

RESEARCH ARTICLE

Pharmacognostical Evaluation of Seeds of *Lepidium sativum* Linn.

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Received 26 Mar 2012; Revised 09 Jun 2012; Accepted 16 Jun 2012

ABSTRACT

Seeds of *Lepidium sativum* Linn. (Brassicaceae), commonly known as Chandrashoor (seeds) is a highly reputed drug in classical text of Ayurveda mainly indicated in the management of hiccough and diarrhea. It is incorporated in the group of Chaturbeeja (Four seeds) which is indicated in the managements of backache, tympanitis pain and nervous disorders. In spite of its popularity, the drug has not been found to be evaluated well as yet. Hence it was thought essential to evaluate the seeds of *Lepidium sativum* Linn. systematically. The present paper deals with the detailed macroscopic and microscopic evaluation of the seed, which includes powder study and preliminary chemical evaluation which deals with the determination of physicochemical constants and T.L.C. separations. The microscopical studies revealed certain common characters of the family like beaker shaped cells of hypodermis of testa and epidermal cells with mucilage content. Physicochemical constants support the results of A.P.I.. Methanolic extract of T.L.C showed 5 spots of different colours when sprayed with vanillin - sulphuric acid, indicating the presence of steroidal, phenolic, alcoholic and terpene types of component.

**Key words:** Macroscopic and Microscopic evaluation, TLC, Beaker shaped cells, API.

INTRODUCTION

*Lepidium sativum* Linn. (Brassicaceae), an annual herb, growing as a weed in and around cultivated fields throughout India<sup>[1,2,3]</sup> commonly known in Sanskrit as ‘Chandrashoor’ in various Ayurvedic texts of medieval India<sup>[4,5,6]</sup> (Fig 1). It is highly reputed for various therapeutic properties and is prescribed in cases of hiccough, diarrhea and also used as tonic<sup>[4-7]</sup>. The most reputed formulation which is commercially available is “Chaturbeeja”; which claims to possess number of therapeutic properties including anti-inflammatory and analgesic activities<sup>[4-6]</sup>. Besides Ayurveda, it is a well known drug amongst various ethnic people residing in countries like Nepal,<sup>[8,9]</sup> Ethiopia,<sup>[10,11]</sup> Morocco,<sup>[12]</sup> Israel,<sup>[13]</sup> Pakistan,<sup>[14,15]</sup> etc. According to them it is an important ethnomedicinal drug of repute, to be explored for its therapeutic properties. Historical background of the drug reveals its entry in India through Muslim invaders, and in view of therapeutic importance, it was incorporated in to classical texts of Ayurveda by well known Nighantu authors like Shodhal and Bhavmisra. In spite of its therapeutical implications the scientific investigation of seed of this plant has not been

carried out. Detailed macroscopic and microscopic studies, which include powder study. The preliminary phytochemical investigation consisting of determination of physicochemical constants and T.L.C. separation of the methanolic extract of the seeds were carried out.

Fig 1: Plant from natural habitate

Figure 1



Photograph of plant from natural habitate

MATERIALS AND METHODS

*Lepidium sativum* plant growing as a weed in the farm in the vicinity of Sirmaur region of Madhya Pradesh was identified correctly with the help of various floras<sup>[2,3]</sup>. The healthy, well matured plants were collected in the flowering and fruiting state, washed well with running fresh water and then were subjected for the preparation of Herbarium to be kept in the Department of Pharmacognosy for documentation (Voucher

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specimen no. - 6040) and its identity was established with the help of scientists of I.P.G.T. & R.A., Gujarat Ayurved University. Seeds were separated from the matured and well developed fruits and stored in a clean and dry container. Powder of the seed (40 mesh) was prepared for studying the microscopic characters, chemical constituents and for preparation of extract to be used for T.L.C. separation.

Sensory and macroscopic characters of the seeds were studied. Diagrammatic Transverse and Longitudinal sections were also studied and drawn with the help of dissecting microscope. Detailed Transverse section of the seed was cleared first with Chloral hydrate and then was stained with Phloroglucinol and Hydrochloric acid for lignified elements. The diagrammatic sections were drawn by using Camera Lucida. The photographs of the sections were also taken by using camera<sup>[17-19]</sup>.

