Pharmacognostic and Preliminary Phytochemical Evaluation of *Ocimum basilicum* L. var. *pilosum* (Willd.) Benth. and *O. tenuiflorum* var. CIM-AYU

Venugopal Gaddaguti*1*, Srinivasa Reddy Ch2, Narasimha Rao Bhogireddy1, Botla Venkat Rao3, Venkateswara Rao Talluri1, Allu Prasada Rao1*.

1*Department of Biotechnology, KL University, Vaddeswaram, Guntur (Dist), A.P, India-522502.
2Department of Botany, P.B.Siddhartha College of Arts & Science, Vijayawada.
3Manphar pharmaceuticals, R&D division, Vijayawada.

ABSTRACT

Background: The genus *Ocimum* comprised of vast number of species and many of them have potential to control wide range of health problems. Objective: The study primarily focused to characterize pharmacognostic and preliminary phytochemical evaluation of hitherto unknown wild *Ocimum basilicum* with *Ocimum tenuiflorum* CIM-AYU. Materials and Methods: A detailed preliminary Phyto pharmacognostic characteristics in fully expanded healthy leaves of two *Ocimum* species (*O. basilicum* and *O. tenuiflorum*) were done including, organoleptic, morphological, microscopical, physicochemical and phytochemical properties in solvent extracts using different solvent fractions according to the Ayurvedic pharmacopoeia. Results: Overall, *O. tenuiflorum* exhibit superior morphological characters than *Ocimum basilicum*. Whereas, organoleptic evaluation confirms that *O. basilicum* exhibit a sharp pungent smell. In *O. basilicum*, the distribution of trichomes found to be relatively more than the plant studied for comparison in the present study. On the other hand, water solubility fraction of *O. basilicum* is 48.84% which twice higher than *O. tenuiflorum* (23.53%). Similarly, the alcohol solubility of *O. basilicum* is significantly higher than *O. tenuiflorum*. The phytochemical screening results with methanolic fraction found to be positive in almost 12 parameters than other solvent fractions in *O. basilicum*. Conclusion: The present studies further validate that, *Ocimum basilicum*, *L. var. pilosum* (Willd.) Benth. a novel and potential medicinal herb with sharp pungent aromatic principles. Further the methanolic soluble leaf fraction draw of all phytochemical constituents of the medicinal herb adds additional pharmacognostic characteristics to plant Indian pharmacopoeia.

Keywords: *Ocimum basilicum*, *L. var. pilosum* (Willd.) Benth, *O. tenuiflorum* var. CIM-AYU, Phyto Pharmacognosy.

INTRODUCTION

Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information (regarding the morphology, microscopical and physical characteristics) of the crude drugs. Pharmacognostic studies have been done for many important drugs, and the resulting observations have been incorporated in various pharmacopoeias [1]. Although number of crude drugs from plant sources was in use, the systematic identification of plant parts used in drug formulation is inadequate. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs [2]. On the other hand, the growth and development and the physiological performance of the source plants is high then they can produce and store high voluminous phytochemicals. Lamiaceae is a large family consisting of 170 genera and about 3000 species widespread in both warm and temperate parts of the world. Of all, the genus *Ocimum* possess around 150 species and almost all species are aromatic in nature with wide range of essential oils and aroma chemicals extensively used in the perfumery & cosmetic industries as well as in indigenous systems of medicine [3, 4].
therapeutic field, wide array of chemical constituents from many species of Ocimum exhibits antioxidant properties and biological activity against insects, fungi and microorganisms. To further validate the medicinal potential of any plant, the phyto chemical screening is prerequisite for identification of new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, oils etc. In addition, the pharmacognostic and preliminary phytochemical studies for the two species of Ocimum have not been reported. In the light of the above fact, the present study aimed to evaluate the Pharmacognastical and preliminary phytochemical investigation were performed such as organoleptic, morphologic, microscopic was and other applicable physico - chemical parameters of leaves of Ocimum basilicum L. var. pilosum (willd.) Benth. and O. tenuiflorum var. CIM-AYU could be used for correct identification of the plants from crude drugs.

