Available online on www.ijppr.com

International Journal of Pharmacognosy and Phytochemical Research 2023; 15(2);17-30

eISSN: 0975-4873, pISSN: 2961-6069

Research Article

Pharmacognostic Standardization, Preliminary Phyto-Chemical Screening and Antioxidant Potential of Root of *Lactuca Dissecta* D. Don

Joshi Amit Kumar*, Wangpo Tenzin, Chachda O. Nilesh, Rawat Geeta

Sunrise University, Bagad Rajput, Tehsil-Ramgarh, Alwar, Rajasthan

Received: 30th March 23; Revised: 20th April 23; Accepted: 24th May 23; Available Online:25th June 23

ABSTRACT

Background: Genus Lactuca L., belonging to the tribe Cichorieae comes in the category of Compositae family which is presently known as Asteraceae family. According to the plant list (TLP) in Asia, Northern America and Europe around 147 species of Lactuca are distributed. The genus Lactuca is derived from Latin word "Lac" meaning milk is native of Southwest Asia and Mediterranean basin. In India in hilly regions and plains of Himanchal Pradesh, Uttar Pradesh, J & K and Arunanchal Pradesh 24 species of Lactuca genus has been reported. Genus Lacuca comprises of various phytochemical and they are utilized in conventional system of medicine for preventing many diseases such as lack of apetite, pain, inflammation UTI and bronchitis. This genus contains sesquiterpenes, lactones, guaianolides, germacranolides, lignans, tannins, flavanoids, flavones and phenolic acid. Despite the known active constituents and conventional application of this herb as a curer still there has been no much experimental results mentioned related to pharmacognostic and phytochemical investigation and antioxidant potential of the plant.

Aim: Thus, the current study was performed to evaluate, investigate and study the pharmacognostical and phytochemical estimation and determination of antioxidant potential of *Lactuca dissecta* D.Don.

Material and Methods: Root samples of *Lactuca dissecta* D.Don were evaluated for macroscopical and microscopical studies. Different physicochemical parameters were also determined and calculated and preliminary phytochemical determination with different solvents like ethyl acetate, acetone, petroleum ether, methanol and aqueous extract of plant root part were carried out in accordance to polarity. Root powder of plant material was treated with different laboratory chemicals used for analysis of powder microscopy and also subjected for florescence analysis under UV light of short and long wavelength and visible light. Antioxidant potential of root extract was also evaluated through DPPH assay.

Results: Transverse section of the root part shows rows of cork cells 7 to 8 tangentially. Under the cortex parenchyma's cells are present which loosely arranged containing prismatic calcium oxalate crystals. Cortical cells consist of brown color content which was embedded with starch grains. Different physicochemical calculation and determinations such as extractive value, loss on drying, swelling, foaming index and ash value were evaluated for root part. Different range of fluorescence color was analyzed by Fluorescence analysis for powdered crude drug. Phytochemical determination shows the presence of chemical constituents such as steroids, proteins, tannins, flavanoids and alkaloids. Antioxidant potential was determined with the help of DPPH assay method in which ethyl acetate and methanol extract showed maximum antioxidant activity.

Keywords: *Lactuca dissecta* D. Don, Florescence analysis, Antioxidant, DPPH, ash value.

INTRODUCTION

Ethanopharmacological or traditional medicine is defined as practices, knowledge and skill which are based on society traditional culture, beliefs, presumptions and experience so as to regulate their hygiene and health. In various ruler areas or indigenous peoples in various underdeveloped countries traditional treatment with herbs are highly effective[1]. System of medicine of health in India which is recognized officially share a large part of herbal drugs including yoga, Unani, Siddha, Ayurvedic, Naturopathy and Homeopathy system. Population of India around 1.1 billion which is around more than 70% uses these systems of medicine[2]. Since primitive time period for the treatment of different diseases various herbs have been utilized as healing agents in numerous formulations.

**Author for Correspondence: amit.joshi.pharmacognosist@gmail.com*

Of the world population around 60 to 80% which resides in developing nations depends essentially on healing or therapeutic plants for their initial health management according to World Health Organization[3]. The foundation of human disease treatment has been the natural compounds from animal, plant and minerals. For primary health care it is approximated that in countries which are developing about 80% of people depends on conventional remedy which is hugely based on species of different animals and plants. At present herbal medicines are in great demand and their popularity is gaining so much faster. At present herbal medicine are in great demand and their popularity is gaining so much faster. In ancient literature around 500 herbs are mentioned for their therapeutic use while in indigenous medicine system around 800 herbs have been utilized[4].

Plant gives a huge and multiple varieties of organic compounds, out of this large majority do not utilized by plants for their development and growth. These constituents conventionally referred to as active constituents or secondary metabolites which are distributed among restricted and taxonomic group in the plant kingdom and have great pharmacological effect[5].

Uttarakhand contains natural beauty which is unique with rich plant diversity which is ranging from high snow zones situated in the north to Terai belt in south. Terai which is low land area and is situated in foothills of Himalayan region it holds on sufficient of surface water. Uttarakhand is well known as "Gods Land". Apart from rich plant resources, Uttarakhand contains various tribal communities as well as ethanobotanical legacy. Asteraceae Berchtold & Presl. (Also known as Compositae Giseke, nom. alt.) is mostly known as thistle, sunflower or daisy family having genera around 134 and containing 370 species constituting the huge dicot family in Uttarakhand state. This family contains herbs, climbers, shrubs and rarely any tree. A huge amount of Asteraceae family-based plants are used for food, healing and other motive around the world[6].

Asteraceae is a family which is important economically as it provides various products including sunflower seeds, lettuce, cooking oils, artichokes, coffee alternatives, sweeteners and herbal tea[7].

