



e-ISSN: 2319-8753 | p-ISSN: 2347-6710

IJIRSET

International Journal of Innovative Research in
SCIENCE | ENGINEERING | TECHNOLOGY

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 11, Issue 8, August 2022

ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA

Impact Factor: 8.118

 9940 572 462

 6381 907 438

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Phytochemical and pharmacological investigation of *Leonotisnepetifolia* (Linn.) R. Br. flowers and roots extracts

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ABSTRACT : Phytochemicals are bioactive compounds obtained from the plants and are widely applied in the traditional herbal medicine. These herbal medicines are used by the local people to cure the various diseases which include the major diseases such as Diabetes Mellitus, Cancer, HIV etc.

Leonotisnepetifolia Family (Lamiaceae) commonly known as 'Klipdagga' which has a long history of several traditional medicinal uses in many countries in the world. A huge number of phytoconstituents have been reported from the plant are 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, arthritic, and many more activities. This literature review presents important species covering phytochemistry and pharmacological activities aspects systematical.

I. INTRODUCTION

Medicinal plants are considered as a rich resource of ingredients which can be used in drug development either pharmacopoeial, non- pharmacopoeial or synthetic drugs. Plants and herbal extracts are well known for their ability to have therapeutic potential and have been exploited worldwide to cure many severe ailments.

They are the basis of Ayurvedic medicine, Unani form of medication and Siddha. From time immemorial, lot of work has been done in this field and still more and more efforts are required to exploit these precious resources for the benefit of human kind.

The recorded use of plants in the treatment of ailments dates back to antiquity. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and protection against predators and infections.

Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines.¹

Plants have provided a source of inspiration for novel drug compounds, as plant- derived medicines have made large contributions to human health and well-being. Their role is two-fold in the development of new drugs: (a) they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; (b) a phytomedicine to be used for the treatment of disease.^{2,3}

Leonotisnepetifolia was first described by Linnaeus (in the genus *Phlomis*), based on an illustration and description of plants growing in the Leiden botanic garden thought to have originated in Surinam. In India it was collected on the Coromandel Coast by the Tranquebar Missionaries, and it may have been one of these, J.G. König, who sent it to Sir Joseph Banks, by whom it was introduced to Kew Gardens in 1778. The plant is considered as a weed of waste lands and cultivated areas.⁴

The genus *Leonotis* has 12 species widely distributed in Pantropics and is represented by one species, *Leonotisnepetifolia* in India.⁵

II. PLANT DESCRIPTION

Leonotisnepetifolia (L.) R.Br (Family-Lamiaceae) is a tall annual herb growing in plains, roadsides, waste places near villages and is often cultivated throughout India. The plant is identified by its finely pubescent obtusely quadrangular stems, long internodes and spinous whorls of orange scarlet flowers with densely wooly upper lip.⁶



Leonotisnepetifolia 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).⁷

Botanical classification of Leonotisnepetifolia

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

[



Fig: - Whole plant of Leonotisnepetifolia

Geographic Distribution and Habitat –

Leonotisnepetifolia is a plant native to tropical and subtropical East Africa and is widely distributed in the tropics in Africa, Southeast Asia, Pacific Islands, Australia, Central and South America, Southern United States, Mexico and Caribbean islands. This plant is present all over the world following the European colonial expansion starting from 17th century. Its natural habitat is that of mixed woods, along the banks of streams and along the roads but it grows in areas where it has been introduced along the roadsides, in over-exploited pastures, in disturbed sites, in uncultivated areas, in waterways and in the floodplains. It is also found in savanna vegetation near streams in Western Australia. It grows up to 2,000 meters of altitude. Leonotisnepetifolia is a perennial and annual herbaceous plant that can reach 3 meters in height.

The stem is branched only at the upper nodes and the plant has an easily extirpable taproot about 10 cm long. The stems are relatively robust, sparsely branched and sparsely covered with small whitish hairs. These stems are four-angled (i.e. quadrangular) and usually have a distinct groove that runs longitudinally along the center of each side. The leaves, 4.5-20 cm long and 2-15 cm broad, are arranged opposite along the stems and carried on 2-10 mm long petioles. The lower leaves, which are somewhat egg-shaped in outline (i.e. oblong-ovate or ovate), are larger and wider than those towards the top of the plant. All the leaves have sharply toothed (i.e. crenate or serrated) margins, pointed tips and are sparsely hairy. The flowers are carried in dense rounded clusters of 5-6 cm in diameter, towards the tops of the stems (usually 2-4 clusters per stem). Each of these clusters is placed directly on the main stem, just above a pair of leaves (ie sessile axillary clusters).

The single flowers (20-40 mm long) are orange or reddish-orange in color and tubular in shape with two main lobes (ie 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 12-15mm long green tube and each flower also has two elongated, pointed green bracts associated with it. Flowering occurs mainly from summer until early winter). The flower clusters form fruit in the form of hard and somewhat pointed balls when ripe, and consist mainly of the persistent tubes of the calyx (which in fruits widen up to 15-25 mm in length). Each individual fruit is a four-lobed "capsule" (schizocarp) which separates into four "seeds" when ripe. These "seeds" are dark brown or dull black, and slightly ovoid or triangular in shape (2.5-4.3 mm in length and 1-1.9 mm in width).