**MATERIALS AND METHODS**

**Collection of plant materials**

Ocimum basilicum L. var. pilosum (willd.) Benth. plant species were collected from Kondapalli reserve forest lies between 16.36° North latitude and 80.30° East longitude at height of 168 meters above Mean Sea Level, Vijayawada of Krishna district, Andhra Pradesh, India. The plant was taxonomically identified up to species level and confirmed by the Botanical Survey of India, TNAU campus, Coimbatore. O. tenuiflorum var. CIM-AYU plant seeds were collected from CIMAP, Hyderabad and planted in university garden for further use.

**Pharmacognostical studies**

**Macroscopic studies**

In organoleptic evaluation, appropriate parameters like taste, odor, size, shape and color of the leaf and leaf powder were studied based on method described by Ayurvedic Pharmacopoeia of India [5,6].

**Morphological Characters**

Morphological investigations of the plant leaves were studied [5,6].

**Microscopic studies**

Thin cross sections of fully expanded healthy leaves of Ocimum basilicum L. var. pilosum (willd.) Benth. and O. tenuiflorum var. CIM-AYU were stained with safranin and fast green and observed under the light microscope. Photomicrographs were taken using Olympus compound microscope attached with digital camera for evaluation of cross sectional details of the two test species.

**Physicochemical Parameters**

Total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value and loss on drying were determined by employing standard methods as given in Ayurvedic Pharmacopoeia of India [7,8]. The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain and the diversity in chemical nature and properties of contents of drug. The percentage values were calculated with reference to the air-dried drug. Identification of various classes of active chemical constituents in leaves of Ocimum basilicum L. var. pilosum (willd,) Benth. and O. tenuiflorum var. CIM-AYU using preliminary and quantitative phytochemical analysis in various solvent extracts (methanol, ethanol, acetone and ethyl acetate) was studied using the standard methods [9,10,11,12].

**Ash values**

The residue remaining left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. It may also include inorganic matter deliberately added for the purpose of adulteration. Hence, an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug. The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash that is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is higher. Indian Pharmacopoeia procedure were used to determine the different ash values (total ash, acid insoluble ash, and water-soluble ash value)

**Determination of Total Ash Value**

Three grams of air dried powdered leaf sample was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon and finally collected and weighed. Later, the percentage of total ash was calculated with reference to the air-dried leaf material of test samples.

**Determination of Acid Insoluble Ash Value**

The ash obtained as directed under total ash was boiled with 25 ml of 2N hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, dried ignited and weighed. Similarly, the percentage of acid insoluble ash with reference to the air-dried leaf sample was also calculated.
Determination of Water soluble Ash Value
The total ash (Known volume of leaf sample) was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash free filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of Alcohol Soluble Extractive Value
5 grams of roots powder was macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to settle for 18 h, and after 24 h, the extract was filtered. From this, 25 ml of the filtrate was evaporated and the extract was dried at 105°C to attain constant weight.

Determination of Water Soluble Extractive Value
Water soluble extractive value was determined using the procedure described for alcohol soluble extractive, except that chloroform was used for maceration.

Determination of Loss on Drying
1.5 gm of the powdered drug in a pre-weighed porcelain dish was dried at 105°C in hot air oven to constant weight and then weighed. The percentage loss on drying of drug was calculated from pre and post heated drug samples.

Preliminary phytochemical studies

Preparation of plant extracts
Fresh leaves of Ocimum species were washed under running tap water and dried for 48hrs in a hot air oven at 60°C. Dried leaf samples were ground using an electric blender to obtain a fine powder and stored in polythene bags until needed for analysis. Fifty gram portions of powdered plant samples were separately dispersed in 500ml of different solvents (Methanol, ethanol, acetone and Ethyl acetate). The solutions were vigorously shaken and then weighed. The difference in weight between the pre and post heated drug samples was calculated from pre and post heated drug samples.

