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Pharmacognostic evaluation of Lens culinaris Medikus seeds

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ABSTRACT

Objective: To present a detailed pharmacognostic study of the *Lens culinaris* Medikus (Fabaceae) seeds, a food grain used as Dhal in India. **Methods:** The macroscopy, microscopy, fluorescence analysis of powdered drug, physicochemical analysis, preliminary testing and other WHO recommended methods for standardization were investigated. **Results:** Seeds are greyish brown in colour. Treatment of powdered drug with various chemical reagents showed the presence of proteins, cellulose, lignins and fixed oils. Microscopy of seeds revealed the presence of starch grains in seed. The colour of seed coat changed from brown to greenish grey in day light and brown to black at 254nm when treated with acetic acid. Total ash value of the seeds was found to be 1.86% w/w whereas foreign organic matter was found to be nil. Qualitative phytochemical analysis revealed the presence of tannins and flavonoids in acetone extract. **Conclusions:** The present study on pharmacognostic profile of *Lens culinaris* Medikus seeds provides an important tool in identification and authentication of this plant to researchers in future.

1. Introduction

Pulses are of great importance to the health of individuals and communities. The nutritional value of these plants lies in the protein content which is often compromised due to the presence of certain anti nutritional fractions[1]. *Lens culinaris* Medikus belonging to family Fabaceae (synonym Lens esculenta Moench.) is commonly called 'masoor dal' in Hindi and "lentils" in English[2, 3]. The genus name Lens is suggestive of the lens-like shape of the lentil seed. It is usually cultivated throughout North India, South-East Europe and in temperate Western Asia. Seeds are a good source of essential minerals such as calcium, iron, vitamin B and constitute an important source of food in many countries. They are consumed as whole grains and reported to have high protein content, carbohydrates and fibers[4, 5]. They are also claimed to have blood purifying property. Lentil paste is commonly used to get rid of old skin marks. It also treats various kidney and gastric ailments[6-8]. The seeds also exhibit antifungal properties[9]. Seeds can be fried and seasoned for consumption whereas flour is used to make soups, stews purees, and mixed with cereals to make bread and cakes, and as a food for infants. Lentil although called as a poor man's meat, is equally liked by all socioeconomic groups in South East Asia[10]. High polyphenolic content present in the seed leads to excellent free radical-scavenging activity[11-13]. As, no reports on the standardization of *Lens culinaris* seeds have been reviewed in literature survey, therefore the present study has been envisaged to explore the pharmacognostic and phytochemical properties of plant seeds.

2. Materials and methods

2.1. Materials and methods

The chemicals used during the study were of analytical grade. The instruments were well calibrated before use.

2.2. Plant material

Seeds of *Lens culinaris* Medik. were purchased from local market, Yamuna Nagar, Haryana, India and authenticated by Mr. S. K Srivastava, Taxonomist, Botanical Survey of India, Dehradun, India. The voucher specimen (No. BSI/NRC/330) of the plant seeds has been retained in

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Pharmacognosy Research Laboratory No.1 in Department of Pharmacognosy, ASBASJSM College of Pharmacy for future reference.

2.3. Macroscopic examination

The macroscopic characters of the seeds were studied with the help of various sensory organs. It refers to evaluation by means of organs of sense and helps to judge various parameters viz. appearance, odour, taste and colour[14].

2.4. Microscopic examination

Microscopic examination of the plants are not only essential to identify the adulterants but also indispensable in the correct identification of the plant[15]. For microscopic studies of the seeds, the procedure recommended by Johansen was followed[16].

2.4.1. Histology

The seeds were taken and thin sections were cut with the help of a sharp blade. Drops of the reagent were placed on one edge of the cover slip and specimen was prepared. A strip of filter paper was placed at the opposite edge of the cover glass for removing the fluid under the cover glass by suction and caused the reagent to flow over the specimen[15-17].

2.4.2. Behaviour of powder with various chemical reagents

The powdered drug was investigated under microscope to study its microscopic characters. The powder of drug was treated with different chemical reagents and its behaviour was studied under day light[17].

2.4.3. Fluorescence analysis of powder

Fluorescence studies were performed with the powder as such and after treating powder with various reagents (picric acid, sodium hydroxide, nitric acid, etc.) under daylight, long UV (365 nm), short UV (254 nm) [18-20]. The studies were performed on the seed coat and cotyledon of the seed separately.

2.5. Physicochemical parameters

The seeds of the plant were subjected for determination of physicochemical parameters including total ash value, acid insoluble ash value, water soluble ash value, foreign organic matter, loss on drying, swelling factor, foaming index[14], determination of crude fibre content[17], determination of fat content[21], alcohol soluble extractives and water soluble extractives[14, 22], pH determination[23].

