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PHARMACOLOGICAL POTENTIAL OF PLANTS FROM HIMALYAN REGION OF PAKISTAN-ASSAY FOR ANTIOXIDANTS INDICES

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ABSTRACT

Objective and layout plan: The project was designed to explore non-enzymatic antioxidants quantities in plants of the Himalayan mountain range, Ayuba, Pakistan. This was aimed at determining their pharmacological potential. After an initial survey, leaves and stem of herbs were collected in three replicas. After proper identification and processing, the extract was prepared in 80 % Methanol. The total alkaloid, Total Phenols, and Flavonoids were determined. The data were subjected to statistical analysis.

Results: The highest amount of total Phenolic contents in leaves of herbs was observed in *Impatiens parviflora* which was 67.79 mg/g of dry weight. The lowest amount of total phenolic contents was observed in *Arisaema wallichianum* 12.72 mg/g. The maximum amount of total phenolic contents was noted in the stem of *Geranium rotun difolium* and the minimum amount of total phenolic contents was found in the stem of *Corydalis lutea*. Total flavonoid contents in leaves of herbs were 43.38 mg/g in *Galium boreale* and 17.83 mg/g in *Arisaema wallichianum*. The maximum quantity of total flavonoids contents in the stem was 43.85 mg/g in *Galium boreale* and a minimum of 6.05 mg/g was in *Impatiens parviflora*. The highest quantity of alkaloids in leaves was 78 mg/g in *Geranium rotundifolium* and the lowest amount of 23.00 mg/g was in *Arisaema wallichianum* and *Artemisia japonica*. The maximum quantity of alkaloids in the stem was 58.0 mg/g in *Ajuga parviflora* and the lowest one was 13 mg/g in *Corydalis lutea*.

Conclusion: *Geranium cotinifolium*, *Ajugareptans, Aesculusindica, Ajugaparviflora, and Ageratum conyzoid*were proved to have more concentration of Phenolics and alkaloids in their leaves.

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Introduction

Several research model revolutions including both experimental and theoretical have made it plausible to fathom the modes of functioning of biological processes at the molecular level [1]. Phytochemicals like phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, phytosterols, etc have been explored for their phytopharmacological properties and utilized for medicinal purposes against many diseases [2-4]. The major bioactive compounds of phenolics, for example, quercetin and kaempferol are responsible for antioxidant activity [5]. These compounds have been used conventionally in therapeutic and curing activities due to having medicinal properties. The significant medicinal properties of many plants are because of the presence of bioactive compounds/phytochemicals [7-9]. Plants are the oldest friends of humans and have been the subject of scholarly researches since ancient times due to their anti-microbial activity and therapeutic properties [10]. The demands for plant-derived alternative medicines in recent year has risen due to their fewer side effects, easy availability and sometimes there is the sole source of cure [11]. This has increased the urge to explore phytochemicals for assessing their medicinal potentials. Many phytochemicals of medicinal plants acting as natural antioxidants can be utilized in the industries for generating pharmaceutical products [12]. Thus, antioxidant related research is a significant topic in the field of medicine. Among the non-enzymatic antioxidants, ascorbic acid, a-tocopherol, carotenoids, and phenols are diet-derived. Other low-molecular-weight antioxidants are serotonin, a-ketoacid, bilirubin, uric acid, coenzyme Q, sex hormones, lipoic acid, etc. [13]. Phenolics and alkaloids are among the most important phytochemicals of medicinal potential.

Phenolics are widely distributed in the plant kingdom and have been reported to have multiple biological effects like antioxidant, anti-inflammatory, free radical scavenging abilities, and anti-carcinogenic potential [14]. Phenolic compounds play a role in the prevention of many diseases such as cardiovascular problems, diabetes, cancer, bacterial and parasitic infections [15]. Flavonoids can decrease oxidative damage to macromolecules by inhibiting the activity of many enzymes such as peroxidase, xanthine oxidase, and nitric oxide synthase, which are supposed to be involved in a free radical

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generation [14]. Many reports are showing that plants possess antioxidant properties due largely to their phenolic compounds [16, 17]. Flavonoids could inhibit blood clotting, can act as an anti-inflammatory, free radical scavenger, and protects from allergic symptoms by inhibiting histamine production [18]. Besides flavonoids, alkaloids have antioxidant, antifungal, anticancer, antiviral, anti-inflammatory, and antiophidic activities [19-21].

