



PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS OF LEAVES OF SOME SELECTED MEDICINAL PLANTS USED BY TRIBAL COMMUNITY OF SANGLA VALLEY, DISTRICT KINNAUR, HIMACHAL PRADESH

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Abstract

The tribal people of Sangla valley in district Kinnaur, Himachal Pradesh have to rely on the traditional health care system involving use of ethnomedicines. The present study deals with the ethnobotanical study and phytochemical screening of leaves of selected medicinal plants like *Asparagus adscendens*, *Argemone mexicana*, *Achyranthes bitdentata*, *Achillea millefolium*, *Betula utilis*, *Betula alnoides*, *Berberis aristata*, *Berberis lycium*, *Cannabis sativa*, *Chenopodium album*, *Dioscorea deltoidea*, *Euphorbia hirta*, *Ephedra gerradiana*, *Ficus religiosa*, *Hippophae salicifolia*, *Heracleum lanatum*, *Hedychium spicatum*, *Lycyesteria formosa*, *Picrorhiza kurrooa*, *Phytolacca acinosa*, *Solanum nigrum*, *Solanum surattense*, *Selinium vaginatum*, *Trillium govanianum*, *Urtica dioica*, *Urtica palviflora*, *Valeriana jatamansi* and *Vitex negundo*. All plant materials were collected from Sangla valley during the period of 2017-2018. The ethnomedicinal data was collected through focus group discussions, participant observation, interviews and by pretested questionnaires. It was observed during 2017-2018, in Sangla valley a total of 28 ethnomedicinal plants were being used by tribal people. It was recorded that herbs species were markedly high (17) followed by shrub (6), tree (4) and climber (1). Ethanolic extracts of leaf powders have been screened for qualitative determination of different secondary metabolites like, Steroids, Glycosides, Saponin, Alkaloids, Tannins, Reducing sugar, Flavonoids and Terpenoids. The present study concluded that these medicinal plants have possessed different vital phytochemicals that helps in the medicinal properties of the studied plants commonly used in Sangla valley of district Kinnaur in Himachal Pradesh.

Key words: Ethnomedicines, Tribal, Biodiversity.

Introduction

India has one of the richest and most varied cultural traditions connected with different medicinal plant species. Medicinal plants are abundant reservoirs of phytochemicals which possess several therapeutic belongings. Phytochemicals present in medicinal plants protect against many diseases (Myres, 1982). Apart from therapeutic purpose, phytochemicals are used as precursors for the synthesis of many drugs in developed pharmaceutical industries and about 80% of medicines are gained from plant species. The important steps in isolating a biologically active phytochemical constituent from plants are isolation, extraction and characterization, of the bioactive groups. The selection of solvent is based on the compounds present in plants. A noble solvent system for plant species should have easy to evaporate, low toxicity, preservative action, inability to cause dissociation of extract, and it should not interfere with the bioassay (Das, 2010; Chukwujekwu *et al.*, 2013). Medicinal plants have been of age long remedies for human ailments because they possess therapeutic value and used for prophylactic purposes. In different country of the World an

increasing interest of traditional used medicinal plants has been observed. Ailments that have been managed traditionally using medicinal plants include malaria, epilepsy, diarrhea, infantile convulsion, bacterial, dysentery and fungal diseases. Medicinal plants are considered to be a chemical industrial unit because it comprises assembly of significant chemical groups such as glycosides, resins, alkaloids, saponins, oleoresins, lactones, and essential oils etc. (Kumar *et al.*, 2013).

Phytochemical study has been done in many parts of the World which involved some plant accessions. The chief chemical groups of interest in these study have been the steroidalm, alkaloids and saponin however, other varied constituents of naturally occurring phytochemicals such as flavonoids, tannins, triterpenoids, essential oils and unsaturated sterols etc. also have been reported by many workers from different countries (Farnsworth *et al.*, 1966). Phytochemical constituents are very significant in medicine and possess most of the valuable drugs. Alkaloid's possess rich medicinal properties. They have physical effect on animals (Edeoga *et al.*, 2001). Throughout the World some workers observed the phytochemical screening of

various medicinal plants (Paris, 1969). Many workers have observed the phytochemical analysis of leaves of medicinal plants from different parts of India (Chandershekar and Rao, 2012). Today all over the World there is rising interest in chemical composition of plant based medicines. Several bioactive constituents have been studied and isolated for pharmacological activity. The pharmaceutical industry has made massive investment in pharmacological and chemical researches during last two decades, all over the World an effort to determine much more effective drugs, rather, a few new drugs. Medicinal plants have positively passed the tests of commercial screenings.