MATERIALS AND METHODS

Plant Collection and Authentification

Specimens of morphological plant were gathered from field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand during the month of July. Sample was identified for different samples taxonomically by Botanical Survey of India botanist, 192, Kaulagarh Road, Northern Regional Center, and Dehradun-248195. Each sample one set was accumulated in the record of Botanical Survey of India herbarium. The plant materials (leaves bark, root) were dried in air completely at normal temperature under shade and were then powdered to a fine quality by using a mixer grinder or laboratory grinding mill. These shaded dried parts of the plant material are finally packed in airtight plastic bag until use.

Morphological Evaluation

Ocular visualization gives the easier and fastest way by which quality, possibly and identity purity of a sample can be established. If there is significant difference in the sample with regard to consistency, color, taste or odor, through the identification, it is regarded that it is not confirming the official necessities. Macroscopical recognization of herbs having medicinal value depends on color, size, shape, surface characters, fracture, surface characteristics and emergence of the cutted surface. Moreover, the features are calculated subjectively and are adulterants and substituents may nearly resemble the original or officially fulfilled required raw material as it is frequently necessary to prove the results by physiochemical or microscopy evaluations[8].

Microscopical Evaluation

Plant materials using for medicine utilization are classified according to their organoleptic and microscopical characteristics. Visible inspection is the quickest and simplest means by which, purity, identity, and most probable drug quality can be established. Microscopical evaluation is a move forward towards identification and authentification of interior structural of crude plant sample to establish original identification by studying the arrangement of tissues. This is performed by recognizing internal cell constants such as vascular bundles, epidermis, collenchymas, schelernchyma, trichomes etc. For this procedure there is a transverse or longitudinal sectioning either by free hand or using microtome may be performed. For the present research work free hand sectioning was performed[8, 9, 10].

Powder Microscopy

Dried leaves and root powder microscopy of plants were performed. The powders of plants were placed on different clean glass slides. Drop of glycerol was added to plant material which were powdered and kept on the slide of glass and it was placed with cover slip was placed over it. The glass slide was then examined below the microscope and various images were clicked at required magnification. For good results various stains were also used to differentiate cellular structure. Each

powder was treated and stained with Iodine, Phloroglucinol, Sudan III, Ruthenium red stain and studied by microscope[8, 9, 11, 12].

Physico-Chemical Parameters

Dried powder of leaves and root were put through to physicochemical examination. Physiochemical constants such as loss on drying, extractive value, ash value, swelling index and foaming index was performed and studied[13].

Fluorescence Analysis of Powders

This analysis is among unique methods used in pharmacognostic methods which are useful in the identification of genuine samples and identifying adulterants[14]. In the process of fluorescence analysis, the morphological part of the plant or crude drug may be examined as such, or in their solution or as extract or in their powdered form. Although, in most of the samples the actual constituents which is responsible for the fluorescence activity has not been identified, the merits of the process are rapidity and simplicity which builds it a precious analytical tool for the recognization of various plant samples and crude drugs[15].

Extraction of Plant Material

The powdered plant leaves which were shade dried undergo successive extraction with various solvents according to the polarity. Plant material which was coarsely powdered around 50g was thoroughly extracted for 3 hours with solvent petroleum ether (50-70ºC) in soxhlet apparatus. Obtained extract was concentrated and solvent is recovered by recovery unit. The plant material which was extracted was then air dried and again packed in soxhlet apparatus and extracted exhaustively with ethyl acetate for 3 hours. Then the extract obtained device was filtered and was evaporated using rota vapor or solvent under reduced pressure and is then recovered through recovery unit. The plant material which was extracted was air dried and is again loaded in the soxhlet apparatus and was drawn out with acetone, methanol and lastly with water and finally filtered, evaporated using rota vapor[16].

Phytochemical Analysis

A concentration of stock concentration of 1% (W/ V) of individual successive extract obtained using petroleum ether, water, acetone, ethyl acetate and methanol was got ready using the particular solvent. Extracts which were having negative and positive controls were tested for the identification of chemical constituents viz: alkaloids, tannins, triterpenoids, phytosterols, falvonoids, cardiac glycosides, anthroquinone glycosides, saponins glycosides, carbohydrates, proteins, amino acids, fats and fixed oils following standard methods[16, 17].

Antioxidant Activity by DPPH Methods

With the help of DPPH (1, 1-diphenyl-2picryl hydrazyl) different extracts of plants morphological parts (leaves, barks and root) were measured and calculated for free radical scavenging activity. In this method in ethanol 0.1mM solution of compound DPPH was prepared. 3 ml of different plant extracts of various morphological parts was added to 1 ml of DPPH solution at distinct congregation (2, 4, 6, 8, 10, 12 µg/ml). By the use of dilution method distinct concentration of only those extracts which were having solubility in ethanol were prepared. The mixture was then permitted to sand for around time period of 30 minutes with strong shaking at normal temperature and with the assist of spectrophotometer (Shimadzu) absorbance of distinct solvents were measured at 517 nm. The experiment and method was performed in triplicate and the compound which was used as standard was ascorbic acid[18, 19, 20].

RESULT AND DISCUSSION

Plant Authentification

Figure 1: Plant authentication certificate and herbarium specimen of *Lactuca dissecta* D.Don

The unknown sample of plant was collected from the field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand throughout the month of July and the sample herbarium were made in 2 sets and

were submitted to Botanical Survey of India botanist, 192, Kaulagarh Road, Northern Regional Center, Dehradun-248195 Sample of the plant was identified taxonomically by taxonomist as *Lactuca dissecta* D. Don one set was accumulated in the herbarium record of Botanical Survey of India. Certificates and identified herbarium samples are mentioned in the above figures.

Macroscopical Evaluation

Figure 2: Macroscopical characters of leaf and bark part of *Lactuca dissecta* D.Don

Morphological study of the root part reveals that it is slightly curved and cylindrical in shape. The size of the root is round around 3 to 9 cm long and 1 to 2 cm in diameter. When dried the color of the rood is brownish white and root part do not have any odour and it is extremely bitter in taste.