#### Physicochemical Constants:

Physicochemical constants of the powder were determined as per the methods described in Ayurvedic Pharmacopoeia of India<sup>[7]</sup>.

#### Phytochemical Screening:

Alcoholic and water extracts of seeds were subjected to preliminary phytochemical qualitative investigations for detecting the presence of various compounds like carbohydrate, protein, alkaloid, glycoside, tannin etc<sup>[16,18]</sup>.

#### T.L.C. Studies:

10 g. powdered seeds were macerated with 100 ml. of methanol in a conical flask for 12 hrs. with shaking. It was filtered and then filtrate was concentrated to 30 ml. under vacuum, 10 microlitre of the filtrate was applied on a pre-coated silica gel 60 F<sub>254</sub> T.L.C. plate (E. Merck) of uniform thickness of 0.2 mm and was developed by using n - Butanol : Acetic acid : Water (2 : 1 : 1) as a solvent system in a twin trough chamber. The solvent was allowed to run up to the distance of 8 cm. The plate was sprayed with vanillin - sulphuric acid and then was heated for 10 minutes in hot air oven at 105<sup>o</sup> C. R<sub>f</sub> values of the resolved coloured spots developed were recorded<sup>[20]</sup>.

### RESULTS

#### Macroscopical: (Fig 2)

1) Seeds oblong to some what cylindrical, slightly broader at the base and notched at the apex, dorsiventrally bulging, occasionally slightly flattened on one surface, measuring 2 to 3 mm in length, about 1 mm in width and in thickness. 2) Surface smooth and appressed with mucilaginous envelope. 3) A narrow, longitudinal shallow groove runs from the apex upto 1/3<sup>rd</sup> to 1/2 portion

of the seed on both the surfaces. 4) Hilum is a whitish protruding spot in the notch at the apex and micropyle lies adjacent to it. 5) Colour is reddish brown, taste mucilaginous, oily and pungent, odour on crushing characteristic.

Figure 2



Seeds

#### Microscopic:

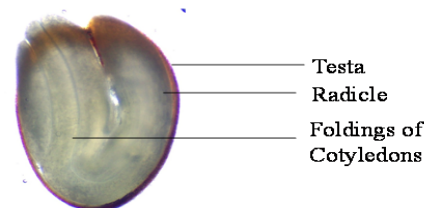
1) Diagrammatic T.S. (Fig 3) passing through the center of the seed is irregularly globular to conical in outline, enclosing a spherical radicle at the narrower end and horizontal folds of cotyledon piled up one above the other at its base, each embedded with rows of vascular bundles, the whole embryo occupying almost the entire inner cavity of the T.S. and encircled by thin outer mucilaginous testa.

Figure 3



2) Diagrammatic L.S. (Fig 4) is broadly oval in outline with a notch at the narrow apical region of the seed and shows an outer narrow mucilaginous testa layer enclosing the vertically running radicle and folded cotyledons lying adjacent to it.

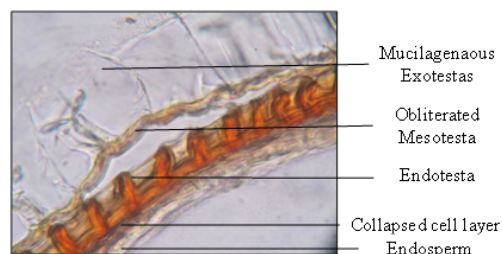
Figure 4



3) Detailed T.S. (Fig 5 (a) & (b)) shows an outer most layer of radially elongated thin walled broad rectangular cells of exotesta embedded with mucilage which escape rapidly on the addition of water breaking its outer cell wall at places and accumulating in the centre to form a vertically running broad pillar shaped column