Materials and Methods

Study Site

The Sangla valley of district Kinnaur has a remarkable terrain of lush green flora, orchards, vineyards and cold desert mountains (Balokhra, 2002). It is rich in vegetation but relatively unexplored and little survey has been done to inventorize, evaluate and explore the medicinal plants that are used by the tribal people of this beautiful valley. Sangla valley is a part of the Greater Himalayas situated at 78° 10' to 79° E Longitude and 31° 06' to 31° 30' N Latitue (Fig. 1). An altitude of valley varies from 1500 to 4500 m. The length of the Sangla valley is about 65 km which accommodating nearly 9,500 populations. The Sangla valley possess a number of watersheds which find their way into the Baspa River. North facing slope of valley is relatively having adequate flora and fauna cover, soil cover, sporadic settlements and farm fields. Uppermost part of the Sangla valley mountain peaks are generally covered with heavy snow for few months with the melting of ice diversity of beautifully colored flowers inhabits these mountains create a rich storehouse of wild medicinal plants and aromatic plants (Chauhan, 2003). Further, its exceptional geographical location and climatic factors make it a biodiversity rich zone covering of different medicinal and aromatic plant species.

Plant Material

For this study, four field trips of the entire area of Sangla valley were undertaken between 2017 to 2018. The information on medicinal plants used by tribal people of Sangla valley was collected by using a pretested questionnaire, interviews, participatory observation and through discussions method (Fig. 2). Only those wild medicinal plants were collected, which were most often used by the tribes of Sangla valley for the treatment of different diseases. Plant species were collected from study sites, shade dried, pulverized and put in storage for further use (Fig. 3). Collection of the plant specimens has been done during field trips to

facilitate the process of identification, dried specimens were identified consulting Botanical Survey of India, Dehradun Uttarakhand and identified plants were deposited to herbarium of Shoolini University, Solan to get voucher number. Information collected during field surveys were consulted from the relevant literature given (Rawat and Kharwal, 2011).

Chemicals and Reagents

All solvents used for extraction method were purchase from Merk, Germany. The different chemicals used for screening of phytochemical groups were bought from Himedia, Mumbai, India.

Preparation of Leaf Extracts

30g of air dried fine leaf powder were infused in ethanol (100ml), up to whole exhaustion. The mixture was filtered with muslin cloth and stored at 4°C temperature for further use.

Phytochemical Test

The screening of the 28 selected medicinal plants for various phytochemical constituents were analyzed (Table 1). Phytochemical screening in medicinal plants is already done by many workers from different countries of the World. We characterized the different chemicals groups such as, Steroids, Glycosides, Saponin, Alkaloids, Tannins, Reducing sugar, Flavonoids and Terpenoids with reference to the technical described in the work (Farnsworth, 1974, Trease and Evans, 1987, Bruneton, 1999, Sharma *et al.*, 2006).

- 1. Glycosides :** First, in a test tube 1 ml of aqueous extract was added with few volume of distilled water. The mixture was shaken and detected for a stable persistent froth for 15-20 minutes.
- 2. Steroids :** Steroids were sought by the reaction of Liebermann. 10 ml of ethanoic extract of leaf was evaporated. The residue of extract was dissolved in 0.5 ml of warm acetic anhydride; we added 0.5 ml of the remains chloroform. It treated with the chemical of Libermann Burchardt. Then appearance, of a ring of blue-green, indicated a positive response.
- 3. Alkaloid :** First, evaporate 10 ml of concentrated etheric solution, the dry filtrate was mixed with 1.5 ml of HCl (2%) acid solution. After that, add two drops of Wagner and Mayer's reagent, and the yellow- white colour precipitate identifies the existence of the alkaloidal base.
- 4. Saponins :** Initial, a test tube 1 ml of aqueous extract was added with few volume of distilled water. The solution was shaken vigorously for few minutes and

observed for a stable persistent froth for 15-20 minutes.

