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# Qualitative and Quantitative Phytochemical Studies of Medicinal Plants of Chithor Valley, Hindukush Range District Swat, KP, and Pakistan

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# **ARTICLE INFO**

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#### **Declaration**

#### **Authors' Contribution**

All authors equally contributed to the study and approved the final manuscript

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# **ABSTRACT**

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain Alkaloids, Flavonoids, Phenol, Saponins, Steroids and Tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti-inflammation activities. The present study involves ten different medicinal plants Adiantum capparis-veneris L, Asplenium dalhouseae, Asplenium trichomanas, Athyrium oxyphyllum, Berberis lyceum, Budhleja crispa, Daphne mucronata, Mentha longifolia, Quercus incana, Rubus sanctus locally available in Chithor Valley, Hindukush range, district Swat, KP, Pakistan. The leaves of the selected medicinal plants were washed, dried and then powdered. The methanolic extract of leaves samples were used for the phytochemical analysis to find out the quantitative and quantitative phytochemical constituents in the plants. The result of the phytochemical analysis of these medicinal plnts showed presence or absence as well as quantitative (mg/g) contain of Flavonoids, Saponins, terpenoids, phlobatannins and sugars in the selected medicinal plants.

# INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits humans further than those attributed macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor [1, 2]. The chemicals that are produced by plants are called as phytochemicals. These are produced by the plant's primary and secondary metabolism. These phytochemicals are important for the plants to thrive or thwart other plants, animals, insects and microbial pests and pathogens. They also help plants and protect them from disease and damage caused by environmental hazards like pollution, UV, stress and draught. They are used as traditional medicine and as poisons from ancient days. Phytochemicals are not the essential nutrients they are rather than the essential nutrients because there is no proof for them to cause any possible health effects in humans are not still established. It is known that they have roles in the protection of human health. More than 4,000 phytochemicals have been catalogued and are classified by protective function,

physical characteristics and chemical characteristics [3, 21, 22, and 23]. A characteristic of plant life is the production of a vast number of natural compounds, often called secondary metabolites. Phytochemicals basically divided in two groups that are primary and secondary metabolites based on the function in plant metabolism. Secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so Secondary metabolites have crucial role in plant development as well as in the interaction of a plant with its biotic and a biotic environment. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries and establishment of crude drugs [4]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates and lead compounds in synthetic drugs [5].

The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value. These compounds are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. The medicinal value of plants lies in some chemical substances (usually secondary metabolites) that produce a definite physiological action as the human body [6]. The world is blessed with natural and unique medicinal plants. A medicinal plant is any plant, which in one or more of its organs contains active ingredients which can be used for therapeutic purposes or contain foundation compounds that can be used for the synthesis of useful drugs. Medicinal plants have invariably been a rich source of new drugs and many drugs in use today were either obtained from plants or developed using their chemical structure as templates. The medicinal value of these plants lies in bioactives phytochemical constituents that produce definite physiological action on the human body [7]. Plants are not only the source of food, feed, energy and raw material, but also a potent source of medicines. The history of using plant based medicines is embedded into the histories of people and the civilizations [8]. In recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems including treatment against hepatocellular carcinoma. Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care. Assessing the current status of health care system, inadequacies of synthetic drugs are likely to be more glaring in the coming years. In the present study, we have concentrated on the preliminary screening, quantitative determination, and the qualitative separation of secondary metabolites from leaves of selected ten different medicinal plants [9, 10]. Plants are used as medicine and food since time immemorial round the globe due to their most valuable properties. Medicinal plants play an important role in drug discovery, and human beings used them for various purposes from ancient time. A trend in phytomedicine is the use of original plant bioactive compounds with the potential for chemical modification, which will broaden phytomedicinal importance [11]. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds. Terpenoids exhibit various important pharmacological activities anti-inflammatory, i.e., anticancer, anti-malarial, inhibition of cholesterol anti-viral and anti-bacterial activities. synthesis, Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anesthetic agents and are found in medicinal plants [12, 13]. Naturally phytochemicals occur in medicinal plants, leaves, grains, vegetables and roots etc. They are primary

and secondary compounds. Primary compound includes chlorophyll, proteins and sugars whereas in secondary compounds flavonoids, alkaloids, sterols, terpenoids, flavonoids, saponins, tannins, volatile oils, etc [14].

