

## Studies on Morphological, Chemical and Molecular Aspects of *Ocimum* species From Central Himalaya, India

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**Abstract:** In the present study, three species of *Ocimum* i.e. *Ocimum sanctum* Linn (Holy Basil), *Ocimum gratissimum* Linn and *Ocimum kilimandscharium* Baker ex Gürke collected from different locations were taken for their taxonomic, chemical and genetic characterization. The volatile constituents identified in the essential oil obtained from *Ocimum sanctum* and *Ocimum gratissimum* were eugenol and (Z)- $\beta$ -ocimene varied in their percentage while the essential oil obtained from *Ocimum kilimandscharicum* has different oil composition and dominated by camphor (80.0%). DNA bands pattern of the three species suggesting that there is a strong relationship between the chemical profile and the genetic variability. Chemical and genetic profiling indicated that *Ocimum sanctum* and *Ocimum gratissimum* are similar in characteristics.

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**Key words:** *Ocimum* species, taxonomic, Chemical profiling, genetic profiling, GC analysis

### 1. Introduction

Medicinal and aromatic plants are of prime economic importance because of continuous and increasing demand for their products by local and foreign markets. Basil is one of the most important plants in this regard. Its essential oil is extensively employed in several countries for flavouring and foodstuffs, confectionary goods, condiments and toiletry products such as mouthwashes and dental creams. It also finds a prime place in the flavouring of foods such as spices, meats, sausages, tomato, potatoes, various kinds of sauces, fancy vinegars, pickles, ketchups and beverages (Kirtikar and Basu, 1933; Samant et al., 1998).

Different parts of the plants are used in endogenous cultures for medicine and homoeopathy. It is also used as a febrifuge and antimalarial plant. The plant infusions are taken for cephalalgia gouty joints and gragee for foul breath. The juice obtained from leaves relieves sore throats and earaches and counteracts ringworm. Seeds are used internally for constipation and piles (Jain, 1968).

*Ocimum* species popularly known as Basil is an annual, aromatic herb of family Lamiaceae that grows all over the world. Basil is one of the species used for the commercial seasoning (Pangtey et al., 2000). It is commonly known that the presence of essential oils and their composition determine the specific aroma of plants and the flavour the condiments. There are usually considerable variations in the contents of the major components within the species.

Three *Ocimum* species were collected from different locations of Uttarakhand Himalaya for studying their morphology, taxonomic character and chemical screening. The species collected were:

*Ocimum sanctum* Linn. (Holy Basil)

*Ocimum gratissimum* Linn.

*Ocimum kilimandscharium* Baker ex Gürke.

### 2. Taxonomic Description

#### 2.1 *Ocimum sanctum* Linn (Holy Basil)-

*O. sanctum* (*syn. O. tenuifolium*) commonly called Holy Basil or Tulsi is a sacred herb in India, used in teas, healing remedies and cosmetics. The plant is worshipped as dear to Vishnu in some section of *vaishnavism* Tulsi (*O. sanctum*) is a widely grown sacred plant of India. It is found growing naturally in moist soil nearly all over the globe (Figure 1 a). In India, Hindus grow Tulsi as a religious plant in their homes, temples and their farms.

#### 2.1.1 Distribution

Throughout India ascending the Himalaya to 6000 ft., Ceylon-common in waste places Malay Islands to Australia and the Pacific, west Asia to Arabia. It is grown in houses temples and gardens. An erect annual grows 0.5-.5 meters in height and has red or purple, Quadrangular branches. The leaves are opposite about 2-4 cm. long and variable in breadth, base narrowed, margins entire or toothed hairy on both the surfaces, dotted with minute glands and are aromatic. Racemes 6-8 inch long. Flowers are tiny, purple and inflorescence is a long spike or 12-14 Cm in length.

The fruits are small, smooth nutlets, reddish gray in colour.

