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PHYTOCHEMICAL AND STANDARDIZATION OF WHOLE PLANT OF *LACTUCA SATIVA* L.

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Abstract

Lactuca Sativa L., known for its ethnomedicinal applications, boasts a plethora of medicinal claims, yet remains largely underexplored. Traditionally, various parts of the plant have been utilized for treating ailments such as skin disorders and leprosy. This study aims to comprehensively investigate the qualitative, quantitative, and antifungal aspects of Lactuca sativa L.

Different tests were conducted to ascertain both qualitative and quantitative parameters, including the presence of protein, glycosides, alkaloids, carbohydrates, and terpenoids. The total ash and acid insoluble ash values were determined to be 12.8% and 10.85%, respectively, providing insight into the mineral composition of the plant material. Furthermore, extractive values for various solvents, such as alcohol and water, were measured at 12.43% and 17.73%, respectively, indicating the solubility of different constituents. Moisture content, calculated through the loss on drying method, yielded a value of 1.5%, crucial for understanding stability and storage requirements. The pH value, determined with a digital meter, was recorded at 6.994, revealing the acidic or alkaline nature of the plant extract.

Preliminary phytochemical screening unveiled that aqueous and methanolic extracts serve as rich sources of amino acids, flavonoids, and proteins. Additionally, the water extract exhibited the presence of carbohydrates and saponins, expanding the repertoire of bioactive compounds present in Lactuca sativa L.

High-Performance Thin-Layer Chromatography (HPTLC) analysis of the hydroalcoholic extract revealed the presence of three peaks at different Rf values at 254 nm, indicating the presence of specific compounds with distinct polarities and absorbance characteristics. Similarly, five peaks were observed at 366 nm, highlighting the complex chemical profile of the extract.

In conclusion, this study provides valuable insights into the qualitative, quantitative, and antifungal aspects of Lactuca sativa L., laying the groundwork for further exploration of its medicinal properties and potential pharmacological applications. Further research is warranted to isolate and identify the bioactive compounds responsible for observed pharmacological activities and validate the traditional uses of this ethnomedicinal plant.

Keywords: Phytochemical, Pharmacognosy, Qualitative tests

I. INTRODUCTION

Lactuca sativa L., commonly known as lettuce, is a widely cultivated leafy vegetable belonging to the Asteraceae family. With its crisp, succulent leaves and mild flavor, lettuce holds a significant place in culinary traditions worldwide, often used in salads, sandwiches, wraps, and garnishes. Beyond its culinary appeal, lettuce is also valued for its nutritional content, providing essential vitamins, minerals, and dietary fiber.

Originating from the Mediterranean region, lettuce has a rich history dating back to ancient civilizations. Its cultivation can be traced as far back as ancient Egypt and Greece, where it was revered for its refreshing qualities and symbolism of prosperity and fertility. Over time, lettuce spread across continents, evolving into numerous varieties with diverse shapes, colors, and textures, each catering to different culinary preferences and growing conditions.

The taxonomy of lettuce encompasses several subspecies and cultivars, reflecting its extensive diversity. Common types include butterhead, romaine (also known as cos), leaf, and crisphead lettuce, each characterized by distinct leaf structures and growth habits. While some varieties thrive in cool climates and require shorter growing seasons, others are bred to withstand heat and resist common diseases, enabling cultivation in various environments throughout the year.

II. Material and methods

1. Material Collection

***Lactuca sativa* L.** was collected from Jamnagar, India, as per standard procedure in the month of June 2020 with assistance of local guide. ***Lactuca sativa* L.** Herbarium was prepared and Authentication was done from Pharmacognosy Laboratory ITRA Jamnagar provided with No. Phm/6314/2020-21 Plant parts like leaf, bark and fruit materials were collected and thoroughly washed further dried under shade at 28 ± 2°C for about 10 days. The dried parts were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50-150mm. The powders were stored in air sealed polythene bags at room temperature.

2. Organoleptic Parameter

The colour, taste and odour of the samples were noted down. These characters were useful to having primary idea about the quality of different formulations without using chemical tests. It includes sensory characters of drug

1. *Sparsha*(Consistency and Texture)
2. *Rupa*(Colour)
3. *Rasa* (Taste)
4. *Gandha*(Odour)

3. Foreign matter

Foreign matter determined by taking 100 g accurately weighed sample and spread in thin layer. The foreign matter should be detected by inspection the unaided eye or by use of a lens. This was separated, weighed and percentage of foreign mater in sample was calculated. The percentage of foreign matter was calculated on the basis of air-

dried sample.

