Mahalakshmi K. *et al*. / *International Journal of Biological & Pharmaceutical Research.* 2013; 4(4): 231-236.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

PHARMACOGNOSTICAL INVESTIGATION OF *FIORIA VITIFOLIA*

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ABSTRACT

Fioria vitifolia. syn. *Hibiscus vitifolius* (Malvaceae) rainy season weed mainly used for the treatment of jaundice in the folklore system of medicine in India. The present study was carried out to investigate organoleptic, morphologic, microscopic and other applicable physico- chemical parameters of whole part of *Fioria Vitifolia*. The plant is an herb or undreshrub grows widely along the roadsides of India and also in the tropical and subtropical regions. The transverse section of the leaf midribs shows upper epidermis, lower epidermis, palisade tissues, spengy tissues, multicellular trichomes, parenchyma and also xylem vessels. Transverse section of leaf a sector enlarged shows upper epidermis, lower epidermis, palisade parenchyma, spengy parenchyma cells, multicellular trichomes. Transverse section of stem shows sclerenchyma cells, trachied, glandular hairs, hairs, trichome, epidermis, cortex, xylem meta vessels, xylem proto vessels and also pith. Transverse section of stem a sector enlarged shows trichome, hair, epidermis, cortex, phloem, sclerenchyma cells, vessels, prota xylem, pith. A pith portions enlarged shows parenchyma cells and cell inclusions and also shows glandular hair. Powder analysis shows parenchyma cells, oil globules, epidermal tissues, bundle of trichome, portion of fiber, sclerenchymatous cells, trachieds, vessel elements. Physico-chemical evaluation includes ash values, extractive values, moisture content was evaluated. The fluorescence analysis of powdered whole part also was evaluated. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

Key Words: *Fioria Vitifolia*, Morphological, Microscopical, Physicochemical, Fluorescence analysis.

INTRODUCTION

Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting observations have been incorporated in various pharmacopoeias (Sharma SK, 2004). There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs (Dhanabal SP, *et al*., 2005). *Fioria vitifolia*. syn. *Hibiscus vitifolius* (Malvaceae) rainy season weed mainly used for the treatment of jaundice in the folklore system of medicine in India. It is commonly known as 'Dhakto Kalo Bhendo' in traditional medicine of Gujarat. The plant having alkaloids, flavones, gossypin, carotenoids, atropine, promethazine, aldose, galactose, bioflavonoids, morphine, pentahydroxy glycosyl flavones.

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The plant is useful in astringent, urinary disinfectant, sedative, analgesic, neuro muscular block properties, anti-inflammatory, anti-pyretic, anti fungal, hypoglycemic and other properties (Anonymous 1). The plant root extract possess potent protective action against anti-tubercular drug induced hepatotoxicity (Samuel AJ *et al*., 2012). Pharmacognostic investigations have not been reported for the whole part of this plant. Therefore the main aim of the present study Pharmacognostical investigation such as organoleptic, morphologic, microscopic and other applicable physico - chemical parameters of whole part of *Fioria Vitifolia* which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Plant material

The species for the proposed study that is *Fioria Vitifolia* were collected from town of Komarapalayam, Namakkal district, Tamilnadu and authenticated as Fioria Vitifolia by Botanical Survey of India, Coimbatore.

Macroscopic features

Macroscopy i.e. evaluation of the drug for the confirmation of its identity, determination of quality and purity and detection of adulteration was done by visual appearance by the naked eyes. Other sensory characteristics like odour, taste, and feel of the drug to the touch were also observed.

Microscopic features

Care was taken to select healthy plant and normal organ. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid-5ml+ 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule (Sass JE, 1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°c) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the section was 10-12 µm. Dewaxing of the sections was by customary procedure (Johanson DA, 1940). The sections were stained with toluidine blue as per the methods (O'Brien TP, 1964). Since toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI (for starch). Glycerin mounted temporary preparation were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaoH and mounted in glycerine medium after staining. Different cell component were studied and measured (Wallis TE, 1985; Evans WC, 1983).

Photomicrograph

Microscopic descriptions of tissue are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy book (Easu K, 1964; Easu K, 1979).

Physico-chemical standards

In the physico-chemical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values, and loss on drying were determined (Kokate CK, 2003; Anonymous 2). The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. The percentage w/w values were calculated with reference to the air-dried drug.

Determination of total ash value

Three gram of whole part powder of *Fioria Vitifolia* was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 °C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

Acid insoluble ash value

The total ash obtained from 3g of root powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water Soluble Ash Value

The total ash obtained from 3g of root powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of Alcohol Soluble Extractive Value

Accurately weighed powder (5 g) of roots was taken and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

Determination of Water Soluble Extractive Value

Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform water was used for maceration.

