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Pharmacognostic Evaluation, Preliminary Phyto-Chemical Screening and Antioxidant Potentiality of Leaf of *Lactuca dissecta* D.Don

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ABSTRACT

In our country India in mountainous places & Uttar Pradesh, Himanchal Pradesh plains, Arunachal Pradesh and Jammu and Kashmir 24 Lactuca genus species have been outlined. Genus Lactuca contains different chemical constituents and they are used in traditional medicine systems for curing various ailments as such pain, appetite lacking, bronchitis and UTI inflammation. This particular genus contains lactones, sesquiterpenes, germacranolides, guaianolides, tannins, lignans and flavonoids, phenolic acid and flavones. **Material and Methods:** Sample of the *Lactuca dissecta* D. Don was graded for microscopical and macroscopical investigation. Various physicochemical specifications were evaluated and computed and initial phytochemical verification accompanied by various solvents such as acetone, ethyl acetate, methanol, petroleum ether, and extract which is aqueous of plant leaf were performed out in concertation accordance to polarity. Powdered leaf material of plant was examined with various chemicals in the laboratory utilized for examination of leaf microscopy which was powdered and also exposed for process of fluorescence analysis below light UV of long and short wave length and visible light. Apart from antioxidant capability of leaf extract was also computed through DPPH assay. **Results:** The transverse section of the leaf part shows epidermal cells showed anomocytic and anisocytic stomata in equal proportion. These cells contain wavy walls. Epidermal cells are straight walled and less wavy towards the coastal region. Epidermis shows non glandular, multicellular trichome having single basal cells. The leaf is dorsiventral, contains mesophyll, and it consists of upper and lower palisade cells in one to three rows. Spongy parenchyma which was 3-4 was covered with cuticles and single layered epidermal cells. Mesophyll tissue contains chloroplast with few calcium oxalate crystals. The transverse section also shows lamina and central vascular bundles. Different physicochemical computation and determinations such as loss on drying, extractive value, ash value, foaming and swelling index were determined for leaf part. For powdered crude drug various scale of fluorescence color were investigated by Fluorescence analysis. Phytochemical diagnosis shows the existence of Phyto- constituents including tannins, flavonoids, saponins, steroids alkaloids, and proteins. The Antioxidant capability was resoluted by the assistance of the method of DPPH assay in that methanol and ethyl acetate extract of the leaf showed highest antioxidant effect.

INTRODUCTION

The word herbal medicine indicates the utilization of therapeutic plants for cure and treatment of different ailments which extends from conventional and commercial medicines of each country. Around 80 % people all over the world are believing on herbal medicine over the past 3 decades as supplements and herbal based medicinal supplements have tremendously increased[1]. In India use of herbal medicine is mentioned in the different system of health viz. yoga, Siddha, Unani, Ayurveda, Naturopathy and Homeopathy which are continuous in use and are safe. Most of the population in India use conventional drugs on regular basis as home-remedies, as spices, as self-medication, as health food as well as over the counter or as drug mentioned in the various nonallopathic systems[2]. Basically, herbal remedies are derived from plants and occur naturally and are used to treat ailments or diseases with regional or local healing applications. These compounds or row morphological part of a herb having organic chemicals with complex nature[3]. In case of Angiosperms i.e flowering plants Asteraceae is the bigger family as it contains about 24000 species belonging to about 1600 genera and are trees, herbs and shrubs. In almost all habitats the plant exists and its distribution is cosmopolitan. Lactuca genus contains herbs either perennial or annual, shrubs, rarely trees as well as climbers[4]. Uttarakhand state of India has environmental beauty which is distinctive with wealthy herb diversity which is extended from north where snow zones located to in south Terai belt. In foot hills of Himalayan region low land area Terai is located as it grasp plenty of surface water. Uttarakhand is famous and known as “Land of Gods”. Aside from wealthy herb resources, Uttarakhand possesses different tribal neighborhood as well as ethnobotanical heritage. Asteraceae previously known as Compositae is commonly known as daisy or sunflower, thistle family containing around 370 plant species belonging to around 134 genera which constitute a big dicot family in Kumaun and Garhwal regions of Uttarakhand. A large quantity of plants which are Asteraceae family based are utilized as healer, food and other purpose all around the world[5]. Asteraceae is a family that is economically important as it gives different products which contains cooking oils lettuce, coffee alternatives, sunflower seeds, sweeteners, artichokes, and herbal tea[6].

MATERIALS AND METHODS

PLANT COLLECTION AND AUTHENTIFICATION

Specimens of morphological plants were gathered from field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand during 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 about consistency, color, taste or odor, through the identification, it is regarded that it is not confirming the official necessities. Macroscopical reorganization 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[7].

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 authentication 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. A 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 was 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 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 that 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 the solvent was 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 were 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 evaporator[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 solvent. Extracts which were having negative and positive controls were tested for the identification of chemical constituents viz: alkaloids, tannins, triterpenoids, phytosterols, flavonoids, 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). Using 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 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 were performed in triplicate and the compound which was used as standard was ascorbic acid[18, 19, 20].