4. **Loss on drying**

The loss on drying was determinate by taking 2 g, accurately weighed sample, in a dried and previously weighed petri-dish; it was spread evenly and dries in an oven at 110°C till constant weight. The weight after drying was noted and loss on drying was calculated. The percentage of loss of drying was calculated on the basis of air-dried sample.

5. Total ash

The ash value of the sample was determined by incinerated about 2 g of accurately weighed drug in a tarred silica crucible at a temperature not exceeding 450°C until free from carbon. Then cooled and weighed. If a carbon free ash were not obtained in this way, then charred mass was exhausted with hot water and the residue was collected on an ash less filter paper. Incinerated the residue and the filter paper, the filtrate was added, evaporated to dryness and ignited at a temperature not exceeding 450°C. The percentage of ash was collected with reference to the air-dried sample.

6. Water soluble extractive

About 5g, accurately weighed sample was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was filtered, taking precaution against loss of solvent and 25 ml of the filtrate was evaporated to dryness in a previously weighed dried evaporating dish. First dried over water bath and then at 110°C in hot air oven, to constant weight and weight was noted down. The percentage of water-soluble extractive was calculated with reference to air-dried sample.

7. Methanol soluble extractive

Methanol soluble extractive value was determine by same procedure as described in water soluble extractive value by taking methanol instead of water. The percentage of alcohol-soluble extractive was calculated with reference to air-dried sample.

8. Hydro-alcohol soluble extractive

Hydro-alcohol soluble extractive value was determine by same procedure as described in water soluble extractive value by taking mixture of methanol and water in ration of 80:20 was used as a solvent. The percentage of alcohol-soluble extractive was calculated with reference to air-dried sample.

9. pH

10% aq. Solution was prepared and check with the digital pH meter.

10. Preliminary Phytochemical screening (Qualitative Study)

Qualitative chemical tests were carried out for identifying various phytoconstituents present in methanolic, water and hydroalcoholic fractions of .[6]

a) **Mayer's reagent for alkaloids:** 2-3 ml filterate with few drops Mayer's reagent gives white ppt.
b) **Wagner's reagent for alkaloids:** 2-3 ml filterate with few drops Wagner's reagent gives reddish brown ppt.

c) **Shinoda test for flavonoids:** 1 g powder of both the leaves, separately, was extracted with 10 ml of ethanol (95%) for 15 minutes on a boiling water bath. To the filtrate, small pieces of magnesium ribbon and 3 drops of hydrochloric acid was added. No dark pink to magenta color was observed indicating the absence of flavonoids in both the leaves.

d) **Lead Acetate test for flavonoids:** To the alcoholic solution of the extract, few drops of 10% lead acetate solution were added. Appearance of yellow precipitate indicated the presence of flavonoids.

e) **FeCl3 test for Phenols:**2 ml of extract in a test tube and ferric chloride solution was added drop by drop. Appearance of bluish black precipitates indicated the presence of phenolics compounds and tannins.

f) **Lignins:** Treat the section of drug with conc. Hydrochloric acid and phloroglucinol solution, pink color is formed.

g) **Borntrager's tests for Anthraquinone Glycosides:** To 3 ml extract, add dil H2SO4. Boil and filter to cold filtrate. Add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

h) **Salkowski test for steroids:** To 0.5 ml chloroform extract in a test tube, 1 ml of concentrated H_2SO_4 was added from the sides of the test tube. Appearance of reddish brown colour in chloroform layer indicated the presence of phytosterols.

i) **Lead Acetate test for tannins:** To 2-3 ml of extract, add few drops of lead acetate solution. White ppt shows presence of tannins.

j) **FeCl3 test for tannins:**2 ml of extract in a test tube and ferric chloride solution was added drop by drop. Appearance of bluish black precipitates indicated the presence of phenolics compounds and tannins.

k) **Foam test for saponins:** Small amount of extract was taken in a test tube with little quantity of water and shaken vigorously. Appearance of foam persisting for 10 min indicated the presence of saponins.

