WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 7, 1048-1060. Research Article ISSN 2277– 7105

PHYTOCHEMICAL, MINERAL COMPOSITION SCREENING AND PHARMACOGNOSTIC EVALUATION OF HIBISCUS ESCULENTUS ROOT FROM MAHARASHTRA

Pradeep C. Dave[1]* and Bharatkumar N. Dave[2]

[1]Department of Biochemistry, Bharati Vidyapeeth Deemeded to be University, Dental College and Hospital, Sec-7, C.B.D Belpada, Kharghar, Navi Mumbai, Maharashtra, India.
[2]Department of Biochemistry, Topiwala National Medical College and Hospital, Mumbai Central, Maharashtra, India.

Article Received on
04 May 2021,
Revised on 24 May 2021,
Accepted on 14 June 2021
DOI: 10.20959/wjpr20217-20846

***Corresponding Author**
Pradeep C. Dave
Department of Biochemistry, Bharati Vidyapeeth Deemeded to be University, Dental College and Hospital, Sec-7, C.B.D Belpada, Kharghar, Navi Mumbai, Maharashtra, India.

ABSTRACT

The different species of *hibiscus* belong to Malvaceae family. These are shrubs found all around the globe. *Hibiscus rosa* and *Hibiscus sabdarrifa* are known to have many medicinal applications but *Hibiscus esculentus* also has medicinal value and is comparatively less studied. This study focuses on Phytochemical, Pharmacognostic, Metal and Heavy metal evaluation of *H.esculentus* roots. This would help further exploration of medicinal properties of plant. This study will contribute to standardization of *H.esculentus* root powder material for ayurvedic pharmacopeia. Tests for Phytoconstituents such as Alkaloids, Flavinoids, Tanins and Phenolics, Saponins and Cardiac glycosides were done using root powder extract. Metal and Heavy metal composition was determined by Atomic Emission Spectroscopy – ICP method. Pharmacognostic evaluation was done by determining physical charecteristics of root powder such as colour, odour, taste and Total Ash content, Acid Insoluble Ash, Water soluble extractives, Moisture and Microscopic study was done. Phytochemical screening showed presence of Flavinoids and Saponins in plant root. Screening for metal composition showed that there was good amount of Calcium, Potassium, Magnesium. Moderate amounts of metals such as Sodium, Iron, Copper, Mangnese, and Zinc. Traces of Nickel, Cobalt, Cadmium and Lead. The heavy metals are within the the range specified by WHO and FDA. Physical examination of *H. esculentus* root powder showed that the powder was light brown in color with sweet odor and tasteless. Also

Pharmacognostic evaluation revealed that the Total Ash Content 3.95 % W/W, Acid insoluble Ash 0.02% W/W, Water soluble extractive 96 % W/W, and Moisture content 6.93 % W/W.

KEYWORDS: *Hibiscus esculentus*, Phytochemical Screening, Mineral composition, Pharmacognostic evaluation.

INTRODUCTION

***Hibiscus esculentus* L. (*Ablemoschus esculentus* (L):** Is valued for its edible green seed pods. The geographical origin of okra is disputed, with supporters of South Asian, Ethopian and West African origins. The plant is cultivated in tropical, subtropical and warm temperate regions around the world.

Okra is an annual, erect, stout-stemmed, 0.6 to 1.5 meters in height, bristly herb, growing upto two meters. The leaves are angular, long-stalked, heart shaped, about 25 cms length, heart shaped base, the margins 3 to 5 lobed and coarsely toothed. Petioles are equilonger. The single flower located in the axiles of the leaves is pale yellow in colour. Fruit 1.5 cms to 3 cms in diameter, tapering in shape with ribs along its length. The plant is aromatic with an odor resembling cloves. The fruit is elongated pod. It is slimy when cut for cooking.[1] Decoction of young *H.esculentus* fruit is used to treat inflammation of mucus membranes, especially of the respiratory tract, accompanied by excessive secretions. The juice is used to treat sore throat associated with coughing. Decoction of leaves and fruits is used to treat urinary problems, such as painful urination and other genitourinary problems including gonorrhea and syphilis. Leaves and roots are used as poultice for wounds. The fruit juice is used to treat diarrhea with fever and related abdominal pain. It contains the special fiber which takes sugar levels in blood under control, providing sugar quantity acceptable for the bowels. The fiber in *H.esculentus* is a valuable nutrient for intestinal microorganisms. It ensures proper intestinal functionality. Okra stimulates the nervous system and ensures recovery from psychological and mental conditions, like anxiety, depression and general weakness.