2.6. Preparation of extracts

The air dried seeds was extracted in Soxhlet assembly with petroleum ether (LCPE), acetone (LCAE) and methanol (LCME). Finally, the drug was macerated with chloroform-water (LCWE). Each time before extracting with the next solvent, the powdered material was dried in hot air oven below 50℃. Each extract was concentrated by distilling off the solvent under vacuum. The extract obtained with each solvent is weighed. Its percentage was calculated in terms of air-dried weight of plant material[24].

2.7. Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out on the extracts (petroleum ether, acetone, methanol, aqueous) to determine the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids, saponins, sterols, mucilage, resins, lipids/ fats etc. by following standard procedures[17,25,26].

3. Results

The results of macroscopic examination, microscopic examination, fluorescence analysis of powder, physicochemical parameters, extractive values and qualitative phytochemical analysis are as follows.

3.1. Macroscopic examination

The colour of the seed was greyish brown with black spots, characteristic in taste and odour with smooth texture (Figure 1). The seeds were orange in colour without seed coat.

3.2. Microscopic studies

3.2.1. Histology

The transverse section of seed revealed the presence of starch grains in the parenchymatous cells (Figure 2A, 2B). The section of seed coat showed the inner and outer integument (Figure 2C). The transverse section of seed, when stained with weak iodine solution, stained the starch grains in violet- purple colour as shown in Figure 2D.

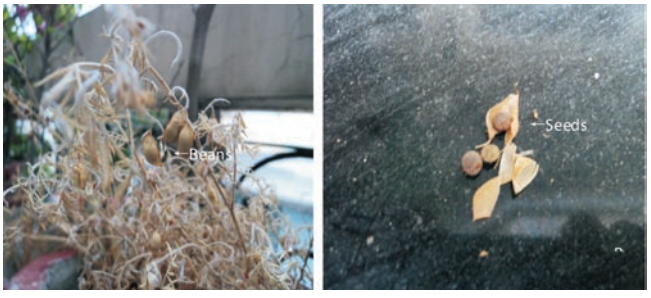

Figure 1. Macroscopy of Lens culinaris Medikus seeds.
A: Beans of Lens culinaris Medikus plant; B: Seeds of Lens culinaris Medikus seeds.

In powder microscopic studies, the unstained and stained (with iodine) starch grains were observed which were oval

Table 1.
Change in nature of powdered drug with chemical reagents.

No.	Reagents	Test	Nature of change	Results
1.	Phloroglucinol–HCl	Lignins	Reddish brown to rose red	+
2.	Iodine soln. followed by H_2SO_4	Cellulose	Black	+
3.	Sudan III	Fixed oil and fat	Pink colour	+
4.	Caustic alkali + HCl	Calcium oxalate	Yellow crystals	–
5.	Weak iodine solution	Starch	Blue colour	+
6.	Lugol's solution	Protein	Black	+
7.	Millon's reagent	Protein	Yellow to brown	+

+: present; – : absent

Table 2.
Fluorescence analysis of powdered Lens culinaris Medik. seed.

Powdered drug	Visible/ Day light		UV 254nm (short)		UV 365nm (long)	
	Seed coat	Cotyledon	Seed coat	Cotyledon	Seed coat	Cotyledon
Powder as such	Brown	Orange	Brown	Orange	Black	Orange
Powder + D.water	Brown	Orange	Brown	Orange	Brown	Orange
Powder + 5% $FeCl_3$	Brown	Orange	Brown	Orange	Black	Orange
Powder + 1 M NaOH	Brown	Light orange	Brown black	Yellow	Black	Orange
Powder + H_2SO_4	Brown	Light orange	Black	Brown	Black	Black
Powder + Acetic acid	Greenish grey	Orange	Black	Light orange	Black	Orange
Powder + Picric acid	Brown	Orange	Black	Yellow	Black	Black
Powder + Nitric acid	Brown	Light orange	Dark brown	Yellow	Black	Dark orange
Powder + I.solution	Brown	Bluish black	Black	Black	Black	Black
Powder + HCl	Brown	Orange	Light brown	Yellow	Black	Orange

D.water: Distilled water; I.solution: Iodine solution

Table 3.
Results of physicochemical parameters of Lens culinaris Medik. seed.

