark brown	Black
Ethyl acetate	Visible	Light green	Brown	Dark green	Dark green
	UV t 254 nm	Light Brown	Dark brown	Brown	Brown
	UV 366 nm	Dark brown	Black	Black	Black
Methanol	Visible	Dark green	Light Brown	Light green	Light green
	UV 254 nm	Brown	Brown	Green	Brown
	UV 366 nm	Dark brown	Black	Dark brown	Dark brown
Ethanol	Visible	Green	Brown	Green	Dark green
	UV 254 nm	Brown	Brown	Brown	Dark green
	UV 366 nm	Dark brown	Dark brown	Dark brown	Brown
Distilled water	Visible	Green	Brown	Green	Dark green
	UV 254 nm	Black	Dark brown	Dark green	Dark green
	UV 366 nm	Brown	Black	Dark brown	Dark brown

***V. leschenaultii* (VLL-leaf, VLB-bark) *R. ellipticus* (REL-leaf) *P. mollissima*(PML-leaf) L.green-Light green, L.yellow-Light yellow, G.brown- Greenish brown, Bl.brown- Blackish brown, Y.green- Yellowish green.**

Table:3 Percentage loss on drying, ash values of different parts of selected medicinal plants

	M. leschenaultii			*B. tinctoria*			*V. Leschenaultii*		*R.ellipticus*	*P. mollissima*
Parameters	**MLL**	**MST**	**MLRT**	**BTL**	**BTS**	**BTR**	**VLL**	**VLB**	**REL**	**PML**
Drying Loss	0.6591	0.5394	0.6923	0.715	0.8012	0.7253	0.7142	0.5481	0.6612	0.6739
Total ash	3.97	3.058	4.29	3.54	3.98	4.23	2.8034	4.63	3.68	3.44
Water soluble ash	0.96	0.82	0.87	1.024	0.87	1.03	1.21	1.33	0.98	1.62
Acid insoluble ash	2.684	1.85	0.91	1.453	1.64	2.48	2.6559	2.047	2.534	1.963

***M. leschenaultii* (MLL-leaf, MST-stem, MLRT-root) *B. tinctoria.* (BTL-Leaf, BTS-stem, BTR-root)**
***V. leschenaultii* (VLL-leaf, VLB-bark) *R.ellipticus*(REL-leaf) *P. mollissima* (PML-leaf**

