

ORIGINAL ARTICLE

Pharmacognostic and physico-chemical evaluation of *Lactuca sativa* L.

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Lactuca sativa L. is an erect, annual herb belonging to the family Asteraceae. The leaves are useful in angina pectoris, ascitis, asthma, constipation, cough, diarrhea, hysteria, gonorrhoea, affections of liver and spleen and pain in pylorus and spinal cord. Leaves are long, thin, orbicular or oblong, obovate, crisp and radical. The trichomes are uniseriate filiform capitate, uniseriate macroform conical and biseriate filiform capitate types; mesophyll is undifferentiated; laticiferous ducts and prismatic crystals are present in ground tissue; midvein bundle is bicollateral. The powder microscopic and organoleptic characters are provided.

Physico-chemical parameters of raw drug viz., extractive values, ash values, formulation, besides weight per ml., total solids, alcohol content along with thin layer chromatography (TLC) and ultraviolet spectroscopic (UV) studies have been undertaken for mother tincture for the first time.

Keywords: Homoeopathy; *Lactuca sativa* L.; Standardization; Pharmacognosy; Physico-Chemical; Pharmacopoeia.

INTRODUCTION

Lactuca sativa L., commonly known as 'garden lettuce' in English and 'salad' locally, is an annual herb belonging to the family Asteraceae. It originated in the warmer temperate parts of Western Asia, including Eastern Mediterranean and is also found in the Himalayas. It is widely cultivated throughout India.¹ The plant is an erect, glabrous, herbaceous annual, 0.5 – 1.2 m high. Leaves 12.5 – 25 cm long, thin, nearly orbicular, oblong or obovate, ligulate, plane, bullate or curled; flower heads of yellow, rays; achenes lenticular-oblong, dark brown or greyish brown, with slender beak and white pappus.¹

The leaves are reported to be useful in angina pectoris, ascitis, asthma, constipation, cough, diarrhea, gonorrhoea, heartburn, hysteria, lactation, affections of liver and spleen, muscae volitantes, noises in ears, pain in pylorus and spinal cord, excessive sleep, whooping cough and yawning. It is also used in poultices for burns and painful ulcers.^{2,3,4}

Its history and authority in Homoeopathy finds

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mention in Jour. f. Arzneim., vol.2, collection of Dr. Ed. Seidel; Austrian Soc. Allopath. Physicians, Zeit der Gesell. Der Aertz. Zu Wien., 1847. Provings with the extract, ½ to 30 grains at a dose; Schroff, Lehrbuch der pharm, experiments with 2/10 dr. of Aust. Lactucarium⁴. (Allen, 1982). French Lactucarium is chiefly prepared from *L. sativa*, From Muller; Die Narkot, Arzeim., 1869.⁴

Chemically, lettuce is the fair source of various minerals and vitamins. The elements present in significant amounts are Mn, Zn, Cu, and Fe.⁵ Fonofos, a thiophosphonate⁶; amyirin, ergosterol, vitamin E and an anti-oxidant¹; lactucin, lactupicrin, sesquiterpene glycoside-lactucide C & A; Besides, carotenoids lactucaxanthin⁷, it also contains an alkaloid, lactucarium, which is a mixture of lactocin and three bitter principles viz., lectucin, lectopicrin and lactucic acid. Besides it also contains lectucerin, a waxy substance and a trace of hyoscyamine.²

Earlier studies on *Lactuca sativa* L. pertaining to pharmacognostic and physico-chemical parameters in general, and in homoeopathic perspective in particular, are not available. Hence, the authors have undertaken detailed pharmacognostic and physico-chemical studies for the first time as per the protocols suggested by Central Council for Research in Homoeopathy (CCRH), Government of India.

MATERIAL AND METHODS

Pharmacognosy

The leaf material of *Lactuca sativa* L. was supplied by Survey of Medicinal Plants and Collection Unit of CCRH, Nilgiris, Tamil Nadu. The leaves were fixed in F.A.A. (Formaldehyde: Acetic acid : Alcohol) and processed for microtomy (paraffin method), sectioned, stained and permanent slides prepared following Johansen.⁸ The epidermal peels were obtained by gently scraping and peeling with razor blade. The powder microscopy characters were studied by boiling the powder drug in distilled water, stained in saffranin and mounted with glycerine. Photomicrography was done with Olympus CH – 2 trinocular research microscope.