III. MATERIALS AND METHODS

Plant Material

The required plant parts were collected from the region of....

Extraction of Plant material:-

The extraction of plant material was done by Hot water extraction method. The plant material was allowed to dry naturally i.e. under shade drying. After completion of drying process, material was ground in a grinder and the powder was kept in an appropriately labeled plastic bottle. 5gm of ground material was weighed using an electronic weighing balance, dissolved in a 25 ml of sterile water and then boiled at 50-60°C for 30 minutes on water bath. The extract was filtered through Whatman No.1 filter paper and centrifuged the filtrate at 2500 rpm for 15 minutes. Resulting extract was stored in sterile bottles at 4-8°C for further analysis⁽²⁵⁾.

Physicochemical Evaluation

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 independently eye or by the use of a lens (6X). Separate and weigh it and the percentage present was calculate.

Determination of moisture content (LOD)

Place about 10 g of drug (without preliminary drying) after accurately weighing in a taped evaporating dish and kept in oven at 105⁰ 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 450⁰C 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 plant parts. Accurately weigh 1 g of the seed and placed in 150 ml measuring cylinder, add 50 ml of distilled water and kept separately 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 4.0 gm 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.

Phytochemical analysis

Preliminary qualitative screening for phytochemicals, of all these plant species was carried out with the following methods.

Test for carbohydrates

Molisch's test

To the sample 2-3 drops of 1% alcoholic - naphthol solution and 2 ml of conc. Sulphuric acid was added along the sides of the test. 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 solution and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

Baljet's test

To the sample add picric acid, orange color shows 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.

Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloride acid and was tested with various reagent for the presence of alkaloids.

The reagents are

Dragendroff's reagent	:	Reddish brown precipitates
Wagner's reagent	:	Reddish brown precipitated
Mayer's reagent	:	Cream color precipitates
Hager's reagent	:	Yellow color precipitates

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 protein 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 protein 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 will be 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 quantity of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloride acid drop wise added and heated. Appearance of pink, Crimson red, green to blue color shows the presence of flavonoids.

Test 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 presence of fixed oil.

Saponification test

Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quality of sample along with a drop of phenolphthlein, 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 oil and fats.

Test 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 drops of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicated 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.²⁶

Pharmacological screening:

Antimicrobial activity

Disc diffusion method will be performed for the screening of antimicrobial property of plant part against four different microorganisms. Disc of whatsmann filter paper of one quarter inch in diameter will prepared and the same was sterilized using autoclave.

Various amount of plant parts extracts will be dissolved in different extracts of different stock solutions (10 - 50 µg/ml) solutions will be prepared. All the dilution prepared will be applied to whatsmann filter paper disc using a micropipette. The disc will be then dried and sterilized. Preparation of culture plate (Sabouraud's agar and Mueller Hinton agar media) will be done by dissolving media in 1000 ml of distilled water and sterilized by autoclave at 121°C for 1 hour. The media will be cooled and poured in sterilized petri plate to solidified at room temperature

The re-cultured anti microbial strains will be used for evaluation. The strains will be streak on the Mueller Hinton media and the drug entrapped patches will be placed. For negative control disc of distilled water and for positive control kanamycin (30µg/ disc) will be used. The petri plates will be incubated at 37 °C for 24 h and the antimicrobial activity will be assessed by measuring the diameter of zone of inhibition. The zone of inhibition will be recorded with the help of zone reader scale. The zone of inhibition will be calculated by subtracting diameter of sample or standard or control by diameter of disc. The more the zone of inhibition the more will be antimicrobial activity.²⁷⁻³⁰

Anthelmintic activity

For the preliminary anthelmintic study, adult earthworms (*Pheretimaposthuma*) will be collected from moist soil and washed with normal saline to remove all fecal material. The earthworms of 3 - 5 cm in length and 0.1 - 0.2 cm in width will be used for all experimental protocols due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

The anthelmintic activity will be estimated by placing the earthworms (*Pheretimaposthuma*) in Petri dishes containing two different concentrations (25 and 50 mg/ ml) of various solutions of the dried plant part. Two worms will be placed in each Petri dish and observed for paralysis or death. The paralysis time will be recorded when any sort of movement of worms will not observed for at least 30 seconds except when they will be shaken vigorously. The death time will be recorded after ascertaining the worms moved neither when shaken vigorously nor when dipped in the warm water (50 °C). The values of test samples will be compared with that of values obtained from the standard drug, albendazole treated group.



IV. RESULTS AND DISCUSSION

It was found that *leonotisnepetifolia* have considerable proportion of important phytochemicals that are easily detected by qualitative tests. In our analysis it was cleared that the *leonotisnepetifolia* is rich in carbohydrates, glycosides, alkaloids, proteins and free amino acids, tannins phenolic compounds, flavonoids, fixed oils and fats, steroids and triterpenoids, mucilage and gums, Antimicrobial activity and Anthelmintic activity.

