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# Preliminary Physiochemical and Phytochemical Profile of *Leonotis nepetaefolia*

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## Abstract

Herbal medicine has many active constituents for many diseases but the proper knowledge must be necessary for the preparationof herbal formulation otherwise active constituents will be damaged. *Leonotis nepetifolia* (L.) is recognized to be native to tropical Africa and southern India. In South Africa and the West Indies it is known as klip dagga, lion's ear, Christmas, and candlesticks. Roots of the plant have been used in the treatment of asthma and bronchitis, fever, and poisoning whereas seeds are recognized in treating burns, and the whole plant is used for menstrual pains. Present investigation includes examination of morphological and microscopic characters; ash value, extractive values and phytochemical evaluations of various root extract. The purpose of research work to generated information of the standardization parameters of selected plant which helps in herbal formulations, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance.

**Keywords:** *Leonotis nepetifolia, Physiochemical parameter & Phtochemical analysis*.

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## INTRODUCTION

Standardization plays a momentous role in the production of phytopharmaceutical of standard quality as the quality standards are based on proper selection of raw materials. As very little specific standards are mentioned in the official monographs evaluation of the crude drugs is of great consequence for the pharmaceutical industry. This involves the determination of identity and purity of quality. Many organic & inorganic contaminants which are virtually impossible to avoid while collecting crude drugs affect the purity of any crude drug which needs proper assessment & detection based on different pharmacognostic & phytochemical parameters [1]. Leonotis nepetifolia is also known as Klip dagga, Christmas candlestick, or lion's ear, is a species of plant in the genus Leonotis and the family Lamiaceae (mint) [2]. The plant is claimed to be used in pain, inflammation, microbial infection, as contraceptives and gynecological disorders.

*Leonotis nepetifolia* plant

**Botanical Description [3, 4]**

| | |
|---|---|
| **Kingdom** | Plantae |
| **Division** | Angiosperms |
| **Class** | Eudicots |
| **Subclass** | Asterids |
| **Order** | Lamiales |
| **Family** | Lamiaceae |
| **Genus** | Leonotis |
| **Species** | Nepetifolia |

## MATERIAL AND METHODS

### Collection and Authentication

The plant parts viz., LNR: *Leonotis nepetaefolia* (L.) R.Br. (Roots) was collected in the months of October 2020 from the various local sites of Malwa region of Madhya Pradesh and identified & authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. JBot/LNR-12 & JBot/LNR-13 was allotted.

### Pharmacognostical study

Various microscopy characters were studied by observing the root.

### Drying and size reduction of plant material:

Root were cut into small pieces, cleaned and shade dried. Then, the root was subjected to physical evaluation with different parameter. The parameters which were used for evaluation are nature color, taste, size, shape, width and length. Finally the root were subjected to size reduction to get coarse powder and then passed through sieve no. - 40 to get uniform powder. Then uniform powder was subjected to standardization with different parameters.

## PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

### Determination of solvent extractive values:

**Determination of water soluble extractive value:** 5 g of the air-dried drug, coarsely powdered were macerated with 100 ml of water in closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. It was filtered rapidly taking precaution against loss of water, then the filtrate was evaporated 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, and dried at $105^0$C then weighed. The percentage of water-soluble extractive with reference to the air dried was calculated.

**Determination of alcohol soluble extractive value:** 5 gm of the air dried and coarsely powdered drug was macerated with 100 ml of ethanol of the specific strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. There after filter rapidly taking precaution against loss of ethanol. Evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dry at $105^0$c and weigh. The percentage of ethanol soluble extractive with reference to the air dried drug has to be calculated.

**Determination of moisture content:** Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Method of determination of moisture content include the loss on drying, the test for loss on drying determines both water and volatile matter in the crude drug. It can be carried out either by heating at $100^0$C-$105^0$C or in a dessicator over phosphorous pentoxide under atmospheric or reduced pressure at room temperature for specific period of time.

**Ash value:** Ash value is helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in case where silica may be present or when the calcium oxalate content of the drug is very high.

**Total ash value:** Weighed accurately about 2 to 3 g of the powdered drug in a tared silica crucible. Incinerated at a temperature not exceeding 450 $^0$C for 4 hr, until free from carbon, cooled and weighed. The percentage of ash with reference to air-dried was calculated following formula.

$$\% \text{ Total ash value} = \frac{\text{Wt. of total ash}}{\text{Wt. of crude drugs}} \times 100$$

**Water soluble ash value:** Boiled the ash with 25 ml of water. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 $^0$C for 4 hr. Cooled in a desiccator and weighed. Substrate the weight of insoluble matter from the total weight of ash. The difference in weight represented weight of water soluble ash. Calculated the percentage

of water soluble ash with reference to the air- dried drug by using the following formula.

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of total ash-Wt.of water insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

**Acid insoluble ash value**: Boiled the ash for 5 min with 25 ml of 2 M HCL. Filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited in a tared crucible at a temperature not exceeding 450 $^0$ C for 4 h. cooled in a desiccator and weighed. Calculated the percentage of acid insoluble ash with reference to the air -dried drug was calculated by using following formula,

$$\% \text{ Acid insoluble ash value} = \frac{\text{Wt. of acid insoluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

**Preliminary qualitative test**

The various extract of *Desmodium gangeticum* was subjected to preliminary qualitative phytochemical investigation. The various tests and reagent used are given below.