#### **MATERIALS AND METHODS**

#### **Collection of Plant Materials**

The plants were collected from Chithor Valley, Hindukush range, district Swat, KP, Pakistan.

The leaves were washed, cleaned and chopped into pieces and dried at 40  $^{\circ}$ C in thermostatically controlled oven until they attained a constant weight. The samples were then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

## **Preparation of Plant Extract**

10g of each powdered leaves were placed in conical flask and 100 ml of methanol was added and plugged with cotton. The powder material was extracted with methanol for 24 hours at room temperature with continuous stirring. After 24 hours the supernatant was collected by filtration and the solvent was evaporated to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use [15].

#### Chemicals

Fehling solution A and Fehling solution B, ethanol, distill water, aqueous HCl, methanol, chloroform, concentrated sulphuric acid, Ammonia solution, picric acid, Hexane.

#### **Preliminary Phytochemical Screening**

The methanolic extracts of following plants was subjected to different chemical tests for the detection of different phytoconstituents using standard procedures.

#### **Test for Phlobatannins**

Plant powder sample was mixed with distill water in a test tube, then shaked it well, and filtered to take plant extract. Then to each plant extract, 1% aqueous hydrochloric acid was added and each plant sample was then boiled with the help of Hot plate stirrer. Formation of

Red colored precipitate confirmed a positive result.

## **Test for Reducing Sugar**

An amount of 0.50~g of selected plant sample was added in 5~ml of distilled water. Then 1~ml of ethanol mixed in plant extract. After that we took 1~ml of Fehling solution A and 1~ml of Fehling solution B in a test tube, heated it to boiling and then poured it in the aqueous ethanol Extract. When color reaction was observed, it shows a positive result.

# **Test for Terpenoids**

An amount of  $0.8\,\mathrm{g}$  of selected plant sample was taken in a test tube, then poured  $10\,\mathrm{ml}$  of methanol in it, shaken well and filtered to take  $5\,\mathrm{ml}$  extract of plant sample. Then  $2\,\mathrm{ml}$  of chloroform were mixed in extract of selected plant sample and  $3\,\mathrm{ml}$  of sulphuric acid were added

in selected sample extract. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

## **Test for Flavonoids**

For the confirmation of flavonoid in the selected plants, 0.5 g of each selected plant extract were added in a test tube

and 10 ml of distill water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H2S04. Indication of yellow color shows the presence of flavonoid in each extract.

[16].

## **Test for Saponins**

1 g of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

- a) **Demonstration of frothing:** 2.5 ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2mins, formation of froth which is stable for some minutes indicate the presence of saponins in the filtrate.
- b) **Demonstration of emulsifying properties:** 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

# Quantitative Determination of Phytochemical Constituents

# **Quantitative Test of Phytochemicals**

Quantitative test of phytochemical was done by the gravimetric method described by Harborne, (1973):

#### Test for Flavonoids

The powdered sample i.e. 5 gram was placed into a conical flask with 100 ml of water and 2ml HCL solution was added. The solution was allowed to boil for 30 minutes and allowed to cool before filtered into Whattman No. 42 filter paper. Aqueous layer was discarded and filtered with preweighted filter paper. Residue of filter paper was dried in an oven for 30 minutes at 600C. Weight of flavonoids was calculated by using following formula.

% Flavonoid =  $W2 - W1 \div Weight$  of Sample × 100 Where,

W1 = weight of empty filter paper

W2 = weight of paper + Flavonoid extract

### **Test for Terpenoids**

Dried plant extract 10 gram (Wi) was taken and soaked in 90 ml of ethanol. The extract after filtration was mixed with 10 ml of petroleum ether and again filtrated using separating funnel. The extract was waited for its complete drying and measurement is taken (Wf). The yield (%) of total terpenoids contents was measured by the formula: Total terpenoids =  $Wi - Wf \div Wi \times 100$ 

Where,

Wi= dried plant extracts,

Wf= extracts after drying

## **Test for Saponins**

The plant extract i.e. 25 ml was placed in a round bottom flask.100 ml of 50% alcohol was added and boiled for 30 minutes and filtered while hot through a filter paper. 2 gram of charcoal was added to the filtrate and it is boiled and filtered while hot. The filtrate was cooled and an equal volume of acetone was added to completely precipitate the

saponins. The precipitated saponins were collected. % of true saponins =  $W2-W1 \div W1 \times 100$ 

Where.

