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Development of quality control parameters for the standardization of *Leonotis nepetaefolia* (L) R.Br.: barchibuti flowers

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Abstract
In traditional system of medicine about 75% of the Indian population depends on this indigenous system for relief. With such a huge section of an everincreasing population relying on herbal remedies, it is imperative that the plant products which have been in use for such a long time be scientifically supported for their efficacy. *Leonotis nepetaefolia* (L) R.Br. belongs to the genus *Leonotis* and family Lamiaceae. It is native to tropical Africa and India. The flowers of the plant are used medicinally for the treatment of several skin infections. The present paper deals with the investigation and development of quality control parameters for standardization of *Leonotis nepetaefolia* (L) R.Br. Flowers. Dried flowers of the plant were evaluated for physicochemical parameters, extraction and preliminary phytochemical screening.

Keywords: *Leonotis nepetaefolia* (l) r.br, flowers, standardization parameters

Introduction
Leonotis nepetaefolia (L) R.Br. commonly known barchibuti as is a less known medicinal plant, belongs to the family Lamiaceae. It is found almost along the hotter parts of India. Traditionally, the all parts of the plant especially roots, leaf and flowers are used for rheumatic problems and also serves as a tonic. Flower heads are used against scalds, burns, ringworm and some skin diseases [1, 3].
In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter. A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems. In Indian systems of medicine most practitioners formulate and dispense their own recipes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [4, 6]. So, far no any systematic study was carried out in evaluating the standardization parameters of flowers of selected plant, therefore, the present work was undertaken to revel and develop the quality control parameters for standardization of selected herb.

Material and Methods
Collection of herbs and their authentication
The flowers of *Leonotis nepetaefolia* (L) R.Br. (LNF) were collected in the months of July-September 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. J/Bot/2020-LNF392 was allotted.

Development of Quality control/Standardization parameters of selected herbs [7, 9]
Pharmacognostical Evaluation
Macroscopic studies
The Macroscopy of different parts of the plant such as color, odor, size, shape, taste, surface characters and fractures were carried out.

Physicochemical Evaluation
The dried parts were subjected to standard procedure for the determination of various physicochemical parameters.

Determination of foreign organic matter (FOM)
Accurately weighed 100 g of the drug sample and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and the percentage present was calculate. The results are given in Table no. 15.

Determination of moisture content (LOD)
Place about 10 g of drug (without preliminary drying) after accurately weighing in a tared evaporating dish and kept in oven at 105^0 C for 5 hours and weigh. The percentage loss on drying with reference to the air dried drug was calculated.

Determination of ash value
The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Total ash
Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

Acid insoluble ash
The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash
The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Determination of swelling index
Swelling index is determined for the presence of mucilage in the seeds. Accurately weigh 1 g of the seed and placed in 150 ml measuring cylinder, add 50 ml of distilled water and kept aside for 24 hours with occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured.

Determination of extractive value
This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.

Cold maceration
Place about 4.0g of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Macerate with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per g of air dried material. For ethanol-soluble extractable matter, use the concentration of solvent specified in the test procedure for the plant material concerned; for water-soluble extractable matter, use water as the solvent.

Successive Extraction of selected herbs
Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered plant material (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

Preliminary phytochemical screening of extracts
The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedures were adopted to perform the study.

Tests for carbohydrates
Molisch's test
To the Sample 2-3 drops of 1% alcoholic - napthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of purple to violet ring at the junction of two liquids shows the presence of carbohydrates.

Fehling test
To the sample add fehling reagent, appearance of brick red precipitate shows presence of carbohydrates.

Test for glycosides
Legal's test
To the sample add 1 ml of pyridine and few drops of sodium nitropruside solutions and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.
Borntrager's test
Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

Baljet's test
To the sample add picric acid, orange color shows presence of glycosides.

Test for alkaloids
A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are

- Dragendroff's reagent- Reddish brown precipitates
- Wagner;s reagent- Reddish brown precipitates
- Mayer's reagent- Cream color precipitates
- Hager's reagent- Yellow color precipitate

Test for proteins and free amino acids
Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

- Million's reagent: Appearance of red color shows the Presence of protein and free amino acid.
- Ninhydrin reagent: Appearance of purple color shows the Presence of Proteins and free amino acids.
- Biuret's test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

Test for tannins and phenolic compounds
A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Blue color or green color
- 10% lead acetate solution: White precipitates

Test for flavonoids

Alkaline reagent test

To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

Shinoda's test

Small quantities of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloric acid drop wise added and heated. Appearance of pink, crimson red, green to blue color shows the presence of flavonoids**.**

Tests for fixed oils and fats

Spot test

A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

Saponification test

Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolpthlein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Tests for steroids and triterpenoids

Libermann-burchard test

Treat the sample with few drops of acetic anhydride, boil and cool. Then add con. Sulphuric acid from the side of test tube, brown ring is formed at the junction two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

Salkowski test

Treat the sample with few drop of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

Test for mucilage and gums

- Small quantities of sample was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.
- To the sample add ruthenium red solution, pink color shows presence of mucilage.

Test for waxes

To the test solution add alcoholic alkali solution, waxes get saponified.