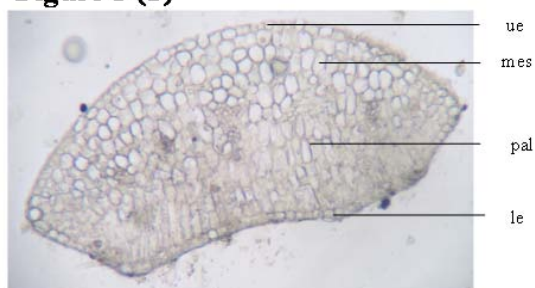
with feathery terminals. Underneath this lies two layers of bright yellow coloured obliterated cells of mesotesta and then, a layer of three sided thickened 'U' shaped cells with a short thread like terminals of endotesta, followed by a tangentially running parenchymatous collapsed celled layer and then a layer of endosperm embedded with aleurone grains and fixed oil globules. Cotyledon consists of upper and lower epidermis covered with thin cuticle and enclosing wide parenchymatous cells of mesophyll. The cells of the lower epidermis are squarish and bigger in size and underneath this lies two rows of palisade cells. The remaining mesophyll cells composed of 8 to 12 rows of spongy parenchyma embedded with fixed oil globules and aleurone grains and rows of procambium vascular bundles of various sizes running in the central region of the tissue. A cotyledon being folded shows slight variations in the shape of their T.S. which may be plano – convex or concavo – convex at places also.

Figure 5 (a)



T. S. of Testa

Figure 5 (b)



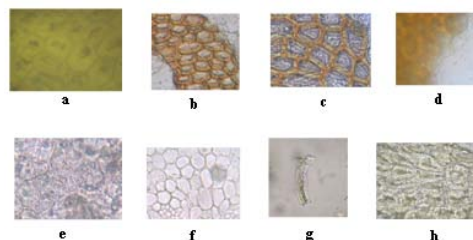
T.S. of Cotyledone

ue- upper epidermis, mes – mesophyll, pal – palisade, le – lower epidermis

- 4) The diagnostic characters of the powder shows (Fig 6) a) fragments of outer thick walled cells of epidermis of testa embedded with mucilage in surface view; b) fragments of endotesta in surface view showing hexagonal thick walled; c) brown coloured cells overlapping with thin walled cells of exotesta; d) fragments of endosperm in surface view with thick walled hexagonal cells embedded with aleurone

grains; e) cells of cotyledon in surface view embedded with oil globules and aleurone grains; f) transversely cut fragments of cotyledon showing mesophyll cells lying underneath the upper and lower epidermis; g) pillar shaped column with feathery terminal of exotesta mucilage cell; h) mucilage cells in surface view.

Figure 6



Powder characters of seeds

**Physicochemical constants:** (Table 1) shows results obtained after performing various physicochemical tests.

Table 1: Phytochemical constants of *Lepidium sativum*

S. No	Test	Mean Value
1	Foreign Matter	2%
2	Total Ash	8%
3	Acid insoluble ash	0.5%
4	Alcohol soluble extractive	13%

**Phytochemical screening :** (Table 2) shows results obtained after performing various qualitative chemical tests.

Table 2: Phytochemical screening tests

S. No	Test	Result
1	Carbohydrate	+
2	Protein	+
3	Fixed oil	+
4	Glycoside	+
5	Alkaloid	+
6	Tannin	+

#### T.L.C. Studies:

(Fig 7 & Table 3) narrate  $R_f$  values and colour reactions of separated compounds with vanilline - sulfuric acid from drugs.

The extract separated using solvent system suggested to separate Flavonoid type of compounds. The spray reagent is selected for triglycerides, phenolic, alcoholic, steroidal and terpene type of molecules. Five compounds separated prominently in above experiment indicating presence of steroidal phenolic compounds.

Table 3: TLC results.

S. No	$R_f$ value	Colour of the band after derivatization
1	0.23	Light violet.
2	0.42	Violet.
3	0.49	Pink.
4	0.77	Brown.
5	0.83	Light brown.



Figure 7

T.L.C. of methanolic extract of *Lepidium sativum* Linn.

## CONCLUSION

*Lepidium sativum* Linn. (Chandrashoora) is attributed with actions like strength promoting, antidiarrheal and blood born diseases. Chandrashoora alongwith Methika (*Trigonella foenum - graceum* Linn.), Kalajaji (*Nigella sativa* Linn.) and Yavani (*Trachyspermum ammi* (Linn.) Spargue) is included in Chaturbeeja group