Herbal products in the form of leaf powder, crushed material, essential oils, extracts, capsules, and tablets are present in markets. Morphological identification of plants for such product preparation is very challenging [22, 23]. Therefore, the exploration of phytochemicals can also provide a useful tool for discrimination and the authentification of related plant species.

Due to topographic, geomorphological, and ecological conditions, mountainous landscapes of Himalaya is more vulnerable to climate change effects. Change in climate and its adverse impacts are rewarding progressively more obvious in fragile ecosystems of Himalaya. [24]. Temperatures are likely to increase more in the high mountain area [25]. Deforestation increases temperature thus it changes the whole global climatic scenario [26]. The altitude in the hilly area represents a complex gradient along which many environmental factors change spatially and temporally [27]. Along with high altitude, light intensity, precipitation, and radiation intensity increase [28, 29], and temperature decreases [30] with rising altitude. Therefore, the impact of altitude on plant growth is the result of the combined action of various factors [29]. Altitudinal gradients significantly affect the physiological attributes of plants in the Himalayan region. Hence, the altitudinal gradients are excellent tools for analyzing plant responses to stresses their adaptation. Plants have evolved several metabolic pathways as a response to environmental stressors [30]. By focusing on these behavioral changes in plants with elevational gradients, we can measure possible impacts that climate change may have on phytochemistry of plants in such varying environmental conditions.

Materials and Methods

The choice of studies

The aim was to explore the methanol soluble antioxidants or secondary metabolites [31] quantity in plants of the Himalayan mountain range. The study can have predictive value for the pharmacological validation of plants. The selection of Himalayan plants is based on arid environmental conditions which enforce plants to synthesize secondary metabolites for adaptation to stressful environment [32]. The climate of the area is cool in the summers, but harsh in the winters. The cold sets in due to monsoons in late July and early August. In the winter severity of cold increases gradually until the west winds bring rains, which eventually turn into snow. The area remains snow-covered through the later part of winters. The average rainfall is 1,644 mm and temperature ranges 3 °C - 11 °C.

Field survey and plant sample collections

In a preliminary survey of Ayubia and its vicinity, meetings with local peoples were arranged to know the geographical area and local plant names. Intact specimens were collected and herborized from the study area for each new plant species present and mentioned by local people, Herborized specimens were identified by specialists, by matching them with the labeled herbarium exsiccates lying in the departmental herbarium (Dr.Mumtaz Bukhari herbarium) of Botany Department BahauddineZakarya University, Multan Pakistan and/or the literature [33]. Data and specimens were collected keeping uniformity among age and size of plants [34, 35].

Determination of Total Phenolic Contents (TPC)

Total phenolic contents were determined by Folin–Ciocalteu reagent, using the method of Lister and Wilson [36]. 2.5 ml of Folin–Ciocalteu's reagent was mixed with distilled water (1:10 v/v) and 4 ml (7.5%, (m/v)) of Na_2CO_3 was added. The mixture was kept in a water bath at 50°C for 30 minutes. 0.5 ml of each sample was introduced into the mixture. The absorbance of all samples was measured at 765 nm using a spectrophotometer. Gallic acid was used as standard and results were expressed as micrograms gallic acid equivalent per milligram of extract (μg GAE/mg).

Determination of Total Flavonoid Contents. (TFC)

Quantification of flavonoids was done by a method used by Quettier-Deleu et al. [37] with little modification. 50.0g of fine powdered plant material was weighed in 1000ml conical flask and was extracted in 100ml of methanol by shaking at 200 rpm at room temperature for overnight. The extract was filtered through Whatman filter paper and the filtrate was evaporated at a rotary evaporator at 50ºC and weighed.