**Alkaloids**

**Preparation of test solution:** The test solution was prepared by dissolving extracts in the dilute hydrochloric acid.

**Mayer' test:** The acidic test solution with Mayer's reagent (Potassium Mercuric iodide) gave cream colored precipitate.

**Hager's test:** The acidic test solution with Hager's reagent (Saturated picric acid solution) gave yellow precipitate.

**Dragendorff's test:** The acidic solution with Dragendorff's reagent (Potassium bismuth iodide) showed reddish brown precipitate.

**Wagner's test:** The acidic test solution treated with Wagner's reagent (Iodine in potassium iodide) gave brown precipitate.

**Tannic acid test:** The acidic test solution treated with Tannic acid gave buff colour precipitate.

**Picrolonic acid test:** Alkaloids gave yellow colour precipitate with picrolonic acid.

**Amino acid:**

**Millon'test:** To the test solution add about 2 ml of millon's reagent white precipitate indicates presence of amino acid.

**Ninhydrine test:** To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid.

**Carbohydrates**

**Preparation of test solution:** The test solution was prepared by dissolving the test extracts with water. Then it was hydrolyzed with 1 volume of 1 N-HCL and subjected to following chemical test.

**Molisch's test:** Test solution with few drops of Molisch's reagent and 2 ml of conc.$H_2So_4$ added slowly from the sides of the test tubes. It showed a purple ring at the junction of two liquids.

**Barfoed's test:** 1 ml of test solution is heated with 1 ml of Barfoed, s reagent on water bath, if red cupric oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharide.

**Benedict's test**: Test solution treated with Benedict' reagent and after boiling on water bath, it showed reddish brown precipitate.

**Fehling's test:** The test solution when heated with equal volume of Fehling's A and B solution, gave orange red precipitate, indicating the presence of reducing sugars.

**Flavonoids**

The flavonoids are all structurally derived from the parent substance called flavones. The flavonoids occur in the free from as well as bound to sugars as glycosides. For this reason, when analyzing flavonoids it is usually better to examine the flavonoids in hydrolyzed plant extracts.

**Preparation of test solution:** To a small amount of extract added equal volume of 2 M HCL and heated in a test tube for 30 to 40 min at 100$^0$C.The cooled extract was filtered, and extracted with ethyl acetate The ethyl acetate was concentrated to dryness, and used to test for flavonoids.

**Shinoda test:** Test solution with few fragments of magnesium ribbon and conc. HCL showed pink to magenta red colour. To a small quantity of test solution when lead acetate solution was added, it formed yellow colored precipitate.

**Alkaline reagent test:** Test solution when treated with sodium hydroxide solution showed increase in the intensity of yellow colour, which becomes colorless on addition of few drops of dilute acid.

**Glycosides**

**Preparation of test solution:** The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

**Test for Cardiac glycosides:**

**Kedde' test:** Add one drop of 90% alcohol and 2 drops of 2 % 3, 5- dinitro benzoic acid in 90% alcohol. Make alkaline with 20 % sodium hydroxide solution, purple colour is produced. The colour reaction with 3, 5-dinitro benzoic acid depends on the presence of α, β-unsaturated lactones in the aglycone.

**Baljet's test:** The test solution treated with sodium picrate gave yellow to orange colour.

**Raymond's test:** Test solution treated with hot methanolic alkali, violet colour is produced.

**Bromine water test:** Test solution dissolve in bromine water give yellow precipitate.

**Keller-killani test for digitoxose:** The test solution treated with few drops of Fecl3 solution and mixed, then $H_2So_4$ containing Fecl3 solution was added, it formed two layers. Lower layer reddish brown, upper layer turns bluish green.

**Legal's test:** Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gave pink to red colour.

**Test for anthraquinone glycosides:**

**Borntrager's test:** Boiled powdered drug with 5 ml of 10 % sulphuric acid for five minutes. Filtered while hot, cooled the filtrate shaken gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution ammonia (10%). Allowed to separate it. The ammonical layer acquired rose pink colour due to presence of anthraquinones.

**Proteins**

**Preparation of test solution:** The test solution was prepared by dissolving the extract in water.

**Millon's test:** Test solution was treated with millon's reagent and heated on a water bath. The proteins were stained red

**Biuret test:** Test solution was treated with 40% sodium hydroxide and dilute copper sulphate solution gave blue colour.

**Xanthoproteic test:** Test solution was treated with conc. $HNO_3$ and boiled which gave yellow precipitate.

**Modified Borntrager's test:** C-glycosides of anthraquinones require more drastic conditions for hydrolysis. Hydrolysis of the drug was carried out with 5 ml of dilute of HCL and 5 ml of 5 % solution of $Fecl_3$. For hydrolyzed extract procedure was carried out as described under Borntrager's test.

**Test for steroids**

**Preparation of test extract solution:** The extract was refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponificable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (saponificable matter) was subjected to the following test by dissolving the residue in the chloroform.