## REFERENCES

1. The Wealth of India, Raw material, vol-VI, L-M, National Institute of Sciences communication, Council of Scientific and Industrial Research, New Delhi, India, Reprint 1998.
2. The Flora of the Presidency of Bombay, Theodore Cooke, Vol – I, Published under the authority of the Secretary of the state for India in Council, Bishen Singh Mahendra pal Singh, 23 A, New Connought Place, Dehradun – 248001.
3. Flora of British India, J. D. Hooker, C. B., Vol-I, Ranunculaceae to Sapindaceae, Bishen Singh, Mahendra Pal Singh, 23-A, Cannought Place, Dehra Dun, 1999. P.159-160.
4. Bhavprakash Nighantu, Commentary by Dr. C.K. Chuneekar, Edited by Dr.G. S. Pandey, Chaukhambha Bharati Prakashan, Varanasi, India, p. 39-40.
5. Gadanigrah , Commentary by Shri Indradev Tripathi, Edited by Sri Ganga Sahay Pandeya, Chaukhambha Sanskrit Sansthan, Varanasi.
6. Dr. K. Nishtesewar, Dravyaguna Vijnana, Chaukhambha Sanskrit Pratishtan, First edition, 2010.
7. The Ayurvedic Pharmacopoeia of India, Part – I, Vol. – I, Government of India, Ministry of Health and Family Welfare, Department of Ayush, Reprint 2001.
8. Birendra Malla *et al.*, Indigenous knowledge on ethnobotanical plants of Kavrepalanchowk district. Kathmandu University Journal of Science, Engineering and Technology, Vol. 5, No. II, September, 2009, p. 96 – 109.
9. N. K Bhattarai, Ethnobotanical studies in Central Nepal: The preservation of plant – foods. CNAS Journal, vol. 18, No. 2 (July 1991).
10. Gidey Yirga *et al*, Ethnobotanical study of Traditional medicinal plants Gindeberet District, Western Ethiopia. Proceedings of the 1<sup>st</sup> International Technology, Education and Environmental Conference (C) African Society for Scientific Research (ASSR).
11. Debela H. *et al*, Use of traditional Medicinal plants by people of ‘BOOSAT’ sub district, Central Eastern Ethiopia, Ethiop. J. Health Sci. Vol. 16, No. 2, July 2006.
12. *Ethnopharmacology forum*, Medicinal plants used in the treatment of diabetes in Morocco. Int. J. Diabetes & Metabolism (2002) 10 : 33 – 50.
13. Efraim Lev, Ethno-diversity within current ethno-pharmacology as part of Israeli traditional medicine – A review, Journal of Ethnobiology and Ethnomedicine, 2006, 2:4.

and it is indicated in the management of indigestion, colic, pain in flanks and flatulence. The seed contains mucilaginous material. The endosperm is embedded with aleurone grains and fixed oil globules. Cotyledons consist of mesophyll like paranchymatous tissue. Phytochemical analysis reveals that all the physiochemical constants like foreign matter, ash values are within normal limites. Phytochemical screening indicates the presence of carbohydrates, proteins, fixed oil, glycosides, alkaloids and tannin contents. The presence of carbohydrate and protein content supports its usage as traditional nutrient supplement. T.L.C. studies indicate five spots after spraying vanillin - sulfuric acid which is light violet, violet, pink, brown and light brown in colour. These reported observations are useful to evolve new leads for establishing the various pharmacological activities attributed to Chandrashoora (*Lepidium sativum* Linn.) in Ayurvedic classics.

14. Sher Wali Khan *at al.*, Ethnobotanical studies on some useful herbs of Harmosh and Bugrote valley in Gilgit, Northern areas of Pakistan, Pak. J. Bot., 40 (1) , 2008. p. 43 – 58.
15. Haider Ali *et al.*, The Ethnobotany of Chitral vally, Pakistan with perticular reference to Medicinal plants, Pak. J. Bot., 41 (4) : 2009 – 2041.
16. Wallis T.E., Text book of Pharmacognosy, 15<sup>th</sup> ed. London, Churchill Publication; 1985.
17. Wallis T. E., Analytical Microscopy, Third Edition, J. & A. Churchill, Ltd., 104 Gloucester Place, W. I., London, 1965.
18. W. C. Evans, Trease and Evans, Pharmacognosy, 15<sup>th</sup> Edition, W. B. Saunders, 2008.
19. Donald Alexander Johansen, Plant Microtechnique, First edition, Sixth impression, McGraw – Hill Book Company, Inc., New York and London, 1940.
20. Egon Stalh. Thin Layer Chromatography, Springer, 2005.