5. **Tannins** : First, in a test tube about 1 ml of the ethanol extract was added with 2 ml of distilled water. Three drops of diluted ferric chloride solution were added and observed for green to blue-green colour specifies the presence of tannin.
6. **Flavonoids** : The ethanol extract 5ml was mixed to a concentrated Sulphuric acid 1 ml and 0.5 gram of Magnesium (Mg). A red or pink coloration that disappear on standing three minutes signifies the presence of flavonoids.
7. **Reducing sugar** : Initial, the ethanol extract 1 ml was mixed to 1 ml of distilled water and added 18-20 drops of boiling Fehling's solution (A and B) in a test tube. The indication of a precipitate red-brick coloration in the bottom of the test tube specifies the presence of reducing sugars.
8. **Terpenoids** : To the test solution add 2 ml of chloroform and 1 ml of concentrated Sulfuric acid. Appearance of reddish brown colour indicates the presence of terpenoids.

Results and Discussion

Plant species which have one or more of its organ containing materials that can be used for the healing purpose, are called medicinal plant species (Driver, 1960). It was observed that in Sangla valley a total of 28 medicinal plants were being used by tribal people a few of these belonged to the same or different families. The observations and inferences made in phytochemical tests of 28 medicinal plants is tabulated (Table 2). These phytochemical tests indicate the presence of several bioactive secondary metabolites which is responsible for medicinal characteristics. It was observed that in Sangla valley a total of 28 ethomedicinal plants were being used by tribal people and a few of these belonged to the same families. It was recorded that herbs species were markedly high (17) followed by shrub (6), tree (4), and climber (1). The preliminary phytochemical screening carried out on ethanolic extracts of *Asparagus adscendens*, *Argemone mexicana*, *Achyranthes bidentata*, *Achillea millefolium*, *Betula utilis*, *Betula alnoides*, *Berberis aristata*, *Berberis lycium*, *Cannabis sativa*, *Chenopodium album*, *Dioscorea deltoidea*, *Euphorbia hirta*, *Ephedra gerradiana*, *Ficus religiosa*, *Hippophae salicifolia*, *Heracleum lanatum*, *Hedychium spicatum*, *Leycesteria formosa*, *Picrorhiza kurroo*, *Phytolacca acinosa*, *Solanum nigrum*, *Solanum surattense*, *Selinium vaginatum*, *Trillium govanianum*, *Urtica dioica*, *Urtica palviflora*, *Valeriana jatamansi* and *Vitex negundo*. Leaves revealed the presence of

phytoconstituents such as flavonoids, alkaloids, tannins, saponins, reducing sugars, terpenoids and flavonoid (Table 2). All phytochemicals i.e. Steroids, Glycosides, Saponin, Alkaloids, Tannins, Reducing sugar, Flavonoids and Terpenoids were present in all selected wild medicinal plants. Tannin were absent in three species i.e. *Cannabis sativa* (Cannabaceae), *Euphorbia hirta* (Euphorbiaceae) and *Leycesteria formosa* (Table 2). Phytochemical groups such as flavonoids, tannins, alkaloids and several other secondary metabolites or aromatic compounds of plants serve as defense mechanism against predation by many insects, microorganism, and herbivores. The therapeutic properties of medicinal plants are may be due to the presence of a number of secondary metabolites such as flavonoids, alkaloids, phenols, glycosides, steroids and saponins (Table 1) etc. Due to the presence of secondary metabolites biological activities of medicinal plant species possess many important activities such as antidiabetic, hypoglycemic, antimicrobial, antioxidant, anti-inflammatory, anticholinergic, antimalarial, activities etc. These ethnomedicinal plants were not only used by tribal peoples for ethnomedicines but they also used them for multipurpose such as ethnoveterinary, food, fodder and fuel etc. The results of this survey propose that ethnobotanical remedies play significant role in tribal people's life. Unfortunately, this traditional knowledge of tribal people is vanishing fast and also there is a lack of proper documentation of ethnomedicine of the study area. There is urgent need to document this vast knowledge and experience of tribal people concerning the use of ethnomedicines for human healthcare. It may be concluded that these medicinal plant species are very useful. These plants may be used to cure some common and other different diseases (Peach and Tracey, 1955). Medicinal plant species have been used in the form of indigenous systems of medicine. Mentioned that the complete phytochemical screenings of medicinal plant species of India should be carried out, because these secondary metabolites are responsible for medicinal activity of the plant species. Number of plant species were determined for secondary metabolites for their medicinal values.