Phytochemicals are non – Nutritive plant chemicals that have protective or disease preventive properties. They are non essential nutrients, meaning they are not required by the human body for sustaining life. It is well known fact that plants produce these chemicals to prevent themselves but recent research demonstrates that they can also protect humans against

disease. There are more than 10,000 phytochemicals having medicinal properties e.g. Lycopene from tomatoes, isoflavones in soy and flavinoids in fruits. Many of these phytochemicals have beneficial effects on long term health when consumed by humans, and can be used to effectively treat human diseases. At least 12000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on human body through processes identical to those already well understood for the chemical compounds in conventional drugs.[2,3] Plant products hold high promise as therapeutic agents. Several plants and isolates have contributed novel compounds possessing promising biological and therapeutic activities. The use of plant products for therapeutic activity is considered as novel approach.[4]

For standardization and quality assurance purposes three attributes viz. authenticity, purity and assays are desirable. Authenticity corresponds to the right identity which involves many parameters like morphology, microscopy, and chemical analysis. Purity pertains to evaluating that there are no adulterants present in plant materials. It can be evaluated by pharmacognostic evaluations like qualitative and quantitative microscopy, physical constants like ash values, extractive values etc.

The plants usually have two types of minerals the Micro nutrients and Macronutients. Micronutrients include cations like Fe, Mn, Cu, Zn, Ni. and anions like B, Mo, Cl. The plant mineral composition is studied to determine any heavy metal ions present in the roots . Since a higher heavy metal concentration is toxic to human beings.

Figure 1: Whole plant of *Hibiscus esculentus.*

MATERIALS AND METHODS

Collection, Processing and Authentication of raw materials

The dried roots of *Hibiscus esculentus* were collected from fields nearby Kharghar (Navi Mumbai). The authenticatication was done at Nicholas Piramal India Ltd, Goregaon, Mumbai and used for the study. The roots were dried in shade.

The root material was cut into pieces and powdered using grinder. The dried materials were powdered (40 mesh) and subjected to soxhlet extraction using methanol as a solvent. The methanolic extracts were concentrated under vacuum at temperature of 50 degrees and used for the final study

Morphological evaluation

Morphological evaluation can be applied for the authentication of crude drug material where such materials are known to occur in a particular form and involves evaluation by color, odour, taste, texture etc.[5,6]

Phytochemical analysis

Table no. 1: Phytochemical screening of *H.esculentus* root powder.

Sr. no	Name of bioactive compounds	Name of the test	Procedure
1	Alkaloids	a)Dragendroffs Test	To 2 to 3 ml of filterate add few drops of Dragendroffs reagent. Orange brown ppt is formed.
		b) Hagers Test	2-3ml filterate with few drops Wagners reagent gives reddish brown ppt.
2	Flavinoids	Shinodas Test	To dry powder or extract add 5 ml of 95% of ethanol few drops of conc HCl and magnesium turnings.Pink colour is observed
3	Tanins and Phenolics	Tanins and Phenolics Test	Add 2 to 3 drops of ferric chloride to 1ml extract for the formation of a dark blue or greenish black coloured product which shows presence of tannins.
4	Saponins	Foam Test	1 ml of extract is diluted by 20 ml of distilled water.and then shake in graduated cylinder for15 mins .A 1 cm layer of foam indicates presence of saponins.

5	Cardiac Glycosides	Killer Killani Test	1 ml of filterate + 1ml of glacial acetiacid+ Fe Cl3 +H_2SO4 Green blue ppt is obtained.

Pharmacognostic evaluation

A) Determination of total ash content

Three grams of crude drug was accurately weighed and placed in a previously ignited and tared crucible. The samples were spread in an even layer and ignited by gradually increasing the heat to 500-600 ˚C until white then cooled in a desiccator and weighed without delay.