RESULT AND DISCUSSION

PLANT AUTHENTIFICATION

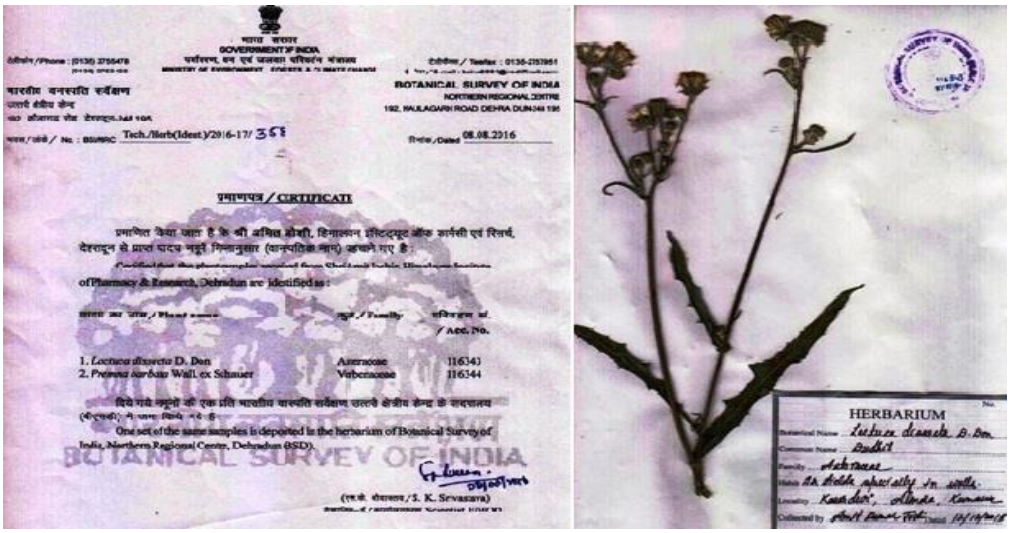


Figure 1: Plant authentication certificate and herbarium specimen of *Lactuca dissecta* D.Don

The unknown sample of plant was collected from the field areas, forest, and adjacent region of Kasardevi, Almora Uttarakhand throughout 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

MICROSCOPICAL EVALUATION

Microscopical Evaluation of *Lactuca dissecta* D.Don

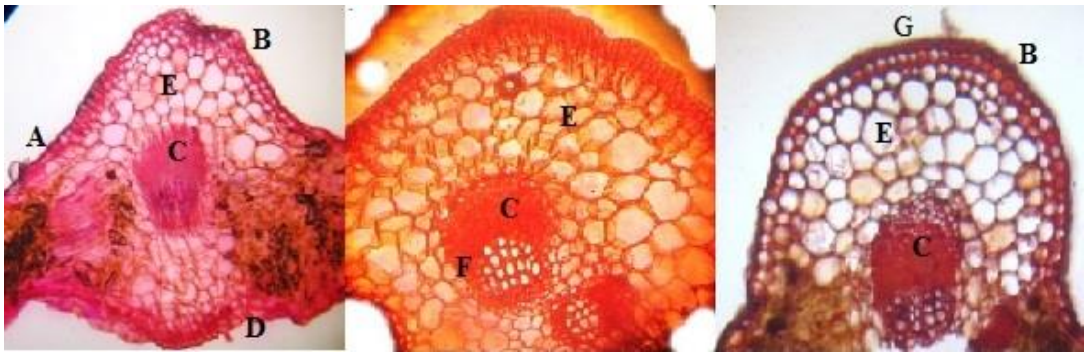


Figure 3: T. S of *Lactuca dissecta* D.Don leaf showing glandular trichome, lower epidermis, vascular bundles, collenchyma's, phloem, uniseriate trichome

Epidermal cells showed anomocytic and anisocytic stomata in equal proportion. These cells contain wavy walls. Epidermal cells are straight-walled and less wavy towards the coastal region. Epidermis shows non glandular, multicellular trichome having single basal cells. The leaf is dorsiventral, contains mesophyll, and it consists of upper and lower palisade cells in one to three rows. Spongy parenchyma which was 3-4 was covered with cuticles and single layered epidermal cells. Mesophyll tissue contains chloroplast with few calcium oxalate crystals. Transverse section also shows lamina and central vascular bundles. Bicolateral vascular bundles were present which have 2 to 5 rows thick sclerenchyma cells. Lower meta-xylem and upper meta-xylem compose xylem. Phloem is present in both sides of lower and upper portion of xylem.