Results and Discussion

The flowers of *Leonotis nepetaefolia* (L) R.Br. (LNF) were collected from local sites of Malwa region of Madhya Pradesh, India and identified morphologically and compared with standard pharmacopoeial monograph. The flowers are borne in dense rounded (i.e. spherical) clusters (5-6 cm across) towards the tops of the stems (usually 2-4 clusters per stem). Each of these clusters is arranged directly on the main stem, just above a pair of leaves (i.e. sessile axillary clusters). The individual flowers (20-40 mm long) are orange or reddish-orange in colour and tubular in shape with two main lobes (i.e. they are two-lipped). The lower of these lobes is further divided near its tip, while the upper lobe is somewhat arched or curved and densely hairy. These flowers are surrounded by a green tube (i.e. calyx tube) 12-15 mm long and each flower also has two elongated (i.e. lanceolate) and pointed green bracts associated with it. Flowering occurs mostly from summer through to early winter (i.e. from December to July).

The flower clusters form a hard and somewhat spiky ball when mature, and consist mainly of the persistent calyx tubes (which enlarge to 15-25 mm long in fruit). Each individual fruit is a four-lobed 'capsule' (i.e. schizocarp) that separates into four 'seeds' (i.e. nutlets) when mature. These 'seeds' are dark brown or dull black in colour, and either somewhat egg-shaped (i.e. oblong-ovoid) or triangular in shape (2.5-4.3 mm long and 1-1.9 mm wide)

Fig 1: *Leonotis nepetaefolia* (L) R.Br. Flowers

The dried plant part of *Leonotis nepetaefolia* (L) R.Br. Flowers (LNF) were subjected to standard procedure for the determination of various physicochemical parameters. The results were presented in table 1.

Table 1: Physicochemical Evaluation of *Leonotis nepetaefolia* (L) R.Br. Flowers

S/No.	Parameters	LNF
1.	FOM	1.19±0.03
2.	LOD	3.13±0.15
3.	TA	7.38±0.08
4.	AIS	1.08±0.12
5.	WSA	2.32±0.11
6.	SI	3.09±0.04
7.	WSEV	19.21±1.02
8	ESEV	7.10±1.22

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

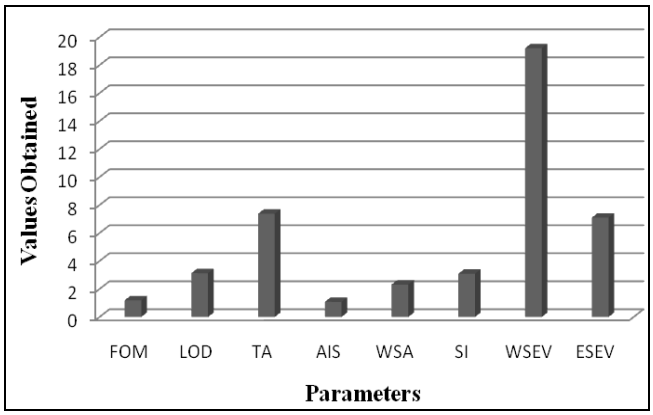

Fig 2: Physicochemical Evaluation of *Leonotis nepetaefolia* (L) R.Br. Flowers

The shade dried coarsely powdered plant material of LNF *Leonotis nepetaefolia* (L) R.Br. Flowers was extracted with petroleum ether, Chloroform, ethanol and water. The extracts obtained were evaluated for pH, color and % yield. The results are presented in table 2.

Table 2: Estimation of % yield of various extract of *Leonotis nepetaefolia* (L) R.Br. Flowers

S/No.	Extract	Parameters			
		Nature of Extract	Color	pH	% Yield (w/w)
1.	PEELNF	Semi Solid	Light greenish	7.0	0.92
2.	CELNF	Semi solid	Greenish Black	7.12	1.89
3.	EELNF	Solid Powder	Light Green	7.12	7.43
4.	AEENF	Solid Powder	Light Green	7.04	11.27

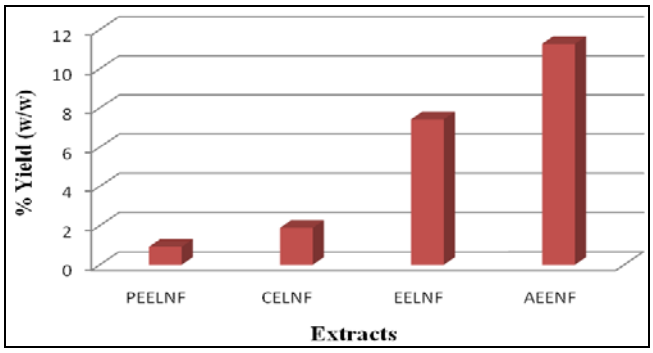

Fig 3: % yield of extract

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure was adopted to perform the study.

Table 3: Preliminary phytochemical screening *Leonotis nepetaefolia* (L) R.Br. Flowers

S/No.	Constituents	Flower Extract			
		Peelnf	Celnf	Eelnf	Aelnf
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	-	-	-	-

Abbr.: +=Present; -=Absent

Conclusion

Development of quality parameters of the medicinal plants is of prime importance in order to reveal safety aspects. In traditional system of medicine herbal healers treat diseases using the plants which have immense medicinal potentiality. But due to lack of standardization parameters correct identification of the plant is lacking, therefore development of QC parameters is of great interest. The present work was undertaken to revel the standardization parameters of *Leonotis nepetaefolia* (L) R.Br. Flowers. In this study morphological, physicochemical, extraction and preliminary phytochemical screening of the selected plant material was done and reported.

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