Microscopical Evaluation

Microscopical Evaluation of *Lactuca dissecta* D. Don

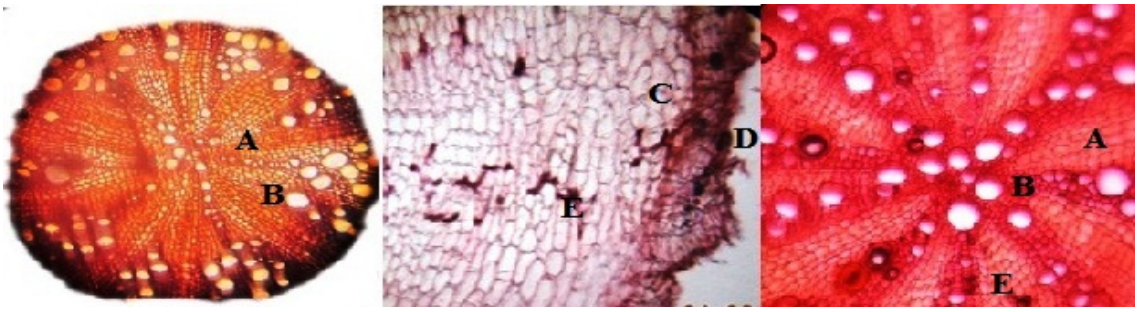

Figure 3: T. S. of *Lactuca dissecta* D. Don root showing Phloem, xylem, cortex, cork and stone cells

Transverse section of the root part shows rows of cork cells 7 to 8 tangentially. Under the cortex parenchyma's cells are present which loosely arranged containing prismatic calcium oxalate crystals. Cortical cells consist of brown color content which was embedded with starch grains. In the cortical zone ring of stone cells were observed. Below the phloem region and the stone cells lignified pericycle fibers were found. Vascular bundles were situated centrally. Above the xylem fiber phloem is situated which contain some sieve tubes. From the central medullary rays were arising which were extended up to cortex region. In the center round around 6 to 8 vascular bundles are situated.

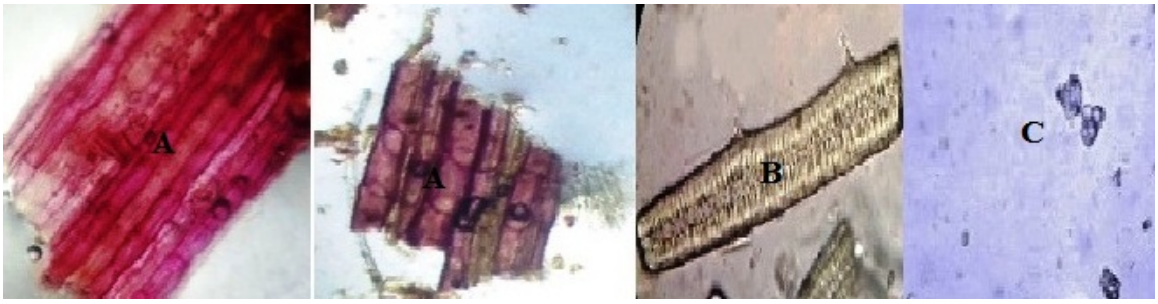

Figure 4: Powder photomicrograph of *Lactuca dissecta* D. Don root showing medullary rays, pitted vessel and starch grains.

The color of the root powder was light brown, having astringent in taste with fibrous texture and no odor. Diagnostic microscopical features of the root powder shows cork cells in surface and pitted parenchyma

cells which were lignified from vascular bundle and cortical. Parenchyma cells were present in cortex region along with simple fibers. In the stiller region pitted vessels are present.

Physico-Chemical Parameters

Physico-chemical parameters of *Lactuca dissecta* D. Don root

Figure 5: Physico-chemical parameters of *Lactuca dissecta* D. Don root mentioning type ash value, loss on drying, foaming index and swelling index

Loss on drying

The value of loss on drying for the root sample of *Lactuca dissecta* D. Don was found to be 7.407%.

Table 1: Loss on drying of *Lactuca dissecta* D.Don root powder

S. No.	Drug wt. + Petri dish wt. before drying A (g)	Drug wt. + Petri dish wt. after drying B (g)	Loss on drying. A-B (g)	% of loss on drying
1	10.046 + 43.023	52.321	0.748	7.445
2	10.045 + 41.155	50.462	0.738	7.346
3	10.039 + 44.528	53.821	0.746	7.431
Mean ± S.E.M (n=3)				7.407 ± 0.030

Ash Value

The values of ash i.e. Total ash, water soluble ash and acid insoluble ash were evaluated as per official process. Existence of inorganic content in a raw drug is a measure of total ash. Total ash greater value gave indication that more inorganic matter is present in plant material. In the drug existence of inorganic matter is a measure of total ash. Large value shows the plant material consists of more inorganic matter. The total ash value of *Lactuca dissecta* D.Don root was calculated as 6.251%. To the total ash concentrated acid was added, the acid combines and reacts with calcium oxalate crystals. If calcium oxalate crystals are large in number in plant material, quantity of substance after acid treatment will remain quite less. Acid insoluble ash lower value denotes the existence of calcium oxalate crystals in large number in plant material. Amount of silica present in given plant material is determined by acid insoluble ash. Value of acid insoluble ash was computed as 2.030%. Another part of total ash is water soluble ash, which dissolves in the drug and is excellent indicator of the water-soluble salts. Water soluble ash was computed as 1.527%. The results were found to be almost within limits.