POWDER MICROSCOPY

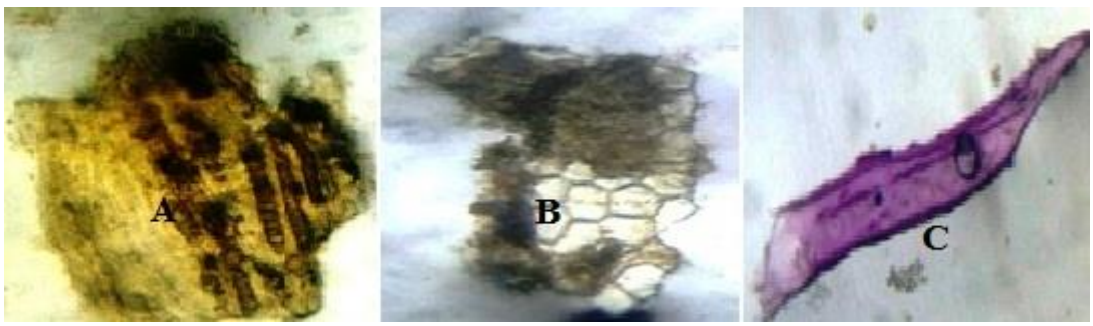


Figure 4: Powder photomicrograph of *Lactuca dissecta* D.Don leaf showing xylem vessels, epidermal cells and covering trichome

Color of the leaf was light green, which was slightly spongy and lightly salty with no odor. When treated with safranin, water, chloral hydrate and various staining agents such as phloroglucinol + HCl, iodine solution, ruthenium red, sudan red etc. The powder characteristic of the leaf shows vascular bundles with simple fibers. Spongy parenchyma along with chloroplast cells was present in the lower portion of mesophyll. Leaves upper portion contains a covering trichome.

DETERMINATION OF LEAF CONSTANTS

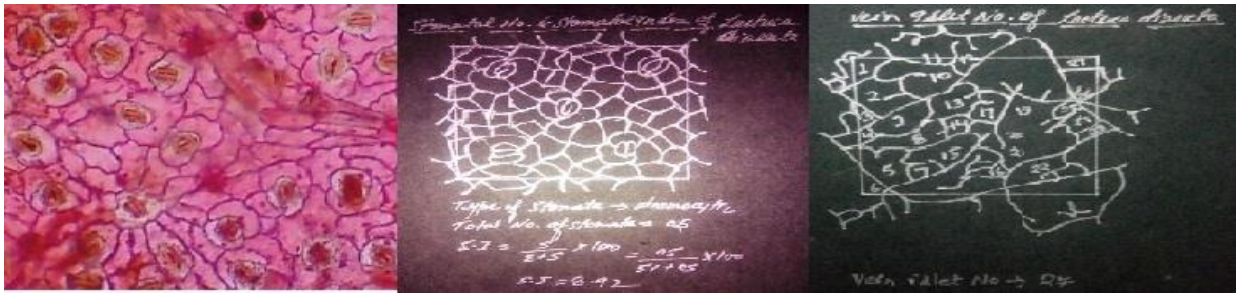


Figure 5: Leaf constant parameters of *Lactuca dissecta* D.Don leaf mentioning stomata type, index of stomata and vein islet number

The stomata type present was anisocytic, values of stomatal index, stomatal number, and vein islet number of upper and lower epidermis of leaf was calculated and results were tabulated in below mentioned table.

Table no. 1: Stomatal no. and stomatal index of *Lactuca dissecta* D.Don leaf

S. No.	Surface Type	No. of stomata (per mm^2)	Epidermal cells total in number	Index of Stomata (I= S÷E+S x 100) (value in $1mm^2$ area)	Vein islet no. (value in $1mm^2$ area)
1	Lower	34	146	23.287	18
		37	117	24.025	16
		35	105	25.000	19
	Mean ± S.E.M	35.333 ± 0.881		24.104 ± 0.496	17.666 ± 0.881
2	Upper	52	156	23.960	21
		54	172	23.893	24
		51	178	22.869	22
	Mean ± S.E.M	52.333 ± 0.879		23.574 ± 0.353	22.332 ± 0.784

PHYSICO-CHEMICAL PARAMETERS

Physicochemical parameters of *Lactuca dissecta* D.Don leaf

Figure 6: Physico-chemical parameters of *Lactuca dissecta* D.Don leaf mentioning type loss on drying, ash value, swelling index and foaming index

Loss on Drying

The value of loss on drying for the leaf sample of *Lactuca dissecta* D.Don was found to be 6.312%.

Table no. 2: Loss on drying of *Lactuca dissecta* D.Don leaf powder

S. No.	Drug wt. + porcelain dish before drying A (g)	Drug wt. + porcelain dish after drying B (g)	Loss on drying A-B (g)	% of loss on drying
1	10.014 + 42.636	51.946	0.704	7.030
2	10.010 + 40.913	50.323	0.600	5.994
3	10.011 + 44.370	53.789	0.592	5.913
Mean ± S.E.M (n=3)				6.312 ± 0.359

Ash Value

The values of ash i.e. Total ash, water-soluble ash and acid insoluble ash were evaluated as per official process. The 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 leaf was calculated as 5.761%. 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 1.022%. 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 1.539%. The results were found to be almost within limits.

