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Pharmacognostical and phytochemical analysis of *Lepidium sativum* L. seeds

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ABSTRACT
Objective of the present study was to carry out the physicochemical and phytochemicals standardization of *Lepidium sativum* L seeds to establish the standard pharmacognostical parameters of this valuable medicinal plant. Many standardization parameters of *Lepidium sativum* were analyzed. Standard method was adopted for the preliminary phytochemicals screening. Analysis of pesticides residues, aflatoxin & heavy metals were also performed. The sections of seeds were prepared for quantitative microscopic parameters. The air dried powdered plant material was subjected for determination of physicochemical standardizations like ash value, Extractive value and fluorescence nature of the powder drug using light of short and long wavelength of 254nm and 366nm respectively. Phytochemical screening was performed for the identification of phytoconstituents in the plant which was helpful in the development of analytical profile. The morphological and microscopic examinations of drug were revealed the presence of endosperm cell which are polygonal in shape and contain alerone grains and oil droplet, cell of testa, yellow colouring matter and starch grains. Preliminary phytochemical screening showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins and lipids in the drug extract and flourescence nature of drug was confirmed by fluorescence analysis in different solvent. Concentrations of heavy metals,ash value and extractive value were determined and found within acceptable Pharmacopoeial limits. Pesticides residues and aflatoxins were also determined but not detected in the tested samples. The physicochemical and phytochemical standards which are outcome of this research may be utilized as substantial data for identification and standardization of *L. sativum* seed.

***Key Words*:** Garden cress, brassicaceae, aflatoxins, pesticides residue, extractive value, heavy metal.

INTRODUCTION

Lepidium sativum Linn (Brassicaceae) is an annual herb locally known as halon in india but commonly known as Garden cress. *Lepidium sativum* is a polymorphous species and its centre of origin is Eritre and Ethiopia. Halon is a fast-growing edible plant. Seeds, roots and leaves of Gardencress are of economic importance; but, the crop is mainly cultivated for seeds. It is a medicinal important herb in India (Paolo Scartezzini, and Ester Speroni, 2004) it is an erect, glabrous, annual, herbaceous plant growing upto 15-45cm in height. It has small white flowers in long racemes. The pods of halon are obovate, rotundate, elliptic, notched at apex emarginated, and winged. It can be grown at all elevations, throughout the year, but the best herb is obtained in the winter season. The leaves of the plant are used in salads, cooked with other vegetables and used to garnish food. Leaves are stimulant and diuretic (Maghrani *et al.*, 2005; Wright *et al.*, 2007). Halon seeds are brownish red in colour and oval in shape (Al-Yahya *et al.*, 1994). Morphologically, *L. sativum* seeds resemble that of seed oil with the dicotyledonous endosperm approximately to 82–85% of the seed content, the seed coat content 12– 18% and the embryo for 2–4% of the seeds, respectively. Seeds contain 27% of protein, 14-26% of lipids, 35-54% of carbohydrates and 8% of crude fibre (Mathews *et al.*, 1993). The carbohydrates of the *L. sativum* seeds comprise of 90.0% non-starch polysaccharides and 10% of starch. The seed bran has high dietary fibre content and also it has high water holding capacity. Seed bran can be used as a rich source of dietary fibre (Gokavi *et al.*, 2004). *L. sativum* seed contains 20-25% semidrying yellowish oil and the mostly fatty acid in it is alpha linolenic acid (32-35%). Garden cress oil has a balanced amount polyunsaturated fatty acids (46.8%) and monounsaturated fatty acids (37.6%) and also contains natural antioxidants *viz.*, tocopherols and carotenoids which protect the oil from rancidity. Seven imidazole alkaloids, lepidine B, C, D, E, and F and two new monomeric alkaloids semilepidinoside A and B, sinapic acid and sinapin were reported in seeds of *L. sativum* (Maier *et al.*, 1998). Benzyl isothiocyanate and benzyl cyanide were reported in colourless volatile oil but α-tocopherol and β-sitosterol were reported in the unsaponifiable matter of halon seeds (Lee *et al.*, 2004). A detailed study on phytochemical properties of halon seed oil has been reported. *L. sativum* seeds have been used in traditional medicine since old times in India (McConnell *et al.*, 2007). The roots of halon are acrid and bitter which are useful in treatment of secondary syphilis and used as a condiment (Welbourne, 1979). The aqueous extract *of L. sativum* seeds promoted hypoglycaemic activity both in normal and diabetic rats without interfering insulin secretion (Eddouks *et al.*, 2005). Standardization of *L.*

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sativum seeds have not been reported so far. In the present investigation, quality standards of *L. sativum* were developed to establish quality and purity of the drug.