W1=Weight of filter paper W2=Weight of residue

[17].

#### RESULT AND DISCUSSION

The presence or absence of phytochemicals was evaluated using qualitative analysis of leaves from selected ten medicinal plants. The results are provided in Table 1. Saponins are found in all ten plants, according to the study [18] also reported. Saponins contain a variety of functions, including the ability to precipitate and coagulate red blood cells, as well as the ability to bind cholesterol. It also shows foam formation in aqueous solutions and hemolytic action, and saponins have traditionally been employed as detergents and molluscicides. In addition to their industrial applications as foaming and surface-active agents, saponins have beneficial health effects against various diseases as according to [19, 20].

**Table 1**Qualitative phytochemical studies of medicinal plants of Chithor Valley, Hindukush range, district Swat, KP, Pakistan.

S. No	Botanical Name	Phytochemical classes				
		Saponins	Sugars	Flavonoids	Terpenoids	Phlobatannins
1.	Adiantum capparis- veneris L.	+	+	-	-	-
2.	Asplenium dalhouseae	+	-	+	+++	-
3.	Asplenium trichomanas	+	-	+	+++	-
4.	Athyrium oxyphyllum	+	-	-	+++	+
5.	Berberis lyceum	+	+	+	+	-
6.	Budhleja crispa	+	-	+	-	-
7.	Daphne mucronata	+	-	+	+++	-
8.	Mentha longifolia	+	-	-	-	+
9.	Quercus incana	+	+	-	++	-
10.	Rubus sanctus	+	+	-	+	-

#### **Keys: +...presence of phytoconstituents**

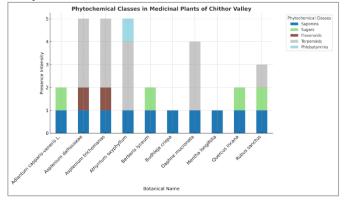
-.... Absence of phytoconstituents

++....Presence with high amount of plants chemical

+++....Presence with abundance of plants chemical

## Figure 1

Stacked bar graph showing the distribution of phytochemical classes in the medicinal plants of Chithor Valley.

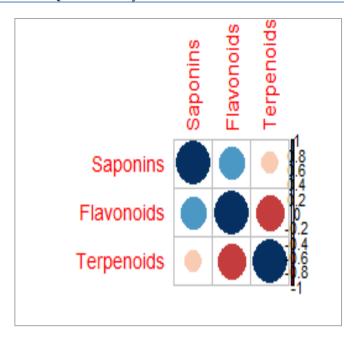


The qualitative phytochemical screening of medicinal plants from Chithor Valley, Hindukush range, district Swat, revealed the presence of important secondary metabolites such as saponins, sugars, flavonoids, terpenoids, and phlobatannins. The results indicate that saponins were present in all the studied plants, which highlights their common occurrence and medicinal importance, since saponins are known for their antimicrobial, antiinflammatory, and immune-boosting activities. Sugars were detected in a few species such as Adiantum capparisveneris, Berberis lyceum, Quercus incana, and Rubus Sanctus, showing their role as primary metabolites and energy sources. **Flavonoids**, important antioxidants, were present in species like Asplenium dalhouseae, Asplenium trichomanas, Budhleja crispa, and Daphne mucronata, which supports their traditional use against oxidative stress-related disorders. Terpenoids were highly abundant in some ferns such as Asplenium dalhouseae, Asplenium trichomanas, and Daphne mucronata (noted as "+++"), while moderate amounts were found in Athyrium oxyphyllum and Quercus incana. Terpenoids are pharmacologically significant for their anti-inflammatory. and anticancer effects. Interestingly. phlobatannins were rarely detected, being present only in Athyrium oxyphyllum, suggesting a species-specific phytochemical profile. Asplenium trichomanas showed the presence of saponins, flavonoids, and an abundance of terpenoids, but lacked sugars and phlobatannins. This diverse phytochemical profile justifies its medicinal use in traditional practices for respiratory and inflammatory ailments. Overall, the study highlights that these medicinal plants are rich sources of bioactive compounds, supporting their ethnomedicinal applications and potential for pharmaceutical exploration.

