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Pharmacognostical and nutritional assessment of *Indigofera cassioides* DC. flowers, an ethnomedicinal plant

Research Article

Nisha Vats[1*], Harisha C R[2], Acharya R N[3]

1. PG. Scholar, Dravyaguna Department, 2. Professor, Head of Pharmacognosy Department,
3. Professor & Head of Dravyaguna Department,
ITRA, Jamnagar. Gujarat. India.

Abstract

Background: *Indigofera cassioides* DC. a deciduous shrub of family *Fabaceae* is an unexplored medicinal plant, being traditionally used in the states of Kerala, Odisha, and Maharashtra. Different parts of the plant have been reported both as a food and in the management of chest pain, cold, asthma, diarrhoea, dysentery and piles. Material and methods: *Indigofera cassioides* after proper authentication was collected from *Paikmal* (Odisha), during the month of March and its flowers were studied for microscopic characters, physico-chemical analysis, secondary metabolites screening, nutritional values and heavy metal analysis following standard protocol recommended by the Ayurvedic Pharmacopoeia of India. Result: Powder microscopy shows the presence of simple trichome, simple starch grain, pollen grain, simple fibers, oil globules, epidermal cells on edge, group of pollen of grains, yellowish brown content, parenchyma cells of corolla, epidermal cells along with stomata, pleuri-cellular trichome. Physicochemical parameters including moisture content, ash value and extractive value of flower powder were reported. Nutritional evaluation shows that sample 1 (one year stored)has the greater nutritive value than the sample 2 (fresh). Flowers tested negative for heavy metals (mercury, cadmium, arsenic and lead). Conclusion: The present findings will be useful in establishing pharmacognostic and phytochemical standards for identification as well as assessment of purity and quality of this plant which definitely gaining the relevance in plant drug research and establishment of plant monograph.

Key Words: *Anukta dravya*, Heavy metal, *Indigofera cassioides*, Nutritional.

Introduction

India is a rich treasure trove of diverse flora along with ethno-medicinal knowledge (1).Plants used as drug and vegetable, are the reservoirs of naturally occurring chemical compounds and of structurally diverse bioactive molecules. *Indigofera cassioides* DC. of Fabaceae family is a deciduous shrub and one among the commonly used folklore medicinal plants of Odisha, Chhattisgarh, Kerala and Maharashtra (2) for the management of cough, cold, asthma, chest pain diarrhoea, piles, etc(3). Its flower and leaves are used as vegetables in Odisha in various forms (4). Even though different parts of the plant have many uses, only few studies related to its pharmacological activities have been reported. Research findings related to pharmacopoeial standards, nutritional values, and purity etc of its flowers are still lacking.

The present paper details the powder microscopic features, preliminary physicochemical and phytochemical characters and nutritional values of *I. Cassioides* flowers.

*** Corresponding Author:**
Nisha Vats
PG. Scholar, Dravyaguna Department,
ITRA, Jamnagar.
Gujarat. India.
Email Id: nishuvats332@gmail.com

Material and methods

Plant Collection and Processing

The *Indigofera cassioides* DC. was identified with the help of different flora and authenticated by the pharmacognosy expert and its flowers were collected by first author from its natural habitat Paikmal, Odisha (Longitude: 83.70371" and Latitude: 19.95432") in March 2020 and March 2021.(Fig 1)

After a through wash with potable water the flowers were dried under shade. The dried samples were coarsely ground into homogenous powder using a mechanical grinder and stored at room temperature until required for use.

Organoleptic evaluations

Flowers were subjected to various organoleptic characters like taste, odor, color and touch (5).

Powder microscopy

The dried powder of flowers was studied by placing small amount of powder on slide and observed under the microscope. Characteristic structure and cell content such as fibers, stone cells, starch grains, calcium oxalate crystals and plant cells were observed at various magnifications and photographs was taken with the help of a Quasmo binocular compound (6).

