

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 antimicrobial 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 world¹⁻⁴ through Ayurveda, Siddha and Unani systems of medicine.⁵⁻⁹ 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.¹⁰⁻¹²

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 opportunistic infections.¹³⁻¹⁷

Major chemical constituents of *Ocimum* species are mono and sesquiterpenes like eugenol, α -pinene, β -pinene, camphene, sabinene, p-cymene, limonene, linalool, camphor, borneol, terpin-4-ol, α -terpineol, methyl chavicol, α -cubebene, α -copaene, β -bourbonene, β -cubebene, α -elemene, methyl eugenol, β -caryophyllene, β -gurjunene, α -humulene, germacrene, germacrene, cubebol and δ -cadinene.¹⁸⁻²¹

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*.²¹⁻²⁴

MATERIALS AND METHODS

Material collection, identification, authentication

Plant material (*Ocimum americanum*, *Ocimum basilicum*) was purchased from local nursery of district Amravati from Maharashtra. Plant material (*Ocimum kilimandscharicum*, *Ocimum gratissimum*, *Ocimum sanctum*) was purchased from Nagarjun Garden Dr. P.D.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.²⁵⁻²⁶

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 mounted in stains, reagents and media. Thus it determines the size, shape, and relative structure of different cell Microscopical parameters

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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.²⁵ Microscopy carried out by using digital microscope (Olympus, Model- U-APT, 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.²⁶

Physicochemical evaluation

Ash values, extractive values and loss on drying determined as per standard procedures mentioned in Indian pharmacopoeia 2010.²⁶⁻²⁷ 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 screening²⁶ for detection of various chemical constituents.

Extraction of essential oil

Extraction of oil was carried out by hydro distillation technique using Clevenger apparatus²⁶⁻²⁷ and stored in dark glass vial in a refrigerator until further testing.

Chromatographic evaluation

Comparative TLC using pre-coated silica gel GF254 plate 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.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 100.²⁶

Antibacterial 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.²⁹⁻³⁰

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.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 palisade 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.














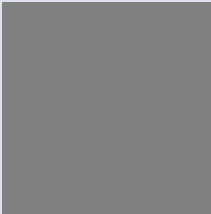

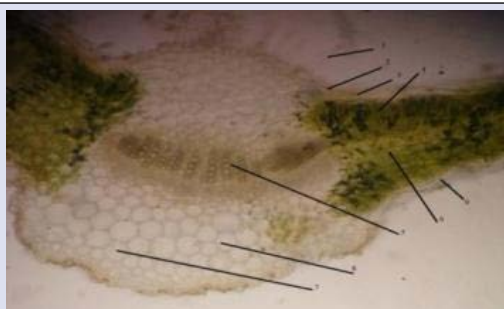
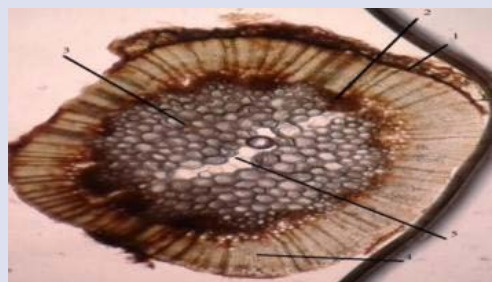
Parameter	<i>O. americanum</i>	<i>O. basilicum</i>	<i>O. gratissimum</i>	<i>O. kilimandscharicum</i>	<i>O. sanctum</i>
Colour	Green to yellow green	Green or some time purple	Light green	Pale green	Green to purple
Odour	Aromatic	Faint	Aromatic	Aromatic	Aromatic
Taste	Characteristic, mint like flavour	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,	Ellipsoid nut lets, And pitted.	Elongated, round at one end and flattened at the other	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.