Determination of alkaloids

Total alkaloid contents were determined by Harborne [38] method with little modification. 50.0g of the fine powdered plant material was weighed into a 1000ml conical flask. The extract was taken by shaking the plant sample in 200ml of 10% acetic acid in ethanol at room temperature at 200rpm for 4h. The extract was passed through the Whatman filter paper and the filtrate was concentrated in a water bath to one-quarter of the original volume at 95ºC and 40% ammonium hydroxide (NH_4OH) was added dropwise for precipitation and allowed to stand for overnight to complete precipitation. The whole solution was filtered through Whatman filter paper, precipitation was oven-dried at 75ºC and weighed.

Statistical analysis

Data obtained was analyzed by using one way ANOVA (Analysis Of Variance) at a 5% level of statistical significance separately for herbs, shrubs, and trees. Means were compared by Duncan's multiple range test [39].

Results

Total Phenolic Contents in leaves of herbs

The maximum amount of total Phenolic contents in leaves of herbs was observed in *Impatiens parviflora* which was 67.79 mg/g of dry weight. The lowest amount of total phenolic contents was observed in *Arwasaema wallichianum*12.72 mg/g of dry weight given in table (1).

Table 1. Total Phenolic Contents (mg/g of dry weight) in leaves and stem of herbs

Sr #	Species	Family	TPC in Leaves of herbs	% Difference	TPC in Stems of herbs	% Difference
1	*Arwasaema wallichianum*	Aricaceae	12.72±1.80 h	81.23	18.20±1.1 gh	56.70
2	*Corydal waslutea*	Papaveraceae	12.73±2.07 h	81.23	8.67±0.80 k	79.37
3	*Ageratum conyzoid*	Asteraceae	61.61±4.9 ab	9.11	41.30±3.6 ab	1.75
4	*Impatiens parviflora*	Balsaminaceae	67.79±5.1 a	0.00	42.16±1.7 a	2.87
5	*Rumex nepalenswas*	Polygonaceae	31.22±2.6 f	53.95	19.31±2.1 g	54.06
6	*Viola odorata*	Violaceae	49.54±3.9 bcd	26.92	35.35±3.1 c	15.90
7	*Artemisia japonica*	Asteraceae	47.24±3.3 bcde	30.31	28.46±2.23 e	32.29
8	*Swertia chirayita*	Gentianaceae	39.80±2.9 e	41.29	39.94±2.4 b	26.39
9	*Mentha arvenswas*	Lamiaceae	25.08±2.1 fg	63.00	12.65±1.2 j	69.91
10	*Geranium rotundifolium*	Geraniaceae	53.45±4.7 abc	21.16	42.03±3.6 a	0.00
11	*Ajuga reptans*	Lamiaceae	53.97±4.5 ab	20.39	30.04±2.6 d	28.54
12	*Aesculus indica*	Spindaceae	39.30±2.9 e	42.12	29.5±2.07 de	30.18
13	*Ajuga parviflora*	Lamiaceae	50.29±3.5 bc	25.81	30.43±2.5 d	27.61
14	*Galium boreale*	Rubiaceae	27.08±2.2 f	60.06	19.24±1.1 g	54.23
15	*Polypodium peltatum*	Polypodiaceae	17.11±2.1 gh	74.76	14.61±1.4 i	65.25
16	*Rumex obtusifolius*	Polygonaceae	41.20±3.6 de	39.22	23.53±2.5 f	44.02
17	*Fragaria Virginia*	Rosaceae	18.20±1.2 gh	73.15	16.86±2 h	59.89
18	*Geranium wallichianum*	Geraniaceae	44.91±3.7 cde	33.76	24.09±2.1 f	42.69

Mean±Standard deviation; Different letters in columns show significant differences; %age difference was calculated from a maximum value

Geranium rotundifolium, Ageratum conyzoid, and Impatiens parviflora had more than 40mg/g phenolic contents while *Viola odorata, Swertia chirayita, Ajuga parviflora*had more than 30 mg/g phenolics in their leaves. The rest of the plant species has less than 30mg/g Phenolics.

