Phytochemical Constituents of *Berberis lycium Royle* and *Justicia adhatoda*

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Summary: The use of leaves and roots of *Berberis lycium* Royle and *Justicia adhatoda* against different human infections is common practice in the North East of Pakistan. A study was conducted to evaluate the leaves and roots of *Berberis lycium Royle* and *Justicia adhatoda* for active compound alkaloids, flavonoids, saponins, tannins, vitamins (Vit. C, niacin, riboflavin, and thiamin) and metal ion contents .Results indicate that alkaloid (2.34 8.35 mg/ 100 g), Flavonoid (1.722 0.40 mg/ 100 g), saponin (3.52-9.24 mg/ 100 g), tannin (0.58-0.02 mg/ g) were present in the roots of these plants. The leaves contain vitamin C (30.12-32.15 mg/ g) niacin (2.60-2.75 mg/ 100g), riboflavin (0.32-0.62 mg/ 100g), thiamin (1.52-1.68 mg/ g). Both roots and leaves of these plants also contained significant quantity of essential minerals. Results revealed that these plants are potential source of phytochemicals those can be used as raw material for pharmaceutical industries for preparation of various therapeutic agents.

Introduction

Ethnobotanical studies conducted in North East of Pakistan show that rural people have been using extracts of root, leaves and fruits of *Berberis lycium* and *Justicia adhatoda* against liver cirrhosis, hepatitis, colic disorder, chest cold, suppressed cough and for healing of wounds. Chewing sticks used in place of tooth paste are very effective against growth of mouth micro flora and reliable in cleaning of teeth [1].

Many indigenous plants are being used by general public against different infectious diseases in Pakistan. People use fruits and vegetable to minimize the risk of cardiovascular disease, cancer and similar other diseases. Medicinal plants exhibited wide range of biological and pharmacological activities, such as anti-inflammatory, antibacterial and antifungal properties. Furthermore extracts of roots, barks, seeds and fruits of these plants used in the preparation of syrups and decoctions commonly used against fever, diabetes, liver cirrhosis and hepatitis [2].

Berberis lycium Royle (Berberidacae) is an important medicinal shrub, native to Pakistan and India, but also found in other parts of world. Inhabitants of these areas use an extract of the root of *Berberis lycium* for the treatment of diabetes, liver and digestive complaints, coughs and colds, sore eyes, broken bones, ulcers and wounds [3].

Justicia adhatoda L is one of the most important species and dominant vegetation of hilly areas of Rawalpindi and Islamabad. It belongs to family *Acanthaceae,* subclass *Asteridae* and species *adhatoda.* The leaves, roots and flowers of this shrub are rich in different phytochemicals. The aqueous and alcoholic leaves extracts of *Justicia adhatoda* have anti diabetic properties and commonly used against type 2 diabetes by inhabitants of North East of Pakistan [4]

It is generally assumed that phytochemicals including alkaloids, flavonoids, saponins, tannin, vitamins and minerals are active constituents and have protective effects against several diseases. Our diet contains vegetable and fruits, which consist of significant quantity of phytochemicals, which protect us from different diseases and infections, particularly cardiovascular diseases [5]. Herbs and spices are also good sources of natural antioxidants. Several types of polyphenoles show anti-carcinogenic and antimutagenic effects [6]. Polyphenols have been reported to have wide range of anti-microbial activities against Gram positive and Gram negative bacteria. *Berberis lycium* and *Justicia adhatoda* are antimicrobial agents, as roots contain significant levels of polyphenolic compounds. Polyphenolic compounds are electron donors, readily oxidized to form phenolate ion or quinine [7].

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Alkaloids and tannins are secondary metabolites found in roots of *Berberis lycium* and *Justicia adhatoda*. Alkaloids are ranked as the most efficient therapeutically significant plant substances. Pure isolated plant alkaloids and their derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects [8]. They exhibit marked physiological activity when administered to animals. Tannin contents are partly responsible for the bitter taste associated with the raw roots of *Berberis lycium* and *Justicia adhatoda* [9]. Therefore keeping in view the importance of medicinal plants the present study was under taken with following aims and objectives.