Table 2: Total ash value of *Lactuca dissecta* D. Don root powder

S. No.	Drug wt. (g)	China dish empty wt. (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of total ash
1	3.054	16.912	17.106	0.194	6.352
2	3.047	17.496	17.682	0.186	6.104
3	3.049	17.564	17.756	0.192	6.297
Mean ± S.E.M (n=3)					6.251 ± 0.075

Table 3: Acid insoluble ash value of *Lactuca dissecta* D. Don root powder

S. No.	Drug wt.	China dish empty	Crucible wt. + Wt.	Ash Wt. (g)	% of acid insoluble

	(g)	wt. (g)	of ash (g)		ash
1	3.022	18.056	18.329	0.064	2.117
2	3.019	17.672	17.918	0.059	1.954
3	3.020	17.870	18.129	0.061	2.019
Mean ± S.E.M (n=3)					2.030 ± 0.047

Table 4: Water soluble ash value of *Lactuca dissecta* D. Don root powder

S. No.	Drug wt. (g)	China dish empty wt. (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of water-soluble ash
1	3.063	16.762	16.958	0.051	1.665
2	3.053	17.239	17.440	0.047	1.539
3	3.047	17.423	17.610	0.042	1.378
Mean ± S.E.M (n=3)					1.527 ± 0.083

Extractive Value

The extractive value of *Lactuca dissecta* D.Don root was determined by the hot extraction method and cold extraction method. The extractive values were calculated for different plant extracts. In this study it was investigated that in hot extraction procedure, methanolic extract shows maximum extractive value of 11.747% while extractive value of petroleum ether was found to be 3.181% which was lower as compared to other extracts. In case of cold extraction method water soluble extract shows the peak extractive value of 15.429% while petroleum ether was less effective with extractive value of 1.659% in contrast to additional solvent extracts. The outcomes are tabulated as follows:

Table 5: Petroleum ether soluble extractive value of *Lactuca dissecta* D. Don root powder (Hot Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.079	56.1239	56.1563	0.0324	3.177
2	4.019	55.9451	55.9770	0.0319	3.174
3	4.021	56.1092	56.1413	0.0321	3.193
Mean± S.E.M (n=3)					3.181 ± 0.005

Table 6: Chloroform soluble extractive value of *Lactuca dissecta* D. Don root powder (Hot Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.013	59.2314	59.2746	0.0432	4.306
2	4.007	57.1432	57.1905	0.0473	4.721
3	4.004	58.7642	58.8134	0.0492	4.915
Mean ± S.E.M (n=3)					4.647 ± 0.179

Table 7: Ethyl acetate extractive soluble value of *Lactuca dissecta* D. Don root powder (Hot Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.007	58.4521	58.5133	0.0612	6.109
2	4.003	55.9926	56.0519	0.0593	5.925
3	4.002	56.0426	56.1045	0.0619	6.186
Mean ± S.E.M (n=3)					6.073 ± 0.077

Table 8: Methanol soluble extractive value of *Lactuca dissecta* D. Don root powder (Hot Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	3.998	57.7652	57.8973	0.1321	13.216
2	4.003	57.9128	58.0282	0.1154	11.531
3	4.005	58.2192	58.3243	0.1051	10.496
Mean ± S.E.M (n=3)					11.747 ± 0.792

Table 9: Water soluble extractive value of *Lactuca dissecta* D. Don root powder (Hot Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.014	57.3481	57.4404	0.0923	9.197
2	4.012	56.9901	57.0847	0.0946	9.431
3	4.012	57.5641	57.6571	0.0930	9.272
Mean ± S.E.M (n=3)					9.300 ± 0.068

Table 10: Petroleum ether soluble extractive value of *Lactuca dissecta* D. Don root powder (Cold Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.001	59.3764	59.3995	0.0231	2.309
2	4.006	60.0012	60.0255	0.0243	2.426
3	4.001	59.1205	59.1459	0.0254	2.539
Mean ± S.E.M (n=3)					2.424 ± 0.066

Table 11: Chloroform soluble extractive value of *Lactuca dissecta* D. Don root powder (Cold Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.013	57.1293	57.1758	0.0465	4.545
2	4.007	56.7856	56.8368	0.0512	5.111
3	4.002	57.0126	57.0622	0.0496	4.957
Mean ± S.E.M (n=3)					4.871 ± 0.618

Table 12: Ethyl acetate soluble extractive value of *Lactuca dissecta* D. Don root powder (Cold Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.009	57.2381	57.2557	0.0176	1.756
2	4.007	57.1724	57.1888	0.0164	1.637
3	4.009	58.0787	58.0946	0.0159	1.586
Mean ± S.E.M (n=3)					1.659 ± 0.050

Table 13: Methanol soluble extractive value of *Lactuca dissecta* D. Don root powder (Cold Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.017	57.2341	57.3264	0.0923	9.190
2	4.015	58.0194	58.1106	0.0912	9.085
3	4.017	57.6721	57.7620	0.0899	8.951
Mean± S.E.M (n=3)					9.075 ± 0.056

Table 14: Water soluble extractive value of *Lactuca dissecta* D. Don root powder (Cold Extraction Method)

S. No.	Drug wt. (g)	Empty Petri plate wt. (g)	Petri plate wt. + Wt. of extractible matter (g)	Wt. of extractible matter (g)	% of extractible matter
1	4.008	56.3642	56.5098	0.1456	14.530
2	4.007	57.1286	57.2848	0.1562	15.592
3	4.008	57.4321	57.5867	0.1546	15.429
Mean ± S.E.M (n=3)					15.183 ± 0.269

Swelling Index

Various healing plants possess a definite healing value due to the presence of fluctuating constituents of hemicelluloses or pectin, gum and mucilage which leads to different swelling properties of diverse plant material. The swelling index parameter was identified and computed to determine the amount of plant crude material shows swelling after treatment with water and to calculate that the plant material contains some content of mucilage. The swelling index of *Lactuca dissecta* D. Don root was found to be 3.466. The results are tabulated as follows:

Table 15: Swelling index value of *Lactuca dissecta* D.Don root powder

S.No	Powdered drug weight (gm)	Stock Volume	Swelling factor
1	1.0	25	3.1
2	1.0	25	3.4
3	1.0	25	3.9
Mean± S.E.M (n=3)			3.466 ± 0.233

Foaming Index

In 10 test tubes decoction of plant material and water was taken in different ratio, foam was measured with the help of scale after shaking the test tube and when the foam in the test tube becomes persistent. Foam X-height in every test tube was measured below 1cm. So the foaming index of *Lactuca dissecta* D. Don root was found to be 120.370 indicating some amount or presence of saponins. The results are tabulated as follows:

Fluorescence Analysis of Powders

The powder of (mesh size 40) of root morphological part of *Lactuca dissecta* D.Don was examined under daylight and UV light. Fluorescence analysis of plants various morphological parts showed various coloration by utilizing distinct chemical reagents under UV and visible light. In case of different natural products UV light produce a fluorescent nature which is important character of fluorescence analysis. The outcomes of fluorescence determination revealed that in visible light various shades were exhibited by plant powder such as yellow, green, cream and brown fluorescence while different shades of yellow, green, cream, brown, black and light red fluorescence were observed in short and long UV. The results are tabulated as follows:

Table 16: Foaming index value of *Lactuca dissecta* D. Don root powder

S. No.	Powdered drug wt. (gm)	Stock Volume (in ml)	Dilution of the test solution (in ml)										Foaming Index
			1	2	3	4	5	6	7	8	9	10	
1	1.0	100	0.4	0.3	0.1	0.5	0.6	0.5	0.7	0.6	1.4	0.8	111.111
2	1.0	100	0.2	0.4	0.3	0.6	0.5	0.7	0.5	1.1	0.9	0.8	125.000
3	1.0	100	0.1	0.5	0.2	0.4	0.6	0.8	0.7	1.3	0.7	0.9	125.000
Mean ± S.E.M (n=3)													120.370 ±4. 629

Table 17: Fluorescence analysis of *Lactuca dissecta D.Don* root powder powders

S. No.	*Lactuca dissecta* (Root)	Visible	Short UV-254 nm	Long UV-365 nm
1	Powder + 1N NaOH in water	Amber	Amber	Amber, green
2	Powder + 1N NaOH in alcohol	Pale yellow	Faint green	Light green
3	Powder + acetic acid	Light yellow	Faint green	Light green
4	Powder + methanol	Faint yellow	Cream	Light green
5	Powder + H_2SO_4	Amber	Black	Black
6	Powder + petroleum ether	No color	No color	No color
7	Powder + HCl	Brown	Brown	Amber, green
8	Powder + water	Yellowish brown	Yellow brown	Light green
9	Powder + nitric acid	Yellow	Yellow	Light yellow
10	Powder + acetone	Greenish yellow	Light green	Light green

Extraction of Plant Material

500 gm coarse powder of root morphological part of *Lactuca dissecta* D. Don was subjected to successive extraction with different solvents like petroleum ether, chloroform, acetone, ethyl acetate, methanol and water in for around 3 hrs per solvent. Results are tabulated in below mentioned table.

Table 18: Data showing successive solvent extraction values and nature of extract of *Lactuca dissecta D. Don* root.

S. No	Solvent Used	Wt. of drug (gm)	Yield (gm)	% Yield	Extract color	Property	Mean ± S.E.M
1	Pet. ether	120	5.216	4.346	Light brown	Semisolid	4.425 ± 0.044
			5.320	4.433			
			5.398	4.498			
2	Chloroform	120	3.429	2.857	Yellowish	Slightly Powder	2.740 ± 0.058
			3.276	2.680			
			3.221	2.684			
3	Acetone	120	4.264	3.553	Dark brown	Sticky	3.558 ± 0.057
			4.112	3.462			
			4.394	3.661			
4	Ethyl acetate	120	6.095	5.079	Reddish brown	Sticky	5.140 ± 0.030
			6.198	5.165			
			6.212	5.176			
5	Methanol	120	7.610	6.341	Reddish yellow	Sticky	6.237 ± 0.054
			7.454	6.211			
			7.391	6.159			
6	Water	120	8.924	7.436	Light reddish	Amorphous powder	7.326 ± 0.065
			8.798	7.331			
			8.654	7.211			

Qualitative Phytochemical Analysis

With the help of screening chemical test the existence in the extracts of root of *Lactuca dissecta* D. Don when tested with various solvent extracts of different polarity shows the existence of different chemical constituents such as alkaloids, flavanoids, tannins, saponins glycoside, sterols as well as proteins as secondary metabolites

.

Table 19: Preliminary phytochemical investigation of various extracts of *Lactuca dissecta* D. Don root

Chemical Constituents	Tests	PEPB	CFPB	ACPB	EAPB	MEPB	WAPB
Alkaloids	Mayer's test	--	--	--	--	--	--
	Wagner's test	--	--	--	++	++	--
	Hager's test	--	--	--	--	--	--
	Dragendroff's test	--	--	--	--	++	--
Flavanoids	Alkaline reagent test	++	--	++	--	++	++
	Shinoda test	--	++	--	--	--	--
Tannins	Ferric Chloride test	--	--	--	++	--	++

	Legal Test	--	--	--	--	--	++
Anthraquinone glycosides	Borntrager's test	--	--	--	--	--	--
Saponin glycosides	Foam test	--	--	--	--	--	--
Steroids	Salkowski test	--	--	++	++	++	--
	Liebermann Burchard	++	++	++	--	++	++
Proteins	Biuret test	--	--	++	--	--	--
	Millon's test	--	++	--	--	++	--
	Xanthoproteic test	--	--	++	--	--	--

[PE=Petroleum ether, CF= Chloroform, AC=Acetone, EA=Ethyl acetate, ME=Methanol, WA=Water]

Antioxidant Activity (Free Radical Scavenging Activity Using DPPH)

