

ISOLATION OF BIOACTIVE COMPOUNDS OF *Justicia heterocarpa* T.
AND *Blepharis ciliaris* L.

BY

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ABSTRACT

In order to explore the medicinal values of plant species like *Justicia heterocarpa* T and *Blepharis ciliaris* L. (*Acanthaceae*), a study was conducted to analyze roots, leaves and fruits of both plants species for identification of various organic compounds. Chemical analysis as well as identification of organic compounds by chromatographic techniques were carried out. Results indicate that both plants 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 *Blepharis ciliaris* and *Justicia heterocarpa* indicated their importance in the form of local medicines. This study will help to increase the importance of raw materials 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 subcontinents since many centuries.

Key words: *Justicia heterocarpa*, *Blepharis ciliaris*, 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 produce 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. (Saak and Forshlich, 1982). It was observed that developed countries mostly import 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.

Blepharis ciliaris is locally known as simbuli or simbulu belonging to family *Acanthaceae*. Leaves and bracts rigid of silvery appearance, 4-rowed, patent, recurved, spiny-tipped and prickly-toothed. Flowers 2cm long consisting of a short tube and a solitary broad flat limb (Tackholm, 1974).

The plant 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 (Ghosh *et al.*, 1990). Whereas, an extract made from its roots (known as 'rasaunt') is being used against many infections including eye's disorders. (Chopra *et al.* 1998). 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 and possesses stomachic, astringent, antipyretic and diaphoretic properties (Shamsa *et al.* 1999).

Justicia heterocarpa belongs to family *Acanthaceae*. Leaves ovate-lanceolate, petioled. Sepals linear, white hairy. Fruits of two kinds, spiny and spineless in the same cluster, rarely all the same (Tackholm, 1974).

Chemical compounds found in leaves and roots of this plant includes essential oils, fats, resins, sugar, gum, amino acids, proteins and vitamin 'C' (Dymock, 1972). The flowers contained -D- glucoside, kaempferol and its glucosides, as well as the biflavonoid namely

quercetin (Rawat *et al.*, 1944).

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*.

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 *Justicia heterocarpa* and *Blepharis ciliaris*.
2. To compare the chemical analysis of compounds found in *Blepharis ciliaris* fruits and *Justicia heterocarpa*.

MATERIALS AND METHODS

Collection of samples

In order to analyze the bioactive compounds present in root and fruit of *Blepharis ciliaris* and leaves, fruit and root of *Justicia heterocarpa*, the plant samples were collected from different localities of Gebel Elba desert road. 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 *Blepharis ciliaris* and *Justicia heterocarpa* were washed, dried and stored at room temperature prior to analysis. Finally the samples were crushed and converted into powdered form and saved for further analysis.

Chemical analysis of root, leaves and fruit samples

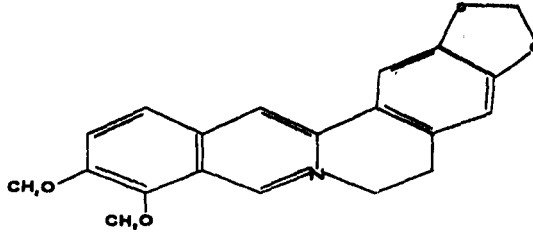
Both roots and fruits samples were analyzed for protein, carbohydrate, lipid,

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.

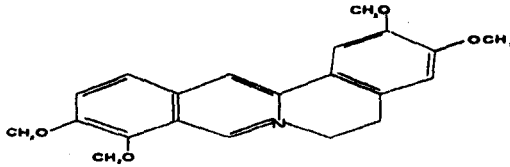
In order to extract and purifying alkaloids from roots, leaves and fruits samples, following procedure were adopted.

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 .

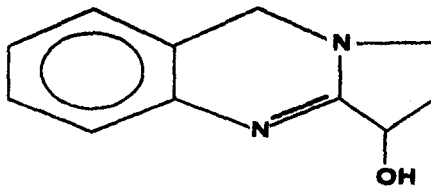


Berberine

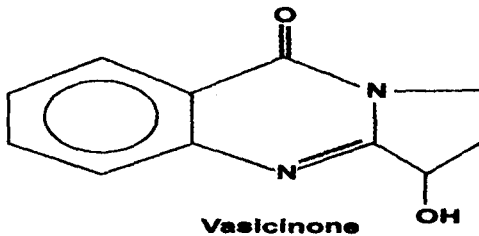


Palmatine

(Source: Vennestrom and Klayman, 1988)



Vasicine



Vasicinone

(Source: *et al.*, 1980)
Structures of alkaloid

RESULTS AND DISSCUSSION

Results of biochemical compounds found in roots, leaves and fruits of *Blepharis ciliaris* and *Justicia heterocarpa* is given in Tables 1-5 .

