

Extraction and Purification of Various Organic Compounds in Selected Medicinal Plants of Kotli Sattian, District Rawalpindi, Pakistan

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ABSTRACT

The medicinal values of roots, leaves and fruits of Funnel (*Foeniculum vulgare* Mill), Berbery (*Berberis lyceum* Royle), Vasaka and (*Justicia adhatoda* L) were explored in this study. The root, leaf and fruit samples of these plant species were collected from hilly areas of Kotli Sattian. Chemical analyses as well as identification of organic compounds by chromatographic techniques were carried out. The results indicate that all three plant species contained Proteins, Sugars, Lipids, Fiber and Vitamin C. Flavonoids and Saponins (Phytohormones) were found only in the fruit and leaf samples of *Foeniculum vulgare*. Palmatine, and Berberine, (Alkaloids) were present in the leaves, and fruits of *Berberis lyceum*. Whereas Vasicine and Vasicinone (Alkaloids) accumulated in the roots and leave of *Justicia adhatoda*. It was observed that roots of *Berberis lyceum* and *Justicia adhatoda* contained higher concentrations of all chemical compounds analyzed as compared to fruits and leave except Sugar, and Vitamin C which were high in the fruit of *Berberis lyceum* . By contrast in case of *Foeniculum vulgare* leaves and fruits of which contained higher concentration of protein, fats, flavonoids and saponin. The extract of roots, leaves and fruits of these plant species are being used against various infections and diseases in rural population of subcontinent since many centuries. This experiment will help to highlight the importance of these valuable organic compounds found in these plant species and their demand in the market will be increased in the future.

Key words: *Berberis lyceum, Justicia adhatoda, Foeniculum vulgare, Natual medicines*

INTRODUCTION

Medicinal plants are a major source of drugs for the treatment of various health disorders especially in rural areas of Pakistan, India, China, Afghanistan, Iran and other countries of this region. The use of plant based medicines (local medicine) dates back to 4000-5000 B.C. Nowadays huge number of allopathic medicines also contain plant based ingredients that are used for their preparation by different companies. There are about 400,000 species of higher plants in the world, as compared to animal's species that are about 5-10 million. The plant materials contain thousands of chemicals which act against diseases and infections of humans and animals when properly used. Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavors, fragrances, Proteins,

Amino acids, bioactive peptides, Phyto hormones, sugar, flavonoids and bio pesticides. Furthermore according to assessment of WHO about 80% of world population depend on medicinal plants for their health care needs, and more than 30% of the pharmaceutical preparations are based on plants (1). Where as some reports indicated that there are 90 popular medicinal plants and different Pharmaceutical companies are using extracts of these plants in various drugs. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity (2). However, the developed countries mostly import raw material from developing countries and after processing export it back as high priced prepared medicines to developing countries (1).

In Pakistan about 2000 plant species have been established of having some medicinal value, out of which only 400 are being used extensively in traditional medicine. Although Pakistan has variety in climate and rich in medicinal plants, but no systematic attempt has been made for utilization of natural resources of this country (3).

Justicia adhatoda is one of the most important plant species and dominant vegetation of hilly areas of Rawalpindi, Islamabad and extended up to NWFP (4). It belongs to family *Acanthaceae*, subclass *Asteridae* and species *Adhatoda*. It is evergreen, gregarious shrub 3-6 m long, large leaves lanceolate 10-20 by 4-8 cm. The flowers are white or purple arranged in short, dense auxiliary pedunculate inflorescence. (5).

The medicinal properties of *Justicia adhatoda* are well known in India, Pakistan and several other countries for many years. It was reported by different authors that roots, leaves and flowers of the plant species contained alkaloids (Vasicine and Vasicinone etc), flavonoids and an essential oil (3)

The leaves of *Justicia adhatoda* are mostly used in the treatment of respiratory disorders in Ayurveda. The alkaloids, vasicine and vasicinone present in the leaves, possess respiratory stimulant activity (6). Vasicine, at low concentrations, induced bronchodilation and relaxation of the tracheal muscle. However, at high concentrations, vasicine offered significant protection against histamine-induced bronchospasm in guinea pigs. Vasicinone, the auto-oxidation product of vasicine has been reported to cause bronchodilatory effects both *in vitro* and *in vivo* (7).

Berberis lyceum is locally known as simbuli or simbulu belonging to family Berberidaceae. It is about 4-6 feet in height with thorny branches. The leaves are somewhat obviate, with ciliated teeth on their margins. The flowers are drooping racemes, with yellow petals. The berries (fruit) grow in loose bunches (8).

Berberis lyceum is valued mainly for its fruits and roots, which contain alkaloids like berberine and palmitine. These alkaloids are effective against eye diseases, febrifuge, and piles (9). Whereas, an extract made from its roots (known as 'rasaunt') is used against many infections including eye's disorders (10). Similarly in some areas of India and Pakistan its fruits are mostly used as a tonic against liver and heart diseases (11). Furthermore it has antihistaminic, stomachic, astringent, antipyretic and diaphoretic properties (12).

