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Phytochemical Screening of the Medicinal Plants of Nepal

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ABSTRACT : *Traditional medicine involves the use of different plant extracts or their bioactive constituents. Medicinal plants are an important source of phytochemicals that offer traditional medicinal treatment of various ailments. This type of study provides the health application at affordable cost. The objective of this research was to test for the presence of phytochemical compounds in thirty two different medicinal plants, which were collected from three different regions of Nepal. Qualitative phytochemical analysis of these plants confirm the presence of various phytochemicals like alkaloids, saponins, glycosides, terpenoids, steroids, coumarins, tannins, flavonoids.*

Keywords - *Screening, Plant extract, Secondary metabolites, Tannins, Steroids.*

I. INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing [2]. The world health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs [3]. Medicinal plants are a source of great economic value all over the world. Around 80% of products were of plant origin and their sales exceeded US $65 billion in 2003 [4]. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Nepal is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In Nepal thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Among the 7,000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are said to be found in Nepal [5].

The medicinal values of the plant lies in some organic compounds and the most important of these bioactive constituents are alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids [6]. These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [7]. Among the 120 active compounds currently isolated from the higher plants that are widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [8]. Plant products have been part of phytomedicines since time immemorial. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances [9,10,11]. There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects [9]. The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants [10, 11]. A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value

in disclosing new sources of such economic materials such as flavoniods, tannins, essential oils, gums, precursors for the synthesis of complex chemical substances, etc.

Despite many studies on medicinal plant resources of Nepal, a large number of medicinal plants and associated indigenous uses still wait proper documentation and evaluation of their therapeutic properties. Due to species climatic and geographical conditions, temperate and alpine plants of the Himalaya offer greater possibilities of having novel molecules and even largest quantities of the active compounds. The main purpose of the present study was preliminary qualitative phytochemical analysis in the methanolic extract of thirty three different medicinal plants of Nepal.

II. MATERIALS AND METHODS

2.1 Collection of plant materials

Thirty three fresh medicinal plants (Table 1) were collected during the month of April-May, 2011 from around Daman and Hetauda of Makwanpur District and Dolalghat locations of Kavre District. The plant materials were shade dried under room temperature until all the water molecules evaporated. After drying, the plant materials were cut into small pieces and grounded well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

TABLE 1: Collected plants for investigation

S.No.	Scientific Name	Local Name	Location	Plant parts used
1.	*Caloptris procera*	aank	Hetauda	Leaf, stem, flower
2.	*Tamarindus indica*	imli	Hetauda	Leaf, stem
3.	*Ricinus communis*	ader	Hetauda	Leaf, stem ,seed
4.	*Catharanthus roseus red*	barha mase rato	Hetauda	Leaf, stem, root
5.	*Bauhinia variegata*	koiralo	Dolalghat	Leaf, stem, flower
6.	*Catharanthus roseus white*	barha mase seto	Hetauda	Leaf, stem, root
7.	*Digitalis purpurea*	digitalis	Daman	Leaf, root
8.	*Piper longum*	pipla	Hetauda	Leaf
9.	*Ageratum conyzoide*	gandhe	Daman	Leaf, stem, flower
10.	*Scoparia dulcis*	chini jhaar	Hetauda	Leaf, stem, root
11.	*Mahonia nepalensis*	jagane mandro	Godawari	Leaf, bark, seed
12.	*Cyprus rotundus*	mothe	Hetauda	Leaf, root
13.	*Moringa olifera*	sajeewon	Hetauda	Leaf, stem
14.	*Vitex negundo*	simali	Hetauda	Leaf, stem
15.	*Datura metel*	kalo dhaturo	Dolalghat	Seed
16.	*Berberis aristata/ asiatica*	chutro	Hetauda	Bark, leaf
17.	*Achyranthes aspera*	datiun	Hetauda	Leaf, stem, root
18.	*Vitex negundo*	simali	Panchkhal	Leaf, stem
19.	*Zanthoxylum armatum*	timbur	Daman	Leaf, stem
20.	*Zanthoxylum armatum*	timbur	Dolalghat	Leaf
21.	*Curculigo orchioides*	syaal dhote musali	Daman	Whole plant
22.	*Bergenia ciliata*	pasaad bedh	Daman	Tuber
23.	*Astilbe rivularis*	thulo akhoti	Daman	rhizome
24.	*Dioscorea deltoidea*	kukur tarul/bhyakur tarul	Dolalghat	Tuber
25.	*Abies spectabilis*	talispatra	Hetauda	Leaf, stem
26.	*Eclipta prostrate/ alba*	bhringa raj	Hetauda	Leaf, Stem, root
27.	*Lobelia pyramidalis*	aclabir	Daman	Leaf
28.	*Terminalia chebula*	harro	Hetauda	Bark
29.	*Acorous calamus*	bojo	Godawari	Tuber
30.	*Dipsacus inermis*	ban mulaa	Daman	Tuber
31.	*Taxas baccata*	loth salla	Panchkhal	stem
32.	*Taxas baccata*	loth salla	Godawari	Leaf