Antioxidant Activity of *Lactuca dissecta* D. Don root extract

In-vitro antioxidant activity of various extract of *Lactuca dissecta* D. Don root was determined and was compared with standard ascorbic acid with the help of DPPH assay. Absorbance of control sample was found to be 0.296. Absorbance of the sample at various concentrations was calculated and measured and inhibition of percentage was computed by drawing and plotting a calibration curve between percentage inhibition and concentration. IC_{50} value obtained for standard (ascorbic acid), methanolic extract, ethyl acetate extract and acetone extract were obtained as 16.040, 21.129, 8.455 and 27.658 respectively. It means that acetone and methanol extract of crude plant material at elevated concentration encapsulate increased number of free radicals produced by DPPH resulting into decrease in absorbance and elevation in IC_{50} value. Results are tabulated as:

Table 20: Percentage scavenging activity of Standard Compound (Ascorbic Acid)

S.No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.256, 0.252, 0.254	13.51, 14.86, 14.18	14.18 ± 0.389
2	4	0.246, 0.249, 0.245	16.89, 15.87, 17.22	16.66 ± 0.406
3	6	0.234, 0.231, 0.233	20.94, 21.95, 21.28	21.39 ± 0.296
4	8	0.215, 0.214, 0.216	27.36, 27.70, 27,02	27.36 ± 0.196
5	10	0.194, 0.197, 0.195	34.45, 33.44, 34.12	34.00 ± 0.297
6	12	0.179, 0.176, 0.175	39.52, 40.54, 40.87	40.31 ± 0.406

Table 21: Percentage scavenging activity of methanolic extract of *Lactuca dissecta* root

S.No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.249, 0.247, 0.241	15.87, 16.55, 18.58	17.00 ± 0.814
2	4	0.236, 0.232, 0.234	20.27, 21.62, 20.94	20.94 ± 0.389
3	6	0.221, 0.224, 0.220	25.33, 24.32, 25.67	25.10 ± 0.405
4	8	0.216, 0.214, 0.212	27.02, 27.70, 28.37	27.69 ± 0.389
5	10	0.209, 0.204, 0.201	29.39, 31.08, 32.09	30.85 ± 0.787
6	12	0.194, 0.197, 0.192	34.45, 33.44, 35.13	34.34 ± 0.491

Table 22: Percentage scavenging activity of acetone extract of *Lactuca dissecta* root

S.No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.272, 0.270, 0.273	8.10, 8.78, 7.77	8.21 ± 0.297
2	4	0.261, 0.264. 0.260	11.82, 10.81, 12.16	11.59 ± 0.405
3	6	0.254, 0.257, 0.251	14.18, 13.17, 15.20	14.18 ± 0.586
4	8	0.246, 0.242, 0.245	16.89, 18.24, 17.22	17.45 ± 0.406
5	10	0.232, 0.229, 0.234	21.62, 22.63, 20.94	21.73 ± 0.491
6	12	0.228, 0.224, 0.221	22.97, 24.32, 25.33	24.20 ± 0.683

Table 23: Percentage scavenging activity of ethyl acetate extract of *Lactuca dissecta* root

S.No.	Concentration (µg/ml)	Absorbance at 517 nm	% Inhibition	Mean ±S.E.M. (n=3)
1	2	0.261, 0262, 0264	11.82, 11.48, 10.81	11.37 ± 0.296
2	4	0.256, 0.257, 0.253	13.51, 13.17, 14.52	13.73 ± 0.405
3	6	0.242, 0.239, 0.238	18.24, 19.25, 19.59	19.02 ± 0.405
4	8	0.224, 0.227, 0.221	24.32, 23.31, 25.33	24.32 ± 0.583
5	10	0.206, 0.201, 0.204	30.40, 32.09, 31.08	31.19 ± 0.491
6	12	0.186, 0.183, 0.181	37.16, 38.17, 38.85	38.06 ± 0.491