Table: 4 Qualitative phytochemical analysis of the selected plants

Plant and parts	Extract	Name of secondary metabolites											
M. leschenaultia		**Alk**	**Tan**	**Phe**	**Qui**	**Sap**	**Fld**	**Flv**	**Gly**	**Car**	**Ter**	**Ttp**	**Pro**
Leaf	Hexane	-	-	-	-		-	-	++	++	-	-	-
	Ethylacetate	-	-	-	-	-	-	-	++	++	-	-	-
	Methanol	+++	+	++	-	-	++	+	+	+++	+	++	++
	Ethanol	+++	+	++	-	-	++	+	+	+++	++	+++	++
Stem	Hexane	-	-	-	-	-	-	-	+	+++	+	+	-
	Ethylacetate	-	-	-	-	-	-	-	++	++	-	-	-
	Methanol	+++	+	++	-	-	++	+	+	+++	+	++	+
	Ethanol	+++	+	++	-	-	++	++	++	+++	+	+	++
Root	Hexane	-	-	-	-	-	-	-	++	++	+	++	-
	Ethylacetate	-	-	-	-	-	-	-	++	++	+	++	-
	Methanol	+++	+	++	-	-	++	+	-	++	++	++	++
	Ethanol	+++	+	++	-	-	++	++	++	+	+++	+	+
B tinctoria.													
Leaf	Hexane	-	-	-	-	-	-	-	++	++	+	+	-
	Ethylacetate	-	-	-	-	-	+	+	++	++	+	+	+
	Methanol	+++	+	++	-	+	++	+	-	++	++	++	++
	Ethanol	+++	+	++	-	+	++	+	-	++	++	++	++
Stem	Hexane	-	-	-	-	-	-	-	-	+	++	++	-
	Ethylacetate	-	-	-	-	-	+	-	++	+	+	+	-
	Methanol	+++	+	++	-	+	++	+++	-	++	++	++	+
	Ethanol	+++	+	++	-	+	++	+++	-	++	++	++	+
Root	Hexane	-	-	-	-	-	-	-	++	++	+	+	-
	Ethylacetate	-	-	-	-	-	+	++	+	+++	+	+	-
	Methanol	+++	+	++	-	+	++	+++	+	+++	++	++	+
	Ethanol	+++	+	++	-	+	++	+++	+	+++	++	++	+
R. ellipticus.													
Leaves	Hexane	-	-	-	-	-	+	-	+	-	++	+	-
	Ethylacetate	-	+	++	-	-	++	-	+	-	++	+	-
	Methanol	-	+++	+++	-	++	+++	+++	++	+	++	+	++
P. mollissima													
Leaves	Hexane	+	-	-	-	-	+	-	++	-	+	+	-
	Ethylacetate	+++	++	++	-	+	++	+	++	-	++	++	-
	Methanol	++	+++	++	-	++	+++	++	++	+	+++	++	+
V. leschenaultia													
Leaves	Hexane	-	-	-	-	-	+	-	+	+	-	-	-
	Ethylacetate	+	++	++	+	+++	+++	+	++	++	+	+++	+
	Methanol	+	++	+++	++	+++	+++	+++	+++	++	++	++	+
Bark	Hexane	-	-	-	-	-	+	-	++	++	++	-	-
	Ethylacetate	++	+++	+++	+	+++	+++	+++	+++	++	+++	+++	+
	Methanol	++	+++	+++	++	+++	+++	+++	+++	+	+++	+++	+

Alk- Alkaloids, Tan- Tannin, Phe- phenols, Qui-quinones, Sap- saponins, Fld-flavonoids, Flv- flavones,Gly-glycosides, Ter-terpenoids, Car-carbohydrates, Ttp-treterpinoids, Pro-proteins.+++ = High concentration; ++ = Moderate concentration; += low concentration.

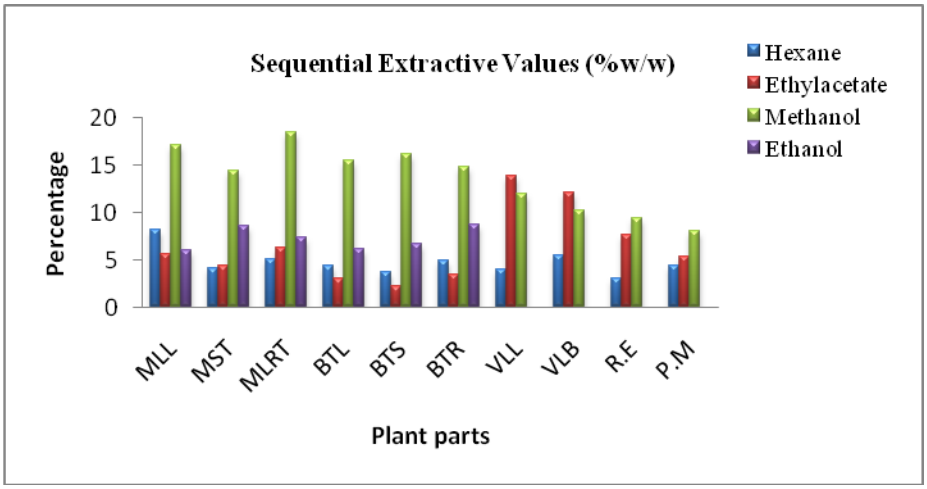
Table:4a Percentage of crude alkaloids, flavonoids, tannin, phenols and saponin