**Salkowski test:** To the test extract solution add few drops of conc. $H_2SO_4$ shaken and allowed to stand, lower layer turned red indicating the presence of steroids.

**Libermann - Burchard test:** The test solution treated with few drops of acetic anhydride and mixed, when conc**.** $H_2SO_4$ was added from the sides of the test tubes, it showed a brown ring at the junction of the two layers and the upper layers turned green. Added few drops of concentrated $H_2SO_4$. Blue colour appeared.

**Sulphur test:** Sulphur test when added in to the test solution, it sank it.

**Tannins and phenol compound**

To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents.

**5% $Fecl_3$ solution:** Deep blue- black colour**.**

**Lead acetate solution:** White precipitate.

**Bromine water:** Discoloration of bromine water

**Acetic acid solution**: Red colour solution.

**Dilute iodine solution:** Transient red colour**.**

One drop of $NH_4OH$, excess 10% $AGNO_3$ solution. Heated for 20 min in boiling water bath. White precipitate was observed, then dark silver mirror deposited on wall of test tube.

**Triterpenoids**

**Preparation of test extract solution:** The test extract solution was prepared by dissolving extract in the chloroform.

**Salkowski test:** Few drops of concentrated sulphuric acid were added to the test solution, shaken and on standing lower layer turned golden yellow [5-9].

## RESULT AND DISCUSSION

The therapeutic efficacy of many indigenous plants, for various diseases has been described by traditional herbal medicinal practitioners [10]. *Leonotis nepetifolia* Family (Lamiaceae) frequently known as 'Klip dagga' which has a long past of numerous traditional medicinal uses in many countries in the world. The plant is rich source of allenic acid, iridoids, glycosides, terpenoids, and many more. This plant exhibited various biological activities and has been attributed to a variety of physiological effects like antifungal, antidiabetic, anxiolytic and arthritic. The macroscopic and microscopic characterstics of *Leonotis nepetaefolia* root was tabulated in table 1. The outcome of the present investigation revealed that the LOD of selected plant was found to be 3.39±0.10 having highly water soluble extractive value (Table 2). The % yield of extracts was tabulated in table 3. The phytochemical analysis showed presence of Alkaloids, carbohydrates, protein and amino acids, flavonoids along with steroids (Table 4).

**Table 1: Macroscopic features of *Leonotis nepetaefolia* (Roots)**

| S/No. | Parameters | Macroscopic | Microscopic (Cross section of Root) |
|---|---|---|---|
| 1. | Color | Dull white | Periderm |
| 2. | Odor | Peculiar | Secondary cortex |
| 3. | Taste | Acrid | Cambium |
| 4. | Shape | Tap root cylinder | Secondary xylem |
| 5. | Size | Variable | Primary cortex |
| 6. | Surface character | Smooth | ----- |
| 7. | Fractures | Absent | ------ |

**Table 2: Physicochemical Evaluation of *Leonotis nepetaefolia* (Roots)**

| S/No. | Parameters | LNR |
|---|---|---|
| 1. | Foreign Organic Matter | 0.32±0.02 |
| 2. | Loss on Drying | 3.39±0.10 |
| 3. | Total Ash | 8.43±0.06 |
| 4. | Acid Insoluble Ash | 2.12±0.03 |
| 5. | Water Soluble Ash | 4.48±0.02 |
| 6. | Swelling Index | 1.10±0.01 |
| 7. | Water Soluble Extractive Value | 18.29±0.09 |
| 8 | Ethanol Soluble Extractive Value | 12.31±0.03 |

**Abbr.**: LNR: *Leonotis nepetaefolia* (Roots)

**Table 3: Estimation of % Yield of Root Extract**

| S/No. | Extract | Parameters | | | |
|---|---|---|---|---|---|
| | | Nature of Extract | Color | pH | % Yield (w/w) |
| 1. | PEELNR | Semi Solid | Light green | 6.8 | 2.89 |
| 2. | CELNR | Semi solid | Green | 7.1 | 4.42 |
| 3. | EELNR | Semi Solid | Pale White | 7.0 | 11.28 |
| 4. | AELNR | Solid Powder | White | 7.0 | 14.38 |

**Table 4: Phytochemical analysis**

| S/No. | Constituents | Root Extract | | | |
|---|---|---|---|---|---|
| | | PEELNR | CELNR | EELNR | AELNR |
| 1. | Carbohydrates | - | - | + | + |
| 2. | Glycosides | - | - | - | - |
| 3. | Alkaloids | + | + | + | + |
| 4. | Protein & Amino acid | - | - | + | + |
| 5. | Tannins & Phenolic compounds | - | - | - | - |
| 6. | Flavonoids | + | + | + | + |
| 7. | Fixed oil and Fats | - | - | + | + |
| 8. | Steriods & Triterpenoids | + | + | + | + |
| 9. | Waxes | - | - | - | - |
| 10. | Mucilage & Gums | - | - | - | - |

**Note:** All values are expressed as Mean±SEM, n=3

## CONCLUSION

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The present study may be useful to appendage information in regard to its characterization and identification of plant.

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