Phytochemical analysis
Chemical tests for screening and identification of bioactive chemical constituents in the two Ocimum (Ocimum basilicum L. var. pilosum (willd.) Benth. and O.tenuiflorum var. CIM-AYU) species were performed in extracts using the standard procedures [9,10,11].

Screening Procedure
Detection of Alkaloids
The individual extracts were dissolved in chloroform. The solution was extracted with dil. H₂SO₄ or dilute HCl and acid layer was taken and tested for presence of alkaloids.

Mayer’s test: To the 1 ml of extract, 1 ml of Mayer’s reagent (Potassium mercuric iodide solution) was added.
Cream colored precipitate indicates the presence of alkaloids.

Wagner’s Test: To 1 ml of the extract, 1 ml of Wagner’s reagent (iodine in potassium iodide) was added. Reddish brown precipitate indicates the presence of alkaloids.

Hagner’s test: To 1 ml of the extract, 1 ml of Hagner’s reagent (iodine in potassium iodide) was added. Brown precipitate indicates the presence of alkaloids.

Dragendroff’s test: To 1 ml of the extract, 1 ml of Dragendroff’s reagent (potassium bismuth iodide solution) was added. Development of orange red precipitate indicates the presence of alkaloids.

Detection of carboxylic acid: One ml of the extract was treated with a few ml of sodium bicarbonate solution. Effervescences indicate the presence of carboxylic acid.

Detection of coumarins: Plant extracts (1 ml) were treated with alcoholic sodium hydroxide. Dark yellow color shows the presence of coumarins.

Detection of flavonoids: 5 ml of leaf (Ocimum basilicum and O.tenuiflorum) extract were separately dissolved in one ml of alcohol and then subjected to the following tests.

Ferric chloride test: A few drops of neutral ferric chloride solution were added to 1 ml of above alcoholic solution. Formation of blackish red color indicates the presence of flavonoids.

Shinoda’s test: To 1 ml of alcoholic extract, 0.5g of magnesium ribbon or magnesium foil was simultaneously added with a few drops of concentrated HCl. Change in color (from red to pink) shows the presence of flavonoids.

Alkaline reagent test: Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of alkaline in the leaf sample.

Lead-acetate test: To 1 ml of alcoholic extract, a few drops of aqueous basic lead acetate solution were added. Reddish brown bulky precipitate indicates the presence of flavonoids.

Tests for Carbohydrates
After gentle shaking, lower layer turning into test solution in test tube were mixed and heated on Benedict’s test: precipitate observed for formation of yellow, then brick red heated in boiling water bath for 5 min. Appearance of green, yellow or red color indicates the presence of reducing sugar in the test solution.

Molish’s test: Few drops of α naphthol solution in alcohol was added to 2-3 ml aqueous extract. To this mixture concentrated H₂SO₄ from sides of the test tube was added and observed for violet ring at the junction of two liquids. Fehling’s test: 1 ml Fehling’s A and 1ml Fehling’s B solutions were mixed and boiled for one minute. To this mixture equal volume of test solution was added and heated in boiling water bath for 5-10 min and was observed for formation of yellow, then brick red precipitate. Benedict’s test: Equal volume of Benedict’s reagent and test solution in test tube were mixed and heated on boiling water bath for 5 min. Appearance of green, yellow or red color indicates the presence of reducing sugar in the test solution.

Detection of Phyto Steroids
Small quantity of extract is dissolved in 5 ml of chloroform. The chloroform solution was subjected to Salkowski and liebermann- Burchard tests.

Salkowski test: One ml of Concentrated sulphuric acid was added to the above solution and allowed to stand for 5 min. After gentle shaking, lower layer turning into golden yellow color indicates the presence of steroids.
Liebermann Buchard Test: 1 ml of sample extract was treated with chloroform, a few drops of acetic anhydride. To this, 1 ml of Concentrated H₂SO₄ was added from the sides of the test tube and allowed to stand for 5 min. Formation of brown ring at the junction of the two layers and the upper layer turning green indicates the presence of steroids.