Table 2. Total Flavonoids Contents (mg/g of dry weight) in leaves and stem of herbs

Sr #	Species	Family	TFC in Leaves of Herbs	% Difference	TFC in Stem of Herbs	% Difference
1	*Arwasaema wallichianum*	Aricaceae	17.83±0.07 g	58.90	13.25±1.1 i	69.78
2	*Corydal waslutea*	Papaveraceae	37.36±0.3 c	13.88	13.61±1.5 hi	68.96
3	*Ageratum conyzoid*	Asteraceae	31.11±0.11 de	28.28	10.64±1.2 j	75.74
4	*Impatiens parviflora*	Balsaminaceae	18.31±0.19 g	57.79	6.05±0.6 k	86.20
5	*Rumex nepalenswas*	Polygonaceae	17.90±0.1 g	58. 74	13.73±0.9 hi	68.69
6	*Viola odorata*	Violaceae	24.27±0.14 f	44.05	19.20±1.6 f	56.21
7	*Artemisia japonica*	Asteraceae	38.38±0.3 bc	11.53	31.77±2.6 c	27.55
8	*Swertia chirayita*	Gentianaceae	19.86±0.3 g	54.22	16.46±0.78 g	62.44
9	*Mentha arvenswas*	Lamiaceae	27.18±0.15 f	37.34	25.58±1.9 e	41.66
10	*Geranium rotundifolium*	Geraniaceae	31.35±0.1 de	27.73	28.97±2.3 d	33.93
11	*Ajuga reptans*	Lamiaceae	26.35±0.15 f	39.26	16.59±0.6 g	62.17
12	*Aesculus indica*	Spindaceae	41.53±0.17 ab	4.26	28.38±3.03 d	35.28

13	*Ajuga parviflora*	Lamiaceae	32.96±0.46 d	24.02	30.64±2.6 cd	30.13
14	*Galium boreale*	Rubiaceae	43.38±0.38 a	0.00	43.85±3.9 a	0.00
15	*Polypodium peltatum*	Polypodiaceae	23.85±0.35 f	45.02	20.82±1.3 f	52.52
16	*Rumex obtusifolius*	Polygonaceae	42.13±0.13 ab	2.88	35.88±2 b	18.18
17	*Fragaria virginia*	Rosaceae	19.00±0.4 g	56.20	16.00±0.9 gh	63.51
18	*Geranium wallichianum*	Geraniaceae	27.77±0.11 ef	28.28	14.09±1.ghi	67.87

Mean±Standard deviation; Different letters in columns show significant differences; %age difference was calculated from a maximum value

Total Phenolic Contents in the stem of herbs

The highest amount of total phenolic contents was observed in the stem of the herb of *Geranium rotundifolium* and the lowest amount of total phenolic contents was observed in *Corydal waslutea* of the stem of herb extracts given in table (1). The stem of *Geranium rotundifolium, Ageratum conyzoid,* and *Impatiens parviflora* were found to contain more than 40mg/g while*Viola odorata, Swertia chirayita,* and *Ajuga parviflora* had less than 30mg/g phenolics in their stem. In other plants, less than 30mg/g Phenolics were detected.

Total Flavonoids Contents in leaves of herbs

Total flavonoid contents in leaves of herbs are 43.38 mg/g in *Galium boreale* and 17.83 mg/g in *Arwasaema wallichianum* given in table (2). Leaves of *Galium boreale* were found to have more than 40mg/g flavonoids.