MATERIALS AND METHODS

Procurement of plant material

Lepidium sativum L seeds were collected from herbal gardenof Hamdard University campus, New Delhi, India, (July -2011) and samples were identified by taxonomist. The voucher specimen was deposited in Pharmacognosy and Photochemistry research laboratory, Vivek College of Technical Education, Bijnor for further reference (voucher no. NISCAIR/RHMD/Consult/-2011-12/1781/81).

Macroscopical and microscopical evaluation

The plant material of halon was subjected to macroscopical and microscopical evaluation. The seeds of *L. sativum* were monitor carefully and preliminary observations were recorded. The seeds were powdered with the help of grinder and stained with different staining reagent to ascertain the presence of particular type of microscopical characters.

Physicochemical standardization

Determination of extractive value

It is the amount of soluble component extracted with different solvents from a given amount of crude drugs (Harborne, 1999).

Cold Extraction

The dried drugs powder (10gm) was macerated with solvent (Petroleum ether, chloroform, methanol and water) of volume 100 mL in a round bottom flask for 24 hours, shaking continuously for six hours and allowing standing for 24 hours. It is filtered rapidly and evaporates the filtrate to dryness in a flat bottom dish and dried at 105°C, to constant weight and calculated percentage yield.

Hot Extraction

The powder material of the halon drug (10gm) was packed in a Soxhlet assembly separately for each solvent like petroleum ether, chloroform, methanol and water. Extract of halon was evaporated to dryness and constant extractive value was calculated.

Successive Extraction

Successive extraction of dried powdered material of halon drug (10gm) was done in a Soxhlet assembly with different solvents like petroleum ether, chloroform and methanol. The extracts of halon were evaporated to dryness and their constant extractive values were recorded.

Determination of ash values

Ash value is a physiochemical parameter of drugs which is helpful in detection of adulteration as well as establishes the quality and purity of the drug.

Determination of total ash values

Ignition of *Lepidium sativum* seed material yields total ash which containing physiological and non-physiological ash. The crude halon drug was incinerated in a crucible at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed to get the total ash content value.

Determination of acid insoluble ash values

Acid insoluble ash represents siliceous earth and sand. Ash is boiled with 25 mL dilute hydrochloric acid (6N) for five minutes. The insoluble content collected on an ash less filter paper, then washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight.

Determination of water-soluble ash values

Ash was dissolved in distilled water and the insoluble portion collected on an ash less filter paper and ignited at 450°C to constant weight. By subtracting the weight of insoluble portion from that of the ash, the weight of soluble part of ash was estimated.

Florescence analysis

The fluorescence character of the halon seed powders was studied in UV and daylight light (366 and 255 nm) by treatment with different reagents like sodium hydroxide, picric acid, hydrochloric acid, nitric acid, iodine acetic acid and ferric chloride etc. (Chase, 1949; Kokoshi, 1958).

Phytochemical screening

The petroleum ether, chloroform, methanolic and aqueous extract of the halon were subjected to preliminary phytochemical investigation for the detection of secondary metabolites (Mukherjee, 2002). The phytoscreening was performed for alkaloids, carbohydrates, protein, saponins, phenolic compounds, flavonoids mucilage, resins and lipids or fats etc.

Heavy metal residues

Determination of heavy metals (cadmium, lead, arsenic and mercury) analysis was carried out in the extracts of *L. sativum* on Atomic Absorption Spectrophotometer according to the American Organization of Analytical Chemists (AOAC) (Anonymous, 2002; Anonymous, 2003).

Pesticide residues

Pesticides (organophosphates organochlorines and pyrethroids) residues in the halon extracts were carried out by GC-MS as per the guideline of American Organization of Analytical Chemists (AOAC) (Anonymous, 2002; Anonymous, 2003).