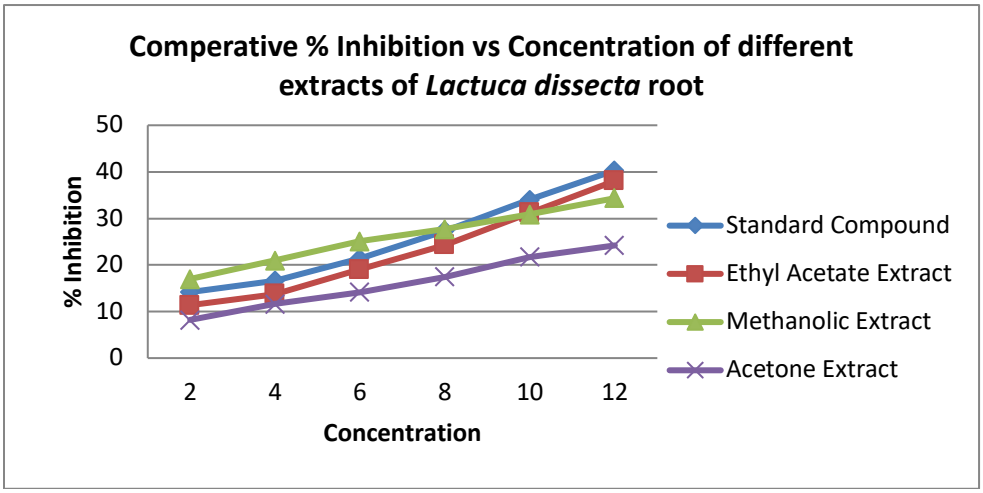

Figure 6: Comparative % Inhibition vs Concentration of *Lactuca dissecta* D.Don

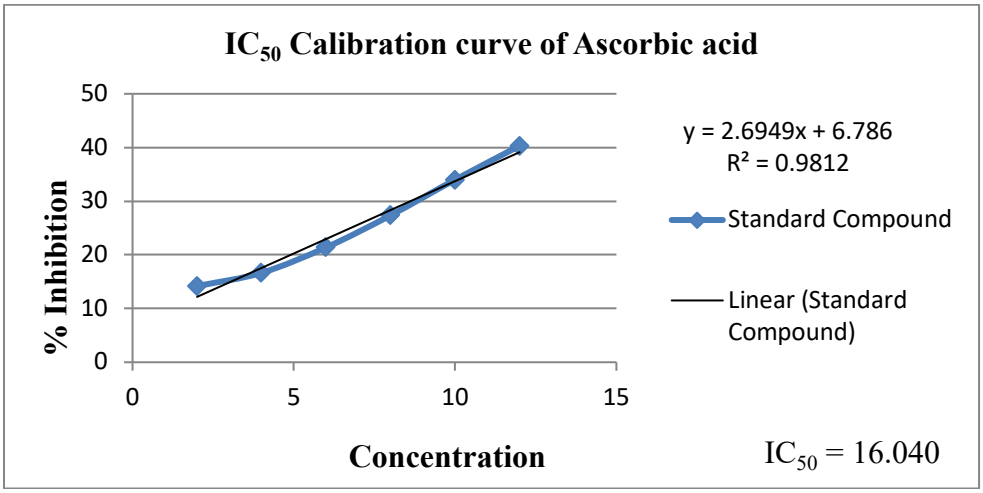

Figure 7: Calibration curve for estimation value of IC_{50} for Ascorbic acid

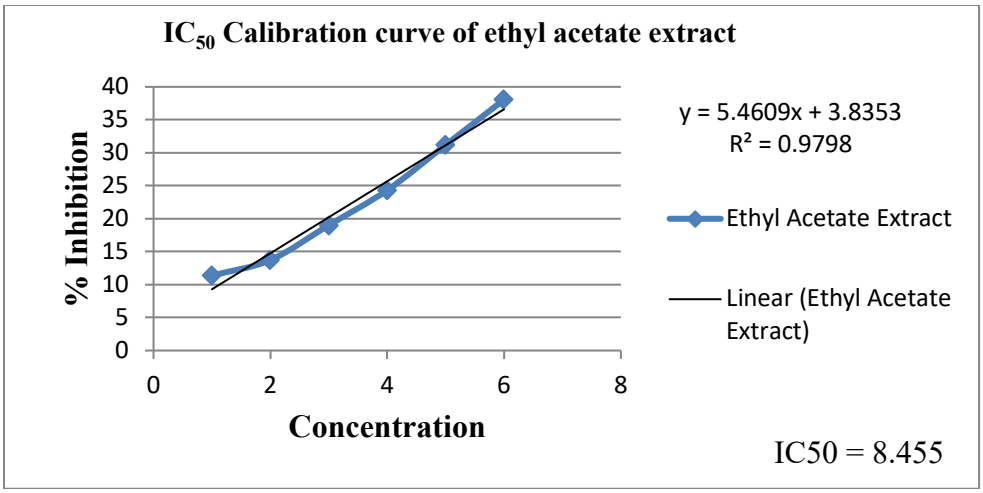

Figure 8: Calibration curve for estimation value of IC_{50} of ethyl acetate extract