Loss on Drying

Weigh accurately about 1.5 gm of the powdered drug in a tared porcelein dish, which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss on drying with reference to the air-dried substance was calculated.

Fluorescence analysis

Fluorescence analysis of the powdered drug with different chemicals were observed in day light and ultra-violet light. The powdered root was treated with various solvents like picric acid, acetic acid, concentrated nitric acid, concentrated sulphuric acid, concentrated hydrochloric acid, ferric chloride, aqueous KOH, Alcoholic KOH, Iodine solution, Ammonia solution 25% v/v and observed under day light and also U.V. 254 nm, U.V. 366 nm (Kokate CK , 1994; Kokoshi CL, 1958; Chase CR and Pratt RS, 1949).

RESULT

Macroscopic features

The macroscopic study of the plant leaves are 2-6cm long, 2-5cm broad, subcordate rounded at base, acute at apex not lobed shallowly. Stalk 1-5cm long. They do look like grape leaves, hence the species name vitifolia. Flowers occur singly in leaf axis. Flower stalk 1.5 -3 cm long in fruit up to 5 cm. sepals fused below the middle. They 1.5 to 2cm long. Flowers are 4-6cm across. Flowers are pale yellow to yellow with a large, purple centre. Petals are 3-5cm long, 2-3 cm board outside with simple and 2 rayed hairs towards top, glabrescent, obovate. Stamina column 1-2cm long, regularly antheriferous all over, glabrous (Fig 1).

Microscopical features

The transverse section of the leaf midribs shows upper epidermis, lower epidermis, palisade tissues, spengy tissues, multicellular trichomes, parenchyma and also xylem vessels (Fig 2). Transverse section of leaf a sector enlarged shows upper epidermis, lower epidermis, palisade parenchyma, spengy parenchyma cells, multicellular trichomes (Fig 3). Transverse section of stem shows sclerenchyma cells, trachied, glandular hairs, hairs, trichome, epidermis, cortex, xylem meta vessels, xylem proto vessels and also pith (Fig 4). Transverse section of stem a sector enlarged shows trichome, hair, epidermis, cortex, phloem, sclerenchyma cells, vessels, prota xylem, pith (fig 5). A pith portions enlarged shows parenchyma cells and cell inclusions (Fig 6) and also shows glandular hair (Fig 7). Powder of the whole part exhibited vessel elements, parenchyma cell, oil globues, epidermal tissues, bundle of trichomes, portion of fibers, sclerenchymatous cells, trachieds are additional features of diagnostic values (Fig 8)

Physico-chemical standards

The physico-chemical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values, and loss on drying were calculated and recorded (table 1).

Fluorescence analysis

Powdered drug under ultra-violet and ordinary light when treated with different reagent emitted various colour radiations with help in identifying the drug in powder form (table 2).