Aflatoxin analysis

Aflatoxins were analyses in *L. sativum* extracts by HPLC method as mention in American Organization of Analytical Chemists (AOAC) (Anonymous, 2002; Anonymous, 2003).

RESULTS AND DISCUSSION

The macroscopical study of the *L sativum* L seeds was done. The seeds were pale brown to brown red in colour, 2-3 mm in size, oval in shape with smooth surface & characteristic odour (table 1). The microscopic examination of powdered material was performed to detect and established various identifying microscopic characters which will be helpful in authentication of the alternative drug supplied in the form of dried powder. The photomicrographs of the identifying features of the plant material are shown in (figure 1-3). The cells of endosperm were seen and the cells are polygonal in shape and contain alerone grains and oil droplet. The cells of testa are longitudinal, elongated and they are closely packed and contain yellow colouring matter. The starch grains were also present which are oval and rounded in shape.The various physiochemical parameter like ash values,

Table 1: Macroscopical characters of *L. Sativum*.

Sr. No	Macroscopic Characters	Observation
1	External Colour	Pale brown to Brownish red
2	Size	2-3 mm
3	Shape	Oval
4	Surface	Smooth
5	Odour	Characteristic
6	Taste	Bitter

Table 2: Summary of results of physicochemical evaluation of drug (n=3).

Parameters			*Lepidium sativum* % w/w (mean ± SD*)
Moisture content			5.8
Ash values	Total Ash		1.57
	Acid insoluble Ash		0.74
	Water soluble Ash		0.83
Successive extraction	Petroleum ether		2.05
	Chloroform		2.67
	Methanol		9.09
	Water: alcohol (50:50)		4.94
	Water		0.294
Detection of contaminants	**Heavy metals****	Cadmium (Cd)	0.24 ± 0.02
		Lead (Pb)	0.42 ± 0.14
		Arsenic (As)	0.48 ± 0.06
		Mercury (Hg)	0.38 ± 0.06
	Aflatoxins (B1,B2, G1 and G2) by HPLC		Aflatoxins, B1, B2, G1 and G2 were analysed by using HPLC method, which were found absent in all samples
	Pesticides (30 pesticides were checked) by GC-MS		The 30 pesticides were analyzed by using GC-MS method, which were found absent in all samples

*Standard deviation, **Limit described by the WHO (Lead- 10ppm; Cadmium-0.30ppm; Mercury-0.50ppm; Arsenic-3.0ppm)

Table 3: Effect of different chemical reagents on the fluorescence behaviour of crude drug powder.

Sr. No.	Treatment	Day light	UV light 254 nm	UV light 366 nm
1	Powder as such	Brownish red	Brownish red	Brown
2	Powder treated with distilled water	Brownish red	Dark brown	Dark brown
3	Powder treated with 1N NaOH in water	Brownish red	Brownish red	Brown
5	Powder treated with HNO_3	Light brown	Dark green	Dark violet
6	Powder treated with H_2SO_4	Green	Black	Blue
7	Powder treated with iodine	Green	Blue	Dark brown
8	Powder treated with conc. HCl	Dark green	Radish brown	Greenish black
9	Powder treated with ammonia	Light green	Dark green	Greenish brown
10	Powder treated with ferric chloride	Green	Radish black	Greenish brown
11	Powder treated with Iodine	Dark brown	Brown	Blue
12	Powder treated with Glacial acetic acid	Yellow	Dark yellow	Yellow
13	Powder treated with Picric acid	Dark yellow	Yellow	Dark yellow
14	Powder treated with Petroleum ether	Dark green	Pale yellow	Dark Brown
15	Powder treated with Chloroform	Dark green	Dark brown	Dark gr

Table 4: Results of Phytochemical screening.

Constituents	Extracts			
	Petroleum ether	Chloro-form	Alcoholic	Aqueous
Alkaloids	-	+	+	+
Carbohydrates	-	-	-	+
Phenolic compounds	-	+	+	-
Flavonoid	-	+	+	+
Proteins and amino-acids	-	-	+	+
Saponins	-	-	+	+
Mucilage	-	-	+	-
Resins	+	+	+	-
Lipids / Fats	+	-	-	-

(-: Absent, +: Present)

Table 5: Determination of heavy metal residues.