S.no	Plant part	Alkaloids	Flavonoids	Phenols	Tannins	Saponins
1	**MLL**	3.58 ± 0.02	1.06 ± 0.02	10.5 ± 0.22	0.81 ± 0.56	0.29 ± 0.12
	MST	5.67 ±0.31	1.92 ± 0.23	12.02 ± 1.03	0.76 ± 0.44	0.46 ±1.03
	MLRT	6.94 ±0.95	1.26 ± 0.33	18 .07 ± 0.38	0.86 ± 0.05	0.54 ±1.23
2	**BTL**	3.29 ± 0.32	1.96 ± 0.28	12.5 ± 0.42	0.43 ± 0.16	0.39 ± 0.22
	BTS	4.49 ±0.34	1.06 ± 0.17	13.03 ± 0.13	0.63 ± 0.217	0.27 ± 0.24
	BTR	5.86 ±0.05	1.56 ± 0.31	19.21 ± 0.14	0.53 ± 0.51	0.27 ± 0.37
3	**VLL**	1.29 ±0.28	9.62 ± 0.13	9.09 ±0.04	16.24 ± 1.11	6.05 ± 0.06
	VLB	1.98 ± 0.02	3.22± 0.01	11.23 ±0.026	18.12 ± 0.34	8.21 ± 0.08
4	**REL**	5.83 ± 0.25	3.56± 0.31	14.73 ± 0.18	15.15 ± 0.19	0.11 ± 0.44
5	**PML**	0.83 ± 0.46	2.97± 0.12	24.09 ± 1.25	25.17 ± 0.28	0.97 ± 0.18

M. leschenaultii **(MLL-leaf, MST-stem, MLRT-root)** ***B. tinctoria.*** **(BTL-Leaf, BTS-stem, BTR-root)**
V. leschenaultii **(VLL-leaf, VLB-bark)** ***R.ellipticus*** **(REL-leaf)** ***P. mollisimma*** **(PML-leaf)**

Figure:1 Selected medicinal plants for the investigations

Figure:2 Sequential extractive values of different parts of selected medicinal plants



M. leschenaultii **(MLL-leaf, MST-stem, MLRT-root)** ***B. tinctoria.*** **(BTL-leaf, BTS-stem, BTR-root)**
V. leschenaultii **(VLL-leaf, VLB-bark)** ***R.ellipticus*** **(REL-leaf)** ***P. mollissima*** **(PML-leaf)**