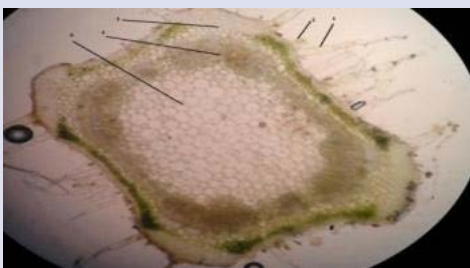
Microscopy leaf of *O. basilicum* where 1-Multicellular curved trichomes, 2- Upper epidermis, 3-Collenchymatous cells, 4- Upper palisade cell, 5-oil globules, 6- Vascular bundle containing xylem & phloem, 7- Spongy tissue, 8- Lower epidermis



Microscopy stem of *O. basilicum* 1-Unicellular covering trichome, 2- Epidermal cell, 3- Collenchymatous cells, 4-Vascular bundle containing xylem & phloem, 5-Spongy tissue, 6- spongy parenchyma



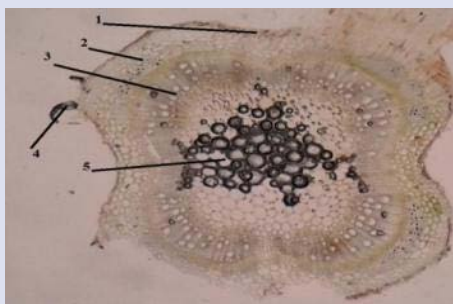
microscopy leaf of *O. gratissimum* where 1-Multicellular covering trichomes, 2- Glandular trichomes, 3- Upper epidermis, 4- Upper palisade cell, 5-Collenchymatous cells, 6- Vascular bundle containing xylem & phloem, 7- oil glands



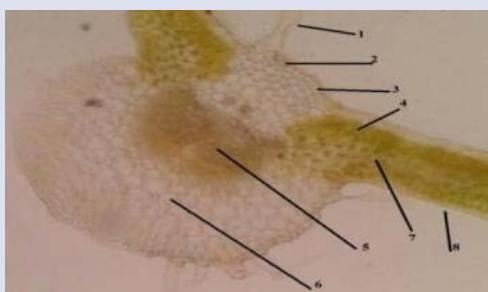
Microscopy stem of *O. gratissimum* Where 1-Multicellular covering trichome, 2- Glandular trichome, 3-Collenchymatous cells, 4-Vascular bundle containing xylem & phloem, 5-Spongy tissue



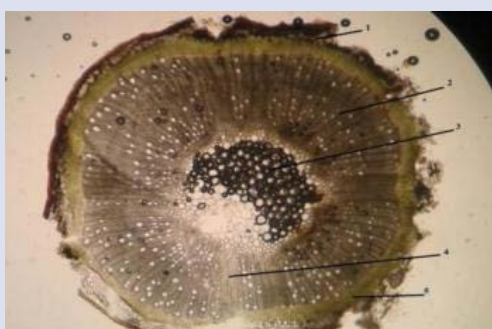
Microscopy of leaf of *O. kilimandscharicum* Where 1-Multicellular covering trichomes, 2- Multicellular curved trichomes , 3- Upper epidermis, 4- Upper palisade cell, 5- Vascular bundle containing xylem & phloem, 6-Collenchymatous cells