### 2.1.2 Uses

The seeds, leaves and the roots of tulsi have great medicinal value. It is used both internally as well as externally. Tulsi has mild antiseptic, analgesic properties and it relieves the swellings also. Hence beneficial externally in various skin diseases. The paste of leaves works well, with marica powder when applied topically in ringworm infestations. The dressing with the pulp of its leaves effectively controls the infections and healing of chronic infected wounds. The leaves when chewed mitigate the infections of the gums. The fresh juice of the leaves in to ears is an effective domestic medicament for ear aches. The massage with the leaves juices improves the circulation beneath the skin and augments the sensation in the skin. In the headache due to sinusitis, the instillation of juice in the nose facilitates the secretion of Kapha and relieves the headache. The dried powder of the leaves can be inhaled like a snuff for the same purpose. Tulsi has specified actions on the respiratory system. It effectively liquefies the phlegm due to its hot and sharp attributes. It gives excellent results in cough due to kapha, allergic bronchitis, asthma and eosinophilia. Combined with honey, the juice works well to control the hiccup. In tubular cough tulsi is also beneficial. It is an effective panacea for fever, especially of Kapha type, while given with honey and marica fruit powder. In such conditions it effectively controls colds and reduces pain. Tulsi juices works as amapacana meaning it digests and destroys the toxins. Tulsi leaves contain a bright yellow volatile oil, which is useful against insects and bacteria. The principal constituents of this oil are eugenol, eugenol methyl ether and carvacrol. The oil is reported to possess anti bacterial properties and acts as an insecticide. The seeds are used in curing urinary problems. Aphrodisiac virtue has been attributed to it, where powdered Tulsi root with clarified butter (Ghee) is prescribed.

### 2.2 *O. gratissimum* Linn.

It is tall extremely fragrant basil, tremendously aromatic plant that adds significance to any garden and known as clove basil. It is brought into the sun-drenched greenhouse during winters, but they are grown in pots and containers outside during the growing season, which is from June to September. It has lime green leaves, which are up to 4 inches long and are put up with intense spikes of tiny, pale flowers of yellow colour. Clove basil is a half-hardy perennial shrub with a licorice-clove flavour and can reach an impressive height of 8 feet (Figure 1 b).

### 2.2.1 Distribution

Throughout India, often cultivated, Ceylon-java, Tropical Africa.

### 2.2.2 Uses

The burning leaves of the clove basil are used to keep away mosquitoes. Clove basil is at times used for cooking purpose as well. In many Italian dishes it is a well-known culinary herb. Tomato sauces, vinegars are some more things in which clove basil is commonly used. Calcium, iron, vitamin C and potassium which are largely beneficial to one's health, clove basil is full of them even small amounts of magnesium manganese and vitamin A are also present. The plant has a pungent taste with some flavour, heating, alexiteric, useful in vomiting fits, "vata" and "kapha", skin diseases, erysipelas, inflammations, strangury, causes insomnia (Ayurveda). They are used as a tonic to help in digestion apart from their culinary use. Clove basil oil, is even used in aromatherapy especially for problems relating to digestion. They have been largely used in Ayurveda, Chinese medicine and dysentery. Clove basil helps relieve cold and flu symptoms and even relieve rheumatic and arthritic conditions. They are even useful in treating chest problems such as coughs or bronchitis. They ease stomach cramps and pain. They also restrain growth of certain bacteria. They are even useful in treating insect bites. They help in relaxing the muscles and blood vessels and thereby increasing the blood circulation. Nervousness, depression, anxiety and tiredness are treated in second by it. And last but not the least, it is real handy in treating insomnia or sleeping disorder.

### 2.3 *O. kilimandscharicum* Baker ex Gürke (Camphor-Scented Basil)

This is one of the species of the genus *Ocimum* Linn. plant that is native in East Africa and was introduced and cultivated in India and some parts of Turkey. It is an evergreen aromatic perennial under shrub belonging to the Lamiaceae family. It thrives as a natural rounded woody shrub that can reach 2 m high in warm temperate regions of the tropics but can be propagated both by seeds and vegetative. The plant has pubescent quadrangular branchlets with simple leaves that are opposite and oblong, narrow at the base and deeply serrated. The leaves contain aromatic oils, which is the essence of the plant. The essential oil is extracted using distillation, expression or solvent extraction methods. The oil constitutes liquid oil and white solid crystals, where the pure crystals possess a characteristic odour and taste of natural Camphor (Figure 1 c).