Higher concentration of alkaloids was found in roots as compared to the fruits (Tables 1 and 2). Furthermore, high concen-

tration 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).

Table (1): Analysis of Bioactive compounds from roots of *Blepharis ciliaris*.

Constituent	Percentage	Constituent	Percentage
Dry matter	62.5	Calcium	2.1
Moisture	20.1	Sodium	1.3
Protein	4.5	Sulphur	0.1
Fat	2.8	Iron	0.3
Sugar	3.1	Zinc	0.2
Fiber	2.5	Berberine	4.5
Palmatine	3.0	Vitamin C	0.3

Table (2): Analysis of bioactive compounds from fruits of *Blepharis ciliaris*.

Constituent	Percentage	Constituent	Percentage
Dry matter	61.5	Calcium	1.5
Moisture	12.9	Sodium	0.7
Protein	3.5	Sulphur	0.1
Fat	1.5	Iron	0.2
Sugar	4.8	Zinc	0.9
Fiber	1.8	Berberine	2.9
Palmatine	1.4	Vitamin C	0.7

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

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.

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 heterocarpa* are commonly used by rural population against diabetes, cough and certain liver disorders. (Sivarajan and Balachandran, 1994).

The roots of *Blepharis ciliaris* 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).

Berberine (a alkaloid) analyzed from root and fruit of *Blepharis ciliaris* can be used to prevent left ventricular hypertrophy development induced by pressure overload, reduce

heart weight and cardiac function (Hong *et al.*, 2002). Furthermore it also effect on the growth of bacteria and protozoa. The alkaloids like vasicine and vasicinone found in the root and leave of *Justicia heterocarpa* have important physiological effects on liver, kidney and stomach problems (Baquar, 1997).

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.

Table (3): Analysis of Bioactive compounds from roots of *Justicia heterocarpa*.

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 heterocarpa*.

Constituent	Percentage	Constituent	Percentage
Dry matter	59.4	Calcium	1.5
Moisture	15.2	Sodium	1.4
Protein	6.5	Sulphur	1.3
Fat	1.4	Iron	1.2
Sugar	16.4	Zinc	0.6
Fiber	6.5	Vasicinone	3.4
Vasicine	4.5	Vitamin C	1.3

Table (5): Spectrophotometric analysis of possible various bioactive compounds (Alkaloids) of two plant species at 470nm and their pH values.

Solvents	Sample		Concentration(mg/L)	pH
	Root of <i>Blapharis ciliaris</i>			
Hexane			0.714	7.8
			0.669	7.9
Root of <i>Justicia heterocarpa</i>				
Cyclohexane			0.161	6.9
			0.570	7.2
Ethanol			0.510	6.7
			0.930	7.6

*Selection of solvents depend on the solubility of various samples

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فصل المركبات الحيوية لنباتي جوستيشيا هيتيروكاربا ونبات شوكة الديب

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العائلة الأكانثية من العائلات التي لها أهمية طبية واقتصادية عالية والنباتات محل الدراسة تم تجميعها من منطقة جبل علية.

ونتيجة لاكتشاف القيمة الطبية لنباتي الجوستيشيا وشوكة الديب أرشدتنا الدراسة لتحليل الجذور والأوراق والثمار لكلا النباتين للتعرف على المركبات العضوية المختلفة و التحليلات الكيميائية مثل التعرف على المركبات العضوية كان عن طريق استخدام طرق الفصل الكروماتوجرافي والتي تم تقديرها.

كما أوضحت النتائج احتواء النباتين على البروتينات والسكريات والدهون وفيتامين سي والصوديوم والكالسيوم والكبريت والحديد والزنك، بينما القلويدات مثل البالميتين والبر برين والفاسيسين والفاسيسينون وكانت موجودة في الأوراق والجذور لهذه النباتات، بينما من الملاحظ أن جذور النباتات محل الدراسة تحتوي على نسبة عالية من هذه المركبات الكيميائية عند مقارنتها بالثمار والأوراق ماعدا السكريات وفيتامين سي فهي موجودة بنسبة عالية في الثمار ووجود مثل هذه المركبات الحيوية في النباتات يوضح أهميتها من الناحية الطبية كما أن مستخلص الجذور والثمار لهذه النباتات يستخدم في علاج العديد من الأمراض المختلفة.