Comparative Pharmacognostical, Phytochemical and Biological Evaluation of Five Ocimum Species

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ABSTRACT
There are about 150 species of Ocimum in the world and out of that 68 species are found in India. Comparative pharmacognostic study of these Ocimum species is unspecified. Growing demand of Ocimum plants demands quality standards for correct identification of desired Ocimum species. Objectives: Hence aim of present study is to establish comparative pharmacognostical, phytochemical and biological standards for most commonly found and morphologically confusing five species of Ocimum. Methods: Macroscopic, microscopic, preliminary phytochemical evaluations, extraction of essential oils, TLC analysis, in vitro antioxidant and antmicrobial potency of selected five species carried out and compared. Results: This comparative study reports that O. sanctum should be preferred in medicine use among selected five species based on phytochemical composition, antioxidant and antimicrobial potency. Key words: Ocimum americanum, Ocimum basilicum, Ocimum kilimandscharicum, Ocimum gratissimum, Ocimum sanctum.

INTRODUCTION
Medicinal properties of Ocimum species belongs to mint or lamiaceae family are known for thousand years to various civilizations of the world through Ayurveda, Siddha and Unani systems of medicine. In the Indian subcontinent, in Ayurveda and Indian mythology, it is commonly called as Tulsi means “matchless one” or “incomparable one” and considered as a sacred plant representing Holy Hindu Laxmi Goddess. All parts of this plant species are of major medicinal use. It is useful in treatment of cold, bronchitis, stress, and digestive disorder. This also possesses anti-oxidant, antimicrobial and mosquito repellent properties. It is strong immunomodulator and adoptogen as per ayurveda as this healing herb has ability to enhance the energetic resonance between the body and the environment to protect body from stress, and digestive disorder. This also possesses antioxidant and antimicrobial potency of selected five species carried out and compared. In India two forms of Ocimum sanctum (synonym O. tenuiflorum) are more common - dark or Shyama (Krishna) Tulsi and light or Rama Tulsi. Ocimum gratissimum is known as Vana (wild/forest) tulsi. Various other species are also commonly found in India like O. canum, O. basilicum, O. kilimandscharicum, O. americanum, O. camphora and O. micranthum.

MATERIALS AND METHODS
Material collection, identification, authentication
Plant material (Ocimum americanum, Ocimum basilicum) was purchased from Nagarjun Garden Dr. P.K.V. Akola. Identification and authentication was made from H.O.D, Botany department, Government Vidarbha Institute of science & Humanities, Amravati (Maharashtra)

Pharmacognostic evaluations
Macroscopic evaluation
Macroscopy of whole plant was studied by observing the organoleptic characters such as color, odor, size, shape, taste and special features including touch and texture etc. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity.

Microscopic evaluation
Microscopic evaluation is very helpful in the initial identification of herbs as well as in detection of adulteration by characteristics tissue features. Every plant possesses a characteristics tissue structure which can be demonstrated through study of tissue arrangement, cell walls and configurations, when properly mountain in stains, reagents and media. Thus it determines the size, shape, and relative structure of different cell Microscopical parameters.
observed were, arrangement of tissues in a transverse section, type of epidermal cells, presence and type of calcium crystals, starch grains, oil globules, aleurone grains and trichomes.\(^\text{25}\) Microscopy carried out by using digital microscope (Olympus, Model- U-APF, Cx31RTSF).

**Powder microscopy evaluation**

The powder of respective plant was used for powder microscopy study. Plain powder and powder treated with specific reagents like phloroglucinol -hydrochloric acid, iodine solution and Sudan red solution were spread as a thin layer on separate glass slides and observed under microscope.\(^\text{26}\)

**Physicochemical evaluation**

Ash values, extractive values and loss on drying determined as per standard procedures mentioned in Indian pharmacopoeia 2010.\(^\text{26}\) These physicochemical standards are helpful in comparative determination quality and purity of the powder of plants of Ocimum genus.