Figure 1. Morphological features of *Fioria Vitifolia*

Figure 2. Transverse section of leaf with mid rib

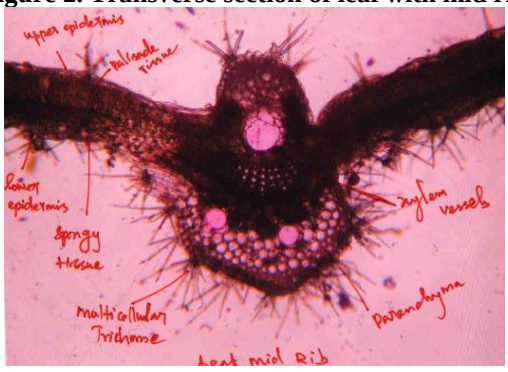


Figure 3. Transverse section of leaf – A sector enlarged

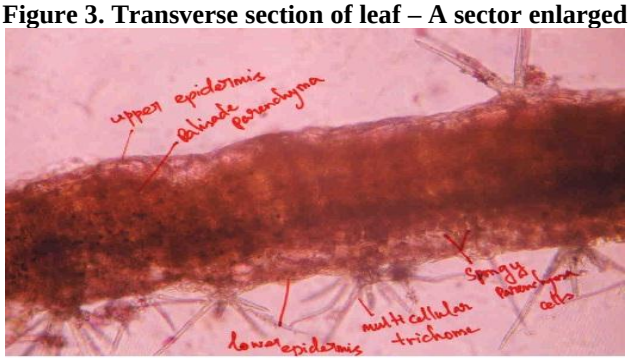


Figure 4. Transverse section of stem

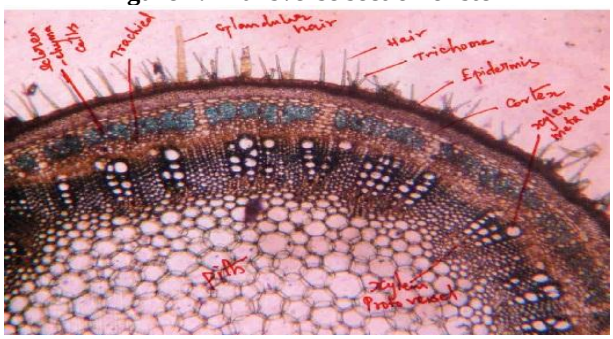


Figure 5. Transverse section of stem –A sector enlarged

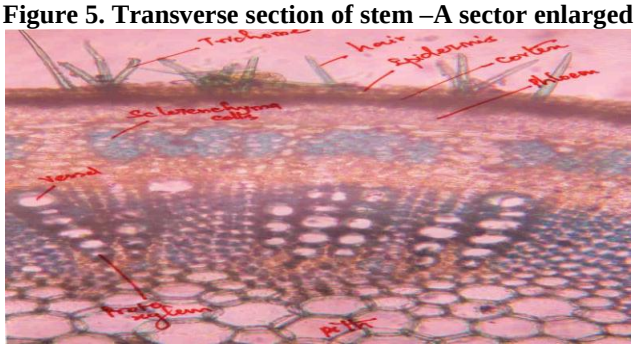


Figure 6. Transverse section of stem - Pith enlarged

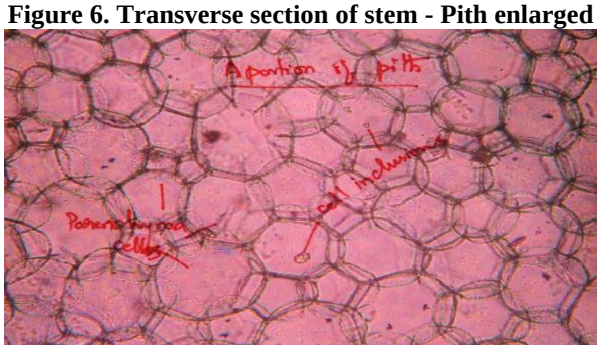


Figure 7. Transverse section of stem – Glandular hair

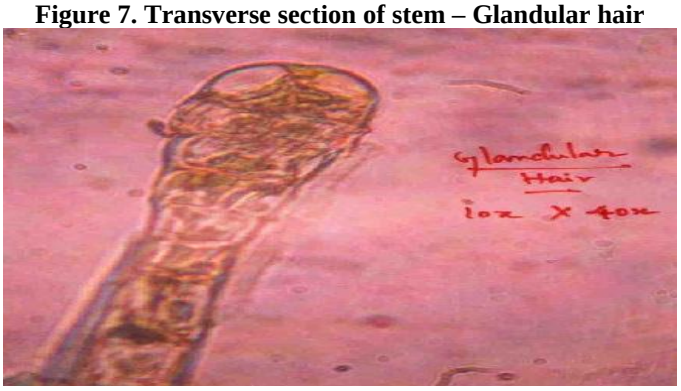
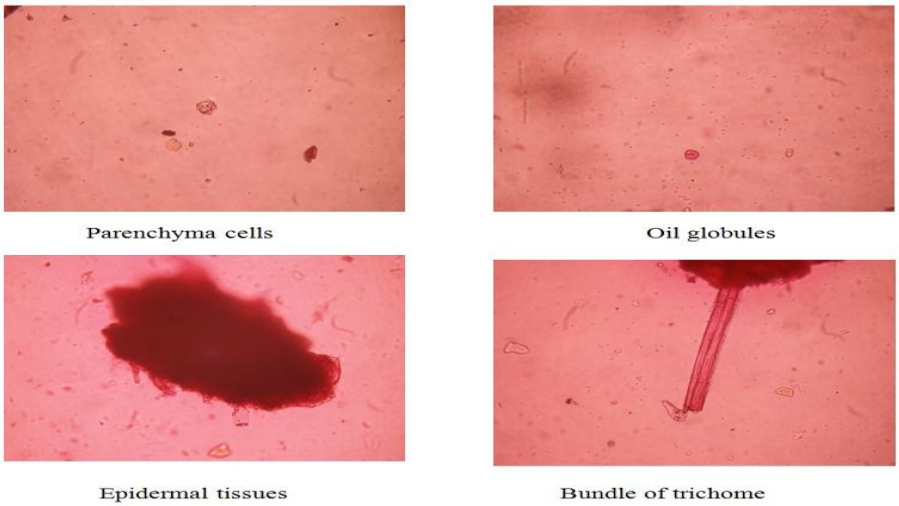
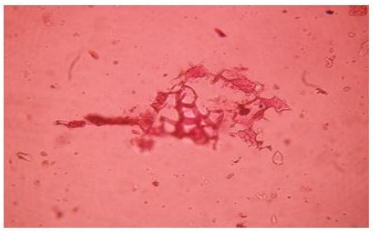


