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**Cyto-Morphological and *In-Vitro* Anthelmintic activity of
Hibiscus esculentus Syn. *Abelmoschus esculentus***

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Abstract

The current study is related to the scientific evaluation of cyto-morphological and anthelmintic studies of species of *Hibiscus esculentus*. The selected plants are either used by the traditional practitioners for its effect or cultivated in large quantity for its medicinal and edible purpose and have been never studied as presented in present investigation. Therefore, the present study was designed to develop the cyto-morphological studies of the plant and to explore the anthelmintic efficacy of the species.

Key-Words: *Hibiscus esculentus*, Cyto-morphological, Anthelmintic Activity

Introduction

Hibiscus is a genus of flowering plants in the mallow family, Malvaceae. It is quite large, containing several hundred species that are native to warm-temperate, subtropical and tropical regions throughout the world. Member species are often noted for their showy flowers and are commonly known simply as hibiscus, or less widely known as rose mallow. The genus includes both annual and perennial herbaceous plants, as well as woody shrubs and small trees.

Bhindi or bende kai or okra Ladies finger (*Abelmoschus esculentus*) is a popular summer vegetable grown extensively along the coast of Mangalore. The tender fruits have rich iodine content which is used in the treatment of goiter, while the leaves are used for treatment of dysentery and inflammation. The dry seeds have rich edible oil content of up to 20 % and protein up to 25%.¹⁻²

The species is an annual or perennial, growing to 2 m tall. It is related to such species as cotton, cocoa, and hibiscus. The leaves are 10–20 cm long and broad, palmately lobed with 5–7 lobes. The flowers are 4–8 cm in diameter, with five white to yellow petals, often with a red or purple spot at the base of each petal. The fruit is a capsule up to 18 cm long, containing numerous seeds.

Abelmoschus esculentus is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds. It is among the most heat- and drought-tolerant vegetable species in the world and will tolerate soils with heavy clay and intermittent moisture but frost can damage the pods.³⁻⁴

Oxalic acid is a naturally occurring colourless organic acid found in many plants including okra. Okra contains 0.05g/100g of oxalic acid. The toxicity of oxalic acid is due to kidney failure, which arises because it causes precipitation of solid calcium oxalate. Oxalic acid can also cause joint pain due to the formation of similar precipitates in the joints. Unspecified parts of the plant were reported in 1898 to possess diuretic properties; this is referenced in numerous sources associated with herbal and traditional medicine.⁵⁻⁶



Fig. 1: *Hibiscus esculentus*: Flowers and Fruits

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Fig. 2: *Hibiscus esculentus*: Seeds

Material and Methods

Selection of Plant

Plants of family Hibiscus (*H. esculentus*) is medicinally important plants, commonly grown in many parts of our country, The various parts of the plant are used in the treatment of various diseases as claimed in folk-lore, till date no any systematic studies were carried out in evaluating the species, therefore, the plants was selected for the present study.

Collection and authentication of Plant Material

The selected plant were collected in the months of June-2013 from the medicinal garden of Ujjain Institute of Pharmaceutical Sciences, Ujjain District of Madhya Pradesh and identified & authenticated in Department of Pharmacognosy, Ujjain Institute of Pharmaceutical Sciences, Ujjain, M.P. and was deposited in our Laboratory, Voucher specimen No., PCog/HE/002.

Morphological Studies

The macroscopy of leaves of Hibiscus species such as color, odor, size, shape, taste, surface characters and fractures were carried out.⁷

Cytological Studies

The specimens of the proposed study were collected, care was taken to select healthy part and for normal organs. Then required samples of organ were fixed in FAA (formalin-5ml+ Acetic acid 5ml+ 70% Ethyl alcohol-90ml). Free hand transverse sections of fresh parts were taken, cleaned in chloral hydrate solution with gentle warming, stained with phloroglucinol and concentrated hydrochloric acid. They were mounted on slide in glycerine and studied under microscope. Microphotographs of sections were documented using microscope with camera, Nikon (14 mp). Descriptive terms of the anatomical features were revealed as given

in the standard anatomy book⁸⁻⁹. The figure and details are given in the results.

Extraction of Plant Juice

The fresh leaves of famil *H. esculentus* were taken, cut in to small pieces, taken 100 gm and grinded with water, filtered with musclin cloth to obtained the fresh juice.¹⁰

Preliminary Phytochemical Screening of Plant Juice

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure was adopted to perform the study¹⁰⁻¹¹.