1. To assess leaves and roots of *Justicia adhatoda* for alkaloid, flavonoid, saponin, tannin and for some selected elements
2. To evaluate leaves and roots of *Berberis lycium* for the determination of various chemical constituents.
3. To compare the levels of phytochemicals in *Berberis lycium* and *Justicia adhatoda* and their uses as therapeutic agents.

Results and Discussion

The phytochemicals found in *Berberis lycium and Justicia adhatoda are* shown in Tables 1-3, whereas comparison of these chemicals in leaves and roots samples are given in Figs. 1-3. The concentration of saponin (9.24 ± 0.35 mg/ 100 g) was higher in *Justicia adhatoda* than *Berberis lycium* (3.52 ± 0.75 mg/ 100 g). *Justicia adhatoda* contained more flavonoids (2.35 ± 0.24 mg) as compared to *Berberis lycium*. Total alkaloid (8.35 ± 1.2 mg/ 100 g) and tannin (0.82 ± 0.10 mg/ 100 g) in the roots of *Berberis lycium* were higher than in *Justicia adhatoda* (Table-1)

Leaves of *Berberis lycium* contained higher quantity of ascorbic acid (32.15 mg / 100 g) than *Justicia adhatoda,* (30.12 mg/ 100 g). Significant amount of riboflavin, thiamin and niacin was also present in the leaves of these plants (Table-2).

The results of the mineral compositions clearly shows that *Berberis lycium* is a rich source of calcium (1.68 ± 0.13 mg/ 100 g) and potassium (1.42 ± 0.10 mg/ 100g) while *Justicia adhatoda* contained 1.60 ± 0.3 mg of calcium and 1.35 ± 0.5 mg of potassium. Phosphorus, magnesium and sodium were also

Table-1: Phytochemical composition of root extract of *Berberis lycium* and *Justicia adhatoda* expressed as mg/ 100 g dry weight.

Phytochemicals	*Berberis lycium*	*Justicia adhotada*
Alkaloids	8.35 ± 1.2	2.34 ± 0.2
Flavonoids	1.72 ± 0.40	2.35 ± 0.24
Saponin	3.52 ± 0.75	9.24 ± 0.35
Tannin	0.82 ± 0.10	0.58 ± 0.20

Results are mean values of 5 determinations on the basis of dry weight ± standard deviation

Table-2: Vitamin composition of the leaves extract of *Berberis lycium* and *Justicia adhatoda* expressed as mg/ 100 g dry weight.

Vitamins	*Berberis lycium*	*Justicia adhatoda*
Thiamin(Vitamin B1)	1.52 ± 0.6	1.68 ± 0.25
Riboflavin(Vitamin B2)	0.32 ± 0.05	0.62 ± 0.3
Niacin(Nicotinic acid)	2.6 ± o.06	2.75 ± 0.4
Ascorbic acid(Vitamin C)	32.15 ± 1.5	30.12 ± 1.7

Results are mean values of 5 determinations on the basis of dry weight ± standard deviation.

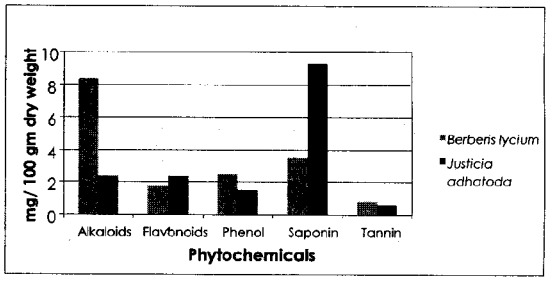

Fig.1: Comparison of phytochemicals found in *Berberis lycium and Justicia adhatoda.*

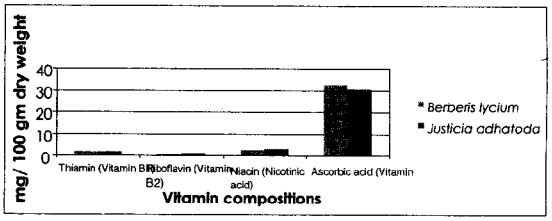

Fig.2: Comparison of Vitamins found in *Berberis lycium and Justicia adhatoda.*

found in roots and leaves samples of these plants (Table-3). Iron (13.21 ± 0.5 mg/ 100 g) and zinc (7.42 ± 0.3 mg/ 100 g) were found in *Berberis lycium* roots while *Justicia adhatoda* root samples contained 16.15 ± 0.4 mg and 8.34 ± 0.5 mg of iron and zinc respectively (Table-3).