Table no. 3: Total ash value of *Lactuca dissecta* D.Don leaf powder

S. No.	Drug wt. (g)	Wt. of empty china dish (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of total ash
1	3.017	18.045	21.062	0.178	5.899
2	3.011	17.094	20.105	0.169	5.612
3	3.013	16.933	19.946	0.174	5.774
Mean ± S.E.M (n=3)					5.761 ± 0.083

Table no. 4: Acid insoluble ash value of *Lactuca dissecta* D.Don leaf powder

S. No.	Drug wt. (g)	Wt. of empty china dish (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of acid-insoluble ash
1	3.033	18.045	18.266	0.027	0.890
2	3.029	17.821	18.017	0.032	1.056
3	3.030	17.786	17.998	0.034	1.112
Mean ± S.E.M (n=3)					1.022 ± 0.069

Table no. 5: Water soluble ash value of *Lactuca dissecta* D.Don leaf powder

S. No.	Drug wt. (g)	Wt. of empty china dish (g)	Crucible wt. + Wt. of ash (g)	Ash Wt. (g)	% of total ash
1	3.029	18.238	18.500	0.044	1.452
2	3.031	17.986	18.233	0.047	1.550
3	3.033	17.119	17.398	0.049	1.615
Mean ± S.E.M (n=3)					1.539 ± 0.082

Extractive Value

The extractive value of *Lactuca dissecta* D. Don leaf 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, water-soluble extract shows maximum extractive value of 7.305% while extractive value of methanol was 1.798% which was lower as compared to other extracts. In case of cold extraction method methanol soluble extract shows the peak extractive value of 7.244% while chloroform was less effective with extractive value of 2.489% as compared to other solvent extracts. The results are tabulated as follows:

Table no. 6: Petroleum ether soluble extractive value of *Lactuca dissecta* D.Don leaf 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	4.0013	58.3219	58.3751	0.0532	5.318
2	4.0009	59.7123	59.7714	0.0591	5.908
3	4.0011	58.5261	58.5772	0.0511	5.108
Mean ± S.E.M (n=3)					5.444 ± 0.239

Table no. 7: Chloroform soluble extractive value of *Lactuca dissecta* D.Don leaf 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	4.0021	56.3417	56.3746	0.0329	3.288
2	4.0013	57.0312	57.0657	0.0345	3.448
3	4.0009	57.0047	57.0344	0.0297	2.969
Mean ± S.E.M (n=3)					3.235 ± 0.140

Table no. 8: Ethyl acetate soluble extractive value of *Lactuca dissecta* D. Don leaf 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	4.0007	57.0423	57.1985	0.0662	6.618
2	4.0003	56.8621	57.0340	0.0681	6.804
3	4.0005	56.4532	56.6054	0.0622	6.122
Mean ± S.E.M (n=3)					6.514 ± 0.203

Table no. 9: Methanol soluble extractive value of *Lactuca dissecta* D. Don leaf 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	4.0028	58.1253	58.1445	0.0192	1.918
2	4.0013	59.0149	59.0324	0.0175	1.749
3	4.0019	58.3421	58.3594	0.0173	1.729
Mean ± S.E.M (n=3)					1.798 ± 0.059

Table no. 10: Water soluble extractive value of *Lactuca dissecta* D. Don leaf 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	4.0006	58.2178	58.2909	0.0731	7.308
2	4.0002	59.0523	59.1292	0.0769	7.689
3	4.0005	58.5427	58.6119	0.0692	6.919
Mean ± S.E.M (n=3)					7.305 ± 0.222

Table no. 11: Petroleum ether soluble extractive value of *Lactuca dissecta* D. Don leaf 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	4.0123	51.2176	51.2632	0.0456	4.546
2	4.0112	52.3489	52.3986	0.0497	4.956
3	4.0099	54.2389	54.2815	0.0426	4.608
Mean ± S.E.M (n=3)					4.703 ± 0.127

Table no. 12: Chloroform soluble extractive value of *Lactuca dissecta* D. Don leaf 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	4.0039	57.2134	57.2368	0.0234	2.337
2	4.0124	56.7632	56.7903	0.0271	2.701
3	4.0012	57.0129	57.0372	0..0243	2.429
Mean ± S.E.M (n=3)					2.489 ± 0.109

Table no. 13: Ethyl acetate soluble extractive value of *Lactuca dissecta* D. Don leaf 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	59.2136	59.2668	0.0532	5.309
2	4.008	58.9478	58.9990	0.0512	5.109
3	4.009	59.1003	59.1570	0.0567	5.657
Mean ± S.E.M (n=3)					5.358 ± 0.160