Table 2 Mean, median, and standard deviation

Compound	Mean	Median	Std. Dev.		
Saponins	3.466	3.065	1.84		
Flavonoids	0.231	0.06	0.31		
Terpenoids	9.405	10.625	7.77		

Figure 2 Inter-relationship among saponins, flavonoids, and terpenoids



ANOVA showing variation between different medicinal plant secondary metabolites

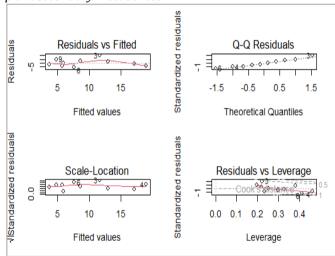


Figure 4 Terpenoids contents in the selected medicinal plants

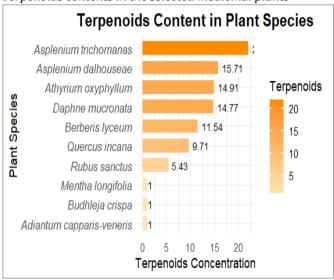
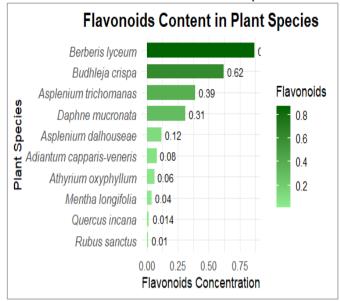
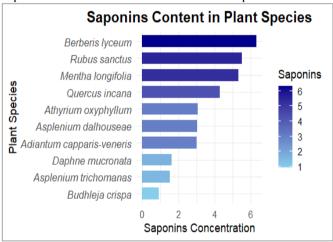


Figure 5

Flavonoid contents in the selected medicinal plants



**Figure 6**Saponins contents in the selected medicinal plants



**Table 3** *Quantitative phytochemical studies of medicinal plants of Chithor Valley, Hindukush range, district Swat, KP, Pakistan.* 

S. No	<b>Botanical Name</b>	Phytochemical classes			
		Saponins (%)	Flavonoids (%)	Terpenoids (%)	
1.	Adiantum capparis-veneris L.	3.01	ND	ND	
2.	Asplenium dalhouseae	3.05	0.12	15.71	
3.	Asplenium trichomanas	1.53	0.39	21.98	
4.	Athyrium oxyphyllum	3.08	ND	14.91	
5.	Berberis lyceum	6.31	0.87	11.54	
6.	Budhleja crispa	0.92	0.62	ND	

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7.	Daphne mucronata	1.64	0.31	14.77
8.	Mentha longifolia	5.31	ND	ND
9.	Quercus incana	4.29	ND	9.71
10.	Rubus sanctus	5.52	ND	5.43

Kevs: ND- Not Detected

The quantitative phytochemical analysis of medicinal plants from Chithor Valley, Hindukush range, district Swat, revealed notable variations in the content of saponins, flavonoids, and terpenoids. Saponins were present in most of the species, with the highest concentrations recorded in Berberis lyceum (6.31%), Rubus Sanctus (5.52%), and *Mentha longifolia* (5.31%), suggesting their potential use in traditional medicine due to known antimicrobial, antiinflammatory, and anticancer activities. Flavonoids, although generally present in lower concentrations, were detected in Berberis lyceum (0.87%), Budhleja crispa (0.62%), and Asplenium trichomanas (0.39%). These compounds are well-recognized for their antioxidant properties, contributing to cardiovascular protection, antimicrobial effects, and cancer prevention. Terpenoids showed the greatest variability, with Asplenium trichomanas (21.98%), Asplenium dalhousae (15.71%), and Athyrium oxyphyllum (14.91%) exhibiting the highest levels. Terpenoids are pharmacologically significant due to their anti-inflammatory, antiviral, antibacterial, and anticancer potential. Interestingly, certain plants such as capparis-veneris and Mentha longifolia Adiantum contained only saponins, while others showed undetectable levels of one or more phytochemicals, highlighting species-specific metabolic differences. Overall, the findings indicate that these medicinal plants are rich reservoirs of bioactive compounds, particularly saponins and terpenoids, which validate their traditional uses and suggest their potential role in the development of novel therapeutic agents.

# CONCLUSION AND RECOMMENDATION Conclusion

From the results of our analysis, it is important to note that the medicinal values of these plants lies in bioactive phytochemical constituents which combined with nutrients and fibres to form an integrated part of human defence mechanisms against diseases and stress.

#### Recommendation

We hereby recommend that further work should be carried out especially on the dosage/response potency of these medicinal plants.

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