Sr. No	Heavy Metals	Concentration
1	Cadmium (Cd)	0.24 ± 0.02
2	Lead (Pb)	0.42 ± 0.14
3	Arsenic (As)	0.48 ± 0.06
4	Mercury (Hg)	0.38 ± 0.06

Table 6: Determination of pesticide residue.

Sr. No	Pesticide	Test method	Results	MDL
1	α-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
2	β-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
3	γ-BHC(Lindanee)	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
4	δ-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
5	Heptachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
6	Heptachlor_Epoxide	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
7	α-Chlordane	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
8	α-Endoulfan	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
9	β -Chlordane	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
10	Endrin	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
11	Total DDE	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
12	Total DDD	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
13	Total DDT	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
14	β -Endoulfan	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
15	Endrin_Aldehyde	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
16	Alachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
17	Butachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
18	Monochlorphos	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
19	Phorate	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
20	Mevinphos	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
21	Dimethoate	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
22	Malathion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
23	Methyl-parathion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
24	Chlorpyrifos	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
25	Ethion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
26	Atrazine	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
27	Simazine	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
28	Diazinone	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
29	Phosphamidon	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
30	Fenitrothion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg

Table 7: Determination of aflatoxin residues.

Sr. No	Test parameter	Test method	Results	MDL
1	AflatoxinB1	AOAC 990.332	Not detected	1.0µg/kg
2	AflatoxinB2	AOAC 990.33	Not detected	1.0µg/kg
3	AflatoxinG1	AOAC 990.33	Not detected	1.0µg/kg
4	AflatoxinG2	AOAC 990.33	Not detected	1.0µg/kg

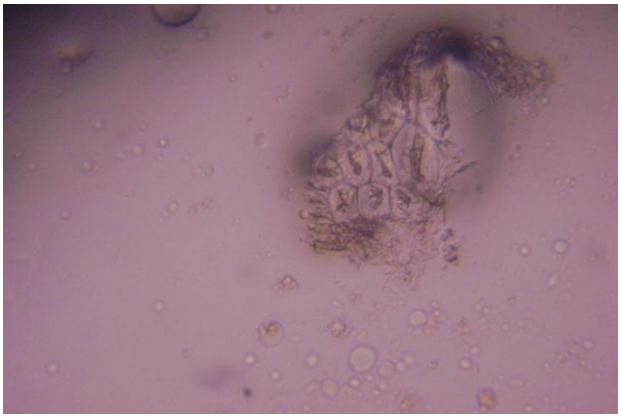

Figure 1: Cells of endosperm in surface view.

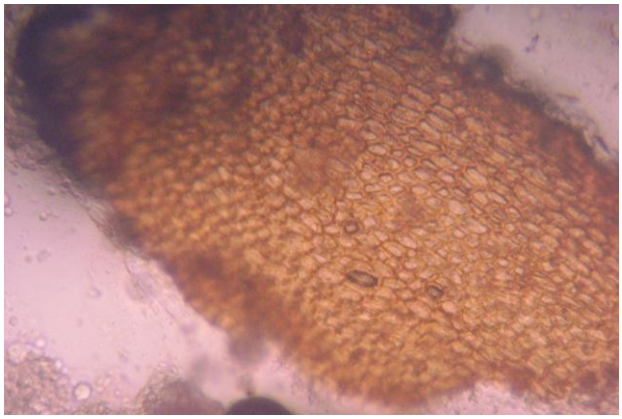

Figure 2: Cells of testa in surface view.

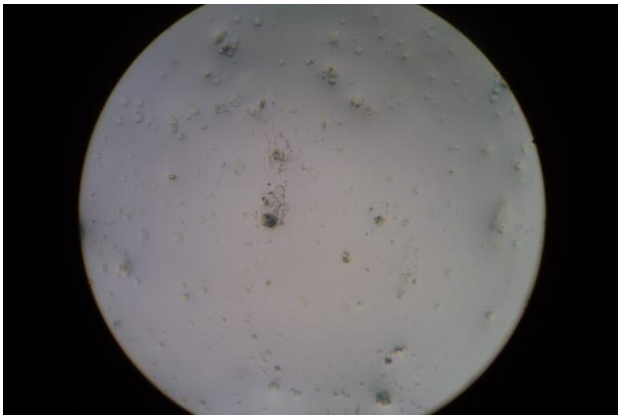

Figure 3: Starch granules.