Table-3: Mineral composition of roots and leaves extracts of *Berberis lycium* and *Justicia adhatoda* expressed as mg/ 100 g dry weight.

Minerals	*Berberis lycium*		*Justicia adhatoda*	
Macro elements	Roots	Leaves	Roots	Leaves
Magnesium	0.56 ± 0.13	0.25 ± 0.10	0.52 ± 0.20	0.62 ± 0.50
Calcium	1.68 ± 0.40	1.42 ± o.30	1.60 ± 0.30	1.35 ±0.30
Potassium	1.42 ± 0.10	0.35 ± 0.20	1.35 ± 0.50	1.15 ± 0.30
Phosphorus	0.42 ± 0.25	0.38 ± 0.30	0.44 ± 0.20	1.21 ± 0.20
Sodium	1.20 ± 0.20	0.10 ± 0.05	0.12 ± 0.10	1.05 ± 0.11
Micro elements				
Iron	13.12 ± 0.50	3.15 ± 0.3	16.15 ± 0.40	7.16 ± 0.20
Zinc	7.42 ± 0.30	4.45 ± 0.2	8.34 ± 0.50	2.15 ± 0.30

Results are mean values of 5 determinations on the basis of dry weight ± standard deviation.

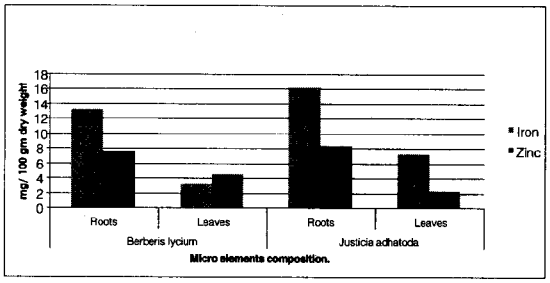

Fig.3: Comparison of trace metals in roots and leaves samples of *Berberis lycium* and *Justicia adhatoda.*

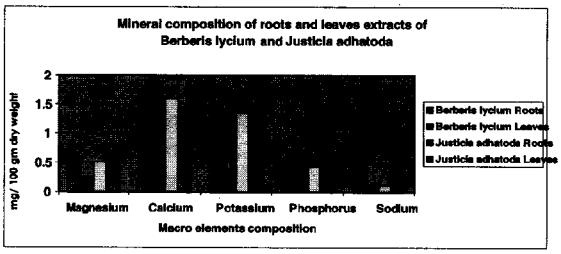

Fig .4: Comparison of macro elements in roots and leaves samples of *Berberis lycium* and *Justicia adhatoda.*

Berberis lycium and Justicia adhatoda roots and leaves are rich source of phytochemicals such as flavonoids, tannins, saponins and alkaloids (Tables 1-3, Figs. 1-3). The biological functions of flavonoids and alkaloids include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcer and similar other disorders [10]. Flavonoids are potent water soluble super anti oxidants and free radical scavengers which prevent oxidative cell damage and have strong anti cancer activity [11]. Flavonoids, in intestinal tract, lower the risk of heart diseases and provide anti-inflammatory action [12]. This is reason for the use of roots of *Berberis lycium* and *Justicia adhatoda* extracts in the treatment of different human diseases [13].

Significant quantity of vitamins were obtained from the leaves of *Berberis lycium* and *Justicia adhatoda* (Table-2, Fig. 2) The vitamins are important for the normal growth of humans and their deficiencies can have adverse effects. Shortage of ascorbic acid in the body impairs the normal formation of intercellular substances, including collagen, bone matrix and tooth dentine. The clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints and defects in skeletal calcification can be related to the association of ascorbic acid deficiency. Furthermore ascorbic acid is also required for normal wound healing [14].

The results of the mineral composition clearly indicates that *Berberis lycium* and *Justicia adhatoda* are rich sources of minerals required for human health (Table-3, Fig. 3). The presence of minerals like phosphorus, calcium, magnesium, potassium, iron and zinc are important for proper function of many enzymes in human body. Zinc is essential for the production of insulin, carbonic anhydrase and other enzymes in the body [2]. Iron is the component of the haemoglobin and helps in the transport of oxygen in the body. Iron together with haemoglobin and ferrodixin plays an important role in human metabolism [15].