l) **Fixed Oils:** To the 5 drops of sample add a pinch of sodium hydrogen sulphate, pungent odour emanates indicating presence of glycerine.

m) **Glycosides:** To 3 ml extract, add dil H2SO4. Boil and filter to cold filtrate. Add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

n) **Biuret Test for proteins:** To 3 ml T.S. add 4% NaOH and few drops of 1% CuSO4 solution, violet or pink colors appears**.**

o) **Ninhydrin Test for amino acids:** Heat 3 ml. test solution and 3 drops of 5% Ninhydrin solution in boiling water bath 10 min. Purple or Bluish color appears.

p) **Molisch's test for carbohydrates:** The extract was mixed with Molisch reagent, and then conc. H_2SO_4 was added along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicated the presence of carbohydrates.

11. High performance thin layer chromatography

7.5 µl of the sample from stock solution was applied on percolated silica gel G on an aluminium plate to a bandwidth of 8 mm using CAMAG LINOMAT 5 thin layer chromatography (TLC) applicator. The plate was

developed in Toluene: ethyl acetate (9:1) as solvent system. The solvent system ratio was designed. The developed plates were visualized at 254 and 366 nm in CAMAG high performance thin layer chromatography scanner 5. From the chromatogram, the number of spots and their R_f values were calculated.

III. RESULTS AND DISCUSSION

Physicochemical parameters of *Lactuca sativa* L.

The results of physico-chemical parameters are depicted in Table 1. The total ash and acid insoluble ash values are 12.8% and 10.85%, respectively. The extractive values for various solvents, such as alcohol and water, were found to be 12.43% and 17.73%. The moisture content was calculated through loss on the drying method, and value was found to be 1.5%.pH value was found to be 6.994 with digital meter.

Table No. 1: Physicochemical parameters of *Lactuca sativa* L.

Powder parameters	**Whole plant**
Loss on drying (%w/w)	1.5±0.25
Alcohol soluble extractive (%w/w)	12.430±0.879
Water soluble extractive (%w/w)	17.733±1.807
Ash value at 450°C (% w/w)	12.8±0.250
Acid insoluble Ash at 450°C (% w/w)	10.850±0.305
pH of 10% w/v aqueous solution	6.994

Phytochemical Screening

The results of the phytochemical screening are shown in Table 2. Preliminary phytochemical screening revealed that aqueous and methanolic extracts are a rich source of Amino acids, flavonoids, protein. In water extract carbohydrates and saponins are also present.

Table No. 2:- Qualitative analysis of *Lactuca sativa* L.

Qualitative test	**Methanol Extract**	**Water Extract**
Carbohydrate	-	+
Steroids & terpinoids	-	-
Amino acid	+	+
Flavonoids	+	+
Tannin & phenol	-	-
Alkaloids	-	-
Saponin	-	+
Glycoside	-	-
Proteins	+	+
Phenols	-	-
Flavanoids	+	+

+ = Present, - = Absent

HPTLC of the extract of Lactuca *sativa* L.

Hydro-alcoholic extract of whole plant of *Lactuca sativa* L. were used for the HPTLC HPTLC analysis of hydro alcoholic extract showed the presence of 3 peaks at different Rf values at 254 nm. Similarly, hydroalcoholic extract showed 5 peaks at 366 nm. The results are summarized in Table 3

Table No.3 :- Hpltc analysis of *Lactuca sativa* L.

Solvent system	**Sample** (MeOH extract)	**254 nm (short UV)**		**366 nm (long UV)**	
		Number of spot	**Rf value**	**Number of spot**	**Rf value**
Toluene: Ethyl acetate (9:1)		3	0.04, 0.18 0.73	5	0.04, 0.18, 0.25, 0.57, 0.80

FTIR analysis:

Powder sample of whole plant were subjected for the FTIR analysis. Analysis was carried out in Virani science college, Rajkot. The result of FTIR analysis revealed the presence of active phytoconstituents.

