

INVESTIGATION FOR BIOACTIVE COMPOUNDS OF *BERBERIS LYCEUM ROYLE* AND *JUSTICIA ADHATODA* L.

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ABSTRACT

Gulfraz, M. and M. Arshad (Associate Professors, Department of Biochemistry, University of Arid Agriculture, Rawalpindi), and **N. Nayyer, N. Kanwal and U. Nisar** (M.Sc. Students, University of Arid Agriculture, Rawalpindi). INVESTIGATION FOR BIOACTIVE COMPOUNDS OF *BERBERIS LYCEUM ROYLE* AND *JUSTICIA ADHATODA* L. *Ethnobotanical Leaflets* 2004. In order to explore the medicinal values of plant species like *Berberis lyceum* and *Justicia adhatoda*, a study was conducted to analyze roots, leaves and fruits of both plant species for identification of various organic compounds. Chemical analysis as well as identification of organic compounds by chromatographic techniques were carried out. Results indicates that both plant species contained Proteins, Sugars, Lipids, Vitamin C, Sodium, Calcium, Sulphur, Iron, and Zinc. Whereas the alkaloids like Palmatine, Berberine, Vasicine and Vasicinone were also found in leaves and roots of these plant species. However, it was observed that roots of both plant species contained higher concentrations of these chemical compounds as compared to fruits and leaves except sugar and vitamin C those were high in fruits. Furthermore presence of such bioactive compounds in *Berberis lyceum* and *Justicia adhatoda* indicated their importance in the form of local medicines. This experiment will help to increase the importance of new raw material found in these plant species and their demand in the market will be increased in the future. The extract of roots and fruits of these plant species are being used against various infections and diseases in rural population of subcontinent since many centuries.

Key Words: *Berberis lyceum*, *Justicia adhatoda*, Chemical analysis, medicine.

INTRODUCTION:

Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed (Edward,2001). Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity. Furthermore, in the world more than 30% of the pharmaceutical preparations are based on plants (Shinwari and Khan, 1998).

However, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants.

The use of medicines from plants in the form of local medicine dates back to 4000-5000 B.C. While the medicinal values of these plants are due to the presence of small doses of active compounds which produces physiological actions in the human and animal body (Zaidi, 1998). Some of the important bioactive compounds found in medicinal plants are alkaloids, glycosides, resins, gums, mucilages etc. (Sack and Forehlich, 1982). It was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries (Shinawie,2002).

In Pakistan there are about 2000 estimated species of medicinal plants out of which 400 are extensively used in traditional medicine. Pakistan has variety in climate and therefore rich in medicinal plants, but no systematic attempt has been made to work and utilize natural resources of this country.

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 (Zaidi,1998).



Berberis lyceum is valued mainly for its fruits and roots, which contain alkaloids like berberine and plamitine. These alkaloids are effective against eye diseases, febrifuge, and piles (Gosh *et al*,1990). Whereas,an extract made from its roots (known as ‘rasaunt’) which is being used against many infections including eye’s disorders. (Chopra *et al*. 1998). Whereas in some areas of India and Pakistan fruits are mostly used as a tonic against liver and heart diseases (Gilani and Janbaz, 1999). Furthermore it showed antihistaminic activity , also possesses stomachic, astringent, antipyretic and diaphoretic properties (Shamsa *et al*.1999).

Justicia adhatoda is one of the most important specie and dominant vegetation of hilly areas of Rawalpindi, Islamabad and extended up to NWFP(Khattack and Gilani,1985). It belongs to family *Acanthaceae*, subclass *Asteridae* and specie *Adhatoda*. It is evergreen, gregarious shrub 3-6 m long, large leaves lanceolate 10-20 by 4-8 cm. Whereas flowers are white or purple in short, dense auxiliary pendunculate (Baquar,1997).

Chemical compounds found in leaves and roots of this plant includes essential oils, fats, resins, sugar, gum, amino acids, proteins and vitamin‘C’ etc. (Dymock,1972).

The medicinal properties of *Adhatoda vasica* Nees are well known in India and several other countries for many years. The leaves contained an essential oil and the alkaloids quinazoline, vasicine, vasicinone and deoxyvasicine(Shinawie,2002). The roots contained vasicinolone, vasicol, peganine and 2’-hydroxy-4-glucosyl-oxychalcone. The flowers contained -D-glucoside, kaempferol and its glucosides, as well as the bioflavonoid, namely quercetin. (Rawat *et al*, 1994).