Conclusion

Thus, from the present study the plant leaf extracts of *Asparagus adscendens*, *Achyranthus bidentate*, *Betula utilis*, *Betula alnoides*, *Berberis aristata*, *Berberis lycium*, *Cannabis sativa*, *Dioscorea deltoidea*, *Euphorbia hirta*, *Ephedra gerardiana*, *Ficus religiosa*, *Hippophae salicifolia*, *Picrorhiza kurroo*, *Phytolacca acinosa*, *Solanum surattense*, *Solanum nigrum*, *Trillium govanianum*, *Valeriana jatamansi* and *Vitex negundo* showed presence of phytochemicals groups and they can

be further used in the pharmaceutical industries for drug production. The review of the past work done and the result of this study suggest that screening of secondary metabolites and ethnomedicines used by tribal people has been already reported from different parts of India. It can be concluded that the source of secondary metabolites like, flavonoids, carbohydrates glycosides, phytosterols, phenols and alkaloids, are present in medicinal plants. Because of the presence of these secondary metabolites they possess high healing potential. These phytochemicals render the medicinal values of the studied plants. According to the results of the present investigation screening of 28 selected medicinal plants clearly reveals that the maximum classes of phytoconstituents are present in medicinal plants extract. Hence, the above mentioned medicinal plant extract could be explored for its highest therapeutic efficacy by pharmaceutical companies in

order to progress safe for many ailments. It can be determined that presence of secondary metabolites might be responsible for their therapeutic effects. It additional reflects a hope for the development of more good chemotherapeutic agents from such plant species which in further may serve for the production of improved therapeutic agents.

Acknowledgment

We express our sincere thanks to Botanical Survey of India (BSI) Dehradun, Uttarakhand for the identification of plant specimens collected from study sites. We also thankful to tribal people of Kinnaur district in Sangla valley to provide us valuable information of ethnomedicines.

Conflict of Interest

Author declare that we have no conflict of interest.



Fig. 1 : Map of Study site (Location of Sangla Valley)



Fig.2: Interaction with tribal people of Sangla valley in district Kinnaur (Himachal Pradesh).

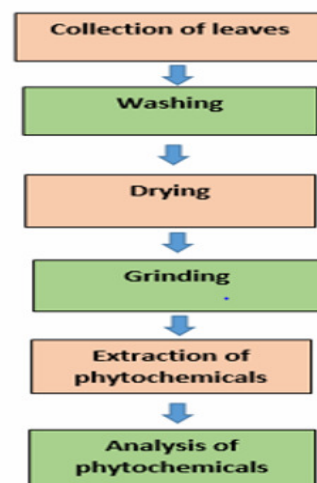


Fig.3: Methodology Summary

Table 1: Qualitative tests for Phytoconstituents.

Sr. No.	Experiment	Observation
1.	Test for Steroids	Appearance of blue green ring.
2.	Test for Glycosides	Appearance red precipitate forms.
3.	Test for Saponins	Appearance foam forms.
4.	Test for Alkaloid	Appearance of white-yellow precipitate.
5.	Test for Tannins	Appearance of blue to green or blue black coloration.
6.	Test for Reducing sugar	Appearance of red brick precipitate.
7.	Test for Flavonoids	Disappear of pink or red coloration on standing indicates the presence of flavonoids.
8.	Test for Terpenoids	Appearance of reddish brown color.

Table 2: Qualitative analysis of selected medicinal plants.