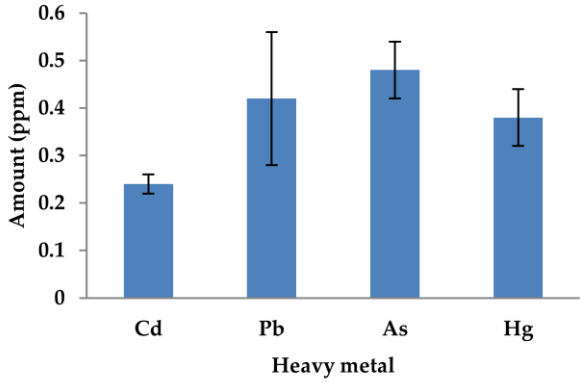

Figure 4: Heavy metal residue for *Lepidium sativum*.

extractive value and loss on drying, were also determined shown in (table 2). Fluorescence nature of the powder gardencress drug in different solvent extracts with different chemicals were analyze using longer light wavelength (366 nm) and shorter light wavelength (254nm) were reported in (table 3). Preliminary qualitative phytochemical screening showed that presence of alkaloids, carbohydrates, flavonoids and saponins. The presence or absence of particular types of phytoconstituents in the plant may be helpful in the development of analytical profile. The preliminary chemical tests of halon extracts were performed to detect the presence and absence of various phytoconstituents. The results of the performed studies are tabulated in table 4. Determination of heavy metals was carried out in the extracts of *L. sativum* on Atomic Absorption Spectrophotometer. The Cadmium was found to be highest in *L. sativum* sample (0.24 ± 0.05 mg/kg) but it was below the permissible limit of 0.3 mg/kg as prescribed by WHO in all the samples. Lead ranged from 0.16 ± 0.06 mg/kg to 0.43 ± 0.05mg/kg in all samples and was far below to the permissible limit of 10 mg/kg as prescribed by WHO. Mercury in all the samples was found to be below the permissible limit. Arsenic and mercury was found in all samples. Both metals were present within permissible limits of 0.5 ppm and 1.0 ppm, respectively (table 5 and figure 4).

Determination of pesticide residue was carried out in extracts of *L. sativum* by standard methods as mention in AOAC guidelines. Total 30 pesticides were tested in all halon samples, none of the pesticides was found in halon extracts (table 6). Mycotoxins are secondary product of metabolites developed by fungus that develop in food products naturally. Toxigenic fungus may contaminate herbal products at different stages of production and processing, mainly due to the presence of humidity and temperature in favorable conditions. Many mycotoxins also have potential stability, which enables their persistence in products after the removal of the fungus by means of the commonly manufacturing and packaging processes.

The most common toxigenic fungi found in plants include species from the genus Aspergillus and Fusarium, mainly *Aspergillus parasiticus Aspergillus flavus*, and *Fusarium verticillioides*. *Aspergillus fungi* produce aflatoxins G_1, G_2, B_1 and B_2, which are considered to be involved in the etiology of human hepatic sarcoma. Aflatoxins G_1, G_2, B_1 and B_2, were determined in the extract of *L. sativum*. No aflatoxin was detected.

CONCLUSION

The generated data of this study will be used to establish its quality and purity and may be utilised to develop pharmacopoeial monograph of this plant. *L. sativum* L seeds have wide range of medicinal values. The outcome of this research work might prove beneficial in herbal industries for herbal industrialization, identification, purification and standardization of *L. sativum* L seeds extracts.

REFERENCES

Al-Yahya *et al.* (1994). Pharmacological and Safety evaluation studies on Garden cress (*Lepidium sativum L.*) seeds. Phytomedicine, Vol 1, Issue 2, Pages 155-159. [DOI]

Anonymous. (2002). Association of Official Analytical Chemists (AOAC), 17th edition, methods AOAC 970.33, methods AOAC 990.33.