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

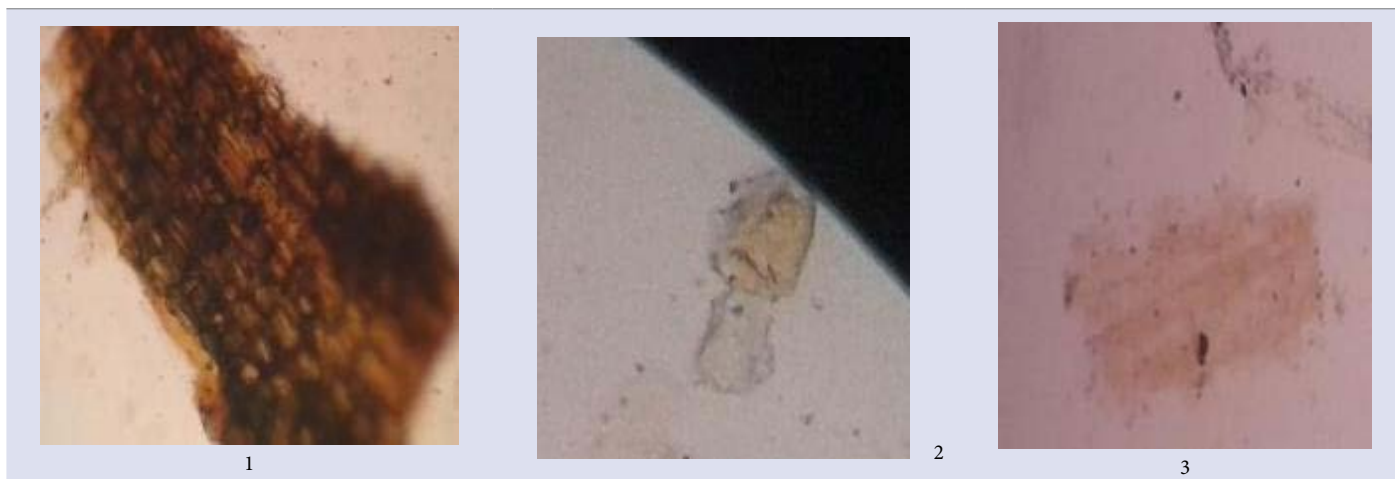


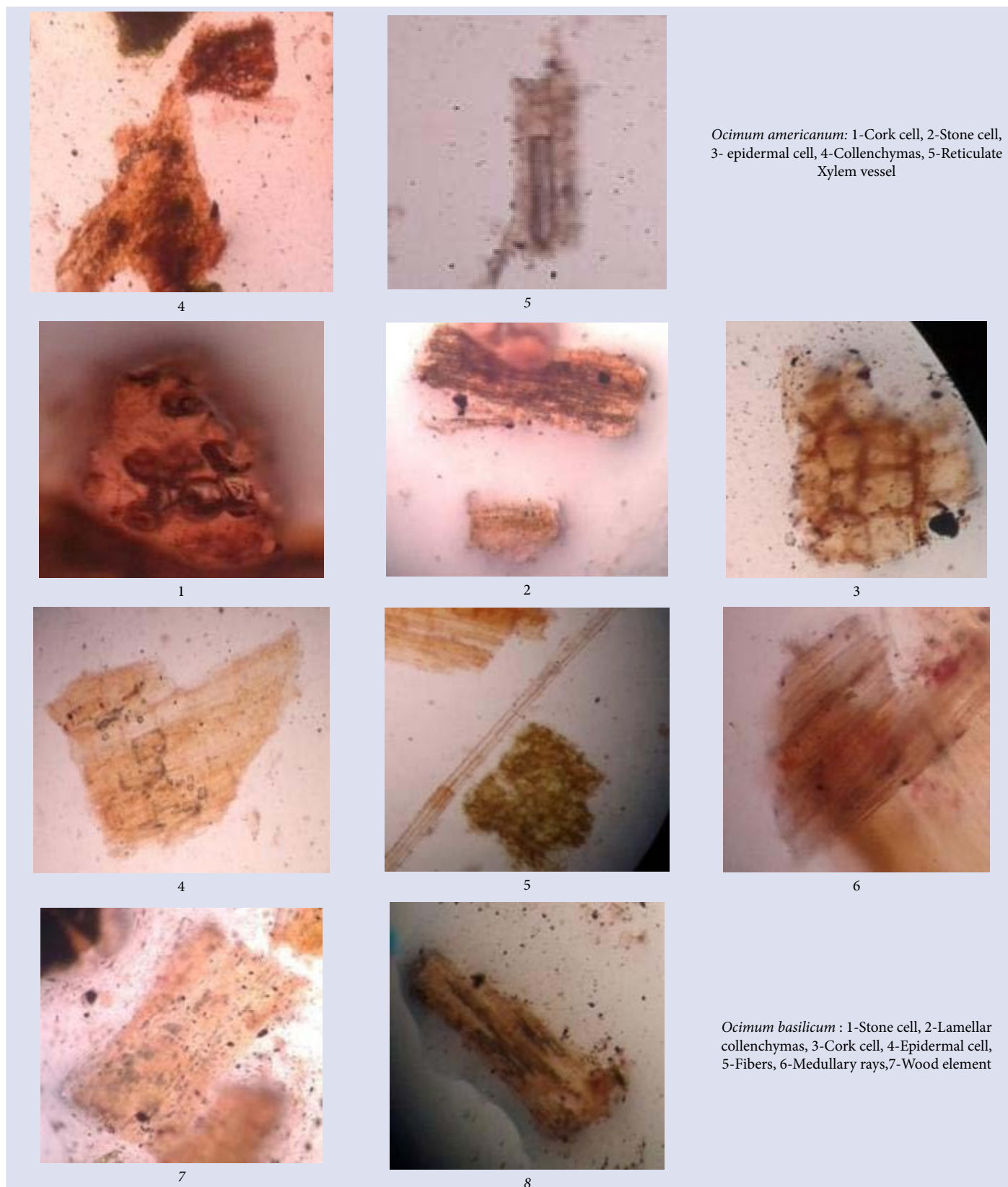
Microscopy of leaf of *O. sanctum* where 1-Unicellular covering trichomes, 2- Glandular trichomes , 3- Upper epidermis, 4- Upper palisade cell, 5- Vascular bundle containing xylem & phloem, 6-Collenchymatous cells, 7-Parenchyma cell, 8- Epidermal cell