### 2.3.1 Uses

Traditionally, extracts of *O. kilimandscharicum* were used to alleviate many ailments in East Africa including treatment of colds, coughs, abdominal pains, measles, diarrhea, insect repellent, particularly against mosquitoes and storage pest control. Toxicity and protectant potential of Camphor has been found to work against product beetles. Research undertaken on this plant's medicinal and insecticide efficacy classifies it as an aromatic plant whose bioactive properties can find use in

pharmaceutical, aroma therapeutic and pesticide. The low boiling point of the oil may be used as a solvent for metallic luster on ceramic bodies. *O. kilimandscharicum* has many different uses, some of them are:

Traditional medicine  
Raw material for commercial production of "Naturub"  
Camphor  
Mosquito repellent  
Source of nectar for bees in apiculture

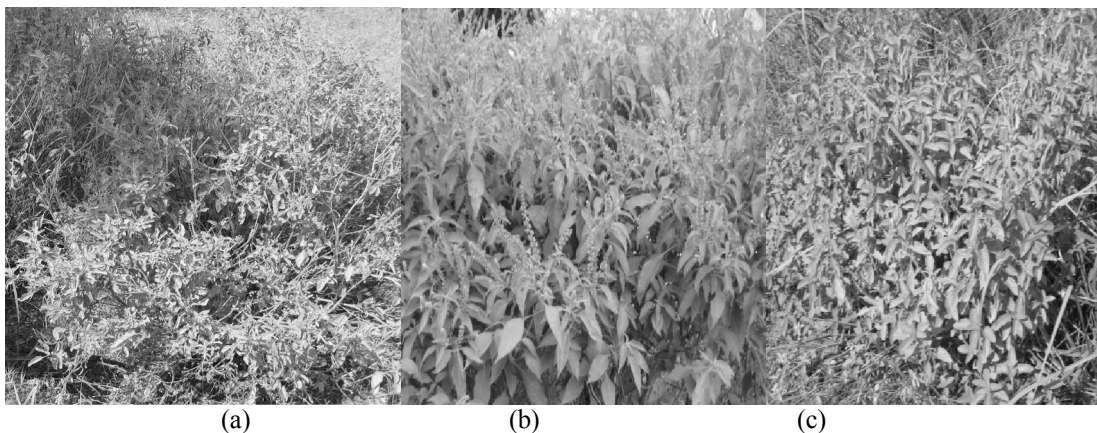


Figure 1. Morphological features of *Ocimum* species: (a) *Ocimum sanctum* Linn. (b) *Ocimum gratissimum* Linn. (c) *Ocimum kilimandscharicum* Baker ex Gürke

## 3. Experimental

### 3.1. Collection of plant materials

The aerial parts of three *Ocimum* species were collected from different locations in Kumaun Himalaya at the flowering stage. *O. sanctum* Linn. was collected from Bhowali, *O. kilimandscharicum* Baker ex Gürke collected from Almora and *O. gratissimum* Linn. was collected from Ranikhet. The identifications were done with floras and literature of Botany Department, Kumaun University, Nainital and confirmed by Prof. Y. P. S. Pangtey, F. N. A. Sc. The voucher specimens have been deposited in Botany Department, Kumaun University, Nainital.

### 3.2. Chemical profiling

#### 3.2.1. Oil isolation

The fresh aerial parts from all three collections (500 g each) were subjected to steam distillation in a copper electric still fitted with spiral glass condensers for two hours obtaining 5 L water distillate. The distillates were saturated with NaCl and the oils were extracted with hexane and dichloromethane. The organic phase was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and the solvent distilled in a thin film rotary vacuum evaporator at  $30^\circ\text{C}$ . The oil yields were 0.3%, 0.4% and 0.4% respectively.

#### 3.2.2. GC Analysis

The oil samples were subjected to GC Analysis on Nucon gas chromatograph model 5765 equipped with FID using stationary phase DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 mm film thickness). Hydrogen was used as a carrier gas at the rate of 1.0ml/min. Injector and detector temperatures were  $200^\circ\text{C}$  and  $230^\circ\text{C}$ , respectively. The column temperature was programmed from  $70^\circ\text{C}$ - $230^\circ\text{C}$  at  $4^\circ\text{C}/\text{min}$ , with the initial hold time of 2 min. The identification was done on the basis of retention time, co injection with standards and known essential oil constituents.