Table: 3 Quantity of Alkaloids (mg/g of dry weight) in leaves and stems of herbs

Sr #	Species	Family	Leaves	% Difference	Stem	% Difference
1	*Arwasaema wallichianum*	Aricaceae	23.00±1.9 h	70.51	20.00±2.2 k	65.52
2	*Corydal waslutea*	Papaveraceae	25.00±2.1 gh	67.95	13.00±0.5 m	77.59
3	*Ageratum conyzoid*	Asteraceae	50.00±5.4 d	35.90	34.00±2.3 g	41.38
4	*Impatiens parviflora*	Balsaminaceae	44.00±3.3 e	43.59	41.00±3.3 e	29.31
5	*Rumexn epalenswas*	Polygonaceae	45.00±3.2 e	42.31	25.00±1.9 i	56.90
6	*Viola odorata*	Violaceae	45.00±3.9 e	42.31	34.00±2.7 g	41.38
7	*Artemisia japonica*	Asteraceae	23.00±1.6 h	70.51	43.00±3.9 d	25.86
8	*Swertia chirayita*	Gentianaceae	25.00±1.9 gh	67.95	16.00±1.4 l	72.41
9	*Mentha arvenswas*	Lamiaceae	49.00±2.4 d	34.62	31.00±2.8h	46.55
10	*Geranium rotundifolium*	Geraniaceae	78.00±5.2 a	0.00	43.00±3.6 d	25.86
11	*Ajuga reptans*	Lamiaceae	70.00±5.3 b	10.26	36.00±3.1 s	37.93
12	*Aesculus indica*	Spindaceae	56.00±4.3 c	28.21	45.00±3.9 c	22.41
13	*Ajuga parviflora*	Lamiaceae	67.00±5.9 b	14.10	58.00±4.4 a	0.00
14	*Galium boreale*	Rubiaceae	37.00±4.0 f	52.56	25.00±1.9 i	56.90
15	*Polypodium peltatum*	Polypodiaceae	27.00±2.1 g	65.38	22.00±1.7 j	62.07
16	*Rumex obtusifolius*	Polygonaceae	47.00±3.9 de	39.74	34.00±2.7 g	41.38
17	*Fragaria Virginia*	Rosaceae	25.00±1.7 gh	67.95	16.00±1.1 l	72.41
18	*Geranium wallichianum*	Geraniaceae	38.00±2.3 f	56.41	48.00±3.9 b	17.24

Mean±Standard deviation; Different letters in columns show significant differences; %age difference was calculated from a maximum value

Artemisia japonica, Ajugaparviflora, and *Rumexobtusifolius*leaves were analyzed as having more than 30mg/g flavonoids.

Total Flavonoids Contents in the stem of herbs

The highest amount of total flavonoid contents in the stem of herbs is 43.85 mg/g in *Galiumboreale*and the lowest amount of total flavonoid contents is 6.05 mg/g in *Impatiens parviflora* given in table (2). When *G. borialе*was taken as standard species then *Impatiens parviflora*show the highest % age difference of 86.20. *Artemisia japonica*, *Ajuga parviflora,* and *Rumexobtusifolius* stem had more than 30mg/g flavonoids.

Quantity of Alkaloids in leaves of herbs

Data in the table (3) showed that the highest quantity of alkaloids in leaves of herbs was 78 mg/g in *Geranium rotundifolium* and the lowest amount was 23.00 mg/g in *Arwasaema wallichianum* and *Artemisia japonica*. When *G.cotinifolium* was taken as standard species then A.*wallichianum* and *A.japonica* showed the highest %age difference of 70.51. The leaves of *Ajuga parviflora* had more than 50mg/g alkaloids. In leaves of *Geranium wallichianum, Impatiens parviflora. Artemisia japonica,*

Geranium rotundifolium, and *Aesculus indica,* the alkaloid contents were more than 40mg/g. More than 30mg/g alkaloids were found in leaves of *Ageratum conyzoid, Viola odorata, Mentha arvenswas, Ajuga reptans,* and *Rumex obtusifolius.*

Quantity of alkaloids in the stem of herbs

Data of table (3) showed that the highest quantity of alkaloids in the stem of herbs was 58 mg/g in *Ajuga parviflora* and the lowest quantity of alkaloids was 13 mg/g in *Corydal waslutea*. When *A.parviflora*was taken as standard species then *C.lutea* show the highest % difference of 77.59. in the stem of *Geranium wallichianum, Impatiens parviflora, Artemisia japonica, Geranium rotundifolium,* and *Aesculu sindica* alkaloid contents of more than 40mg/g quantity were found. *Ageratum conyzoid, Viola odorata, Mentha arvenswas, Ajuga reptans,* and *Rumex obtusifolius* stem contains more than 30mg/g alkaloids.