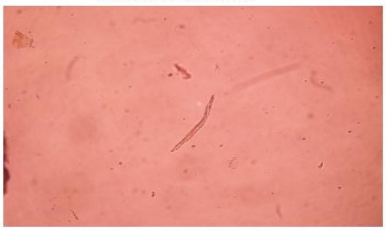
Figure 8. Powder microscopy of *fioria vitifolia*



Portion of fiber



Sclerenchymatous cells



Trachieds

V essel elements

Table 1. Physico-chemical standards of powdered *Fioria Vitifolia*

S.no	Parameter	%w/w
1.	Total ash	13.3
2.	Water soluble ash	7.3
3.	Acid insoluble ash	10.3
4.	Water soluble extractive	32.0
5.	Alcohol soluble extractive	48.0
6.	Loss on drying	20.0

Table 2. Fluorescence analysis of the powdered *Fioria Vitifolia*

S.no	Treatment of powder	Visible light	UV light	
			Short wave (254 nm)	Long wave (366 nm)
1	Picric acid	Pale Green	Pale Green	Dark Green
2	Acetic acid	Pale Brown	Pale Brown	Dark Green
3	Conc. Nitric acid	Pale Yellow	Pale Yellow	Dark Black
4	Conc. Sulphuric acid	Pale Black	Pale Black	Dark Black
5	Conc. Hydrochloric acid	Light Green	Light Green	Dark Green
6	Ferric chloride solution	Pale Brown	Pale Brown	Dark Green
7	Aqueous KOH	Pale Green	Pale Green	Dark Green
8	Alcoholic KOH	Pale Brown	Light Brown	Dark Brown
9	Iodine solution	Light Brown	Pale Brown	Dark Brown
10	Ammonia solution 25% v/v	Light Yellow	Light Green	Dark Green

DISCUSSION

This is the first report of pharmacognostical studies on the whole part of *Fioria Vitifolia*. The plants were collected from town of Komarapalayam. The macroscopic study of the plant leaves are 2-6cm long, 2-5cm broad. They do look like grape leaves, hence the species name vitifolia. Flowers occur singly in leaf axis. Flower stalk 1.5 -3 cm long in fruit up to 5 cm. Microscopical studies indicate that the presence of upper epidermis, lower epidermis, palisade tissues, spengy tissues, multicellular trichomes, parenchyma, xylem vessels, sclerenchyma cells, trachied, glandular hair, clear hair, trichome, cortex, xylem meta vessels, xylem proto vessels, pith, spongy parenchyma cells, prota xylem, cell inclusions and glandular hair. Powder of the whole part exhibited vessel elements, parenchyma cell, oil globues, epidermal tissues, bundle of trichomes, portion of fibers, sclerenchymatous cells, trachieds are additional features of diagnostic values. In ultra-violet and ordinary light analysis with different reagent is useful in identifying the drug in powder form. Physico-chemical evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as

metallic salts and/or silica. The ash values viz., total ash, acid insoluble ash and water soluble ash were 13.3%, 10.3%, 7.3% respectively. Extractive values viz., alcohol soluble extractive value,water soluble extractive were 48.0%, 32.0% respectively. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14%w/w (Anonymous 3). The moisture content (Loss on drying) was 20.0%. These observations will help in the Pharmacognostical identification and standardization of the drug and also contribute towards establishing pharmacopoeial standards.

CONCLUSION

In present investigation various standization parameter such as macroscopy, microscopy, some other pharmacognostic characters and physicochemical standards was carried which could helpful in authentification of *Fioria Vitifolia*. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

ACKNOWLEDGEMENT

The authors are very much thankful to Dr. J.K.K. Munirajah, M.Tech (Bolton), D.Litt, Chairman, J.K.K. Munirajah Educational Institutions, B.Komarapalayam, Tamilnadu. For providing the necessary facilities for carrying out this research work.

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