The important thing is that plant samples contain one abundant secondary metabolite, flavonoid. From the literature survey it was found that flavonoids have wide range of biological properties such as anti-inflammatory, antibacterial, antiviral, anti-allergic, cytotoxic antitumor properties.

Tannins are also involved in treatment of noninsulin dependent diabetes mellitus by enhancing the glucose uptake and inhibiting adipogenesis.

The most striking feature about quinones is its pharmacological properties that make it different from other secondary metabolites. It inhibits HIV 1 reverse transcriptase and shows antitumor and immunomodulatory activities. It also has antimicrobial, anticancer, antiviral and antibacterial properties. Moreover, saponins exhibit various biological activities like, it gives a permeability to the cell membrane, helpful in lowering the serum cholesterol levels, it possess abortifacient properties, it has immunomodulatory property, it has cytotoxic effects on malignant tumor cells and is involved in synergistic enhancement of the toxicity of immunotoxins⁽⁸⁾.

Saponins also show antidiabetic property⁽⁹⁾. Coumarins, a major class of flavonoids, possesses pharmacological properties like antidiabetic, antioxidant, hepato-protective, anticoagulant, antimicrobial, anti-inflammatory, analgesic, antioxidant, anticancer, antiviral, antimalarial activities etc⁽¹⁰⁾. Tannins are reported to have a cardio-protective, anti-inflammatory, anticarcinogenic and antimutagenic properties. Tannins are also involved in treatment of noninsulin dependent diabetes mellitus by enhancing the glucose uptake and inhibiting adipogenesis⁽¹¹⁾. The most striking feature about quinones is its pharmacological properties that makes it different from other secondary metabolites. It inhibits HIV 1 reverse transcriptase and shows antitumor and immunomodulatory activities⁽¹²⁾.

Phytochemical analysis of leonotisnepetifolia

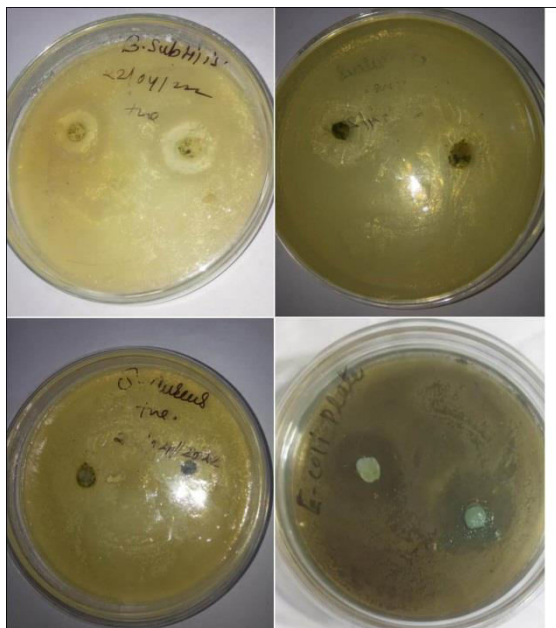
S.NO	Test	Results
1	Carbohydrates	+
2	Glycosides	+
3	Alkaloids	+
4	proteins and free amino acids	+
5	tannins phenolic compounds	+
6	Flavonoids	+
7	fixed oils and fats	+
8	steroids and triterpenoids	+
9	mucilage and gums	+
10	Waxes	+

Physicochemical Evaluation

S.NO	Parameters	Observation of Flowers	Observation of root
1	Foreign organic matter	0.92 % w/w	1.93% w/w
2	Moisture content	8.3% w/w	5.5% w/w
3	Total ash	5.95% w/w	4.076 % w/w
4	Acid insoluble ash	1.006 % w/w	0.7084% w/w
5	Water soluble ash	4.7 % w/w	3.25% w/w
6	Swelling index	Less than 100	Less than 100

Pharmacological screening:

Antimicrobial activity



Four pathogen selected for antimicrobial activity these pathogen are E.coli, Staphylococcus aureus, bacillus subtilis and salmonella. *leonotisnepetifolia* have a antimicrobial property of against four different microorganisms.

Anthelmintic activity

At concentration of 50mg /ml of dry part of plant extract in comparison to albendazole showed insignificant result. The *leonotisnepetifolia* extract showed highest anthelmintic activity at 50mg/ml concentration as compared to 25mg/ml solution.

Time for 25mg/ml- 25 second

Time for 50mg/ml- 12 second

It has been concluded plant exhibit anthelmintic activity and further evaluation of these *leonotisnepetifolia* should be carried out to purify the active ingredient for anthelmintic activity.

V. CONCLUSION

From the overall scenario, it is concluded that as the plants studied, found to rich in phytochemicals,are full of pharmacological and medicinal significance. Out of all secondary metabolites flavonoid is found to be abundant in the everywhere almost plant species studied. Further study is required to find their potentials in the mentioned biological properties such as antidiabetic, anti-tumor, etc.

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SJIF Scientific Journal Impact Factor
Impact Factor: 8.118



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