**Preliminary phytochemical evaluation**

Preliminary phytochemical evaluations is the step to identify different classes of constituent that are primary constituents like carbohydrate, proteins, and lipids or secondary metabolite like glycosides, alkaloids, volatile oil, tannins etc. of great. The compounds that are responsible for medicinal efficacy are usually secondary metabolite. Hence plant material is subjected to preliminary phytochemical screening\(^\text{26}\) for detection of various chemical constituents.

**Extraction of essential oil**

Extraction of oil was carried out by hydro distillation technique using Clevenger apparatus\(^\text{26}\)\(^\text{,27}\) and stored in dark glass vial in a refrigerator until further testing.

**Chromatographic evaluation**

Comparative TLC using pre-coated silica gel GF254plate as stationary phase, toluene: ethyl acetate (93:7) as mobile phase and Anisaldehyde-sulphuric acid as spraying reagent. Sprayed plate heated on hot plate at 110°C till color developed and intensified.\(^\text{28}\)

**Antioxidant activity**

Free radical generation due to oxidative stress is one of the major causes of many diseases in human body. Ocimum species are known to be a very good free radical scavenger hence it is decided to know comparative antioxidant potential of selected Ocimum species. DPPH (1, 1- diphenyl-2- picryl-hydrazyl) is a stable free radical and methanolic solution of it is used to evaluate the anti oxidant activity of several natural compounds. To 1ml of DPPH solution, 3ml of oil sample added. The same reaction mixture without sample but equivalent amount of standard phosphate buffer served as control. Well mixed solution allowed to stand at room temperature for 30 min. Absorbance of reaction mixture was measured at 517 nm. Percentage scavenging activity at different concentrations was calculated by using formula: % scavenging activity = 1 - Absorbance of test/ Absorbance of control x 100.\(^\text{29}\)

**Antimicrobial activity**

Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibacterial in the last years, antimicrobial resistance has now become a global concern. The global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure. In vitro antibacterial potential of essential oils of selected Ocimum species against Escherichia coli was determined by agar well method using nutrient agar media. At the end of incubation, zone of inhibition formed measured in millimeter. Gentamicin is used as standard.\(^\text{20-30}\)

**Antifungal activity**

In vitro antifungal potential of essential oils of selected Ocimum species using YEPD agar media against fungi Candida albicans was determined by using agar well method. All steps are same as procedure given in antibacterial activity, only the incubation period for antifungal activity was for 48 hours. At the end of incubation, zone of inhibition formed measured in millimeter. Fluconazole was used as standard.\(^\text{29-30}\)

**RESULT AND DISCUSSION**

Medicinal use of Ocimum species is abundantly increasing due to its immunomodulator and antioxidant potential. It is commonly used in many marketed or even in homemade herbal tea formulas. There are about 150 species of Ocimum in the world and out of that 68 species are found in India. Comparative pharmacognostic study of these Ocimum species is unspecified. Hence, present research work generates comparative pharmacognostic data of selected five species of ocimum found in Vidarbha region of Maharashtra state.

Total five species of Ocimum i.e. Ocimum sanctum Linn, Ocimum americanum Linn, Ocimum basilicum Linn, Ocimum gratissimum Linn, Ocimum kilimandscharicum guerke are evaluated comparatively for their morphological, microscopical, physiochemical, and phytochemical parameters and also evaluated for their antioxidant and antimicrobial activity by in vitro methods. The whole plants are shade dried and powdered using grinding mill. The powder was stored in airtight container.

Comparative morphological evaluations of all selected five Ocimum species are summarized in Table 1. Microscopy of leaves and stem as well as powder of whole plant of all selected five Ocimum species studied in detail and comparisons are summarised in Table 2 and Table 3.