Physico-chemical

The air-dried sample of the leaves was coarsely powdered and was subjected to determination of moisture content (loss on drying at 105°C), total ash, water soluble ash, acid insoluble ash, extractability in water and alcohol for raw drug and weight per ml, total solids and alcohol content for the finished product. The above parameters were determined as per procedure given in Homoeopathic Pharmacopoeia of India.⁹ The mother tincture was prepared as per H.P.U.S.¹⁰

Mother tincture (alcoholic extract) was studied for its physico-chemical characters, chromatographic and spectroscopic absorbance. All chemicals and solvents used were of Analytical Grade (AR). Silica gel 'G' (E Merck, India) was used for thin layer chromatography (TLC) and the work was carried out at the room temperature. The TLC plate was developed using hexane : ethyl acetate (9:1, v/v) as mobile phase; vanillin - sulphuric acid was used as spraying reagent.^{11,12} The mother tincture was diluted with methanol and UV spectroscopy was done.¹³ The maximum absorption was recorded.

OBSERVATIONS AND RESULTS

Macroscopic

Leaves 12.5 – 25 cm long, thin, nearly orbicular, oblong or obovate, ligulate, plane, bullate or curled, crisp and radical.

Microscopic

Epidermal cells in surface 5 or 6 sided, polygonal, isodiametric to anisodiametric, sides straight to curved and wavy on adaxial, and sinuate on the abaxial side; surface smooth and at places striated (adaxial). Contents slightly dense. Costal cells parallelly oriented, on primary and secondary veins (adaxial) also on tertiary veins (abaxial). Stomata anomocytic, tetracytic

and a few anisocytic, Stomatal Index (SI) 6.25 (adaxial) and 11.76 (abaxial), size 22-33µm (27) long and 16-25µm (20) wide (abaxial) and 19-27 µm (23) long and 16-22µm (19) wide (adaxial). Trichomes: (1) Uniseriate macroform conical, few, more on veins on adaxial surface; (2) Uniseriate filiform glandular capitate, few, all over, more on veins; (3) Biseriate filiform glandular capitate, few, all over (Figure.1, 1-5).

In vertical section midvein is shield-like, tapering, ribbed on either side, more prominently on abaxial and as a small cone on the adaxial. Secondary and tertiary veins also ribbed. Midvein 962 – 1104µm (1040) thick. Lamina uneven, undulated, 140 – 216µm (168) thick (Figure.2).

Epidermis is 1-layered and 2-layered in some places at midvein; cells adaxially large, radially long and few papillate near midvein, cells over the lamina tabular to barrel-shaped and spherical, walls slightly thick on adaxial; oval to spherical, few barrel-shaped, interspersed with stomata on abaxial (Figure.2,1-2).

Mesophyll is poorly differentiated and 6-8 celled, with 3-4 layers of closely packed cells towards adaxial and spongy-like on abaxial; cells oval, oblong and dumbbell-shaped containing chloroplasts. At some places appear as palisade (Figure.2.2).

The ground tissue of midvein consists of collenchyma, parenchyma and sclerenchyma tissues. Collenchyma as a group of cells in the adaxial and abaxial hypodermis at the tip, cells lamellar, angular, 16 – 33µm (24) in diameter. Parenchyma on adaxial side is 6-8 layered and 10-12 layered on the abaxial side; cells polygonal to spherical, 11-41µm (28) in diameter, contents scanty with prismatic crystals of calcium oxalate in a few and interrupted by laticiferous ducts. Sclerenchyma as a cap enclosing vascular bundles. Centrally, a single vascular bundle is ovate, 764-934µm in diameter, endarch, conjoint, bicollateral; secondary vein bundles 538-651µm in diameter. A 2-4 layered vascular cambium is present. Xylem elements in rows, interspersed with fibers and xylem parenchyma. Tracheary elements 8-35µm (21) in diameter. Secondary walls of vessels/tracheids mostly helical, a few scalariform. Phloem is scanty with phloem parenchyma, bast fibers and sieve elements (Figure.2).

Powder microscopy

Pieces of adaxial epidermis with straight to curved sides and stomata. Leaf fragments, light brown with attached epidermis with conical trichomes and mesophyll; Vessel elements with helical thickenings. Prismatic crystals of calcium oxalate. Chlorenchyma tissue of mesophyll. Fragments of lower epidermis with sinuate sides and anomocytic stomata. Some capitate hairs.