The leaves are mostly used in the treatment of respiratory disorders in Ayurveda. The alkaloids, vasicine and vasicinone present in the leaves, possess respiratory stimulant activity (Baquar, 1997). Whereas, 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* (Shinawie, 2002).

Therefore 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* royle and *Justicia adhatoda*.
2. To compare the chemical analysis of compounds found in *Berberis lyceum* fruits and *Justicia adhatoda*.
3. To highlight the values of these bioactive compounds against certain infections in human population.

MATERIALS AND METHODS

Collection of samples

In order to analyze the bioactive compounds present in root and fruit of *Berberis lyceum* and leaves, fruit and root of *Justicia adhatoda*, the plant samples were collected from different localities of Murree, and surrounding hilly areas. Whereas chemical analysis of the root, leaves and fruit samples were carried out with following procedures.

Preparation of samples

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

Chemical analysis of root, leaves and fruit samples

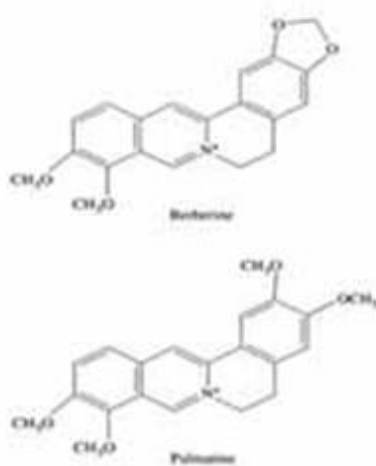
Both roots and fruits samples were analyzed for protein, carbohydrate, lipid, vitamins, and fibers. Sodium, Potassium, Calcium, phosphorus, Sulphur, Iron and Zinc by routine chemical analysis. Whereas alkaloids of these valuable plants species were separated by chemical extraction methods followed by Column and thin layer chromatography.

Instrumentation

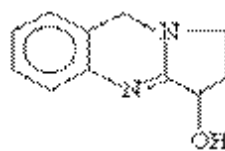
In order to extract and purifying alkaloids from roots, leaves and fruits samples, following procedure were adopted.

Fifty grams (each of roots, fruits and leaves) samples were soaked in the ethanol (80%) for 24 hours and filtered. The ethanol was evaporated and half volume of 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 through column chromatography, those were further identified on thin layer chromatography using reference standards.

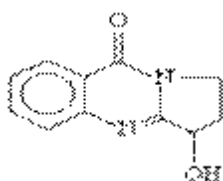
The concentration level of these alkaloids was determined with the help of spectrophotometer at 650 nm and that was compared with standard alkaloid compounds. Finally the PH of alkaloids were obtained and were compared with standard alkaloids .



(Source: Vennestrom and Klayman,1988)



Vasicine



Vasicinone

(Source: Atal,1980)

Structures of alkaloids

RESULTS AND DISSCUSSION:

Results of biochemical compounds found in roots, leaves and fruits of *Berberis lyceum* and *Justicia adhatoda* is given in tables 1-5 whereas comparison of these Bio active compounds are given in Figure 1 and 2.

Higher concentration of alkaloids was found in roots as compared to the fruits (Tables 1 and 2). Furthermore, high concentration of proteins (4.5 %), fat (2.6 %), fiber (2.5%) ,sodium (1.5%) calcium (2.2%), sulphur (0.2%), iron (0.3% zinc (0.3%), palmitine (3.1%) and berberine (4.5% were present in the roots. However, the level of these chemicals was low in fruits except sugar (4.5 %), and vitamin C (0.8%) was high in fruits as compared to roots (Tables 1 and 2). Furthermore, the comparison of these compounds are presented in figure 1.

The pH values and concentration level (mg/l) of various bioactive compounds (Alkaloids) are given in table 5. Which shows that bioactive compounds observed in higher amount in these valuable plants and could be used against various infections and diseases.

The data obtained about various compounds found in roots and leaves of *Justicia adhatoda* are given in the tables 3 and 4. While, comparison of these chemical compounds are presented in figure 2. It was observed that roots of *Justicia adhatoda* contained higher concentration of protein fat and alkaloids like vasicine and vasicinone.(Table 3).The leaves of *Justicia adhatoda* contained higher concentration of sugar and vitamin C (Table 4).

It was observed that roots, leaves and fruit of both plant species contained higher concentrations of chemicals those can be used against various disorders in human population. The extract of roots and leaves of *Justicia adhatoda* are commonly used by rural population against diabetes, cough and certain liver disorders. (Sivarajan and Balachandran, 1994).