Table no. 14: Methanol soluble extractive value of *Lactuca dissecta* D. Don leaf 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	4.004	56.4523	56.5242	0.0719	7.182
2	4.006	56.7843	56.8566	0.0723	7.219
3	4.004	57.1023	57.1757	0.0734	7.332
Mean ± S.E.M (n=3)					7.244 ± 0.045

Table no. 15: Water soluble extractive value of *Lactuca dissecta* D. Don leaf 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	58.2367	58.3029	0.0662	6.618
2	4.003	57.8732	57.9355	0.0623	6.225
3	4.001	58.2136	58.2779	0.0643	6.428
Mean ± S.E.M (n=3)					6.423 ± 0.113

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 that 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 leaf was found to be 6.333. The results are tabulated as follows:

Table no. 16: Swelling index value of *Lactuca dissecta* D.Don leaf powder

S.No	Powdered drug weight (gm)	Stock Volume (in ml)	Swelling factor
1	1.0	25	6.1
2	1.0	25	6.5
3	1.0	25	6.4
Mean± S.E.M (n=3)			6.333 ± 0.120

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 height in every test tube was measured below 1cm. So the foaming index of *Lactuca dissecta* D. Don leaf was found to be more than thousand indicating good amount or presence of saponins. The results are tabulated as follows:

Table no. 17: Foaming index value of *Lactuca dissecta* D. Don leaf 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	1.2	1.3	1.6	1.4	1.7	1.9	1.5	2.1	2.3	2.7	≤ 1000
2	1.0	100	1.4	1.6	1.2	1.6	1.8	1.7	1.9	2.2	2.4	2.1	≤ 1000
3	1.0	100	1.1	1.5	1.3	1.3	1.7	1.9	2.0	2.1	2.4	2.7	≤ 1000
Mean± S.E.M (n=3)													1000 ±0.000

FLUORESCENCE ANALYSIS OF POWDERS

The powder (mesh size 40) of leaf 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 no. 18: Fluorescence analysis of *Lactuca dissecta* (leaf)

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

EXTRACTION OF PLANT MATERIAL

500 gm coarse powder of leaf 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 for around 3 hrs per solvent. Results are tabulated in below mentioned table.

Table no. 19: Data showing successive solvent extraction values and nature of extract of *Lactuca dissecta D. Don* leaf

S. No	Solvent Used	Wt. of drug (gm)	Yield (gm)	% Yield	Extract color	Property	Mean ± S.E.M
1	Pet. ether	120	3.457	2.955	Dark Green	Slightly sticky	2.889 ± 0.053
			3.384	2.820			
			3.423	2.852			
2	Chloroform	120	1.672	1.393	Light green	Slightly amorphous	1.418 ± 0.037
			1.791	1.492			
			1.645	1.370			
3	Acetone	120	2.416	2.013	Light green	Sticky	1.976 ± 0.022
			2.323	1.935			
			2.378	1.981			
4	Ethyl acetate	120	5.245	4.370	Greenish Black	Slight sticky	4.492 ± 0.093
			5.319	4.432			
			5.612	4.676			
5	Methanol	120	8.017	6.680	Greenish Brown	Sticky	6.732 ± 0.026
			8.102	6.751			
			8.119	6.765			
6	Water	120	10.104	8.420	Light Green	Sticky	8.448 ± 0.032
			10.216	8.513			
			10.094	8.411			

QUALITAIVE PHYTOCHEMICAL ANALYSIS

The extracts of leaves of *Lactuca dissecta* D. Don when tested with various solvent extracts of different polarity show the existence of different chemical constituents such as alkaloids, flavonoids, tannins, saponins, steroids and proteins as secondary metabolites.

Table no. 20: Preliminary phytochemical investigation of various extracts of *Premna barbata* Wall. Ex Schauer leaves

Chemical Constituents	Tests	PEPB	CFPB	ACPB	EAPB	MEPB	WAPB
Alkaloids	Mayer's test	-ve	-ve	-ve	-ve	-ve	+ve
	Wagner's test	-ve	-ve	+ve	-ve	+ve	-ve
	Hager's test	-ve	+ve	-ve	-ve	+ve	-ve
	Dragendroff's test	-ve	-ve	-ve	-ve	-ve	+ve
Flavanoids	Alkaline reagent test	+ve	-ve	+ve	+ve	+ve	+ve
	Shinoda test	-ve	+ve	+ve	-ve	-ve	+ve
Tannins	Ferric Chloride test	+ve	-ve	-ve	-ve	-ve	+ve
	Lead acetate test	+ve	-ve	-ve	-ve	+ve	-ve
Cardiac glyco.	Keller Killiani test	-ve	-ve	-ve	-ve	-ve	-ve
	Legal Test	-ve	-ve	-ve	-ve	-ve	-ve
Anthraquinones	Borntrager's test	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	Foam test	+ve	+ve	-ve	+ve	-ve	+ve
Steroids	Salkowski test	-ve	-ve	+ve	+ve	+ve	-ve
	Liebermann Burchard	+ve	-ve	-ve	-ve	+ve	+ve
Proteins	Biuret test	-ve	-ve	-ve	-ve	+ve	-ve
	Millon's test	-ve	+ve	-ve	-ve	+ve	-ve
	Xanthoproteic test	-ve	-ve	+ve	-ve	-ve	-ve

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

THIN LAYER CHROMATOGRAPHIC STUDIES

TLC profile of *Lactuca dissecta* D. Don revealed the chloroform, methanol and water extracts of leaves, and methanolic and aqueous extracts of leaves gave an excellent observation and results which was directing towards the confirmation of various chemical constituents. At various solvent systems chemical constituents gave different value of R_f.