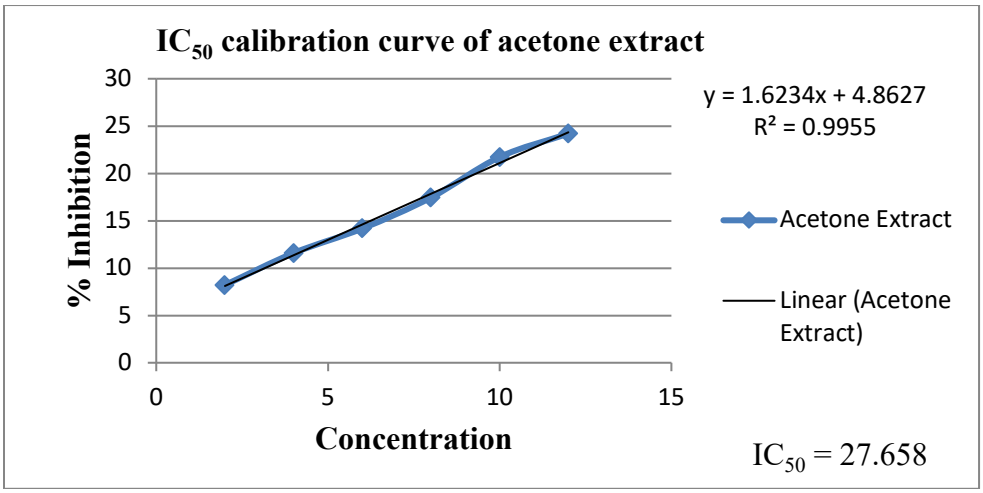

Figure 9: Calibration curve for estimation value of IC_{50} of acetone extract

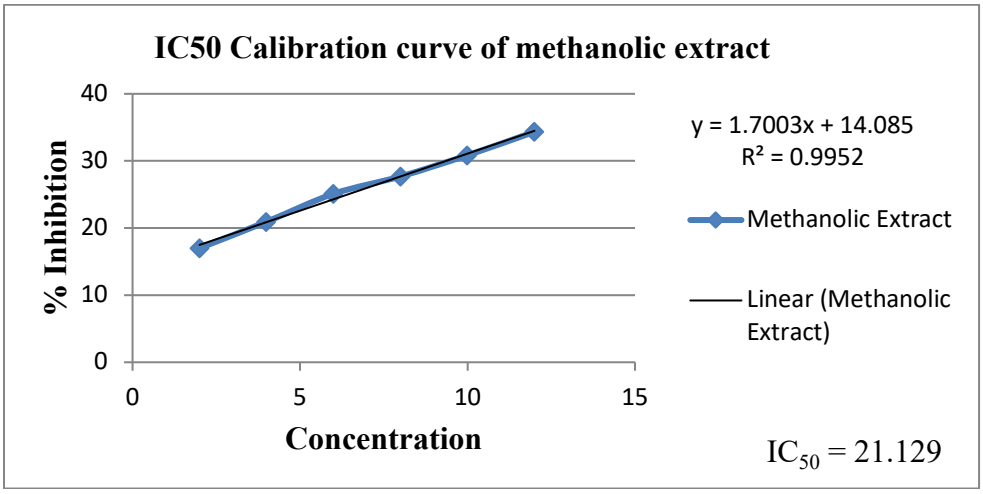

Figure 10: Calibration curve for estimation value of IC_{50} of methanol extract

DISCUSSION

The current study mainly focuses to explore the flora diversity of Kumaun and Garhwal region of Uttarakhand for their healing value. In this investigation the root part of *Lactuca dissecta* D. Don root part was experimented and studied for its therapeutic qualities which were mentioned in conventional system of medicine. In current research investigation it is fully focused on the database knowledge of healing plants in terms of its organoleptic, microscopic, physicochemical, and phytochemical as well as antioxidant potency of *Lactuca dissecta* D. Don. Pharmacognostic evaluation showed that shape of the root is slightly curved and cylindrical in shape with whitish brown color without odor and having bitter taste. Transverse section of root shows 7 to 8 tangential cork cells. Parenchyma's cells are present under the cortex having prismatic calcium oxalate crystals. Below the phloem region and the stone cells lignified pericycle fibers were found and vascular bundles were situated centrally. Preliminary phytochemical analysis of root gives an idea for the presence of some chemical constituents such as sesquiterpenes, lactones, guaianolides, germacranolides, lignans, tannins, flavanoids, flavones and phenolic acid which in future will play important role in the formulation of herbal medicine. Antioxidant activity of the leaf extract of acetone and methanol showed that the plant contains potent antioxidant activity. As not so much experimental work is done and published in research journals on this plant, the result outcomes from this current study serve as a standard in further experimental investigation of this plant. Also, phytoconstituent obtained from this herb will serve as an important source of information and finding and also establishes quality of the herb in pharmaceutical and medicinal studies by providing suitable standard.

REFERENCES

1. Singh A. A Review of various aspects of the Ethnopharmacological, Phytochemical, Pharmacognostical, and Clinical significance of selected Medicinal plants. Asian Journal of Pharmacy and Technology, 2022; 12(24):349-360.
2. Vaidya A.D.B, Devasagayam T.P.A. Current Status of Herbal Drugs in India: An Overview Journal of Clinical Biochemistry and Nutrition, 2007; 41(1): 1-11.
3. Pallie M.S, Perera P.K, Kumarsinghe N, *et. Al*. Ethnopharmacological Use and Biological Activities of Tragiain volucrataL. Hindawi, 2020; 1-17.
4. Verma S,Singh SP. Current and future status of herbal medicines. Veterinary World, 2008; 1(11):347-350.
5. Croteau R, Kutchan T.M and Lewis N.G. Natural Products (Secondary Metabolites). Biochemistry & Molecular Biology of Plants, 1250-1318.
6. Sharma S and S Lata. Ethnobotanical importance of Asteraceae plants among Tharu tribe in Udham Singh Nagar, Uttarakhand, India. Ethnobotany Research and Applications, 2022; 23(34): 1-17.
7. Rahman M AHM and Easmin F. Asteraceae: A taxonomically and medicinally important sunflower family. American International Journal of Biology and Life Sciences, 2021; 3(1): 1-18.
8. World Health Organization, Quality control methods for medicinal plant material, WHO Library.
9. Kokate CK, Practical Pharmacognosy, fourth edition, Vallabh Prakashan, Delhi, 2001, 108-111,125.
10. Trease. GE & Evans WC, Pharmacognosy, Bailleire tindall East bourne. UK.
11. Mestry D, Dighe V. Pharmacognostic evaluation and preliminary phyto-chemical screening of the dried powder of stem bark of *Mimusops elengi* Linn. and leaf of *Jasminum sambac* Ait. Journal of Pharmacognosy and Phytochemistry. 2013; 2(4):107-12.
12. Adhyapak S and Dighe V. Investigation of Microscopical and physicochemical characteristics of plants with anti-diabetic activity. International Journal of Pharmaceutical Science & Research. 2014; 5(3):882-88.
13. Evans WC. Trease and Evans Pharmacognosy. 15th edition, London, United Kingdom: Saunders, 2002, p. 245-47.
14. Committee BHMAS. British Herbal Pharmacopoeia: 1996; British Herbal Medicine Association; 1996.
15. Denston, TC. "A textbook of Pharmacognosy". Sir Isaac Pitman & Sons, Ltd, London, 1946, p. 46-51.
16. Tyler VE, Brady LR and Robbers JE. "Pharmacognosy". Lea & Febiger, Philadelphia, 1976, p. 24.
17. Har Harborne JB, Phytochemical method, A Guide to Modern technique of Plant Analysis. 3rd Edition, Chapman and Hall. New York, 1998.
18. Kokate CK. A textbook of Practical Pharmacognosy. 5th Edition, Vallabh Prakashan New Delhi, 2005, 107-111.
19. Ghosh MN, Fundamental of Experimental Pharmacology, 2nd edition, Scientific Book Agency, Calcutta, 1998, 5-32.

20. Wagner H, Bladet S *et. al.,* Plant Drug Analysis-A TLC Atlas, 1st Edition, Springer verlag Berlin, Heidel berg, New York, 1996; 195-214.

21. Handa SS, Vasisht K, *et. Al*, Compendium of Medicinal and Aromatic Plants-Asia, II, ICS-UNIDO, Area Science Park, Padriciano, Trieste, Italy, 2006; 79-83.