2.2 Preparation of plant extracts

2.2.1 Solvent extraction
Crude plant extract was prepared by Soxhlet extraction method. 10gm of powdered plant material was uniformly packed into a thimble and extracted with 100ml of methanol. The process of extraction continued for 18- 24 hours. Chlorophyll was removed where necessary by treating methanol extract with hexane in separating funnel. The extracts were concentrated by keeping beakers in water bath set at 55ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysis.

2.3 Qualitative phytochemical analysis
Preliminary qualitative phytochemical screening was carried out following standard protocols [12,13,14].

2.3.1 Test for reducing sugars (Fehling's test)
Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

2.3.2 Test for glycoside
4 ml of extract solution was dried till 2 ml. To it was added 1-2 ml of Ammonium Hydroxide and shaken. Appearance of cherish red color indicates the presence of glycosides.

2.3.3 Salkowski's test
Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

2.3.4 Keller-Kilani test
Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of cardiac glycosides.

2.3.5 Test for polyphenols and tannins
Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or blue- black coloration indicated the presence of polyphenols and tannins.

2.3.6 Test for flavonoids
Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink or magneta red colour appeared after few minutes which indicated the presence of flavonoids.

2.3.7 Test for saponins
Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously for 30 seconds. The formation of stable foam (1 cm height) even after 30 minutes was taken as an indication for the presence of saponins.

2.3.8 Test for steroids
Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

2.3.9 Test for terpenoids
Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added, a reddish brown coloration at the interface indicated the presence of terpenoids.

2.3.10 Test for alkaloids
Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.3.11 Test for coumarins
Extract solution is concentrated to yield a residue. Dissolve residue in hot water. After cooling divide solution in two test tubes. To one test tube add 10% (w/v) Ammonium Hydroxide. Other test tube is used as control. Fluorescence color indicates the presence of coumarins.

2.4 Data analysis
The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test. All experiments were done in triplicates.

III. RESULTS AND DISCUSSION

TABLE 2 Result of preliminary qualitative phytochemical analysis

Plants	Alkaloid	Saponin	Coumarin	Glycoside	Tannin and Phenol	Reducing sugars	Flavonoid	Steroids	Triterpenoid
Caloptris procera	-	+	+	-	+	+	-	-	+
Tamarindus indica	+	-	+	+	+	+	+	+	+
Ricinus communis	+	-	+	+	+	+	+	-	+
Catharanthus roseus red	+	+	-	+	+	+	-	-	+
Bauhinia variegata	+	+	+	+	+	+	+	-	+
Catharanthus roseus white	+	+	-	+	+	+	-	-	+
Digitalis purpurea	+	+	+	+	+	+	-	+	+
Piper longum	+	+	+	-	+	+	-	+	+
Ageratum conyzoide	-	+	+	-	+	+	-	+	+
Scoparia dulcis	+	+	+	-	+	+	-	+	+
Mahonia nepalensis	+	-	-	+	+	+	+	-	+
Cyprus rotundus	+	+	+	-	-	+	-	-	+
Moringa olifera	+	+	+	-	+	-	-	+	-
Vitex negundo (Hetauda)	+	+	+	-	-	+	-	+	-
Datura metel	+	-	-	-	-	+	-	-	+
Berberis aristata/ asiatica	+	+	+	+	+	+	-	+	+
Achyranthes aspera	+	+	+	-	+	+	+	+	+
Vitex negundo (Panchkhal)	+	+	+	-	+	+	+	+	+
Zanthoxylum armatum (Daman)	+	+	+	-	+	+	+	+	+
Zanthoxylum armatum (Dolalghat)	+	-	+	+	+	+	+	-	+
Curculigo orchioides	-	+	-	+	-	+	+	-	+
Bergenia ciliata	+	+	-	+	+	+	+	-	-
Astilbe rivularis	+	+	+	-	-	+	+	-	+
Dioscorea deltoidea	+	+	-	+	-	+	+	-	+
Abies spectabilis	+	+	+	-	+	+	+	+	+
Eclipta prostrate	+	+	+	-	+	-	-	+	+
Lobelia pyramidalis	+	-	-	-	-	+	-	-	-
Terminalia chebula	+	+	+	-	+	+	+	+	+
Acorous calamus	+	-	-	+	-	+	+	+	+