The roots of *Berberis lyceum* are commonly used by people for their body pain to repair cut, wounds and also against high grade fever (Ivanovska and Philipov,1996). Similarly fruits of this plant also have various medicinal values (Chopra , 1998).

Table 1. Analysis of Bioactive compounds from roots of *Berberis lyceum*.

Constituent	Percentage	Constituent	Percentage
Dry matter	61.2	Calcium	2.2
Moisture	20.5	Sodium	1.5
Protein	4.5	Sulphur	0.2
Fat	2.6	Iron	0.3
Sugar	3.5	Zinc	0.2
Fiber	2.5	Berberine	4.5
Palmatine	3.1	Vitamin C	0.3

Table 2. Analysis of bioactive compounds from fruits of *Berberis lyceum*.

Constituent	Percentage	Constituent	Percentage
Dry matter	62.5	Calcium	1.8
Moisture	12.5	Sodium	0.6
Protein	2.5	Sulphur	0.1
Fat	1.8	Iron	0.2
Sugar	4.5	Zinc	0.8
Fiber	1.5	Berberine	2.9
		Vitamin C	0.8

Table 3. Analysis of Bioactive compounds from roots of *Justicia adhatoda*.

Constituent	Percentage	Constituent	Percentage
Dry matter	66.4	Calcium	3.1
Moisture	24.6	Sodium	2.4
Protein	8.5	Sulphur	1.2
Fat	2.5	Iron	0.7
Sugar	2.6	Zinc	0.5
Fiber	5.2	Berberine	0.3
Vasicine	7.5	Vitamin C	5.2

Table 4. Analysis of Bioactive compounds from leaves of *Justicia adhatoda*.

Constituent	Percentage	Constituent	Percentage
Dry matter	50.4	Calcium	1.5
Moisture	15.3	Sodium	1.4
Protein	6.5	Sulphur	1.3
Fat	1.6	Iron	1.2
Sugar	16.4	Zinc	0.6
Fiber	6.4	Vasicinone	3.5
Vasicine	4.5	Vitamin C	1.5

Table 5. Spectrophotometric analysis of various alkaloids at 470nm and their PH values.

Alkaloids	Concentration	PH
1	0.724	7.8
2	0.668	7.9
3	0.716	7.7
4	0.163	6.9
5	0.113	7.0
6	0.097	6.9
7	0.58	7.2
8	0.51	6.7
9	0.95	7.6

Figure 1: Comparison of bioactive compounds in roots and fruits of *Berberis lyceum*.

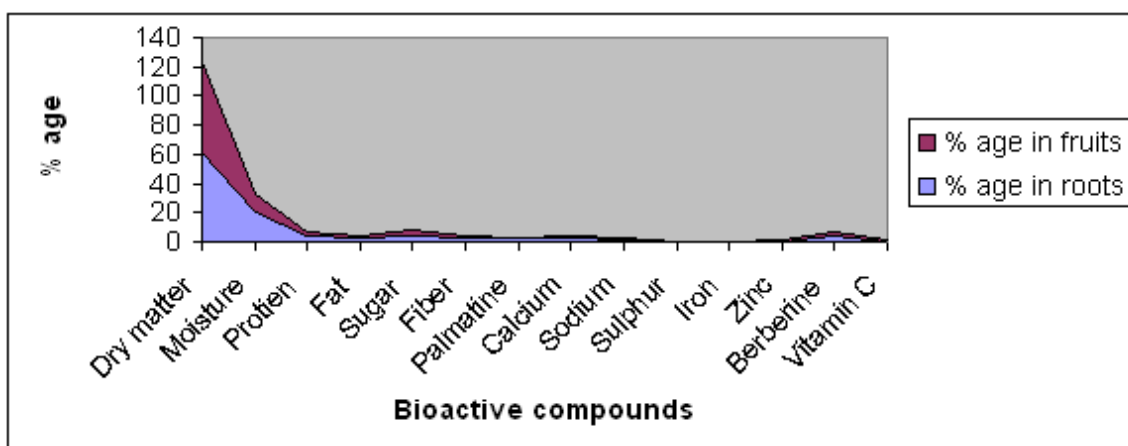
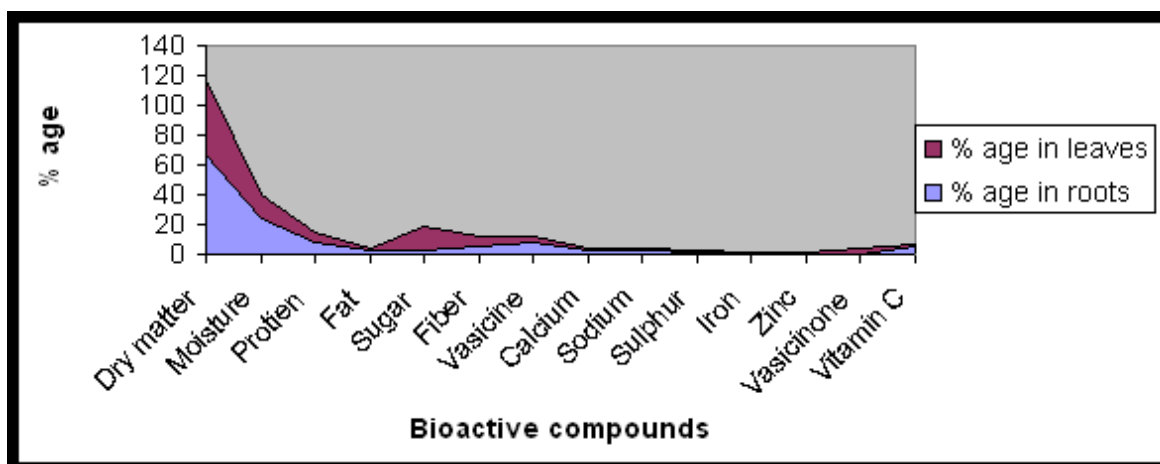