Physico-chemical analysis

Flower powder was subjected to physico-chemical parameters such as moisture content, ash value, acid insoluble ash; water soluble extractive value, alcohol soluble extractive value and pH were carried out by the Ayurvedic Pharmacopoeia of India (7).

Preliminary phytochemical screening

Methanolic and water extract of flower was screened for the presence and absence of the major class of compound by the standard method (8).

Nutritional evaluation

Details of standard operative procedures (SOP) adopted for each parameter and adopted standard test methods are mentioned in table 4 & 5.

Heavy metal analysis

Flowers and leaves were analysed for mercury, lead, arsenic, cadmium heavy metals following standard operative procedures (SOP) and standard methods.

Results Macroscopic study

Organoleptic characters

The observed organoleptic characters of the flowers are given in table 1

Table 1: Observed organoleptic characters of *Indigofera cassioides* flowers

Characters	Flower
Taste	Astringent and sweet
Odor	Characteristic
Texture	Woody
Color	Muddy brown

Powder microscopy

The dried powder of flowers were studied by placing small amount of powder on slide and observing under the microscope Characteristic structure and cell content such as fibers, stone cells, starch grains, calcium oxalate crystals and plant cells was observed at various magnifications and photographs was taken with the help of a Quasmo binocular compound microscope (6).

Physico-chemical parameters

Various physico-chemical parameters like ash value, moisture content, extractive values were investigated and the results are presented in table 2.

Table 2: Physico-chemical parameters of *I. cassioides* flower

Sr no.	Parameters	Results
1	Loss on drying (% w/w)	12.56
2	Ash value (% w/w)	4.40
4	Water soluble extractive value (% w/w)	13.34
5	Alcohol extractive value (% w/w)	2.78
6	pH (5% aqueous)	7

Preliminary phytochemical screening

Methanolic extract of it's flower was used to assess the Qualitative analysis. The methanolic and aqueous extract of test samples were appraised for steroid, glycosides, saponins, alkaloid, tannin, flavonoids etc. their results are as quoted in table no 3.

Table 3: Preliminary phytochemical screening of flower of *Indigofera cassioides*

Sr. No.	Phytochemical parameters	Test	Flower
1	Steroids	Salkowski reaction	+
2	Glycosides	Keller Killiani test	+
3	Saponins	Foam test	+
4	Alkaloids	Dragendorff's	-
5	Tannins	Lead acetate	-
6	Flavanoids	Lead acetate	-

Nutritional evaluation

Nutritional values of the fine powder of flower of *Indigofera cassioides* out of fresh sample and one year old sample are presented in Table no.4 & 5 respectively.

Table 4: Obtained results of nutrition value of fresh *Indigofera cassioides* flower

Sr no.	Quality characteristics	Result	Test method
1	Energy, Kcal/100g	318.94	AL/SOP/6.4/C-035, UNFAO Chapter 2-2003
2	Energy from fat, Kcal/100g	22.86	Chapter 3, calculation of the energy content of foods-Energy conversion factors -FAO
3	Total fat gm/100g	2.54	(AL/SOP/6.4/C-157)Fassai manual for oil & fat Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
4	Saturated fat gm/100g	0.81	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
5	Polyunsaturated fat gm/ 100g	1.02	Fassai manual for oil & fat Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
6	Monounsaturated fat gm/ 100g	0.73	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
7	Carbohydrate gm/100g	55.41	AL/SOP/6.4/C-036, UNFAO Chapter 2-2003
8	Sugar	6.22	AL/SOP/6.4/C-157, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)
9	Sodium	13.88	AL/SOP/6.4/C-088, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)
10	Protein	18.61	AL/SOP/6.4/C-156-IS 7219-1973, Indian standard methods for determination of protein in foods and feeds, December 1974
11	Dietary fibres gm/100gm	10.48	IS 11062: 2019- Indian standard methods for estimation of total dietary fibers in food stuff, February 1985
12	Trans fat gm/100 gm	0.0	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015