In the variation of R_f in secondary metabolites gives important observation and rule for observing their polarity and for suitable selection of particular solvent system which can be used by column chromatography for the, dissociation of natural compounds which are pure.

With different polarity at various ratios of solvent mixture is used for compounds which is to be separated and is pure can be further isolated from plant extract. Using various solvent systems compounds R_f value was calculated by the selection of a suitable system of solvent for a peculiar plant extract. The results for R_f values of leaf extracts are tabulated in table no.

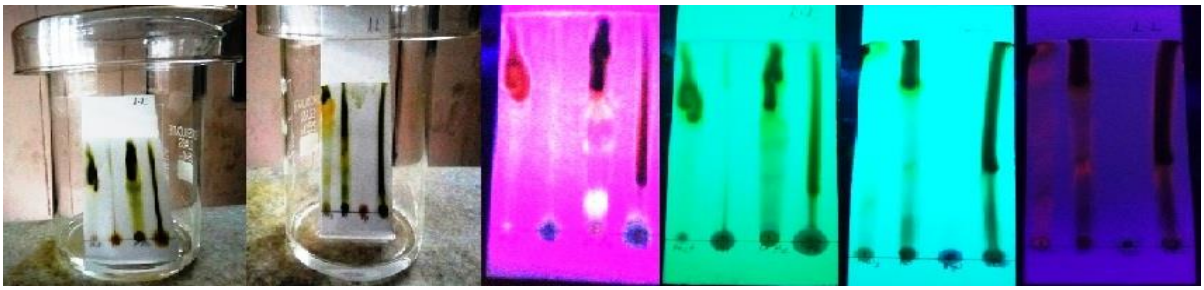


Figure 7: TLC profile of *Lactuca dissecta* D.Don leaf

Table no. 21: TLC solvent system along with spraying reagent and R_f value for determination of secondary metabolites in *Lactuca dissecta* D. Don leaf

S. No	Secondary metabolites	Solvent System	Spraying reagent	Extract type	R_f Value
1	Alkaloids	EtOAc: $CHCl_3$: H_2O (5:3:1)	Mayer's reagent	Chloroform	0.34, 0.38,0.46
				Methanol	0.27, 0.31, 0.35
				Water	0.32, 0.31, 0.41
2	Flavanoids	Butan-1ol: EtOAc: H_2O (5:10:15)	3 % boric acid & 10 % oxalic acid	Chloroform	0.52, 0.57, 0.62
				Methanol	0.48, 0.53, 0.55
				Water	0.41, 0.39, 0.47
3	Tannins	$CHCl_3$: H_2O (6:4)	Ferric Chloride	Chloroform	0.37, 0.31, 0.38
				Methanol	0.45, 0.47, 0.59
				Water	0.56, 0.51, 0.62
4	Saponins	$CHCl_3$: MeOH: H_2O (60:30:4)	Antimony trichloride	Chloroform	0.24, 0.27, 0.31
				Methanol	0.41, 0.43, 0.47
				Water	0.52, 0.56, 0.59
5	Steroids	1-Hexanol: PET:AcOH (65:35:1)	3 % Sulphuric acid	Chloroform	0.17, 0.19, 0.24
				Methanol	0.47, 0.52, 0.57
				Water	0.26, 0.29, 0.24
6	Amino acids	Butan-1ol: AcOH: H_2O (3:1:1)	Ninhydrine Reagent	Chloroform	0.35, 0.45, 0.39
				Methanol	0.27, 0.32, 0.38
				Water	0.57, 0.61, 0.54

ANTIOXIDANT ACTIVITY (FREE RADICAL SCAVENGING ACTIVITY USING DPPH)

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

In-vitro antioxidant activity of various extract of *Lactuca dissecta* D.Don leaf 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. The 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 17.296, 19.592, 18.678 and 22.345 respectively in leaf case extract respectively. It means that ethyl acetate and acetone extract of leaf extract of crude plant material at elevated concentration encapsulate increase number of free radicals produced by DPPH resulting into decrease in absorbance and elevation in IC_{50} value. Results are tabulated as:

Results are tabulated as:

Table no. 22: 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 no. 23: Percentage scavenging activity of methanolic extract of *Lactuca dissecta* leaf				
S. No.	**Concentration (µg/ml)**	**Absorbance at 517 nm**	**% Inhibition**	**Mean ±S.E.M. (n=3)**
1	2	0.267, 0.262, 0.265	9.79, 11.48, 10.47	10.58 ± 0.491
2	4	0.260, 0.256, 0.259	12.16, 13.51, 12.50	12.72 ± 0.405
3	6	0.252, 0.250, 0.254	14.86, 15.54, 14.18	14.86 ± 0.392
4	8	0.234, 0.240, 0.237	20.94, 18.91, 19.93	19.92 ± 0.586
5	10	0.212, 0.217, 0.215	28.37, 26.68, 27.36	27.47 ± 0.491
6	12	0.184, 0.189, 0.183	37.83, 36.14, 38.17	37.38 ± 0.627

Table no. 24: Percentage scavenging activity of acetone extract of *Lactuca dissecta* leaf				
S. No.	**Concentration (µg/ml)**	**Absorbance at 517 nm**	**% Inhibition**	**Mean ±S.E.M. (n=3)**
1	2	0.261, 0.259, 0.257	11.82, 12.50, 13.17	12.49 ± 0.389
2	4	0.256, 0.254, 0.255	13.51, 14.18, 13.85	13.84 ± 0.193
3	6	0.248, 0.244, 0.245	16.21, 17.56, 17.22	16.99 ± 0.405
4	8	0.234, 0.239, 0.233	20.94, 19.25, 21.28	20.49 ± 0.627
5	10	0.219, 0.223,0.221	26.01, 24.66, 25.33	25.33 ± 0.398
6	12	0.204, 0.206,0.203	31.08, 30.40, 31.41	30.96 ± 0.297

Table no. 25: Percentage scavenging activity of ethyl acetate extract of *Lactuca dissecta* leaf				
S. No.	**Concentration (µg/ml)**	**Absorbance at 517 nm**	**% Inhibition**	**Mean ±S.E.M. (n=3)**
1	2	0.252, 0.254, 0.252	14.86, 14.18, 14.86	14.63 ± 0.226
2	4	0.230, 0.232, 0.235	22.29, 21.62, 20.60	21.50 ± 0.419
3	6	0.224, 0.224, 0.221	24.32, 24.32, 25.33	24.65 ± 0.336
4	8	0213, 0.215, 0.216	28.04, 27.36, 27.02	27.47 ± 0.299
5	10	0.210, 0.207, 0.209	28.08, 30.06, 29.39	29.17 ± 0.581
6	12	0.199, 0.192, 0.194	32.77, 35.13, 34.45	34.11 ± 0.701

Figure no. 8: Comperative % Inhibition vs Concentration of different extracts of *Lactuca dissecta* leaf