Figure 2: Comparison of bioactive compounds in roots and leaves of *Justicia adhatoda*.



References

- Atal, C.K.** 1980. *Chemistry and Pharmacology of Vasicine: A New Oxytoxic and Abortifacient*. RRL, Jammu, India.
- Baquar, S.R.** 1997. Medicinal and Poisonous of Pak.J.Med., 95-96, Karachi, Pakistan.
- Chopra, R. N., S. L, Nayar and I.C. Chopra.** 1998. The wealth of India. Raw Materials, 2 (B): 114-115.
- Dymock, W.** 1972. India pharmacographia of plants. Hamdard National Foundation Pak., 3:343-344.
- Edward, A.** 2001. Pathogenesis *Justicia adhatoda* (ed) New, Old and Forgotten remedies .pp 210-220.
- Ghosh, A. K., F. K. Bhattacharyya and D. K. Ghosh.** 1990. Leishmania donovani: A mastigote Inhibition and mode of action of berberine. Exp. Parasitd. 60 (3): 404-413.
- Gilani, A. H. and K. H. Janbaz.** 1999. Possible mechanism of selective intropic activity of the n- butanolic fraction from *Berberis aristata* fruit. Pharmacol.33 (5): 407-414.
- Ivanovska, N. and S. Philipov.** 1996. Study on the anti- inflammatory action of *Berberis vulgaris* root extract , fractions and pure alkaloids. Immunopharmacol. 18 (10) : 553-61.
- Khattak, S. G. and S. N. Gilani.** 1985. Antipyretic studies on some indigenous Pakistani medicinal Plants. Ethnopharmacol. 14 (1): 45-52.
- Rawat, M. S. M., G. Pant, S. Badoni, Y.S. Negi.** 1994. Biochemical investigation of some wild fruits of Garhwal Himalayas. Prog. Horticult. 26(1-2): 35-40.
- Sack, R.B. and J.L. Froehlich.** 1982. Berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins. Infect Immune, 35:471-475.
- Shamsa, F., A. Ahamadiani and R. Khosrokhavar.** 1999. Antithistaminic and anticholinergic activity of Berbery fruit (*Berberis vulgaris*) in the guinea pig ileum. Ethanopharmacol. 64 (2): 161-166.
- Shinawie, A.** 2002. Wounder drugs of medicinal plants. Ethnobotany. ISSN 025-039X Vol 29, Issue 1. Singh R. P and Padmavathi, 2000. Modulatory influence of *Adhatoda vasica* leaf extract on the Enzymes of xenobiotic metabolism, antioxidant status and lipid per oxidation in mice. Mol. Cell Biochem. 213 (1-2): 99-109.
- Shinwari, M.I. and M.A. Khan.** (1998). Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. Pak.J.Forest.48(1-4): 63-90.
- Sivarjan, V. and V. Balachandran.** 1994. Ayurvedic Drugs and their plant sources, Int. Sciences Publ. PP 503.
- Vennerstrom, J.L. and D.L. Klajmas.** 1988. Protoberberine alkaloids as antimalarials. Med. Chem. 31:1084-7.
- Zaidi, S.H.** 1998. Existing indigenous medicinal plant resources of Pakistan and their prospects for utilization. Pakistan Forest Jour. 48(2): 5