Detection of saponins: 1 ml sample extract was mixed with 20 ml of distilled water and agitated in a graduated cylinder for 15 min. Formation of white froth indicates the presence of saponins.

Tests for Proteins and Amino acids

Biuret test: 4% NaOH and a few drops of 1% CuSO₄ was added to 3 ml of test solution and observed for development of violet or pink color.

Million’s test: 3 ml of test solution mixed with 5 ml Million’s reagent which turnn forms white precipitate. On warming, the precipitate turn into brick red or red color

Xantho protein test: 3 ml test solution mixed with 1 ml of concentrated H₂SO₄ for testing the presence of Xantho protein.

Ninhydrin test: 3 ml test solution and 3 drops 5% Ninhydrin solution were heated on water bath for 10 min. for development of purple or bluish color.

Detection of Glycosides

5 ml of leaf extract hydrolyzed with 5 ml each of Conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test:

Legals Test: To 1 ml of hydrolysate, 1 ml of pyridine and a few drops of sodium nitroprusside solution added and finally made with NaOH.

Borntrager’s Test: To 1 ml of hydrolysate, 1 ml of chloroform was added and the chloroform layer was separated. To this, an equal quantity of diluted. NH₃ solution was added. Change in color indicates the presence of glycosides.

Detection of fixed oils: 2 ml of leaf extract was pressed between two filter papers. Formation of transparent, spot indicates the presence of fixed oils. A few drops of 0.5 N alcoholic potassium hydroxide was added to 1 ml of the sample extract. To this, few drops of phenolphthalein indicator was added and the mixture was heated for 1-2 hrs. Soap formation confirms the presence of fixed oils in the test sample.

Detection of quinones: One ml of the sample extract was treated with alcoholic potassium hydroxide solution. Quinones give coloration ranging from red to blue.

Detection of resins: One ml of extract was treated with few drops of acetic anhydride solution followed by one ml of conc. H₂SO₄. Resins give colorations ranging from orange to yellow. One ml of extract was diluted with water. Formation of bulk black precipitate indicates the presence of resins.

Detection of Phenols: 1 ml of the various leaf extract dissolved in alcohol or water was treated with a few ml of neutral ferric chloride solution. Any change in color indicates the presence of phenols.

Detection of tannins: 5 ml leaf extract was dissolved in minimum amount of water and filtered. Then the filtrate was subjected to the following tests:

Ferric Chloride test: To the filtrate, a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins.

Gelatin Test: To the filtrate, gelatin solution was added. Formation of white precipitate indicates the presence of tannins.

Lead acetate Test: To the filtrate, a few drops of aqueous basic lead acetate solution were added. Reddish brown bulky precipitate indicates the presence of tannins.

Presence or absences of various phytochemicals were done for Ocimum basilicum and Ocimum tenuiflorum separately.

RESULTS

Morphological characters

Table 1: depicts organoleptic properties of Ocimum basilicum L. var. pilosum (willd.) Benth. & O. tenuiflorum var. CIM-AYU. Ocimum basilicum relatively small leaves with highly aromatic odor with sharp pungent smell than O. tenuiflorum. However both plant species typically possess aromatic properties which are a characteristic feature of almost all Ocimum species.