Microscopical examination of leaf O. americanum shows isobilateral lamina covered with cuticle; glandular trichomes with multi-cellular head and multicellular warty covering trichomes. Mid rib with arc shaped vascular bundle consisting of xylem and phloem, three to four layers of collenchymatous tissue present on upper side of vascular bundle whereas stem part shows cork, vascular bundle containing xylem & phloem, spongy tissue, collenchymatous cells, pith. Powder microscopy shows reticulate xylem vessel, stone cell, epidermal cell, collenchymas, cork cell.

Ocimum basilicum leaf shows the presence of multicellular curved trichomes, Upper epidermis, collenchymatous cells, upper palisied cell, vascular bundle containing xylem & phloem, spongy tissue, lower epidermis, stem section shows unicellular covering trichome, epidermal cell, collenchymatous cells, vascular bundle containing xylem & phloem, spongy tissue, pith, spongy parenchyma. Powder microscopy shows presence of medullary rays lamellar collenchymas, Cork cell, Epidermal cell, Fibers, Stone cell.

Ocimum gratissimum showed Single layered epidermis, multicellular covering trichomes and glandular trichomes, collenchymatous cells, Vascular bundle consisting of xylem and phloem, Palisade cells and spongy tissue A transverse section of stem showed Shape of section was rectangular Compressed bark cells followed by single layered epidermis Multicellular covering trichomes and glandular trichomes, Collenchymatous cells, Vascular bundle contains xylem and phloem, Spongy tissue was present at centre. Powder microscopy glandular trichomes with multi-cellular head, Thin-walled fiber with pointed...
Table 1: Comparative morphological evaluated parameters of selected five *Ocimum* species.

| Parameter          | O. americanum          | O. basilicum       | O. gratissimum     | O. kilimandscharicum | O. sanctum            |
|--------------------|------------------------|--------------------|--------------------|----------------------|-----------------------|
| **Colour**         | Green to yellow green  | Green or some time | Light green         | Pale green            | Green to purple       |
| **Odour**          | Aromatic               | Faint              | Aromatic            | Aromatic             | Aromatic              |
| **Taste**          | Characteristic, mint like | Characteristic | Oily and sharp, tingling taste like cloves, pungent. | Aromatic camphor like | Warm & pungent, aromatic and sharp. |
| **Height**         | 30-60 cm               | 60-80 cm           | 1 to 1.5 m         | 15 to 30 cm          | 20-60 cm              |
| **Herb**           | Branched herb, branches are sub-quadrangular striate; light puff colored stem | Erect, strongly aromatic, nearly glabrous branching herb, covered with soft spreading hairs | Stem and branches are green | Perennial aromatic evergreen under shrub Stems are brownish green, | Much branched, stems and branches usually purplish, sub-quadrangular, woody, Covered with soft spreading hairs |
| **Leaves**         |                        |                    |                    |                      |                       |
| **Shape**          | Elliptic – lanceolate  | Elliptical         | Ovate, pointed and sharp | Ovate or oblong      | Ovate, elliptical, oblong obtuse or acute |
| **Venation**       | Pinnate                | Pinnate            | Pinnate            | Pinnate              | Pinnate               |
| **Margin**         | Serrate                | Lobed              | Entire             | Serrate              | Entire                |
| **Fruit**          |                        |                    |                    |                      |                       |
| **Shape**          | Small; nut lets are pitted, And pitted. | Ellipsoid nut lets, Elongated, round at one end and flattened at the other | Ovoid, smooth or minutely tuberculate, | Ovoid, smooth or minutely tuberculate, | Caeruleus |
| **Colour**         | Black                  | Black              | Black              | Black                | Brownish Black        |
Table 2: Comparative microscopical evaluated parameters of selected five *Ocimum* species.