13	Iron mg/100 gm	1.39	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
14	Calcium mg/100 gm	89.52	IS 5949: 1990, Indian standard methods for volumetric determination of calcium and Magnesium using data, 2nd revision Feb 1991
15	Potassium mg/100 gm	467.99	AL/SOP/6.4/C-088-Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)
16	Cholesterol mg/100g	0.0	Dr Jeorge W.Jr, Official methods of Analysis AOAC 19th edition, 2012
17	Vitamin D mcg/100g	0.0	AL/SOP/6.4/C-172, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India (version 1.0.0)
18	Added sugar gm/100g	0.00	AL/SOP/6.4/C-109, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)

Table 5: Obtained nutritional value of one year old preserved *I. cassioides* flower

Sr no.	Quality characteristics	Result	Test method
1	Energy, Kcal/100g	348.13	AL/SOP/6.4/C-035, UNFAO Chapter 2-2003
2	Energy from fat, Kcal/100g	14.49	Chapter 3, calculation of the energy contentof foods-Energy conversion factors -FAO
3	Total fat gm/100g	1.61	AL/SOP/6.4/C-157, Fassai manual for oil &fat Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
4	Saturated fat gm/100g	0.0	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
5	Polyunsaturated fat gm/100g	0.0	Fassai manual for oil & fat-Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
6	Monounsaturated fat gm/100g	0.0	Fassai manual for oil & fat-Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
7	Carbohydrate gm/100g	60.49	AL/SOP/6.4/C-036, UNFAO Chapter 2- 2003
8	Sugar	1.44	AL/SOP/6.4/C-157, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)
9	Sodium	0.00	AL/SOP/6.4/C-088, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)
10	Protein	22.92	AL/SOP/6.4/C-156-IS 7219-1973, Indian standard methods for determination of protein in foods and feeds, December 1974
11	Dietary fibres gm/100gm	12.05	IS 11062: 2019- Indian standard methods for estimation of total dietary fibers in food stuff, February 1985
12	Trans fat gm/100 gm	0.0	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
13	Iron mg/100 gm	3.86	Fassai manual for oil & fat, Ministry of Health and Family welfare, Gov. Of India, New Delhi, 2015
14	Calcium mg/100 gm	59.34	IS 5949: 1990, Indian standard methods for volumetric determination of calcium and Magnesium using data, 2nd revision Feb 1991
15	Potassium mg/100 gm	1375.97	AL/SOP/6.4/C-088-Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India (version 1.0.0)
16	Cholesterol mg/100g	0.0	Dr Jeorge W.Jr, Official methods of Analysis AOAC 19th edition, 2012
17	Vitamin D mcg/100g	0.0	AL/SOP/6.4/C-172, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India (version 1.0.0)
18	Added sugar gm/100g	0.00	AL/SOP/6.4/C-109, Standard Operating procedures, Part 2, 3rd August 2015, Ministry of rural development of India(version 1.0.0)

Heavy metal analysis

Fine powder of the flower and leaf of the *I. cassioides* was subjected for the heavy metal Arsenic (As), mercury (Hg), lead (Pb) and Cadmium (Cd) by using inductively coupled plasma atomic emission spectroscopy (9).Obtained results are shown in table no.6. Both samples leaves and flower are devoid of all four heavy metals.

Table 6: Heavy metals analysis of leaves and flower of the *I. cassioides*

Sample	As	Hg	Pb	Cd
	ppm	ppm	ppm	ppm
Leaves	ND	ND	ND	ND
Flower	ND	ND	ND	ND

ND means less than 0.01 ppm

Discussion

The standardization and quality control of the herbal medicines is necessary to assure the quality of the drug because substitute or counterfeit herbal materials are often found in the market. This analysis helps to ensure the identity, quality, purity and safety of drug for the human use (10). According to the World Health Organization, the macroscopic and microscopic description of a plant is the first step to establish the identity and the degree of purity of such materials and should be carried out before any test are undertaken(11).