Foeniculum vulgare is commonly known as fennel and develops an edible bulb (containing leave and formed thick base), which is becoming popular as a vegetable. The leave, stalks and seeds (fruits) of this plant are edible. It is used as carminative, lactagogue and diuretic (13). *Foeniculum*

vulgare is an aromatic herb whose fruits are oblong, ellipsoid or cylindrical, straight or slightly curved and greenish or yellowish brown in color. The weight of seeds can be between 6 and 7 mg where as length is 6 mm and width 2mm. The dried, aromatic fruits are widely employed in culinary preparations for flavoring bread, pastry and candies. It is also used in alcohol liqueurs, as well as in cosmetic and medicinal preparations. (13). This herb has finely out feathery foliage, umbels of mid summer flowers, curved, ribbed seeds and a thick root. It is used as an expensive and extravagant spice and vegetable in different parts of the world. Its seeds contain essential oil, which is used for many purposes by human population (14).

The oil of *Foeniculum vulgare* regulates the peristaltic functions of the gastrointestinal tract, thereby reducing emptying time and increasing the passage of gas. It also relieves the spasm of intestines. It was experimentally observed that *Foeniculum* seeds are effective against hernias and hydrocele when used with other salts or ingredients. (15).

Keeping in view the importance of these valuable medicinal plants, the present study was undertaken with the following aims and objectives:

1. To assess the bioactive compounds of *Berberis lyceum*, *Justicia adhatoda* and *Foeniculum vulgare*
2. To compare the chemical compounds found in *Berberis lyceum* roots and fruits with leaves and roots of *Justicia adhatoda* and *Foeniculum vulgare*
3. Assessment of chemical compounds found in leaves and seeds of *Foeniculum vulgare*

MATERIALS AND METHODS

Collection of samples

The samples (roots, leaves and fruits) of *Berberis lyceum*, *Foeniculum vulgare* and *Justicia adhatoda* were collected from different localities of hilly areas of Kotli Sattian, District Rawalpindi, Pakistan during March and May, 2005. The samples of root and fruit (*Berberis lyceum*), leave and fruit (*Foeniculum vulgare*) and root and leaf of *Justicia adhatoda* were collected in clean plastic bags and labeled with date, number and location of samples.

Preparation of Samples

After collection the roots, leaves and fruits samples of *Berberis lyceum*, *Foeniculum vulgare* and *Justicia adhatoda* were washed and sun dried, followed by oven drying. Finally the samples were crushed and converted into powdered form and stored for further analysis.

Chemical analysis of Plants

The root, leaf and fruit samples of these plants species were analyzed for protein, carbohydrate, lipid, Amino acids, Vitamin C Calcium, phosphorus and Sulphur , Protein flavonodis , saponin, and alkaloids of these valuable plants species were separated by using techniques of one and two dimension thin layer and Column chromatography, followed by spectrophotometric analysis (16, 17). All chemicals used in this study were analytical grade (Sigma and Merck).

Experimental

In order to extract and purify alkaloids from root, leaves and fruits samples, following procedures were adopted: About 100 gram (each of roots, fruits and leaves) samples were soaked in solvents like Ethanol for 24 hours and filtered. The Solvent was evaporated and half volume of the solvent, NaOH (3-4%) was added. The pH of the mixture was adjusted to 10 with NaOH. The mixture was run through a column using silica gel to separate the alkaloids, flavonoids and saponin, which were further identified on thin layer chromatography using reference standards whereas for protein, sephadex (G 20 and G 50) was used. The concentration level of these compounds was determined with the help of spectrophotometer at 470 650 nm.

RESULTS AND DISCUSSION

Results of biochemical analysis of different compounds found in roots, leave and fruits of *Berberis lyceum*, *Foeniculum vulgare*, and *Justicia adhatoda* are given in tables 1-5. Higher concentration of alkaloids and other compounds was found in roots as compared to the leaves and fruits of *Berberis lyceum* and *Justicia adhatoda* (Tables 1 and 2). The results obtained after analysis of *Berberis lyceum* indicated that concentration of Proteins (8.5 %) and Fat (6.5 %) was found in roots as compared to leaves (Protein 5.6% and Fat 4.5%). Whereas concentrations of alkaloids like palmatine and Berberine (5.6%) was higher in roots as compared to leaves (Table 1).

The pH values and concentration level (mgL^{-1}) of various bioactive compounds (Alkaloids) are given in table 5. which shows that bioactive compounds observed in higher amount in these valuable plants and can be used against various infections and diseases. The extracts of roots of *Berberis lyceum* are commonly used by people to repair cut, wounds or injuries and get relief from body pain. These are also used against high grade fever and liver jaundice (18). Similarly fruits of *Berberis lyceum* have high medicinal values and it is delicious dish of various animals and birds due to sweet taste (10).