#### 3.2.3. Identification of the components

Identification of constituents was done on the basis of Retention Index, MS Library search (NIST and WILEY) and by comparing with the MS literature data (Adams, 2007). The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

### 3.3. Genetic Profiling

#### 3.3.1. DNA isolation

The present study was conducted at the Molecular Biology Laboratory of Dept. of

Biotechnology, Kumaun University, Bhimtal. The isolation of genomic DNA was done by CTAB method (Riberio and Lovato, 1987).

### 3.3.2. Quality analysis and Quantification of DNA

Quality and quantity of genomic DNA isolated was checked in UV spectrophotometer and on 0.8% agarose gel, stained with ethidium bromide (EtBr.) and documented using BIORAD gel documentation System.

## 4. Results and discussion

### 4.1. Chemical Profiling

The volatile constituents identified in the essential oil obtained from *O. sanctum* Linn. were eugenol (45.0%), (*Z*)- $\beta$ -ocimene (5.5%) and sabinene (5.0%). Interestingly the essential oil obtained from *O. gratissimum* Linn. was also dominated by eugenol (78.0%.0%) and (*Z*)- $\beta$ -ocimene (5.5%). The qualitative and quantitative composition of essential oils from *O. sanctum* Linn. and *O. gratissimum* Linn. are almost same. While the essential oil obtained from *O. kilimandscharicum* Baker ex Gürke has different oil composition and dominated by camphor (80.0%). The chemicals constituents indicated that among the three species two are similar in their essential oil composition. In a study of essential oils of different geographical origins, Lawrence *et al.* (1980) found that the main constituents of the essential oil of basil are produced by two different biochemical pathways. The phenylpropanoids (methyl chemical, eugenol, methyleugenol and methyl cinnamate) by the shikimic acid pathway and the terpenes (linalool and geraniol) by the mavalonic acid pathway (Yusuf *et al.*, 1994).

### 4.2. Genetic profiling

#### 4.2.1. Quality and Quantity of DNA

DNA could be successfully isolated from all the three species of *Ocimum*. Good quality DNA in *O. gratissimum* L. was obtained when extracted from frozen leaf samples using detergents (CTAB 2%). The DNA bands extracted with modified CTAB method did not show any smearing or RNA contamination. The DNA bands (Figure 2) of the species suggested that *O. sanctum* and *O. gratissimum* have similar genetic character while that of *O. kilimandscharicum* has different. This result is supported by their chemical constituents. The ratio of optical density at 260 nm and 280 nm (A<sub>260</sub>/A<sub>280</sub>) for ideal DNA should be around 1.6 – 1.8. Table 1 indicates the ratio of ODs 260/280 and DNA concentration in ( $\mu$ g/ml).

Cleaning of leaves would be recommended to swab the leaves with cotton wool wet with alcohol prior to preserving in order to dust off the leaf. Collecting conditions and preservation of samples are

important for the quality of DNA (Riberio and Lovato, 2007) as also observed in this study.

Although the spectro-photometric determination results were lower than the expected (1.8 – 2.0), the DNA obtained worked well for the subsequent studies in RAPD studies.

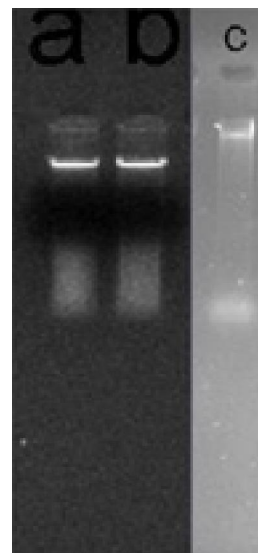


Figure 2. DNA bands of *Ocimum* species

- (a.) *O. sanctum*
- (b.) *O. gratissimum*
- (c.) *O. kilimandscharicum*

Table 1: Quality of DNA extracted from *Ocimum* samples

SAMPLES	RATIO (260/280)
<i>O. gratissimum</i>	1.65
<i>O. kilmandisacharicum</i>	1.52
<i>O. sanctum</i>	1.61

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