Microscopic study of flower powder revealed thepresence of simple trichome, simple starch grain, pollen grain, simple fibers, oil globules, epidermal cells on edge, group of pollen grains, yellowish brown content,

parenchyma cells of corolla, epidermal cells along with stomata, pleuri-cellular trachoma. The salient diagnostic characters can be used for standardization of drug and also used for the preparation of plant monographs.

The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. Flower powder was found to be devoid of foreign matter, which may be due to the good harvesting practice followed during the collection of the drug. The moisture content is a vital parameter for evaluating the efficacy of a drying process and as guide to determining the possibility of deterioration due to high moisture content during process and storage (12).

The determination of ash value has equal importance in the evaluation and identification of inorganic impurities in crude drugs (13). Ash value of flower was found to be 4.40(% w/w). The flower found to have more extractive value in water 13.34(% w/w) than methanol 2.78 (% w/w), which indicates the probability of the presence of high-water soluble constituents in flower. The pH of the aqueous extracts of flower is 7 which indicate the neutral nature of the crude drug.

The preliminary screening tests are useful in the detection of the chemical groups and subsequently may lead to the drug discovery and development (14). Preliminary phytochemical analysis made for flower of *I. Cassioides* revealed the presence of steroids, glycosides and saponin.

Nutritional evaluation of flower revealed that sample 1 has the higher nutritive values than the sample 2 like energy, carbohydrate, protein, dietary fibres, iron and potassium then the sample 2. Carbohydrate acts as a fuel source of brain high energy demand and essential for the fat oxidation. Protein are basic building materials for the growth and maintenance of the body, act as messenger for the hormones, maintain proper pH and also necessary for the immune health. High dietary fibre content shows that it helps in maintaining the bowel health and decrease the constipation problem, balance intestinal pH, absorb more water, improves cholesterol and blood sugar level.Iron is the essential element for blood production which regulates body temperature, thyroid function, brain development and functioning.Due to its high iron content it might be very beneficial in many diseases like anaemia, thyroid disorder, improper functioning of the brain and also provides oxygenation and nutrition to the skin.

Presence of heavy metals gives severe and chronic toxic effects in different organs of the body like gastro-intestinal and kidney dysfunction, nervine disorders, skin lesions, vascular damage and many more complications. *I. cassioides* is devoid of such harmful toxic heavy metals therefore it can be consumed internally as a food or medicine.

Conclusion

Standardization is essential measure for quality, purity and sample identification. The present study on Pharmacognostical, Phytochemical evaluation and nutritional analysis of flower *I. Cassioides* of family Fabaceae will provide useful information for its identification. Generated data can be used for determining correct identity and purity of plants part and detection of adulteration as well as may be useful to establish certain botanical standards.The present research finding justifies the traditional uses of ICF as a vegetable. Flower of the *I. cassioides* plant when collected from natural sources following food collection practice possess high nutritive value along with lots of other basic and essential microelements and maybe a great source of food in nutritional deficiency diseases especially in remote areas. One year old preserved sample is having more energy value than the fresh sample. So, collected samples indicate that active ingredients remain intact. One year old can be consumed up to one year if stored in good storage conditions.