| Microscopy leaf of *O. americanum* where 1-Multicellular covering trichomes, 2- Glandular trichomes, 3- Upper epidermis, 4- Upper palisade cell, 5- Vascular bundle containing xylem & phloem, 6- Collenchymatous cells, 7- Oil globules, 8-Spongy tissue, 9- Lower epidermis |
|---|
| Microscopy of stem of *O. americanum* where 1-Cork, 2- Vascular bundle containing xylem & phloem, 3-Spongy tissue, 4-Collenchymatous cells, 5-pith. |
| Microscopy leaf of *O. basilicum* where 1-Multicellular curved trichomes, 2- Upper epidermis, 3-Collenchymatous cells, 4- Upper palisade cell, 5-oil globules, 6- Vascular bundle containing xylem & phloem, 7- Spongy tissue 8- Lower epidermis |
| Microscopy stem of *O. basilicum* 1-Unicellular covering trichome, 2- Epidermal cell, 3- Collenchymatous cells, 4-Vascular bundle containing xylem & phloem, 4-Spongy tissue, 5-Pith, 6- spongy parenchyma |
| microscopy leaf of *O. gratissimum* where 1-Multicellular covering trichomes, 2- Glandular trichomes, 3- Upper epidermis, 4- Upper palisaded cell, 5-Collenchymatous cells, 6- Vascular bundle containing xylem & phloem, 7- oil glands |
| Microscopy stem of *O. gratissimum* 1-Multicellular covering trichome, 2- Glandular trichome, 3-Collenchymatous cells, 4-Vascular bundle containing xylem & phloem, 5-Spongy tissue |
Microscopy of leaf of *O. kilimandscharicum* Where 1-Multicellular covering trichomes, 2-Multicellular curved trichomes, 3-Upper epidermis, 4-Upper palisaded cell, 5-Vascular bundle containing xylem & phloem, 6-Collenchymatous cells

Microscopy of stem of *O. kilimandscharicum* where 1-Cork, 2-Collenchymatous cells, 3-Vascular bundle containing xylem & phloem, 4-Multicellular curved trichomes, 5-Pith

Microscopy of leaf of *O. sanctum* where 1-Unicellular covering trichomes, 2-Glandular trichomes, 3-Upper epidermis, 4-Upper palisaded cell, 5-Vascular bundle containing xylem & phloem, 6-Collenchymatous cells, 7-Parenchyma cell, 8-Epidermal cell

Microscopy stem of *O. sanctum* 1-Cork, 2-Collenchymatous cells, 3-Pith, 4-Medullar rays

Table 3: Comparative Powder Microscopical evaluated parameters of selected five *Ocimum* species.
Ocimum americanum: 1-Cork cell, 2-Stone cell, 3-epidermal cell, 4-Collenchymas, 5-Reticulate Xylem vessel

Ocimum basilicum: 1-Stone cell, 2-Lamellar collenchymas, 3-Cork cell, 4-Epidermal cell, 5-Fibers, 6-Medullary rays, 7-Wood element
Ocimum gratissimum: 1-Reticulate xylem vessel, 2-Fiber, 3-Cortical cell, 4-Stone cell, 5-Multicellular trichome, 6-Epidermal cell, 7-Vessels, 8-Oil glands
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Ocimum sanctum: 1- Multicellular curved trichomes, 2-Glandular trichomes, 3-Scleranmatous fiber, 4-Epidermal cell, 5-Cork cell, 6-Bicuspid stomata

Ocimum kilimandscharicum: 1- Stone cell, 2-Reticulate xylem vessel, 3-multi cellular curved trichomes, 4-Storage parenchyma,

end, multicellular covering trichome, Diacytic stomata, Wavy walled epidermal cells, Collenchymatous cell with intercellular spaces, Vessels. This species can be identified as highly multi-cellular trichome containing species. This is only one species which contain trichomes on the stem.

Ocimum kilimandscharicum leaf part shows multi-cellular covering trichomes, multi-cellular curved trichomes, upper epidermis upper palisied cell, vascular bundle containing xylem & phloem, Collenchymatous cells, and stem part shows cork. Collenchymatous cells, vascular bundle containing xylem & phloem, multi-cellular curved trichomes, pith and powder microscopy shows Storage parenchyma, Reticulate xylem vessel, multi cellular curved trichomes, Stone cell.