In-Vitro Anthelmintic Activity¹²

Selection and collection of earthworm

For the anthelmintic activity, Indian adult earthworms (*Pheretima posthuma*) in 6-8 cm in length and 0.1-0.2-3 cm in width were used. The earthworm were collected (due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human being) from moist soil and washed out of sand.

Sample preparation

Fresh plant juice of *H. esculentus* was used for present investigation.

Standard preparation

100 mg of standard drug albendazole was dissolved in 100 ml of distilled water to prepare the stock solution (1000 microgm/ml). From the above stock 100 microgm/ml were prepared.

Control preparation

100ml of water was taken in a beaker taken as a control solution.

Anthelmintic investigation

The earthworms were divided in five groups of six earthworms in each group having uniform size. Each group was treated with fresh plant juice and standard drug albendazole. Observation were made for time taken to paralyze (paralysis was said to occur when earthworms did not revive in normal saline and shows no motility) and death (death was concluded when earthworms lost their motility and followed with their body colors fading away. The result was reported in results. For the different extract graph was plotted between concentration Vs time of paralysis and time of death, and was reported in graph.

Results and Discussion

The plants *H. esculentus* belongs to genera Hibiscus family Malvaceae is an indigenous plants grown in many parts of our country and was chosen for the present investigation. The scanty availability of information on this plant facilitates the study on it. The

attempt was made to study the anthelmintic activity of these species. The morphological features of leaves of *H. esculentus* were studied. The morphological features were given in table 1 and shown in Fig. 3.

Table 1: Morphology features of *H. esculentus*

S/No.	Features	HE
1.	Colour	Light Green
2.	Odour	Odorless
3.	Taste	Mucilaginous
4.	Shape	Deltaoid
5.	Surface	Rough
6.	Texture	U=Smooth: L=Rough
7.	Veneation	Reticulate



Fig. 3: Leaves of *H. esculentus*

The anatomical features of the leaves of *H. esculentus* were investigated and were summarized in Table 2 and illustrated in Fig. 4. The leaf anatomy (Fig. 4) of the taxa showed the mesophyll which is confined to the center of the lamina is composed of 2-3 epidermal layers of cells and are regular in shape. There are also well developed sclerenchymatous and parenchymatous cells and calcium oxalate crystal in the leaves. The *Hibiscus* species investigated possesses features in their leaf anatomy that could be vital in their description and in their taxonomy. The variation in the

epidermal cells: long chains, big and numerous. The nature of xylem vessels in taxa, very small few and ovoid in shape xylem cells.

This study is therefore based on the principles that leaf anatomy has played a major role in the identification, characterization and delimitation of plants. Hence the need to incorporate information from root and leaf anatomy with data derived from other botanical disciplines remains vital when formulating conclusions on the systematic of the taxa investigated.

Table 3: Anatomical characters of the leaves of the *H. esculentus*

S/No.	Characters	HE
1.	Nature of central cells	Large without stains of Calcium Oxalate
2.	Mesophyll	3- 4 layers with regular shapes
3.	Xylem vessels	Very small and few
4.	Sclerenchymatous and Parenchymatous	Well developed
5.	Crystals	Present
6.	Phloem	Present
7.	Trichomes	Small and Multicellular

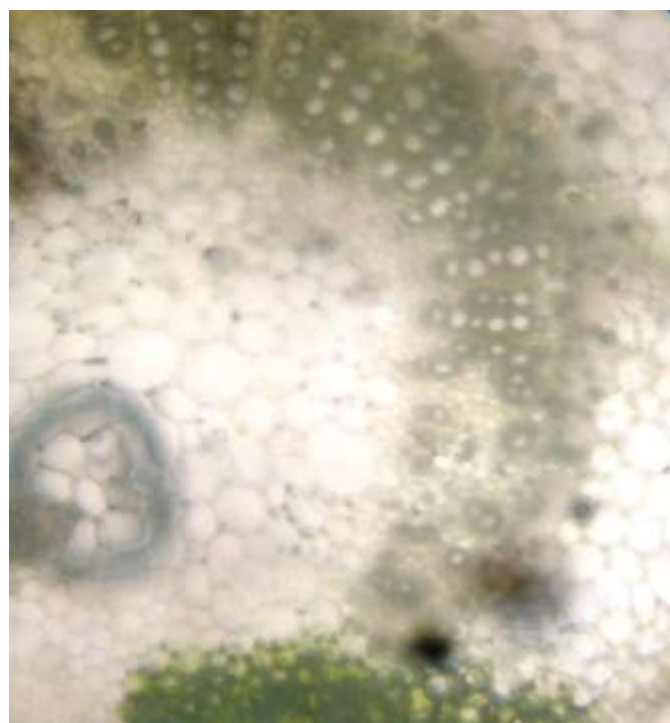


Fig. 4: Anatomical features of *H. esculentus*

The fresh juices of plants were subjected to phytochemical screening which reveal the various pharmacological active constituents. The result was reported in table 3.

