Fingerprinting and quality control of *Guizotia abyssinica L.F. Cass*

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Abstract: *Guizotia abyssinica L.F. Cass* L (family: Asteraceae) is a commonly used plant known as Niger or Birdseed or Ramtil or Karale. This plant is dietetically and economically important. It is used as spices in India. Seeds of this plant contain 37% to 50% of edible oil with a pleasant nut-like taste. The seeds of Niger have a high amount of protein (20 to 25 % albuminoids). *G. abyssinica* has ethnobotanical importance. It is used as a supplement towards the balanced diet of tribals. Niger seeds are used as a substitute for ghee by poor people. The seeds of this plant are used to make chutney. *G. abyssinica* is mainly used for treating gastro-intestinal, respiratory and allergy problems. It is a rich source of phytochemicals viz. alkaloids, essential oils, glycosides, saponins, flavonoids, polysaccharides, steroids and tannins. In the present investigation exomorphic, anatomical, pharmacognostic, physicochemical and chromatographic (fingerprinting) standards had been evolved for *G. abyssinica*. The quantitative microscopic studies have shown that the vessels are with helical to spiral thickenings. Physico-chemical parameters such as total ash, water-soluble ash and acid insoluble ash values were recorded as 15.68, 12.14 and 3.40 % w/w respectively of seed powder. The extractive values of seed powder with ethanol, chloroform, petroleum ether, and water yielded 8.10, 3.20, 8.50, and 8.70% w/w respectively. The fluorescence studies of seed showed a distinctive pattern. The characteristic TLC pattern of seed serves as a fingerprint for *G. abyssinica*. The fingerprint (TLC) developed using chloroform: Ethyl Acetate: Formic Acid 5:4:1 solvent system. In view of the importance of *G. abyssinica* as a nutraceutical, tribal supplement, and export potential, it needs an extensive investigation. The fingerprinting and quality control is important for the safety and efficacy of this traditionally important plant.

Index Terms - Ash values, *Guizotia abyssinica*, finger printing, quality control, pharmacognostic standards.

1. INTRODUCTION

Guizotia abyssinica Cass. (Asteraceae) is one of the important plants used as spices. It is an oil yielding plant native to Ethiopia and Malawi. The seeds of this plant are oil yielding and used by the tribal and rural people of our country as a source of edible oil [1]. *G. abyssinica* is known by different common names such as Niger, Bird seed, Ramtil and Karale. This crop is cultivated in the southern parts of India as well in the Central India, especially of Madhya Pradesh. It is a lifeline of Madhya Pradesh tribes and cultivated mainly by marginal farmers. The seeds are called Niger seeds in Karnataka, Andhra Pradesh and Maharashtra. It is called 'Karale' in Marathi.

G. abyssinica is mainly grown for its edible oil and seeds. It is used to make dry chutney that is an accompaniment with bread (Chapati). The seeds of this plant are used as spices. The seed oil can be used directly for cooking as it is with similar fatty acid composition to sunflower oil. *G. abyssinica* seed contains about 37-50 % oil, 20-25 % protein (Albuminoids), 10-18% sugars, and 10-15% crude fiber [2]. It has good nutritional and medicinal value. It is used in various microbial and inflammation conditions. The seed oil of this plant is polyunsaturated, clear, edible, and a nutty taste and sweet odor. This oil is valuable dietetically and economically. Niger seeds are generally used to make chutney in Maharashtra and other parts of India. The oil extracted from Niger seeds is used as a substitute for ghee. The oil of this plant is important for the tribals as it serves an important ingredient in a balanced diet. Niger oil has a variety of uses viz. cooking oil, lighting, anointing, painting and cleaning of machinery [3], [4], [5]. Niger oil is used as a substitute for sesame oil, especially for pharmaceutical purposes. It is in the preparation of various types of foods, paints and soaps. The remains after oil extraction are used in animal feed preparations. The seeds of Niger have been extensively studied for its nutritional value, biological activities, and antioxidative properties [6]. Niger has many medicinal uses such as rheumatism, cough, birth control, syphilis, gastrointestinal problems, asthma, allergy and cancer. The demand for *G. abyssinica* is ever increasing in the national and international market due to its nutritional, therapeutic and economic value. A review of literature has shown that very scanty work on the standardization of *G. abyssinica* has been carried out so far [7]. In view of this, a detailed investigation has been carried to evolve macroscopic, microscopic, phytochemical, physicochemical and chromatographic standards for quality control, safety and efficacy of *G. abyssinica* products.