Our results revealed that root and leaves extracts of *Berberis lycium* and *Justicia adhatoda* contained important bioactive compounds required for human and animal health. Therefore, roots and leaves of these plants can be used both for nutritional as well as medicinal purposes. More studies are recommended in future for isolation, characterization and the structure determination of industrially important phytochemicals from these medicinal shrubs.

Experimental

Sample Preparation

Roots or leaves samples (500 g) of *Berberis lycium & Justicia adhatoda* were sun dried followed

by oven drying at 80 °C overnight and finely ground to a fine powder form and stored in air tight bottles for further analysis.

Chemical Analysis

The powder form of plant sample was sieved with 2 mm pore size siever and 2 g of plant sample was subjected to dry ashing in a well cleaned porcelain crucible at 550 °C in a muffle furnace. The resultant ash was dissolved in 5ml of mixture of HNO_3/ HCL/ H_2O (1: 1: 1) and heated gently on hot plate until brown reaction fumes disappeared. Added 5ml of deionized water and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask filtered and volume was raised to the mark with deionized water. This solution was used for analysis of metal ions by atomic absorption spectrophotometer, a 10 cm long cell was used and concentration of each element in the sample was calculated on the basis of weight of sample used.

Phytochemical Determination

Preparation of fat free samples: Two g of plant sample was defatted with 100 ml of diethyl ether using soxhlet apparatus for two hours.

Alkaloid Determination

Root sample (2 g) was added to 200 ml of 20 % acetic acid in ethanol and allowed to stand for five hours. The reaction mixture was filtered and extract was concentrated to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected by filtration and weight was calculated [9], which indicated amount of alkaloid in sample.

Tannin Determination

Total 500 mg of sample was added with 50 ml of distilled water and shaken for 1 hour in a mechanical shaker followed by filtration. Five ml of filtrate was mixed with 3 ml of 0.1M $FeCl_3$, 0.1N HCl and 0.08M potassium ferrocyanide. A blank sample and 100 ppm of standard (analyzing for tannin acid) was prepared and absorbance was measured in a spectrophotometer at 120 nm within 10 minutes of standard preparation [10].

Determination of Phenol in Plant

For the extraction of the phenolic component, the fat free sample (5 g) was boiled with 100 ml of ether for 15 minutes. Five ml of the extract was pipette into 50 ml flask, and then 10 ml of distilled water was added, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were left to react for 30 minutes for color development. The absorbance of solution was measured using a spectrophotometer at 505 nm [10].

Saponin Determination

The level of saponin in plant samples were measured by dispersion of 20 g of plant sample in 200 ml of 20 % ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55 °C, the mixture was filtered and residue re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The extract was transferred into 250 ml separating funnel and 20 ml diethyl ether was added. The aqueous layer was recovered while the ether layer was discarded and process was repeated by adding 60 ml of n-butanol. The combined n-butanol extracts were washed repeatedly with 10 ml of 5 % of aqueous sodium chloride. The remaining solution was heated in water bath. After evaporation sample was dried in the oven to a constant weight. The saponin content was calculated in % age by comparing weight of sample used [9].

Flavonoid determination: Total 100 g of root sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The solution was evaporated to dryness over a water bath and weight [11].

Determination of Riboflavin

About 5 g of sample was extracted with 100 ml of 50 % ethanol solution and shaken for 1 hour and filtered. Ten ml of extract was mixed with 5 % potassium permanganate and 10 ml of 30 % H_2O_2 and allowed to stand over a hot water bath for 30

minutes. Then 2 ml of 40 % sodium sulphate was added and volume was raised upto 50 ml and the absorbance was measured at 510 nm in a spectrophotometer.

Determination of Vitamins

Total 5 g of the sample was added in 100 ml of EDTA/ TCA (2: 1) extracting solution mixture was shaken for 30 minutes and centrifuged at 3000 rpm for about 20 minutes, 20 ml of the extract was pipetted into a volumetric flask and 1 % starch solution as indicator was added and titrated with 20% copper sulphate solution to get a dark end point for vitamin C, however, for other vitamins absorbances of similar reaction mixtures were measured by spectrophotometer with little modification [12].

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