Anonymous. (2003). Quality Standards of Indian Medicinal Plants. Vol- I, Indian Council of Medicinal Research, New Delhi. ISBN-0972-721

Chase *et al.* (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J of American Pharma Asso, Vol 38 Issue 6, Pages 324- 331 [DOI]

Eddouks *et al.* (2005). Study of the hypoglycaemic activity *of Lepidium sativum* L. aqueous extract in normal and diabetic rats. Journal of Ethnopharmacology, Vol 97 Issue 2, Pages 391–395. [DOI]

Elisabetta.etal. (2007) Growth and protein profile changes in *Lepidium sativum* L. plantlets exposed to cadmium. Environmental and Experimental Botany, Vol 59 Issue 2, Pages 179–187. [DOI]

Gokavi *et al.*, (2004). Chemical composition of garden cress (*Lepidium sativum*) seeds and its fractions and use of bran as a functional ingredient. Plant Food. Hum. Nutr, Vol 59, Issue 3,Pages 105–111. [DOI]

Harborne (1999). Phytochemical methods. A guide to modern technique of plant analysis. Plant Pathology, Vol 48, Issue 1, Pages 199. [DOI]

Herlich K. (1991) Official method of analysis of the Association of official analysis chemists AOAC. Analytica Chimica Acta Arlington USA, Vol 242, Pages 302. [DOI]

Julian, S, Pflugmacher, S. (2007). Antioxidative stress response of *Lepidium sativum* due to exposure to cyanobacterial secondary metabolites. Toxicon, Vol 50, Issue 1, Pages 85–93. [DOI]

Kokoshi *et al.* (1958). Fluorescence of powdered vegetable drugs under UV radiation. J American Pharma Asso, Vol 47, Issue 10, Pages 715-717. [DOI]

Koleva, I.I. *et al.* (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, Vol 13 Issue 1, Pages 8-17. [DOI]

Lee *et al.*, (2004). Reactive oxygen species, aging and antioxidative neutraceuticals. Comp Rev Food Sci Food Safety, Vol 3, Issue 1, Pages 21-33. [DOI]

Maghrani, *et al.* (2005) Antihypertensive effect of *Lepidium sativum* L. in spontaneously hypertensive rats. Journal of Ethnopharmacology, Vol 100, Issue1-2, Pages 193–197. [DOI]

Maier *et al.* (2002). A process for the preparation of dietary fibre from garden cress seeds. Indian Patent No.242/DEL.

Mathews *et al.* (1993). Some physicochemical characteristics of *Lepidium sativum* (haliv) seeds. Die Nahrung, Vol 37 Issue 1 Pages 69–71. [DOI]

McConnell *et al.* (2007). Physical characteristics of vegetable foodstuffs that could influence bowel function. J Sci Food Agric, Vol 25, Issue 12, Pages 1457–1464. [DOI]

Mughal *et al.*, (1999) A steryl ester from *Lepidium sativum*. Phytochemistry, Vol 50, Issue 8, Pages 1375-1377. [DOI]

Mukherjee (2002). Quality control of herbal drugs. Business Horizons pharmaceutical Publishers New Delhi. ISBN 81-900788-4-4.

Paolo Scartezzini, Ester Speroni *et al.*, (2004) Review of some plants of Indian traditional medicine with antioxidant activity. Journal of Ethno pharmacology, Vol 71, Issue 1-2, Pages 23-43. [DOI]

Samson *et al.* (2001). Mycotoxins and phycotoxins in perspective at the turn of the millennium. Chemistry International - Newsmagazine for IUPAC Vol 24, Issue 1, Pages 23–23. [DOI]

Shoba, F.G., Thomas, M. (2001) Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhea. Journal of Ethnopharmacology, Vol 76, Issue 1, Pages 73–76. [DOI]

Ulrich *et al.*, (1998) Seven Imidazole Alkaloids from *Lepidium sativum*. Phytochemistry. Vol. 49, Issue 6, Pages 1791-1795. [DOI]

Welbourne, T. C. (1979) Ammonia production and glutamine incorporation into glutathione in the functioning rat kidney. Can J Biochem, Vol 57, Issue 3, Pages 233–237. [DOI]

Wright *et al.*, (2007). Herbal medicines as diuretics: A review of the scientific evidence. Journal of Ethnopharmacology, Vol 114, Issue 1, Pages 1–31. [DOI]