Discussion

The result of the present study revealed that the quantity of alkaloids is higher in all of the plants and plant parts such as leaves and stems of different plants as well the same plant part. The quantity of alkaloids in leaves of all of the plants is higher as compared to that of stems. Similar results have also been reported by Kayani [40] and Cirak [41]. It is reported that young plant parts contain a greater level of alkaloids over old parts [42]. It might be due to direct and frequent exposure of leaves to an environmental factor. Leave are the main metabolic sites and also sensitive sites of plants which are affected by herbivores, parasites, pathogen such as bacteria viruses. A large number of compounds are present in external layers of different plant parts. Their Level is different from plant to plant depending upon variety [43]. Variations in alkaloid concentration among plant species might be due to the reason that alkaloids could be used by plants to protect their organs from the harm of herbivores. Alkaloids are utilized in homeostasis, in the storage of ions and nitrogenous wastes, and in protecting against parasites.

Our results revealed that the amounts of phenolics and flavonoids in leaves of all of the plants are higher as compared to that in the stems. This can be due to a higher number of chloroplasts in their leaves as compared to the stem. Phenolic substances are synthesized in the chloroplast of plants in the presence of light [44]. In the present study, the phenolic contents varied from species to species. The difference in accumulation and concentrations of different phenolic contents in different plants during their life span is also reported by Cirak et al. [45]. Similarly, the difference in the amount of total flavonoids contents in leaves and stem show that the flavonoids might be converted into secondary phenolic compounds or their degradation occurs by enzymes. Flavonoid's losses during maturation of different plant parts may reflect the conversion of flavonoids into secondary phenolic compounds or they become degraded by the action of the enzyme [46, 47].

The variations in the number of antioxidants were detected among different species. This might be due to their presence at different altitudes as along altitudinal variations plant responses to stress and plant adaptation to abiotic factors vary due to variability in environmental factors such as precipitation, light intensity, temperature, and radiation intensity [28, 30]. Environmental variables also influence the growth conditions of plants [Ahmad et al. 2016]. In changing climate conditions plants have to evolve several metabolic pathways as a response to environmental stressors [30]. Similar results were reported by Radawan et al. [48] for the presence ofvariable antioxidant compounds including phenolics and flavonoids in different specimens.

The difference of antioxidant concentration in plants might be due to their experiencing different phenological behavior and exposure to different environmental factors which enable them to synthesize compounds according to their need. The bioactive metabolites that belong to phenolic compounds are involved in plant growth, reproduction, and protection against pathogenic microorganisms and predators [49]. Variation in the distribution of antioxidants in plants might be also due to their geographical distribution [50] and species-specific responses [51]. The concentration difference of antioxidants might be also due to their less or more utilization in activity via scavenging or stabilizing free radicals through hydrogenation or complexion with oxidizing species [52].

In the present study, the quantification of phytochemicals like alkaloids, flavonoids, and phenolics determine the pharmacodynamic properties of plants. A species having more Phenolics have the phytomedicinal potential for many diseases. Polyphenols derived medicine have antimicrobial, anticancer, antimutagenic, astringent, and anti-diarrheal properties. These have also been reported as healing agents in different inflammatory conditions, gonorrhea, burns and to promote blood clotting, reduce blood pressure, and modulate immune responses. The presence of a high quantity of phenolic compounds reveals that these can be used for protection against pathogenic microorganisms and predators. At the same time, these have many other medicinal properties such as antimicrobial and anti-inflammatory agents [49]. Plants with a high concentration of flavonoids can be utilized in the synthesis of drugs for antibacterial, antifungal, and antiviral activities [53].

Authors contribution: GhualYasin and Mumtaz Hussain conducted the experiments. Muzammil Hussain worked for writing and proofreading lab experiments.

Conflict of interest: Authors have no conflict of interest.

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