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

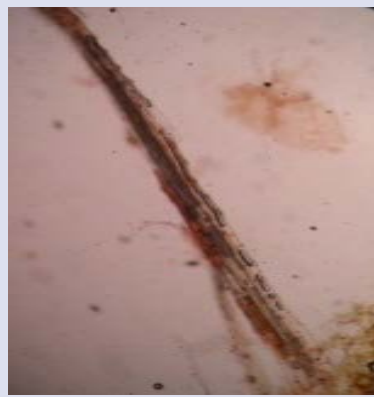
Table 3: Comparative Powder Microscopical evaluated parameters of selected five *Ocimum* species.







1



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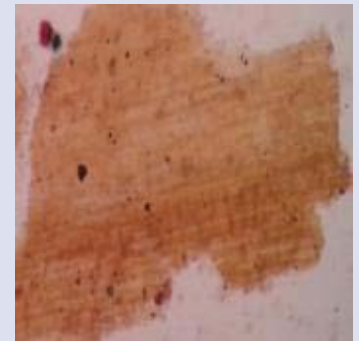
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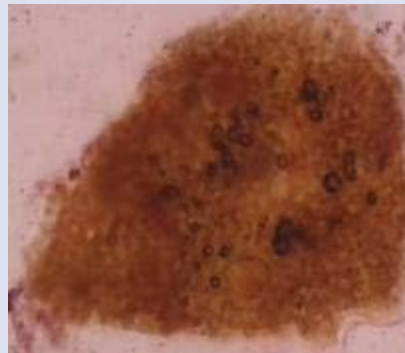
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6



7



8

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



1



2



3

Table 4: Comparative ash values of selected five *Ocimum* species.

Ocimum species	Total Ash (%w/w)	Acid Insoluble (%w/w)	Water Soluble (%w/w)
<i>Ocimum americanum</i>	8.1	0.4	3.8
<i>Ocimum basilicum</i>	8.7	0.2	3.5
<i>Ocimum gratissimum</i>	7.9	0.3	3.8
<i>Ocimum kilimandscharicum</i>	7.6	0.3	3.0
<i>Ocimum sanctum</i>	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
<i>Ocimum americanum</i>	Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Tannins, Phenolic compounds, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids
<i>Ocimum basilicum</i>	Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Tannins, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids
<i>Ocimum gratissimum</i>	Alkaloids, Proteins, Amino acids, Phenolic compounds, Tannins, Triterpenoids, Volatile oils, Steroids, Sterols, Saponins, Flavones, Flavonoids
<i>Ocimum kilimandscharicum</i>	Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Triterpenoids, Volatile oils
<i>Ocimum sanctum</i>	Alkaloids, Glycoside, Gums mucilage, Proteins, Amino acids, Tannins, 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	Essential Oils		Methanolic extract	
	Total Separated constituents	Rf Value	Total Separated constituents	Rf Value
<i>Ocimum americanum</i>	07	0.23, 0.33, 0.45, 0.51, 0.73, 0.84, 0.98	03	0.1, 0.2, 0.3
<i>Ocimum basilicum</i>	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
<i>Ocimum gratissimum</i>	00	Nil	02	0.15, 0.3
<i>Ocimum kilimandscharicum</i>	02	0.3, 0.6	01	0.15
<i>Ocimum sanctum</i>	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 [$\mu\text{g}/\text{ml}$]	Antioxidant Activity (%)
1.	Ascorbic acid (10 $\mu\text{g}/\text{ml}$)	1.83	54.5
2	<i>Ocimum americanum</i>	3.1	36.3
3	<i>Ocimum basilicum</i>	3.1	36.3
4	<i>Ocimum gratissimum</i>	1.8	18.1
5	<i>Ocimum kilimandscharicum</i>	1.8	18.1
6	<i>Ocimum sanctum</i>	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 (<i>E. coli</i>)	20mm	19mm	31mm	10mm	13mm	33mm
Fungus (<i>Candida albicans</i>)	-	31mm	-	-	34mm	32 mm