B) Determination of acid insoluble ash

The crucible containing the total ash was added with 25 ml of HCl (70g/l),covered with a watch glass and boiled gently for five minutes .Then rinse the watch glass with with 5 ml of hot water and add this liquid to the crucible. The insoluble matters were collected on an ashless filter-paper and washed with hot water until the filterate was neutral. The filter paper containing the insoluble matters was transferred to original crucible, dried on a hot plate and ignited again. The crucible was then cooled in a dessicator and weighed without delay.

C) Determination of water soluble extractive

5 grams of ground crude drug were macerated with 100 ml of distilled water in shaking bath and allowed to stand for 18 hrs. They were filtered and 20 ml of filtrate were evaporated to dryness in a tared small beaker and dried at 105 ˚C to constant weight.

D) Determination of moisture

Fifty grams of ground crude drug were added with 200 ml of water saturated toluene and distilled by azeotropic distillation. As soon as water was completely distilled, rinsed the inner side of the condenser tube with toluene and continued the distillation for 5 more mins. Allow the receiving tube to cool to room temperature. When water and toluene layers were separated read of the volume of water.[7,8]

E) Microscopic study

Fully grown normal healthy plants were selected. The roots of these plants were cut and thoroughly washed with water to remove the adherent impurities and dried in sunlight. Selected samples of the dried root were stored in a solution containing formalin (5ml), acetic acid (5ml), and 70% v/v ethyl alcohol(Formal Acetic Alcohol) 90ml. This step is called as

fixation. Specimens for fixation should not be more than 5mm thick, and no greater than 10mm at the widest point. Hard material like roots and woody stems should not exceed 5mm if using FFA (Formal Acetic Alcohol) or FPA Formal Propionic Alcohol) as fixative., The specimens can be kept stored quite safely in a fixative for months. 28 ml Mc Cartney bottles, which have rubber, lined metal screw caps, half filled with fixative, are an ideal way of taking the fixative into the field. The specimens should be left in the fixative for at least 24 hrs after they have sunk to the bottom before dehydrating. After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the method described by Johansen 1940.[152] Slow dehydration of the root is done to remove all the water. In order not to destroy the delicate tissues, this is done gradually in a series of Alcohol /Water solutions of increasing strengths.

Table no. 2: Dehydration steps for microscopic study of *H. esculentus* root.

	Step -1	Step-2	Step-3	Step-4	Step-5	Step-6	Step-7	Step-8
Approx Total % Alcohol	50	70	85	95	100	Pure TBA	Pure TBA	Pure TBA
Time	2 hrs	o/night	1 hr	1 hr	1 hr	1 hr	1 hr	1 hr

These are average times and may be extended for harder tissue. The material is placed on top of cooled paraffin wax (just sufficiently solidified – not cooled completely). Cover with the TBA of step 8 of dehydration, 1 hr after material has sunk to the bottom. Infilteration of the specimens was carried by gradual addition of paraffin wax(50 -60˚C mp) until Tertiary Butyl alcohol solution attains supersaturation. The specimens were casted into paraffin blocks.

F) Sectioning

The paraffin embedded specimens were sectioned with the help of Senior rotary microtome, RMT-30. The thickness of the section was kept between 10 to 12 µm. The dewaxing of the section was carried by the procedure described by Johanson.[8] The section was stained with Toluidine blue. Toluidine blue is a polychromatic stain. The dye renders pink colour to cellulose walls, blue to lignified cells, dark green to suberin, violet to mucilage, and blue to protein bodies. Stain preparation:- 0.1 g of Toluidine blue in 100ml of 0.1M benzoate buffer p.H 4.4. If benzoate is not available water can used for general purpose. Distilled water was used for preparation of Toluidine blue as benzoate buffer was not available and general structural details were described.[9]

The staining was done as follows

1. Place the section on a clean slide.
2. Flood the section with aqueous solution of 0.1% Toludine blue solution for 1 min.
3. Gently remove the stain by using a filter paper. Wash the sections by flooding them with water followed by its removal. Repeat unless there is no excess stain around the section.
4. Add a drop of clean water over the sections and apply a cover glass.