Sr. No.	Name of the Plant	Steroids	Glycoside	Saponins	Alkaloids	Reducing sugar	Tannin	Flavonoids	Terpenoids
1.	<i>Asparagus adscendens</i> Roxb	+	+	+	+	+	+	+	+
2.	<i>Argemone Mexicana</i> L.	+	+	+	+	+	+	+	+
3.	<i>Achyranthes bitdentata</i> Blume	+	+	+	+	+	+	+	+
4.	<i>Achillea millefolium</i> L.	+	+	+	+	+	+	+	+
5.	<i>Betula utilis</i> D. Don	+	+	+	+	+	+	+	+
6.	<i>Betula alnoides</i> Buch.- Ham. ex D. Don	+	+	+	+	+	+	+	+
7.	<i>Berberis aristata</i> DC.	+	+	+	+	+	+	+	+
8.	<i>Berberis lycium</i> Royle	+	+	+	+	+	+	+	+
9.	<i>Cannabis sativa</i> L.	+	+	+	+	+	-	+	+
10.	<i>Chenopodium album</i> L.	+	+	+	+	+	+	+	+
11.	<i>Dioscorea deltoidea</i> Wall	+	+	+	+	+	+	+	+
12.	<i>Euphorbia hirta</i> L.	+	+	+	+	+	-	+	+
13.	<i>Ephedra gerradiana</i> Wall	+	+	+	+	+	+	+	+
14.	<i>Ficus religiosa</i> L.	+	+	+	+	+	+	+	+
15.	<i>Hippophae salicifolia</i> D. Don	+	+	+	+	+	+	+	+
16.	<i>Heracleum lanatum</i> Michx	+	+	+	+	+	+	+	+
17.	<i>Hedychium spicatum</i> Sm	+	+	+	+	+	+	+	+
18.	<i>Leycesteria formosa</i> Wall.	+	+	+	+	+	-	+	+
19.	<i>Picrorhiza kurroa</i> Royle ex Benth	+	+	+	+	+	+	+	+
20.	<i>Phytolacca acinosa</i> Roxb	+	+	+	+	+	+	+	+
21.	<i>Solanum nigrum</i> L.	+	+	+	+	+	+	+	+
22.	<i>Solanum surattense</i> Burm. F.	+	+	+	+	+	+	+	+
23.	<i>Selinium vaginatum</i> C.B. Clarke	+	+	+	+	+	+	+	+
24.	<i>Trillium govanianum</i> D. Don	+	+	+	+	+	+	+	+
25.	<i>Urtica dioica</i> L.	+	+	+	+	+	+	+	+
26.	<i>Urtica palviflora</i> Roxb.	+	+	+	+	+	+	+	+
27.	<i>Valeriana jatamansi</i> D. Don	+	+	+	+	+	+	+	+
28.	<i>Vitex negundo</i> L.	+	+	+	+	+	+	+	+

Note: (-) Absence; (+) Presence.

Table 3: Ethno-medicines used by tribal people of Sangla valley in higher altitude of Kinnaur District.