Microscopical studies

T.S of the leaves

Transverse Section of leaf passing through midrib in both plant species exhibit pot shaped, convex lower surface. However, the cross sectional view of Ocimum basilicum L. var. pilosum appears to exhibit flat upper surface than O. tenuiflorum. The upper epidermal lamina groove of O. basilicum L. var. pilosum shown to possess more number of sessile glandular trichomes than O. tenuiflorum. O. basilicum possess short and multi cellular trichomes when compared to O. tenuiflorum. In addition, O. tenuiflorum exhibit unicellular long trichomes which are exclusively distributed on the upper surface of the leaf. Whereas trichome distribution is predominantly seen on upper and lower surface of O. basilicum appears to be multi cellular in nature. In cross sectional view of O. basilicum leaf exhibit 8-9 rows of collenchymatous tissue underneath of upper surface. In contrast to O. basilicum, O. tenuiflorum found to possess 4-5 rows of collenchymatous tissue is seen in between vascular tissue and epidermis. However, a centrally
located vascular tissue composed of 8-12 uniseriate rows of metaxylem with vessels and parenchyma towards adaxial side and an arc of phloem towards abaxial side is prominently seen in cross sectional view of both plant species. Mesophyll tissue is composed of 1 to 2 rows of perpendicularly arranged well-developed palisade parenchymatous tissue towards upper epidermis and 5 to 6 rows of spongy parenchyma towards lower epidermis. However, vascular tissue is surrounded by bundle sheath is visible in O. tenuiflorum, where as it not clearly seen in O. basilicum leaf. In surface view, both the epidermal layers are traversed with diacytic stomata and with stomatal index being 26 to 33/mm\(^2\) and 18 to 21/mm\(^2\) on lower and upper sides respectively.

**T.S. of the stem**

Transverse section passing through stem of both species is quadrangular in outline. Anatomically stem is well demarcated as epidermis, cortex and vascular region. Epidermis is uniseriate with stalked glandular trichomes on ridges and uniseriate multicellular trichomes with tapering cell in the furrow region. Collenchyma is well developed in ridge region below the epidermis and poorly developed in the furrow region in the narrow cortex. Stone cells were observed in the cortical region. However endodermis is not clear. Pericycle is heterogeneous with well-developed sclerenchyma over vascular tissues altered with parenchyma. Vascular region with 6-7 well developed vascular bundles in eustele. Vascular cylinder has a narrow secondary phloem and a wide, dense cylinder of secondary xylem. Secondary xylem consists of metaxylem with thin wide vessels in radial multiples and thick walled xylem fibers. The pith is wide and parenchymatous with starch and sclereids. The old stem has wider secondary phloem and xylem. Phloem consists of slightly dilated rays and tangentially oblong, radial rows of sieve elements and parenchyma cells. Secondary xylem has solitary or radial and multiples of vessels which are thick walled and angular. Xylem rays are narrow and straight. Xylem fibers are thick walled. Growth rings are absent.

**Physicochemical Parameters**

Phytochemical parameters of *O. basilicum* L. var. *pilosum* (wild) Benth & *O. tenuiflorum* var. CIM-AYU in methanolic, ethanolic, acetone and ethyl acetate leaf extracts were presented in Table 4. Overall, methanolic leaf fraction of *O. basilicum* exhibit positive results in almost all parameters except quinines and resins. However, only acetone fraction of the *O. basilicum* leaf exhibit positive result for quinines and remaining three tests failed to detect quinines. Carboxylic acids and Coumarins are present only in *O. basilicum* and are absent in *O. tenuiflorum*. Presence of coumarins are reported that insecticidal properties. The present data further strengthens that, methanolic leaf extracts found to be the better choice to extract all important chemical constituents in the leaf material. In addition, for further application purposes, the methanolic fractions can be used to obtain high purity phytochemicals from this plant. From the results it is further evident that, the newly reported *Ocimum* species is potential candidate for exploring solutions to various health problems like other known *Ocimum* species. Due to its sharp pungent smell, the essential oil of this plant may have the potential to use combat microbial infections. In addition the aromatic principles of the plant may have mosquito properties against and commercially viable aromatic principles and phytochemicals offer wide applications in human health care. However, when compared to *O. tenuiflorum*, the plant of wild in nature, with limited biomass production with poor seed germination. Importanty the plant posses novel aromatic oils which offer commercial applications. Further to cater the growing needs of present demand, the useful characteristic may be transferred through conventional breeding / biotechnological principles for development of high yielding *Ocimum* species offer promising revenue to the farming community.

**CONFLICT OF INTEREST**

None declared

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