Dipsacus inermis	+	+	+	-	-	+	-	-	+
Taxas baccata (Panchkhal)	+	-	+	+	+	+	+	+	+
Taxas baccata (godawari)	+	-	+	+	+	+	+	+	+

Note: '+' indicates presence and '-' absence

The result of preliminary phytochemical analysis of thirty two medicinal plants tested is tabulated in Table 2. The phytochemical study revealed the presence of various phytochemicals in the methanolic extracts of different medicinal plants. None of the tested plant had all the phytochemicals i.e Alkaloid, Saponin, Coumarin, Glycoside, Tanin, Reducing Sugars, Flavonoid, Steroids and Triterpenoid . Unlike the other tested plants no alkaloids were found in *Caloptris procera, Ageratum conyzoide and Curculigo orchioides*. Reducing sugars were found to be present in all the plants but absent in *Moringa olifera and Eclipta prostrate*. Triterpenoids were found to be present in all the plants except *Moringa olifera, Vitex negundo* (Hetauda), *Bergenia ciliate and Lobelia pyramidalis.* All the phytochemicals were present in *Tamarindus indica, Bauhinia variegate* and *Digitalis purpurea* except saponin, steroids and flavonoid respectively. In plants like *Berberis aristata* and *Digitilas purpurea,* flavonoids were absent, however the rest of the phytochemicals were present in all the selected plants. *Abies spectabilis* and *Terminalia Chebula* showed common phytochemical characteristics because both were tested negative only for glycoside. *Achyranthes aspera, Vitex negundo* (panchkhal) *and Zanthoxylum Armatum* (Daman) showed the presence of all the compounds except glycosides. Both *Taxas baccata* (Panchkhal) and *Taxas baccata* (Godawari) showed similar phytochemical characteristics. Infact, only saponin was found to be absent in *Taxas baccata*. Both the species of *Catharanthus roseus*, white and red, showed similarities in the presence and absence of the phytochemicals tested. *Vitex negundo* (Daman) showed the presence of tannins, flavonoids and triterpeniods where as the similar plant of Hetauda did not. In *Zanthoxylum Armatum* (Daman), saponin and steroids were found to be present where as in *Zanthoxylum Armatum* (Dolalghat) those were absent . However, glycoside was present in *Zanthoxylum Armatum* (Dolalghat) but not in *Zanthoxylum Armatum* (Daman).

Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids, which are known to exhibit medicinal as well as physiological activities. Several workers have reported the analgesic [15], antispasmodic and antibacterial [16, 17] properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports [18]. Phenolic compound possess biological properties such as apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [19]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation [20]. Steroids have been reported to have antibacterial properties [21] and they are very important compounds especially due to their relationship with compounds such as sex hormones [22]. The growth of many fungi, yeasts, bacteria and viruses can be inhibited by tannins [23]. Terpinoids are found in 29 medicinal plants out of 32 plants selected. Terpenoids and tannins are attributed for analgesic and anti-inflammatory activities.

Although, the absence of certain phytochemicals in one sample and its presence in the other can be safely attributed to the various physiological and biosynthetic reactions taking place inside the plant, the effect of the environment should not be neglected, as the environment always modify the things. The preliminary phytochemical tests are therefore significant and helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds [24].The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

IV. CONCLUSION

The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to see whether these plants have said health benefits, especially as anti cancer drugs, and elucidate the possible mechanism of action of these extracts.

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