No.	Parameters	Results
1.	Total ash value (% w/w)	1.858
2.	Acid insoluble ash value (% w/w)	0.046
3.	Water soluble ash value (% w/w)	1.562
4.	Foreign organic matter	–
5.	Loss on drying (% w/w)	9.204
6.	Alcohol soluble extractive value (% w/w)	6.664
7.	Water soluble extractive value (% w/w)	11.598
8.	Cold extraction alcohol soluble extractable matter (% w/w)	1.75
9.	Hot extraction alcohol soluble extractable matter (% w/w)	8.75
10.	Cold extraction water soluble extractable matter (% w/w)	5.5
11.	Hot extraction water soluble extractable matter (% w/w)	14.0
12.	Crude fibre content (% w/w)	5.695
13.	Fat content (% w/w)	1.512
14.	Swelling index	1.83
15.	Foaming index	Less than 100
16.	pH 1% solution	7.88
17	pH 10% solution	6.35

in shape, striated with fissured hilum (Figure 3A, 3B). The Figure 3C showed the parenchymatous cells. The pieces of seed testa with bluish black spots were also detected at 10X and 40X (Figure 3D, 3E).

3.2.2. Behaviour of powder with various chemical reagents

The powder behaviour after treatment with different chemical reagents has been provided in Table 1.

3.2.3. Fluorescence analysis

The colour of seed coat changed from brown to greenish grey in day light and brown to black at 254nm when treated with acetic acid. The colour of cotyledon changed from orange to brown (254nm) and orange to black (365nm) when treated with concentrated sulphuric acid. The results of fluorescence studies of seed powder using different reagents are given in Table 2.

3.4. Physicochemical parameters

The results of ash values, foreign organic matter, percentage weight loss on drying, pH of 1% and 10% solution,

Table 4.
Results of phytochemical screening of successive extracts of Lens culinaris Medik. seed.

No.	Constituents	FCPE	FCAE	FCME	FCWE
1.	Alkaloids	–	–	–	–
2.	Carbohydrates	–	–	+	+
3.	Glycosides	–	–	–	–
4.	Flavonoids	–	+	+	–
5.	Saponins	–	–	+	+
6.	Tannins	–	+	–	–
7.	Proteins and amino acids	–	–	–	+
8.	Steroids	–	–	–	–
9.	Triterpenoids	–	–	+	–
10.	Fats and fixed oil	+	–	–	–

+;present; –: absent; LCPE: Petroleum ether extract; LCAE: Acetone extract; LCME: Methanol extract; LCWE:Aqueous extract

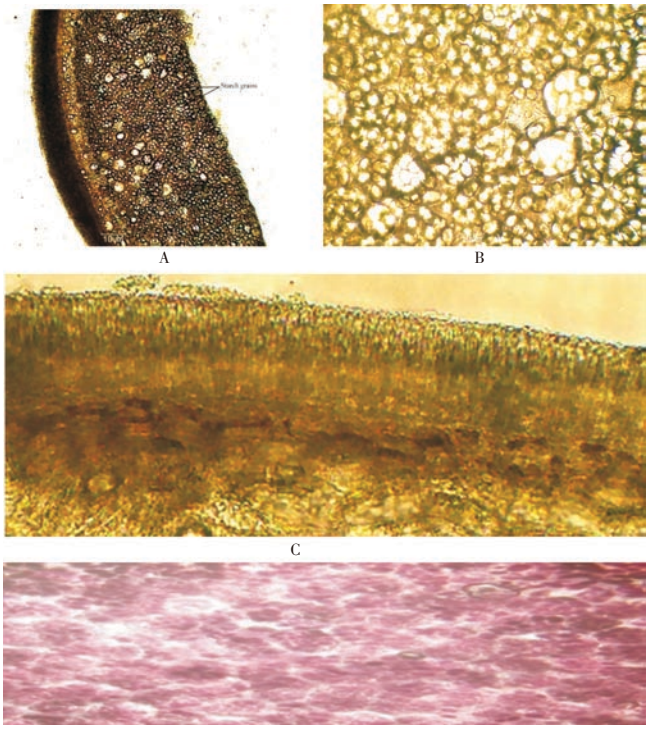

Figure 2. Histology of Lens culinaris Medikus seeds.
A: Transverse section of the seed (10X); B: Parenchymatous cells(40X); C: Transverse section of the seed coat(40X); D: Stained section of seed (10X).