Figure:3 Phytochemical screening test

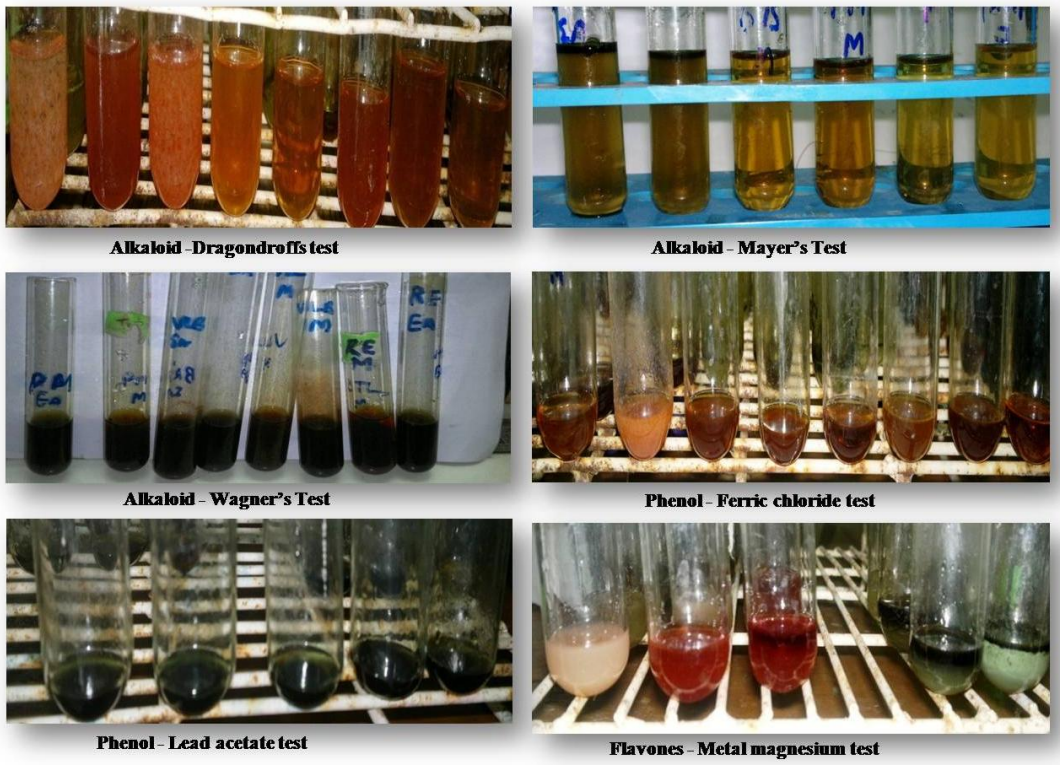
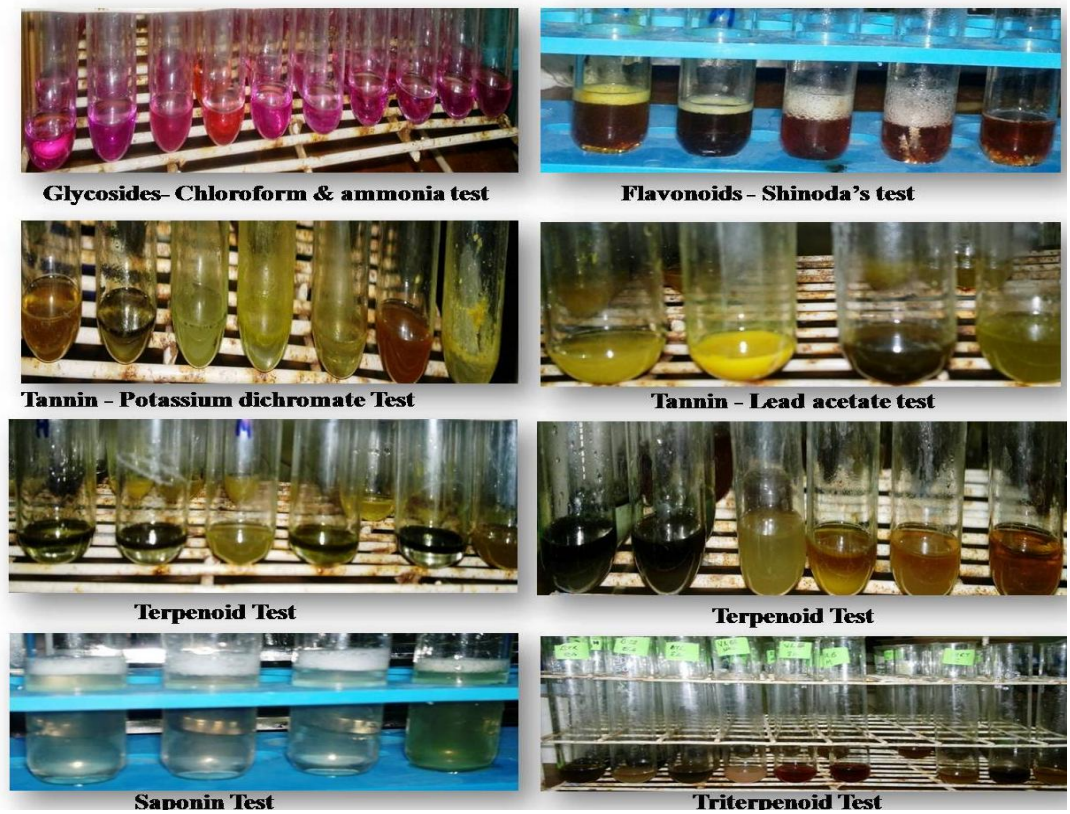


Figure:3a Phytochemical screening test



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