G) Photomicrographs

Microscopic description of the root structure were supplemented with micrographs. Photo graphs were taken with Nikon Lab Photo -2 microscopic unit. For normal observations bright field was used.

Estimation of Metals and Heavy metals in the sample

The estimation was done at Envirocare labs, Thane, Maharashtra by Atomic Emission Spectroscopy.

Inductively coupled plasma –Atomic emission spectroscopy

Inductively Coupled Plasma -Atomic Emission Spectrometry (ICP- AES) is an emission spectrophotometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation, $E = hc/\lambda$ ... (where h is Planck's constant, c the velocity of light and λ is wavelength), and hence the wavelength of light emitted is also unique. Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is most common to select a single wavelength (or a very few) for a given element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the sample being analyzed. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, the analyst can qualitatively and quantitatively find the elements from the given sample relative to a reference standard.

The wavelengths used in AES ranges from the upper part of the vacuum ultraviolet (160 nm) to the limit of visible light (800 nm). As borosilicate glass absorbs light below 310 nm and

oxygen in air absorbs light below 200 nm, optical lenses and prisms are generally fabricated from quartz glass and optical paths are evacuated or filled by a non absorbing gas such as Argon.

Table 3: Mineral composition of *H. esculentus* root powder.

Sr. no	Name of the element/ Heavy metal
1	Calcium as Ca
2	Potassium as K
3	Cadmium as Cd
4	Cobalt as Co
5	Magnesium as Mg
6	Sodium as Na
7	Lead as Pb
8	Iron as Fe
9	Copper as Cu
10	Mangnese as Mn
11	Nickel as Ni
12	Zinc as Zn

RESULTS AND DISCUSSION

Physical characteristics of *H.esculentus* root powder:- The Physical characteristics of the powder were recorded**.** Also Total Ash content, Acid Insoluble Ash, Water soluble extractive and moisture content of the powder were determined for the purpose of standardization.

Table no. 4: Physical characteristics of *hibiscus esculentus* powder.

Sr. no	Character	Observations
1	Colour	Light brown
2	Odour	sweet
3	Taste	Tasteless

Table no. 5: Extractive values of *Hibiscus esculentus* root powder.

Sr. no.	Name of the test	Values % w/w
1	Determination of Total Ash	3.95
2	Determination of Acid insoluble Ash	0.02
3	Determination of water soluble extractive	96
4	Determination of Moisture	6.93

Table no. 6: Metal and Heavy metal analysis of *Hibiscus esculentus* root powder.

Sr. no	Name of the element/ Heavy Metal	mg/kg	Permissible limits of Heavy metal conc as per WHO and FDA authorities for herbal drugs in mg/Kg / ppm
1	Calcium as Ca	5660.41	-
2	Potassium as K	1593.73	-
3	Cadmium as Cd	<0.50	0.3
4	Cobalt as Co	<0.50	-
5	Magnesium as Mg	1130.29	-
6	Sodium as Na	199.23	-
7	Lead as Pb	0.66	10
8	Iron as Fe	216.74	-
9	Copper as Cu	1.93	20
10	Mangnese as Mn	41.59	-
11	Nickel as Ni	0.50	-
12	Zinc as Zn	12.92	50

The concentration of metals and especially heavy metals in ayurvedic drugs is important, to determine the toxicity of drugs.

From the above information it is clear that concentration of heavy metals in the powder is within the normal limits of heavy metal concentration prescribed by WHO and FDA. Also the powder is good source of Calcium, Potassium, Magnesium, and Iron.

Microscopic study

The root shows secondary growth. The secondary vascular tissues form a continuous cylinder. Primary phloem is crushed. Broad medullary rays are seen which are traversed in the xylem and phloem through the cambium. In extra stellar region, periderm is seen which comprises of Phellum and pheloderm.