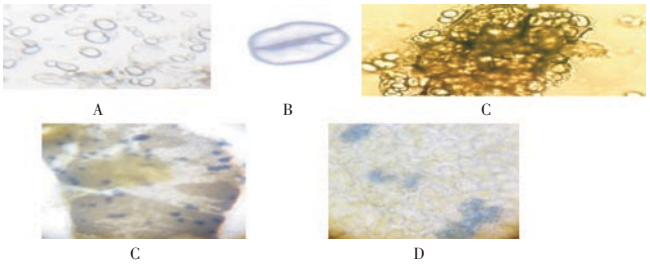

Figure 3. Powder microscopy of Lens culinaris Medikus seeds
A: Unstained starch grains(10X); B: Stained starch grain(40X);C: Parenchymatous cells above Palisade cells(40X); D: Seed Coat (10X); E: Seed Coat (40X).

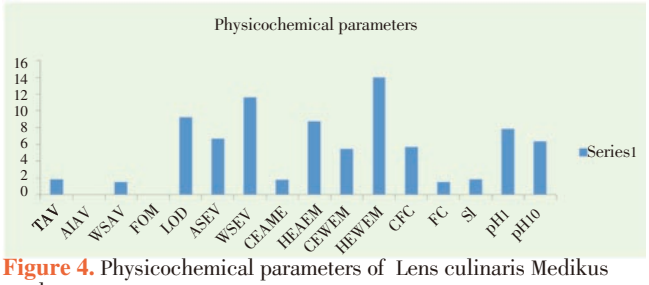

Figure 4. Physicochemical parameters of Lens culinaris Medikus seeds.
TAV: Total ash value; AIAV: Acid insoluble ash value; WSAV: Water soluble ash value; FOM: Foreign organic matter; LOD: Loss on drying; ASEV: Alcohol soluble extractive value; WSEV: Water soluble extractive value; CEAEM: Cold extraction alcohol soluble extractable matter; HEAEM: Hot extraction alcohol soluble extractable matter; CEWEM: Cold extraction water soluble extractable matter; HEWEM: Hot extraction water soluble extractable matter; CFC: Crude fibre content; FC: Fat content, SI: Swelling index; pH 1: pH 1% solution; pH 10: pH 10% solution

determination of crude fibre and fat content, extractive values, foaming index, swelling index is provided in Table 3 and Figure 4. Foreign organic matter was found to be nil due to the proper cleaning of the material by the sellers. Percent loss on drying was not too high, hence could discourage bacterial, fungal or yeast growth. Swelling index was observed due to presence of considerable amount of mucilage in seeds.

3.5. Plant drug extractives

The extractive values (%w/w) of plant drug when treated with petroleum ether, acetone, methanol and aqueous extract were found to be 1.512, 2.713, 8.896 and 4.874 respectively.

3.6. Qualitative phytochemical analysis

Preliminary phytochemical screening of acetone extract indicated high concentration of tannins and flavonoids but methanol extract contained triterpenoids, flavonoids, saponins, carbohydrates and proteins. The results of phytochemical screening of plant extracts have been shown in Table 4. These secondary metabolites are known to possess various pharmacological effects and may be responsible for various pharmacological effects of *Lens culinaris* Medik. seeds.

4. Discussion

Standardization of a plant material refers to its quality, purity and identity. As no standardization of this plant material has been recorded, this study was an attempt to lay down the standards for further authentication of this plant part. The organoleptic studies indicate some important characteristics which are useful diagnostic characters whereas microscopy is the simplest and cheapest method to identify the plant material. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products, which do not fluoresce in daylight. If the substance themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is important parameter of pharmacognostic evaluation. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards [27, 28]. Determination of crude fibre is useful in distinguishing between similar drugs or in the detection of adulteration. It also helps to remove the more resistant parts of plant organs which can be used for microscopic examination. The extractive value is useful to ascertain the chemical constituents of the crude drug [29, 30]. The phytochemical screening reveals the chemical nature of the plant. The plant material contains saponins, flavonoids and tannins as the major bioactive phytoconstituents. They have already reported to exhibit various biological properties. Saponins show wide ranging cytostatic effects against cancer cells[31]. The ability of saponins to lower the serum cholesterol level of animals has also been reported[32, 33]. The flavonoids possess cardioprotective, lipid lowering, antiulcer, hepatoprotective, anti-inflammatory, antineoplastic, antibacterial, antifungal, antiallergic, antiviral and antioxidant properties[34]. The tannins possess antimicrobial activity, antioxidant activity and antihypertensive properties[11,35-37]. Triterpenoids possess wound healing, anti-inflammatory, anti-bacterial, antiviral and hepatoprotective effects[38].

The parameters which are reported here can be considered distinctive enough to identify and decide the authenticity of this drug in herbal industry trade and this can be included as microscopic standards in Indian Herbal Pharmacopoeia.

Conflict of interest statement

We declare that we have no conflict of interest.

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