The concentration level of protein (8.5 %), vasicine (5.5 %), vaicinone (3.8%), fat (3.5%) and fiber (1.8%) was found in roots samples of *Justicia adhatoda*. Where as level of such compounds was low in leaves except sugar (4.5%) and vitamin C (1.1%)

It was observed that roots and leave this plant specie contained higher concentrations of chemicals that can be used in drugs required against various disorders of human population. The extract of roots and leaves of *Justicia adhatoda* is commonly used by rural population against diabetes, cough and certain liver disorders. (6).

Analysis of leaves and fruits of *Foeniculum vulgare* shows that higher concentration of flavonoids , saponins, proteins, amino acid (especially Isoleucine) and fats were present in the both leaves and fruits samples (Tables 3-5) The leaves contained higher concentration of flavonoids and fat, whereas the level of saponins, protein and other organic compounds were high in seeds (Table 3) . The seeds of *Foeniculum* are considered as essential ingredients for many local medicines that are used against stomach, kidney and liver infection and disorders (15). The organic compounds obtained from seeds will further increase the market value of these valuable medicinal plants. The younger and fresh leaves are considered as delicious and traditional vegetables in many areas of this region (19) .

Furthermore Seeds (fruits) are being used in almost all houses of this region for many purposes of human population, whereas leaves are mostly used as vegetables either cooked or in the form of salad.(8). Data given in table 4 represents the variation in absorbance in pH due to change in solvent system for extraction of chemical compounds.

Berberine (a alkaloid) analyzed from root and fruit of *Berberis lyceum* can be used to prevent left ventricular hypertrophy development induced by pressure overload, reduce heart weight and cardiac function (20).Furthermore it also effect on the growth of bacteria and protozoa. The alkaloids like vascine and vasicinone found in the root and leave of *Justicia adhatoda* have important physiological effects on liver, kidney and stomach problems (5). Whereassaponins and flavonoids found in seed and leaves of *Foneculum vulgare* have important medicinal values and used in different drugs.

Therefore it is recommended that extraction and purification of such alkaloids are very valuable in the preparations of drugs of various types. The assessment of various effects of such compounds on animals and human health are required in the future studies.

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Table 1. The percentage (%) of various Chemical compounds analyzed from Edible parts of *Berberis lyceum*.

Constituent in Root	Percentage	Constituent in Leaves	Percentage
Protein	8.5	Protein	5.6
Fat	6.5	Fat	4.5
Sugar	2.1	Sugar	6.5
Fiber	3.5	Fiber	2,5
Palmatine	4.5	Palmatine	0.8

Calcium	1.8	Calcium	0.9
Sulphur	2.7	Sulphur	0.8
Berberine	5.6	Berberine	2.5
Vitamin C	1.2	Vitamin C	1.1

Table 2. The percentage (%) of various Chemical compounds analyzed from Edible parts of *Justicia adhatoda*.

Constituent in Root	Percentage	Constituent in Leaves	Percentage
Protein	8.5	Protein	6.6
Fat	3.5	Fat	2.5
Sugar	2.1	Sugar	4.5
Fiber	3.5	Fiber	2.5
Vasicine	5.5	Vasicine	1.8
Vasicinone	3.8	Vasicinone	0.5
Sulphur	2.1	Sulphur	0.4
Calcium	1.6	Calcium	1.7
Vitamin C	1.1	Vitamin C	3.4

Table 3. The percentage (%) of various chemical compounds analyzed from Edible parts of *Foeniculum vulgare*.

Chemical Compound	Seeds	Leaf
Protein	16.5	8.5
Fat	13.25	7.5
Fiber	5.1	6.2
Mineral	5.2	4.1
Moisture	18.5	25.5
Saponin	16.5	12.3
Flavonoids	9.5	21.6

Table 4. Spectrophotometric analysis of possible alkaloids of three plant species at 470nm and their pH values.

Solvents*		Sample	Concentration (mg/L)	PH
Hexane		Root of <i>Berberis lyceum</i>		
		1	0.613	7.9
		2	0.656	7.8
		3	0.463	6.9
Cycle hexane		Root of <i>Justicia adhatoda</i>	0.413	7.4
		1		
		2	0.397	6.5
		3	0.158	7.2
Ethanol		1	0.692	6.5
		2	0.711	7.6
		3	0.683	6.9

*Selection of solvents depend on the solubility of various samples

Table 5. The percentage of Amino acids in leaves and seeds of *Foeniculum vulgare*.

Amino acids	Seeds	Leaves
Glycine	0.55	0.65
Lecusine	0.63	0.59
Isoleucine	0.73	0.71
Proline	0.53	0.50
Phenylalaine	0.45	0.47
Tryptophane	0.53	0.52