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Conflict of Interest

None

Fig.1:(A) Plant in natural habitat; (B) Flower in dry form; (C) Infloresence; (D) Flower's vegetable

Fig.2: (A)Flower powder; (B) Fibres with brown content; (C) Starch grain; (D) Pollen grain; (E) Simple fibres; (F) Oil globules

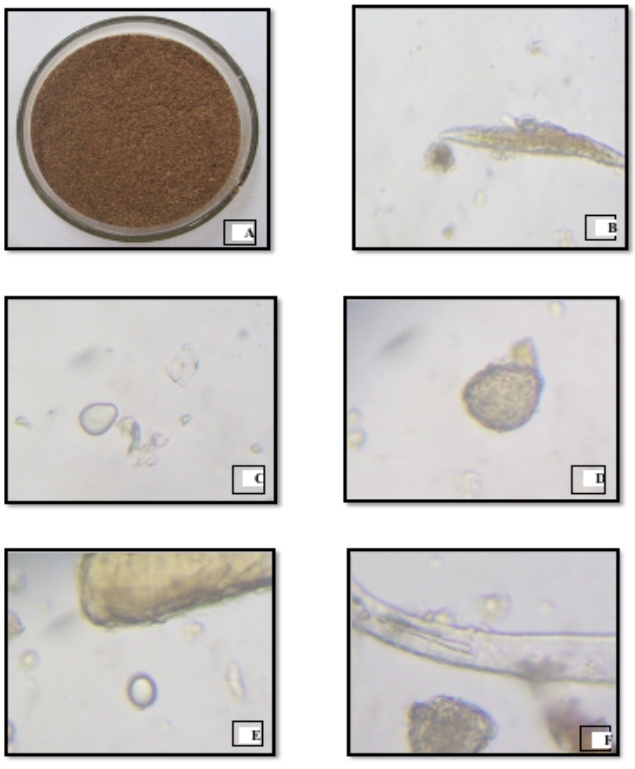
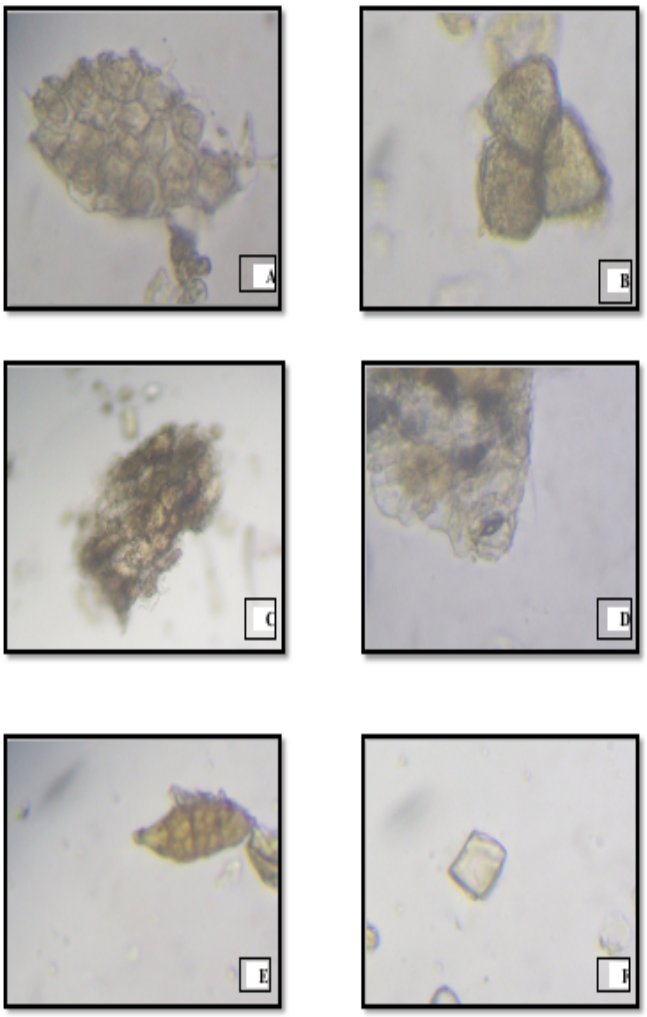


Fig. 3. (A) Epidermal cells; (B) Trihyderal group of pollen grain; (C) Hypodermal cells; (D) Epidermal cells with stomata; (E) Pleuri-cellular trichome; (F) Prismatic crystals



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