Ocimum sanctum leaf microscopy shows Unicellular covering trichomes, Glandular trichomes, Upper epidermis, Upper palisied cell, Vascular bundle containing xylem & phloem, collenchymatous cells, Parenchyma cell, Epidermal cell. Stem shows cork, collenchymatous cells, pith, and medullar rays. Powder microscopy shows glandular trichomes, fiber multi-cellular curved trichomes, sclerenchymatous fiber, and stomata cell cork cell bicuspid epidermal cells.

Physicochemical evaluation (Tables 4 & 5) was carried out which shows that total ash values ranges from 7.6 (O. basilicum) to 8.7 (O. sanctum) % w/w, acid insoluble ash ranges from 0.2 to 0.4% w/w, water soluble ash ranges from 3 to 3.7% w/w. Extractive values are found as: alcohol soluble extractive value ranges from 2.9 (O. sanctum) to 5.7 (O. kilimandscharicum) % w/w, water soluble extractive value which ranges from 3.7 (O. americanum) to 6.9 (O. sanctum) % w/w and ether soluble extractives which ranges from 2.1 (O. kilimandscharicum) to 3.8 (O. basilicum) % w/w.

The phytochemical studies were carried to collect the comparative phytochemical analysis of selected species of Ocimum. Phytochemical tests were carried for presence of alkaloids, glycoside, anthraquinone glycosides, gums mucilage, proteins, amino acids, tannins, phenolic compound, triterpenoids, steroids, sterols, saponins, flavones, flavonoids. Results are summarised in Table 6.

For the extraction of essential oils, shaded dried powder of whole plant was used. Essential oil was extracted by using Clevenger apparatus and oil was stored in air tight amber colored bottle.

Comparative chromatographic evaluation (Table 7 and Figure 1) was carried out for methanolic extract and essential oils of all five species using silica gel GF 254 as a stationary phase and toluene: ethyl acetate (93:7) as mobile phase.

In-vitro Antioxidant activity was carried out by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) method. Antioxidant activity observed as shown in
Table 4: Comparative ash values of selected five Ocimum species.

| Ocimum species     | Total Ash (%w/w) | Acid Insoluble (%w/w) | Water Soluble (%w/w) |
|--------------------|------------------|-----------------------|----------------------|
| Ocimum americanum   | 8.1              | 0.4                   | 3.8                  |
| Ocimum basilicum    | 8.7              | 0.2                   | 3.5                  |
| Ocimum gratissimum  | 7.9              | 0.3                   | 3.8                  |
| Ocimum kilimandscharicum | 7.6          | 0.3                   | 3.0                  |
| Ocimum sanctum      | 8.3              | 0.4                   | 3.7                  |

Table 5: Comparative extractive values selected five Ocimum species.

| Extractive values | Ocimum americanum | Ocimum basilicum | Ocimum gratissimum | Ocimum kilimandscharicum | Ocimum sanctum |
|-------------------|-------------------|------------------|---------------------|--------------------------|----------------|
| Water soluble     | 3.7               | 6.9              | 6.4                 | 4.0                       | 4.5            |
| Alcohol soluble   | 2.9               | 4.2              | 3.0                 | 5.7                       | 2.7            |
| Ether soluble     | 3.5               | 3.8              | 3.2                 | 2.1                       | 3.1            |

Table 6: Comparative preliminary phytochemical screening results of selected five Ocimum species.

| Ocimum species          | Phytochemicals                                                                                                                                                                                                 |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ocimum americanum       | Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Phenolic compounds, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids                                                      |
| Ocimum basilicum        | Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Tannins, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids                                                                         |
| Ocimum gratissimum      | Alkaloids, Proteins, Amino acids, Phenolic compounds, Tannins, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids                                                                          |
| Ocimum kilimandscharicum| Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Triterpenoids, Volatile oils                                                                                                                     |
| Ocimum sanctum          | Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Phenolic compounds, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids                                                          |