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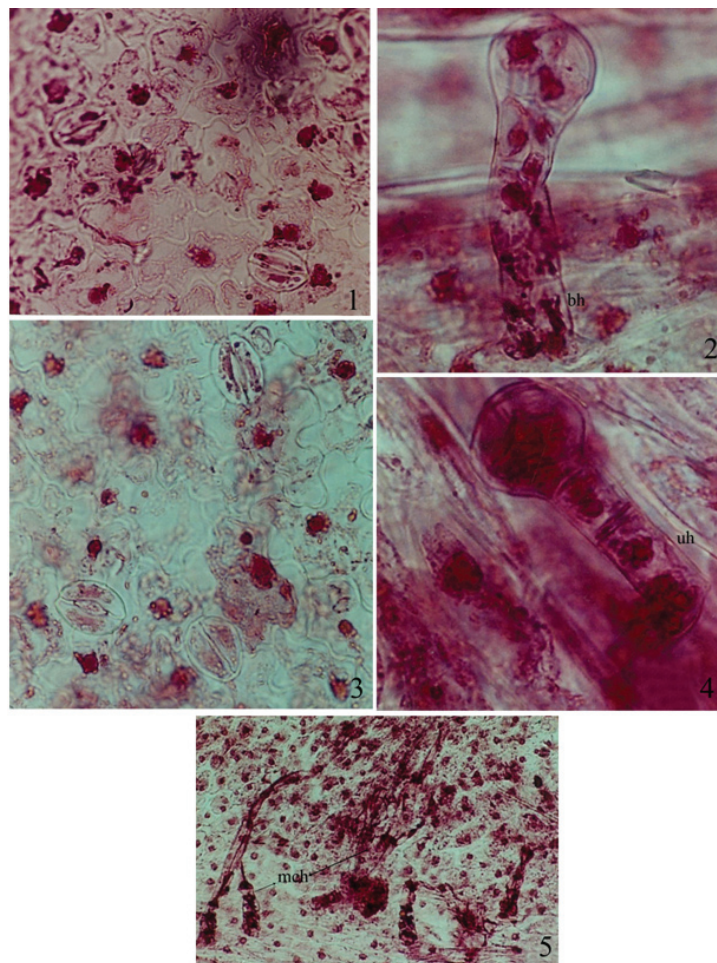


Figure.1

1. Adaxial epidermis in surface X 536.
2. Biseriate capitate hair X 573,
3. Abaxial epidermis in surface X 565
4. Uniseriate capitate hair X 555,
5. Adaxial epidermis with uniseriate macroform conical hair X 93 bh – biseriate capitate hair; mch – uniseriate macroform conical hair; uh – uniseriate capitates hair.

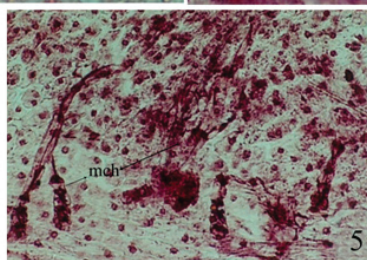


Figure. 2

1. V.S. of leaf midvein X 57
2. V.S. of leaf lamina X 342 C – collenchyma; e – epidermis; le – lower epidermis; ph – phloem; Ue – upper epidermis; um - undifferentiated mesophyll; vb – vascular bundle; X – xylem

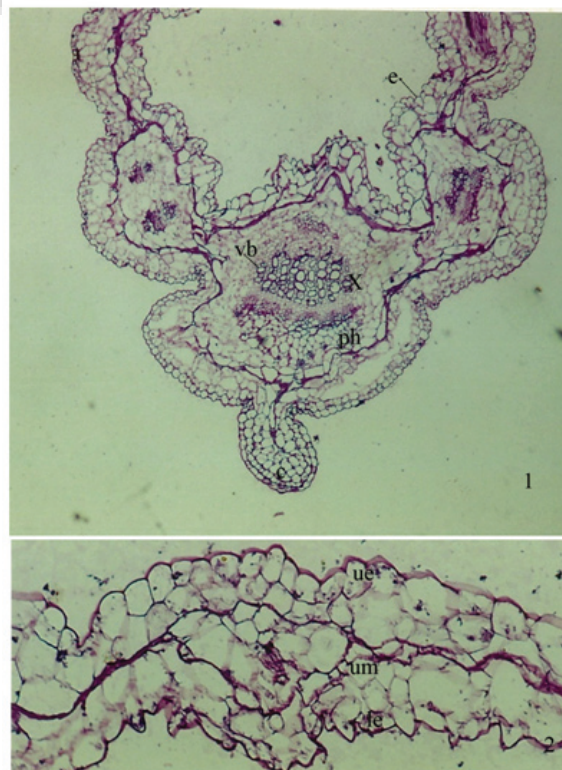


Table 1: Physico-chemical Standardisation of Raw Drug of *Lactuca sativa*

S.No	Parameters	Quantitative values
1	Moisture content (Loss on drying at 105° C)	Not more than 11.8 % w/w
2	Total ash	Not more than 19.98 % w/w
3	Acid insoluble ash	Not more than 2.76 % w/w
4	Water soluble ash	Not more than 7.1 % w/w
5	Alcohol soluble extractive	Not less than 7.5 % w/w
6	Water soluble extractive	Not less than 27.5 % w/w
7	Extractive values in	
	a. Hexane	Not less than 0.3 % w/w
	b. Chloroform	Not less than 0.75 % w/w
	c. Methanol	Not less than 19.25 % w/w