Sr. No.	Name of the Plant	Family	Local name	Habit	Voucher Number	Ailments/diseases treated
1.	<i>Asparagus adscendens</i> Roxb	Asparagaceae	Safed musali	Shrub	SUBMS/BOT-1011	Cough, Cold Fever, Reduce high blood pressure
2.	<i>Argemone Mexicana</i> L.	Papaveraceae	Satayanashi	Herb	SUBMS/BOT-1023	Cough, Cold, Fever, Jaundice, Asthma
3.	<i>Achyranthes bitdentata</i> Blume	Amaranthaceae	Putkanda	Herb	SUBMS/BOT-1002	Reduce high blood pressure, Skin infection, Wound healing, Joint pain
4.	<i>Achillea millefolium</i> L.	Asteraceae	Bhutkshi	Herb	SUBMS/BOT-1002	Cough, Cold, Fever, Skin infections, Joint pain
5.	<i>Betula utilis</i> D. Don	Betulaceae	Bhojpatra	Tree	SUBMS/BOT-1003	Cough, Cold, Fever, Asthma
6.	<i>Betula alnoides</i> Buch.- Ham. ex D. Don	Betulaceae	Bhojpatra	Tree	SUBMS/BOT-1004	Dysentery, Diarrhea, Throat complaints
7.	<i>Berberis aristata</i> DC.	Berberidaceae	Kashmal	Shrub	SUBMS/BOT-1005	High Blood Pressure, Body pain, Respiratory infection
8.	<i>Berberis lycium</i> Royle	Berberidaceae	Kashmal	Shrub	SUBMS/BOT-1006	Headache, Skin allergy, Cough, Cold, Reduce high blood pressure
9.	<i>Cannabis sativa</i> L.	Cannabinaceae	Bhang	Herb	SUBMS/BOT-1007	Urinary infections, Dysentery, Sun burn, Reduce high blood pressure
10.	<i>Chenopodium album</i> L.	Chenopodiaceae	Bathua	Herb	SUBMS/BOT-1021	Asthma, Cold, Cough, Jaundice, Reduce high blood pressure
11.	<i>Dioscorea deltoidea</i> Wall	Dioscoreaceae	Shingli-mingli	Climber	SUBMS/BOT-1008	Sprain of foot, Asthma, Cold, Cough, Jaundice
12.	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Bara dudhi	Herb	SUBMS/BOT-1009	Stone in bladder
13.	<i>Ephedra gerradiana</i> Wall	Ephedraceae	Somlata	Shrub	SUBMS/BOT-1010	Asthma, Cold, Cough
14.	<i>Ficus religiosa</i> L.	Pipal	Moraceae	Tree	SUBMS/BOT-1011	Headache, Wounds
15.	<i>Hippophae salicifolia</i> D. Don	Achok	Elaeagnaceae	Shrub	SUBMS/BOT-1012	Sprain of foot, Asthma, Cold
16.	<i>Heracleum lanatum</i> Michx	Patrala	Apiaceae	Herb	SUBMS/BOT-1020	Headache, Skin allergy, Cough, Cold, Reduce high blood pressure
17.	<i>Hedychium spicatum</i> Sm.	Zingiberaceae	Kapurkachri	Herb	SUBMS-BOT-1027	Skin allergy, Cough, Cold, Reduce high blood pressure
18.	<i>Leycesteria formosa</i> Wall.	Caprifoliaceae	Piralu	Shrub	SUBMS/BOT-1026	Cough, Cold
19.	<i>Picrorhiza kurroa</i> Royle ex Benth	Kour	Scrophulariaceae	Herb	SUBMS/BOT-1013	Headache, Skin allergy, Cough, Cold, Joint pain
20.	<i>Phytolacca acinosa</i> Roxb	Jalga	Phytolacceae	Herb	SUBMS/BOT-1014	Skin allergy, Cough, Cold, Joint pain
21.	<i>Solanum nigrum</i> L.	Solanaceae	Mokoi	Herb	SUBMS/BOT-1015	Skin allergy, Joint pain
22.	<i>Solanum surattense</i> Burm. F.	Kateli	Solanaceae	Herb	SUBMS/BOT-1016	Body pain, Respiratory infection
23.	<i>Selinium vaginatum</i> C.B. Clarke	Apiaceae	Apiaceae	Herb	SUBMS/BOT-439	High blood pressure, Body pain
24.	<i>Trillium govanianum</i> D. Don	Trilliaceae	Nagchhatri	Herb	SUBMS/BOT-1017	Headache, Skin allergy, Cough, Cold
25.	<i>Urtica dioica</i> L.	Urticaceae	BichhuBooti	Herb	SUBMS/BOT-1024	Joint pain, Fever
26.	<i>Urtica palviflora</i> Roxb.	Urticaceae	Kandali	Herb	SUBMS/BOT-1025	Cough, Fever
27.	<i>Valeriana jatamansi</i> D. Don	Valerianaceae	Mushkbala	Herb	SUBMS/BOT-1018	Headache, Skin allergy, Cough, Cold, Asthma
28.	<i>Vitex negundo</i> L.	Lamiaceae	Nirgundi	Tree	SUBMS/BOT-1019	Headache, Skin allergy, Cough, Cold, Skin infections

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