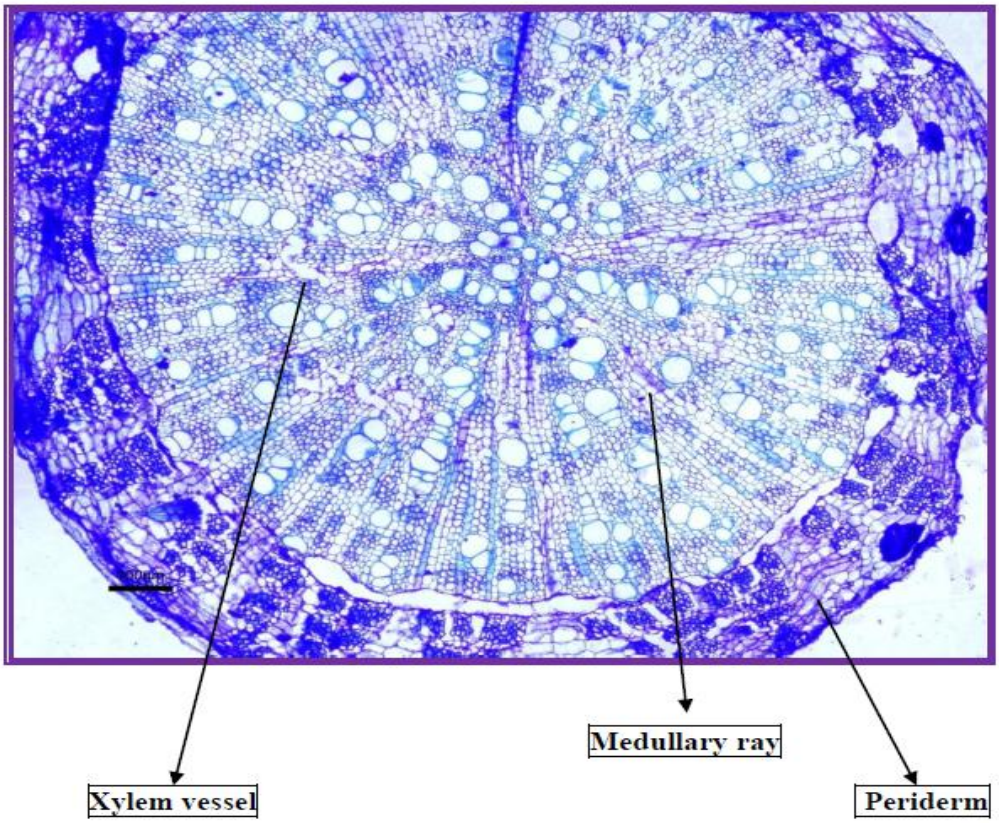

Fig. 2: Microscopic structure of H.*esculentus* root.

Table no. 6: Phytochemical evaluation of *H.esculentus* root powder.

Sr. no	Name of Bioactive compounds	Name of the Test	Observation	Inference
1	Alkaloids	a)Dragendroffs Test	No Orange brown ppt formed	Alkaloids Absent
		b) Hagers Test	No reddish Brown ppt	Alkaloids Absent
2	Flavinoids	Shinodas Test	Pink colour obtained	Flavonoids Present
3	Tanins and Phenolics	Tanins and Phenolics Test	No blue ,green or black colour observed	Tannins and Phenolics absent
4	Saponins	Foam Test	1 cm layer of foam observed	Saponins present
5	Cardiac Glycosides	Killer Killani Test	No greenish blue ppt obtained	Cardiac glycosides absent

CONCLUSION

The *Hibiscus esulentus* plant is a very common and easily available plant. A lot of research is being carried out to reveal the bioactive compounds and medicinal properties of genus *Hibiscus*. Our study forms a part of this immense work.

In this project we have tried to conduct Biochemical and Pharmacognostic analysis of the *Hibiscus esculentus* root powder. The mineral composition along with heavy metal concentration was also determined. This was done to provide a data for standardization of the herbal powder and prevent adultration.

ACKNOLEDGEMENTS

I am greatful to Department of Biochemistry, Topiwala National Medical College and Hospital, Mumbai Central, Mumbai., Department of Pharmacology, Bombay Veternary College, and C. K. Thakur College, New Panvel for allowing me to use their facilities. I am thankful to DR.Vinayak Naik Sr.Scientist (Botanist) at Piramal Life Sciences for Plant Identification. Iam thankful to all who have contributed to this work directly or indirectly.

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