Table 2: Formulation of mother tincture of *Lactuca sativa* (by Percolation technique⁹)

Alcohol	55% v/v
Drug strength	1/10
Preparation :	
<i>Lactuca sativa</i> leaves in coarse powder	100 g
Strong alcohol	578 ml
Purified water	450 ml
To make one thousand milliliters of the mother tincture	

Table 3: Standardisation of Mother Tincture

S.No.	Parameters	Observations
1.	Organoleptic profile	
	a. appearance	clear, non-viscous, producing foam
	b. colour	Reddish brown
	c. odour	pleasant and aromatic
2.	Sediments	absent
3.	Weight per ml	Not more than 0.91 g
4.	Total solids	Not more than 2.8% w/v
5.	Alcohol content	52 - 55 % v/v
6.	pH	4.0 - 4.5 % v/v
7.	λ max	231.244, 295 and 326 nm
8.	Refractive index at R.T	1.411

Table 4: Chromatographic results of *Lactuca sativa*

Solvent system	Detecting agent	No. of spots	Rf values	Colour of spots
Hexane : Ethyl acetate (9 : 1, v/v)	Vanillin-sulphuric acid	4	0.26	Purple
			0.47	do
			0.66	do
			0.94	do

Organoleptic characters

- Colour - light green
- Touch - smooth
- Odour - not characteristic
- Taste - slightly bitter

Physico-chemical studies

The determined data under the physico-chemical study for the raw drug is summarised in Table 1 and that of mother tincture preparation and its standardisation in Table 2 & 3 respectively. The results of TLC studies are presented in Table 4.

TLC was carried out on silica gel 'G' coated plates (E Merck, India) and hexane : ethyl acetate (9 : 1, v/v) was the developing solvent system, the spots were detected by spraying with vanillin-sulphuric acid reagent.

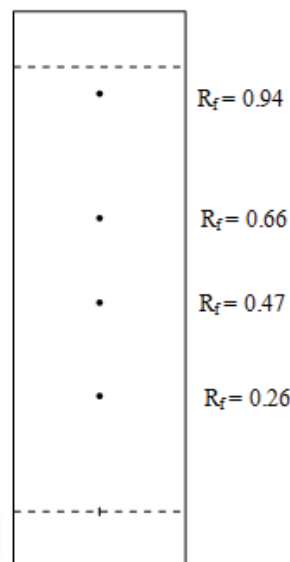


Figure. 3: TLC retention behavior of *Lactuca sativa*

Pharmacognosy

General features

Lactuca sativa L., popularly known as 'garden lettuce', is an erect, annual herb, belonging to the family Asteraceae. Morphologically the leaves are 12.5 – 25 cm long, thin, oblong, obovate, plane, curled, crisp and radical.

Epidermal cells in surface have thin straight to curved or wavy sides on adaxial and sinuate on abaxial. Leaves are amphistomatic and possess anomocytic, tetracytic and a few anisocytic stomata. The stomatal index (S.I.) is 6.25 on adaxial and 11.7 on abaxial. Trichomes are uniseriate macroform conical, uniseriate filiform glandular capitate and biseriate filiform glandular capitate hair types. The presence of glandular hairs in *Lactuca* as reported earlier is presently confirmed.

In V.S. leaf, midvein is shield-like, 764 – 934µm in diameter, ribbed on either sides, more prominently on abaxial and tapering below. Lamina is uneven, undulated, 140 – 216µm thick. Mesophyll is poorly differentiated, 6-8 layered and at places appears as palisade.

The ground parenchyma is dispersed with prismatic crystalliferous cells and laticiferous ducts.

Centrally, an ovate vascular bundle, 764-934µm in diameter, is present, which is bicollateral. A 2-4 layered cambium is present. The secondary walls of vessels/tracheids have helical and scalariform thickenings.

The powder microscopic features and organoleptic characters along with the anatomical studies are diagnostic and establish the standards for the drug.

Physico-chemical

The observed physico-chemical data for the raw drug and finished product are summarized in Tables 1-3. Under the raw drug studies, the higher total ash content recorded indicates the presence of various elements in significant proportion. The rest of the values falls under acceptable range.

The results of TLC studies presented in Table 4 and Fig.3 reveal four distinct spots and UV spectrophotometric study exhibits (maximum absorption) four prominent peaks which can be taken as characteristic standards for the drug.

The determined physico-chemical data, macro and microscopical characters and the methodology employed in the study will help in identification, authentication and ensures quality, purity and efficacy of the drug.

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