2. MATERIALS AND METHODS

Plant material: The plant specimens for the study were collected from Katraj region Pune. The healthy and fully mature seeds were taken for the investigation. This plant is an erect, stout, branched annual herb with 1-3 feet height and bearing numerous yellow flowers [8]. Niger seed resembles sunflower seeds in shape, but is smaller in size and black, bears a fairly thick, adherent seed coat.

Microscopic studies: Free-hand sections of seeds of 15 µm-25 µm were taken, stained with phloroglucinol-hydrochloric acid (1:1), and mounted in glycerin. Microphotography of T.S. of seed was made. The permanent slides were made as per the standard procedure [9]. The starch grains were stained with an Iodine solution.

Powder analysis: Seed powder was prepared using sieve mesh 60 (Sixty) for the observation of microscopical characteristics. The powdered drug was separately treated with phloroglucinol-hydrochloric acid (1:1) solution and iodine solution [10].

Fluorescence studies: The fluorescence analysis carried out as per the standard method [11]. The mountant medium viz. distilled water, 1 N NaOH, 1 N HCl, 1 N H_2SO_4, and 1 N HNO_3 were used, and the fluorescence at ordinary light and UV light (254nm and 366nm) were recorded. The colour for fluorescence analysis was confirmed from 'A Mycological Colour Chart' of Rayner [12]. In the present investigation detailed analysis of the seed powder and seed solvent extracts of water, ethanol, methanol, Petroleum ether, chloroform and ethyl acetate was done.

Physico-chemical analysis: It was carried out as per the WHO guidelines [13]. The parameters viz. total ash, water-soluble ash, and acid-insoluble ash values were determined. Ethanol, water, methanol, petroleum ether, chloroform, ethyl acetate soluble extractive values were determined. The seed extractives of *G. abyssinica* were used for chemical analysis. The different phytochemicals tests were performed to record the phytochemicals present in the seed [14].

Thin Layer Chromatography (TLC): TLC studies were carried out as per the standard method [15].

Solvent system: Chloroform: Ethyl Acetate: Formic Acid 5:4:1was used for TLC of methanol seed extract. TLC was carried out on precoated E. Merk silica gel plates of 0.30 mm thickness. After air-drying, the plate was visualized in UV 254 nm, 366 nm, and iodine vapour. Rf values were recorded under standard conditions.

Photomicrographs: Microscopic descriptions of seeds were supplemented with micrographs. Photographs of different magnifications were taken with Canon Microscopic unit.

3.RESULTS AND DISCUSSION

Macroscopical characteristics: The seed, technically a fruit called an achene. The characteristics of seeds have been tabulated in Table no.1.

Table no.1 Characteristics features *Guizotia abyssinica L.F. Cass* **L. *seed***

Characteristics	Observations
Type	Achenes are black with white to yellow scars on the top and base with hard testa
Seed dimensions (mm)	11.0-25.0-150 X 0.5-1.2.-2.5
Seed colour	Black glossy
Odour	Significant
Taste	Sweet
Texture	Smooth and uniform
Shape	Obovoid and narrowly long like a needle
Embryo	White
No of seeds in 1g	393.0

Figure 1. Seeds of G. abyssinica.