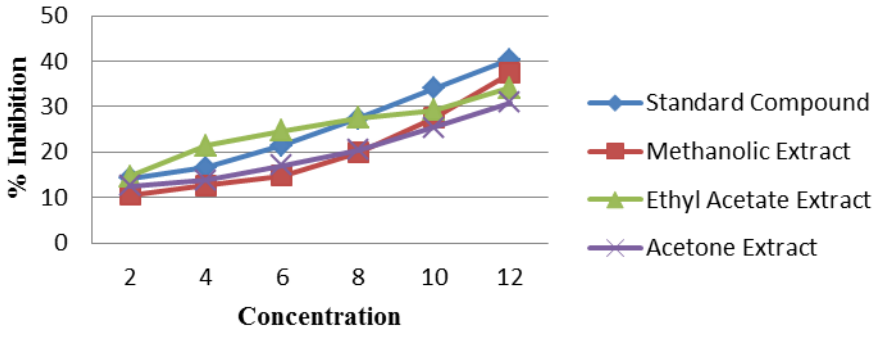


Figure no. 9: IC_{50} calibration curve of ascorbic acid

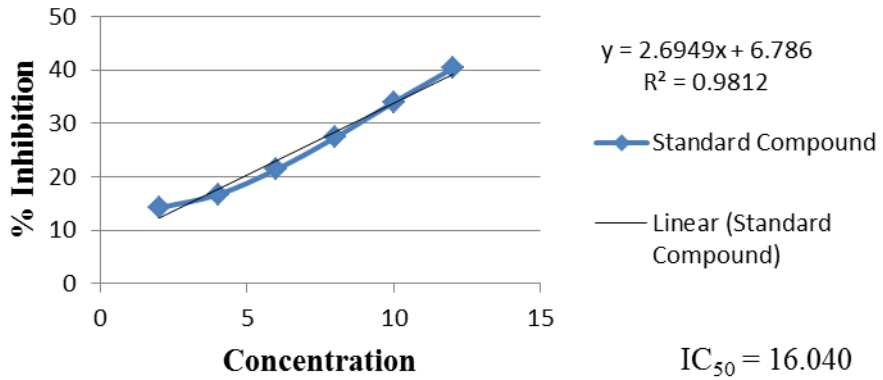


Figure no. 10: IC_{50} calibration curve of methanol extract

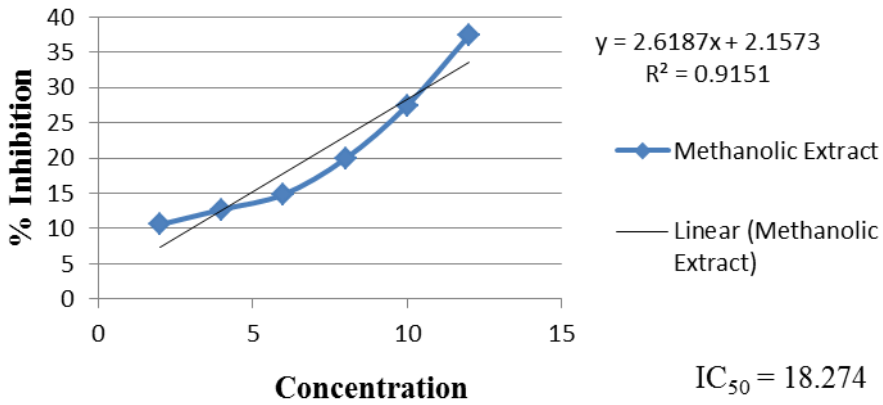
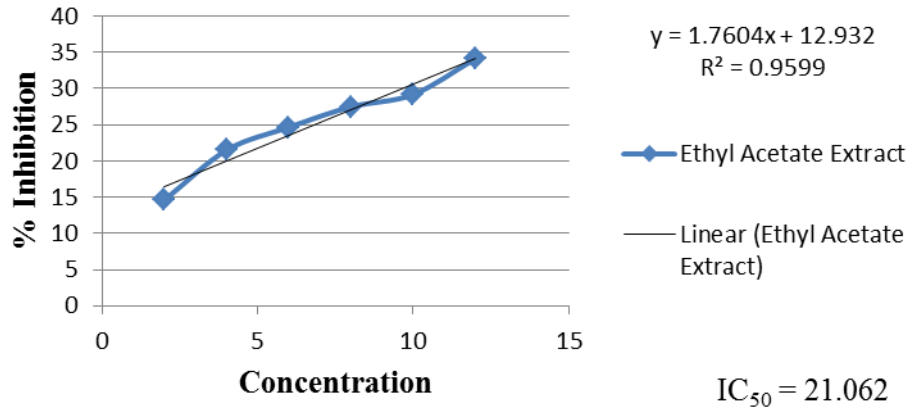


Figure no. 11: IC_{50} calibration curve of ethyl acetate extract



DISCUSSION

The present investigation mainly focus point to investigate the greenery variety of the Garhwal and Kumaun region representing Uttarakhand for their rehabilitation value. In present study the leaf part of *Lactuca dissecta* D. Don was researched and calculated for its healing properties which were determined in conventional medicinal system. In present research examination it is totally concentrated on the knowledge of database for curing plants in terms of its microscopic, organoleptic, phytochemical and physicochemical, as well as potency of antioxidant of *Lactuca dissecta* D. Don leaf. Pharmacognostic authentication represented that the leaf shape is Sagittate with Wavy & Serrate margin with Wavy & Serrate margin and venation Pinnate having 5 to 10cm long & 1 to 2cm wide in size, the color of the leaf is light green without any odor having Acrid & bitter taste. Transverse section of leaf showed epidermal cells showed having anomocytic and anisocytic stomata in equal proportion. These cells contain wavy walls. Epidermal cells are straight walled and less wavy towards the coastal region. Epidermis shows non glandular, multicellular trichome having single basal cells. The leaf is dorsiventral, contains mesophyll, and it consists of upper and lower palisade cells in one to three rows. Spongy parenchyma which was 3-4 was covered with cuticles and single layered epidermal cells. Mesophyll tissue contains chloroplast with few calcium oxalate crystals. Transverse section also shows lamina and central vascular bundles. Bicolateral vascular bundles were present which are having 2 to 5 rows thick sclerenchyma cells. Lower meta-xylem and upper meta-xylem composes xylem. Phloem is present in both sides of lower and upper portion of xylem.

Preliminary phytochemical analysis of leaf gives an idea for the presence of some chemical constituents such lactones, sesquiterpenes, germacranolides, guaianolides, tannins, lignans and flavonoids, phenolic acid, and flavones which in further will have important character in the role making of traditional medicine. Antioxidant activity of the extract of leaf showed ethyl acetate and acetone extract of plant contains potent antioxidant activity. As not so much experimental work is performed and mentioned in journals for research on this herb, the outcomes of result from this present research be employed as a standard in future innovative investigation of this herb. Also, chemical constituents extracted from this plant will be used as an important beginning of information and discovery and indicated quality of the plant in medicinal studies and pharmaceutical by providing suitable parameters of standard.

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