Table 7: Comparative TLC results of essential oil and methanolic extract of selected five Ocimum species.

| Ocimum species          | Total Separated constituents | Rf Value                                      | Total Separated constitutes | Rf Value                                      |
|-------------------------|------------------------------|-----------------------------------------------|-----------------------------|-----------------------------------------------|
| Ocimum americanum       | 07                           | 0.23, 0.33, 0.45, 0.51, 0.73, 0.84, 0.98       | 03                          | 0.1, 0.2, 0.3                                |
| Ocimum basilicum        | 10                           | 0.1, 0.18, 0.19, 0.22, 0.33, 0.41, 0.48, 0.70, 0.8, 0.9 | 04                          | 0.08, 0.15, 0.17, 0.24                       |
| Ocimum gratissimum      | 00                           | Nil                                           | 02                          | 0.15, 0.3                                    |
| Ocimum kilimandscharicum| 02                           | 0.3, 0.6                                      | 01                          | 0.15                                         |
| Ocimum sanctum          | 09                           | 0.08, 0.1, 0.22, 0.21, 0.3, 0.4, 0.7, 0.8, 0.9 | 02                          | 0.1, 0.25                                    |

Table 8: Comparative Antioxidant potential of selected five Ocimum species.

| No. | Extracts            | IC 50 [µg/ml] | Antioxidant Activity (%) |
|-----|---------------------|---------------|--------------------------|
| 1   | Ascorbic acid (10µg/ml) | 1.83          | 54.5                     |
| 2   | Ocimum americanum   | 3.1           | 36.3                     |
| 3   | Ocimum basilicum    | 3.1           | 36.3                     |
| 4   | Ocimum gratissimum  | 1.8           | 18.1                     |
| 5   | Ocimum kilimandscharicum | 1.8          | 18.1                     |
| 6   | Ocimum sanctum      | 1.2           | 39.2                     |

Table 9: Comparative antimicrobial potential of selected five Ocimum species.

| Bacteria          | Ocimum americanum | Ocimum basilicum | Ocimum gratissimum | Ocimum kilimandscharicum | Ocimum sanctum | Standard |
|-------------------|-------------------|------------------|---------------------|--------------------------|----------------|----------|
| Bacteria (E coli) | 20mm              | 19mm             | 31mm                | 10mm                     | 13mm           | 33mm     |
| Fungus (Candida albicans) | -             | 31mm             | -                   | -                        | 34mm           | 32 mm    |
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**Figure 1:** TLC of essential oil and extract where OA: Ocimum americanum, OB: Ocimum basilicum, OG: Ocimum gratissimum, OK: Ocimum kilimandscharicum, OS: Ocimum sanctum.

**Figure 2:** Comparative antimicrobial potential of selected five Ocimum species.
Table 8 more in *O. sanctum* while *O. basilicum* and *O. gratissimum* also showed good anti-oxidant activity.

Antimicrobial potential (Table 9 and Figure 2) was evaluated by agar well method against *E. coli* and *Candida albicans* *O. kilimandscharicum* has more potent antifungal activity compared to *O.americum*, *O. basilicum*, *O.gratissimum*, *O.sanctum* while later species have more antibacterial activity.

**CONCLUSION**

Present work has provided useful information to identity, differentiate and evaluate most commonly used and confusing species of genus Ocimum-*O. americum, O. basilicum, gratissimum, O. kilimandscharicum, O. sanctum*. Finally it can be concluded that *O. sanctum* should be preferred among five selected species based on comparative phytochemical composition, antioxidant and antimicrobial activity.

**CONFLICTS OF INTEREST**

There is no conflict of interest.

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