Table 3: Preliminary phytochemical screening of fresh leaves juice of *H. esculentus*

S/No.	Constituents	Hibiscus species Fresh Leaves Juice
		HE
1.	Carbohydrates	+
2.	Glycosides	+
3.	Alkaloids	+
4.	Protein & Amino acid	+
5.	Tannins & Phenolic compounds	-
6.	Flavonoids	-
7.	Fixed oil and Fats	-
8.	Steroids & Triterpenoids	+
9.	Waxes	-
10.	Mucilage & Gums	-

Abbr. - = Absent, + = Present

Anthelmintic activity

Gastrointestinal parasites create a serious threat to the production of livestock in developing nations. Despite the fact of development of anthelmintic resistance in parasites of high economic significance, chemotherapy is still the most widely used option for the control of helminthes. Helminthes parasite infections are global problems with serious social and economic repercussions in the Third World countries. The diseases affect the health status of a large fraction of the human population as well as animals. Some types of dangerous helminthes infections like filariasis have only a few therapeutic modalities at present. The continuous and long-term reliance on a small range of compounds has led to the development of drug resistance in many helminthic strains. In addition, after treatment with albendazole or mebendazole, several side effects have been reported in hosts such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting), nervous system symptoms (headache, dizziness), and allergic phenomena (edema, rashes, urticaria). Some anthelmintic drugs, such as praziquantel and albendazole, are contraindicated for certain groups of patients like pregnant and lactating woman. These drugs have also to be used with caution in hepatitis patients and in children below 2 years of age. Keeping all these aspects in mind the search of natural anthelmintic is essential.

H. esculentus is an indigenous and medicinal plant grown under cultivation in many parts of our country and was chosen for the present investigation. The scanty availability of information on this plant facilitates the study on it. The attempt was made to study *in vitro* anthelmintic activity of fresh leaves juice. The anthelmintic activities of the leaves juice were determined and were reported and found to be significant when compared with the standard drug (Table 4). The anthelmintic activities were determined and it was found that FJHEL possesses comparative good and significant when compared with the standard drug.

Table 4: Comparative Anthelmintic activity of three species of *Hibiscus* taxa

S/No.	Treatment	Paralysis Time	Death Time
1.	Control	-	-
2.	Standard	11.29±0.22	22.38±0.33
3.	FJHEL	15.66±0.29	48.16±0.18

Values are expressed as X (Mean) ± SEM, n=6. (One way ANOVA followed by Student t-test). Statistically significance of P<0.001 in comparison to standard.

Abbr.: C=Control (Water only), SD=Standard drug (Albendazole), FJHEL= Fresh juice of *Hibiscus esculentus* leaves

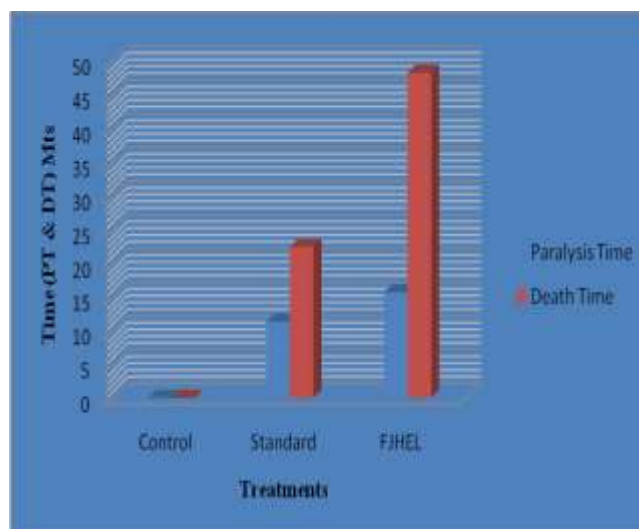


Fig. 5: Paralysis and Death time of fresh leave juice of *Hibiscus esculentus* leaves

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