Microscopic study of seed: The anatomical peculiarities of T.S. of seed have been tabulated in Table no.2

Table no.2. Anatomical peculiarities of *G. abyssinica*

Anatomical parameters	**Important peculiarities are seen in** *G. abyssinica*
Outline	Round in transverse section
The overall structure in T.S.	Distinct testa, thin endosperm, and bulky cotyledons.
Outer integument	It shows well-developed epidermis. The epidermal cells are thin-walled, single-layered, and made up of polygonal and tabular cells full of mucilage. Sub-epidermis is 2-3 layered, brown with thick-walled parenchyma.
Inner integument	The cells are thick-walled with the small lumen and pitted with tangentially elongated, collapsed parenchymatous cells of 1-2 layers. The pigment layer is brown or chocolate brown, single-layered, thick-walled with pitted rectangular cells.
Endosperm and cotyledons	It is narrow, thin, and surrounded by cotyledons. The cells of endosperm and cotyledons are parenchymatous, colorless, polyhedral, somewhat thick-walled containing aleurone grains, and abundant oil globules.

Fig 2. T.S. of seed Guizotia abyssinica

Quantitative microscopy: In view of the importance of quantitative microscopy, the details are tabulated in Table no. 3.

Table no.3. Quantitative microscopic studies of *G. abyssinica* **seed**

Cells and cell contents	**Dimensions**
Parenchyma cells (length)	145 µ
Vessels (with helical to spiral thickenings) length	120.0µ
Stone cells (diameter)	32.5µ
Phloem fibres (length)	410µ
Starch grains (simple)	18-35µ

Powder microscopy: Seed powder has shown the presence of fragments of thin-walled parenchyma cells with simple starch grains. (It also showed characteristic sclerenchyma fibres, a bundle of fibres, sclerenchyma with pigments, stone cells, and pitted sclerenchyma cells. The spiral to helical elongated xylem vessels and parenchymatous tissues had been recorded (Fig. 3).

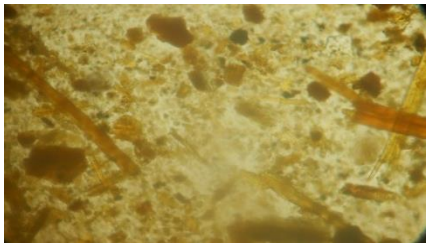

Fig. 3. Microscopic characteristics of seed powder (X100)

Physico-chemical characteristics of seed: The physical constant such as total ash of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. The results of ash values are tabulated in Table no. 4.

Table 4. Physico-chemical parameters of *G. abyssinica* seed.

Sr no	Parameters	Results (with range)
1	Foreign matter	2.0%
2	Total ash	15.78% w/w (14.40-16.30)
3	Water soluble ash	12.14% w/w (10.2-14.3)
4	Acid-insoluble ash	3.40% w/w (2.92-3.67)
5	Ethanol extractives	9.20% w/v (9.0-9.5)
6	Chloroform extractives	4.0% w/v (3.10-4.80)
9	Water extractives	9.30% w/v (8.60-9.87)
10	Petroleum ether extractives	1.80% w/v (1.40-1.90)
11	Ethyl acetate exctractives	1.20% w/v (1.05-1.35)

In the present investigation values of total ash, acid insoluble ash, water-soluble ash, ethanol-soluble extractives and water-soluble extractives have been recorded 15.78%, 3.40%, 12.14%, 9.20% and 9.30% respectively while the earlier researchers recorded these values as 3.98%, 0.2%, 0.60%, 26.94% and 9.4% w/w respectively [16]. The values recorded total ash, acid insoluble ash, and water-soluble ash are higher than the values recorded by earlier workers.

Fluorescence analysis: It is very useful for easy identification of adulteration or admixtures. The fluorescence characteristics of seed powder have been recorded in table no. 5.

Table no.5 Fluorescence characteristics of seed powder of *G. abyssinica*

Sr no	Mountant medium	254nm	366nm	Natural daylight
1	Dry powder	Isabellite	Vinaceous grey	Grey
2	P + D.W.	Brown vinaceous	Purple slate	Isabellite
3	P+1N HCl	Violaceous grey	Greenish grey	Mouse grey
4	P+1N HNO_3	Purplish grey	Olivaceous grey	Pale mouse grey
5	P+O.1 N. NaOH	Honey	Dark bluish green	Grey

Table no.6. Fluorescence characteristics of extractives *G. abyssinica*

Sr no	**Extracts**	**254nm**	**366nm**	**Natural day light**
1	Water	Brown vinaceous	Dark livid	Hazel
2	Ethanol	Greyish flax blue	Glacicous grey	Greyish skyblue
3	Methanol	Greyish lavender	Sky grey	Glacious grey
4	Petroleum ether	Greyish violet	Livid violet	Greyish lavender
5	Chloroform	Vinaceous purple	Livid violet	Sky grey
6	Ethylacetate	Greyish violet	Sky grey	Glacious grey

Preliminary phytochemical study: Results of phytochemical tests have been tabulated in Table no.7.