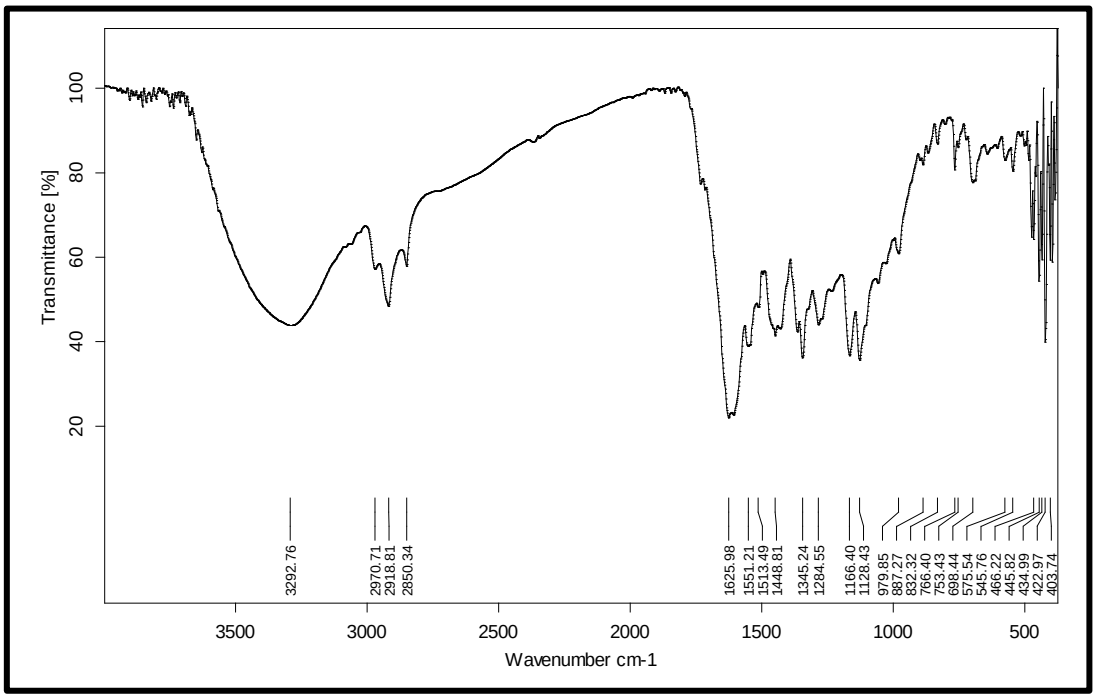

Table No.4: FTIR analysis of *Lactuca sativa* L.

Obtained Peak	**Bond**	**Functional Group**	**Appearance**
3292.76	C-H	Alkyne	Strong

2970.71	O-H	Alcohol	Strong
2918.81	C-H	Alkyl	Weak
2850.34	C-H	Aldehyde	Weak
1625.98	C=C	Benzene Ring	Medium
1551.21	C=C	Aromatic	Strong
1513.49	C=C	Alkene	Medium
1448.81	C-H	Alkyl Halide	Variable
1345.24	CH_2	Alkyl Halide	Variable
1284.55	CH_3	Alkyl Halide	Variable
1166.40	C-H	Alkyl Halide	Variable
1128.43	C-H	Alkyl Halide	Variable

Conclusion

The preliminary phytochemical screening of Lactuca sativa L. aqueous and methanolic extracts has unveiled a rich reservoir of bioactive compounds, including amino acids, flavonoids, proteins, carbohydrates, and saponins. These findings underscore the potential of lettuce as a valuable source of nutrients and phytochemicals with diverse health-promoting properties.

Furthermore, the HPTLC analysis of the hydroalcoholic extract has provided valuable insights into its chemical composition. The presence of distinct peaks at different Rf values, observed at both 254 nm and 366 nm, indicates the presence of specific compounds with varying polarities and absorbance characteristics. These peaks may correspond to individual phytoconstituents or chemical compounds present in the extract, warranting further investigation to elucidate their identities and potential pharmacological activities.

Overall, the combined results of phytochemical screening and HPTLC analysis highlight the complexity and potential therapeutic relevance of Lactuca sativa L. extracts. Further studies, including isolation, identification, and bioactivity assays of individual compounds, are warranted to explore the pharmacological applications and therapeutic benefits of these phytoconstituents. Such investigations could pave the way for the development of novel pharmaceuticals, nutraceuticals, and functional food products harnessing the diverse bioactive compounds present in lettuce extracts.

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