Table no. 7. Preliminary phytochemical tests *G. abyssinica*

Srno	Chemicals	Test Performed	W	C	P.E.	EA	EtOH
1	Alkaloids	Dragendorff's test	-	-	-	+	+
2	Flavone	Shinoda test	+	+		-	+
3	Steroid	Liebermann-Burchard reagent	+	-	+	-	-
4	Tannins	Neutral $FeCl_3$	+	-	-	-	-
5	Sugar	Molisch's test	+	-	-	-	-
6	Terpenes	Noller's test	+	+	+	-	+
7	Glycosides	Berlin-blue reaction	-	+	+	-	-

W= water, C= chloroform, P.E. Petroleum ether, EA= ethyl acetate, EtOH= ethanol

'+' sign indicates the presence of a particular phytochemical while '-'indicates that the particular phytochemical is not detected.

In the present investigation, phytochemical tests showed the presence of alkaloid, glycoside, saponins, flavonoids, polysaccharides, steroids, sugar, terpenes, and tannin in the seed.

Thin Layer Chromatography (TLC): This technique has applications in standardization, determination of the ingredients in drug formulations, and detection of adulterants or substitutes. TLC is also practiced as a qualitative tool for the study of admixtures. In the present investigation, TLC was performed using Chloroform: Ethyl Acetate: Formic Acid (5:4:1) solvent system.

Figure 4 G. abyssinica TLC pattern (Ethyl acetate extract)

Table no. 9. TLC Fingerprint of *G. abyssinica* seed chloroform **extract (**chloroform: Ethyl Acetate: Formic Acid, 5:4:1**).**

Table no. 8. TLC pattern of various extracts of *G. abyssinica seed*

Sr	Extractives	Adsorbent	Solvent system	Viewing	Rf Values (Retention factor)
1	Ethyl acetate	Precoated silicagel	Chloroform: Ethyl Acetate: Formic Acid, 5:4:1	Iodine vapor	0.12, 0.23, 0.92
2	Distilled water	Precoated silicagel	Chloroform: Ethyl Acetate: Formic Acid, 5:4:1	Iodine vapor	0.12, 0.22, 0.32,0.92
3	Petroleum ether	Precoated silicagel	Chloroform: Ethyl Acetate: Formic Acid, 5:4:1	Iodine vapour	0.12, 0.22, 0.32, 0.54, 0.92

Plates were observed under CAMAG UV cabinet.
*Mean of 10 observations
With Iodine developer, 3 prominent bands were visible in all the extracts with Rf values 0.12, 0.22, 0.92

Table no. 10. TLC Fingerprint of *G. abyssinica* seed Ethyl acetate **extract.**

(Solvent system- Chloroform: Ethyl Acetate: Formic Acid, 5:4:1)

No. of spots	Rf value	254nm	366 nm	Iodine developer
1	0.12	Faint blue	Intense blue	Faint yellow
2	0.23	Faint blue	Faint blue	Yellow
3	0.32	Faint blue	Faint blue	Faint yellow
4	0.92	Intense blue	Intense blue	Yellow

Earlier workers have done carried out HPTLC study using Toluene: Ethyl Acetate: (9:1) solvent system and observed prominent spots with Rf values 0.54, 0.58, 0.70 under short UV, 0.45, 0.82 under long UV [16].

CONCLUSION

Guizotia abyssinica Cass is an edible oilseed with therapeutic applications. In the present investigation, exomorphic, microscopic, quantitative microscopy, pharmacognostic, preliminary phytochemical, and TLC studies have been carried out for authentication, detection of adulteration, and quality control of *G. abyssinica.* The work carried out in the present investigation will be beneficial to carry out further researches.

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