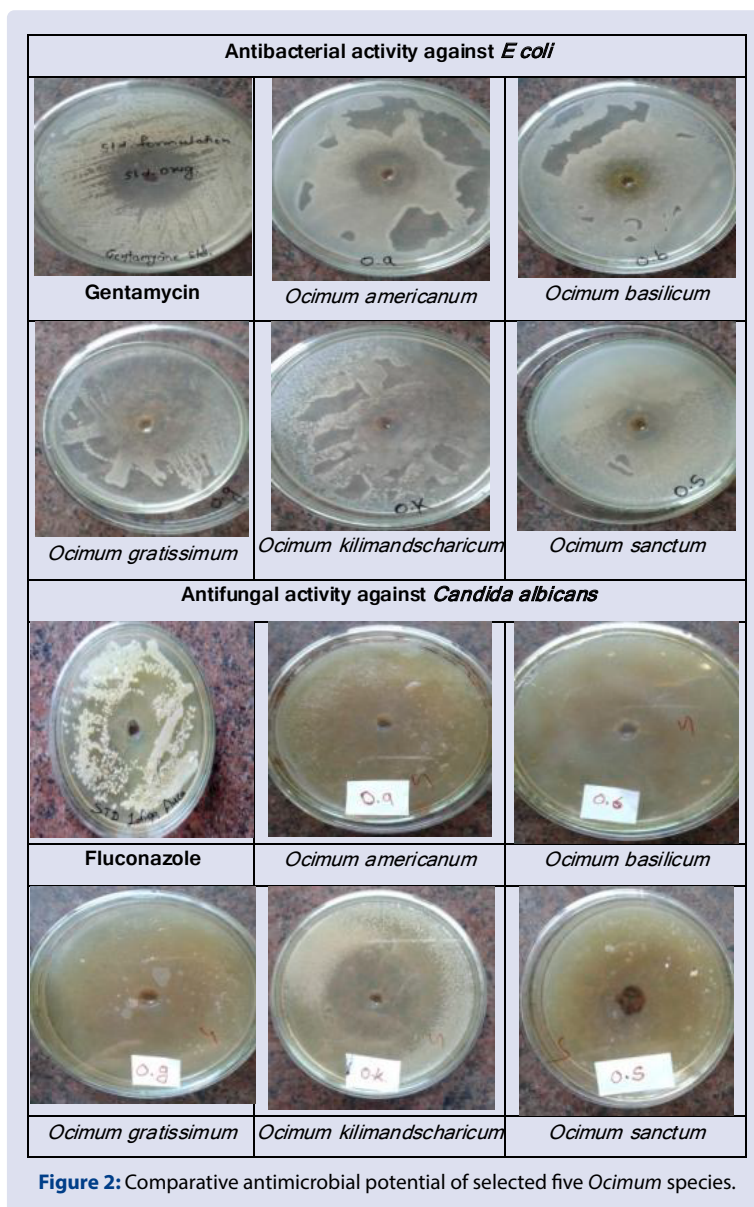
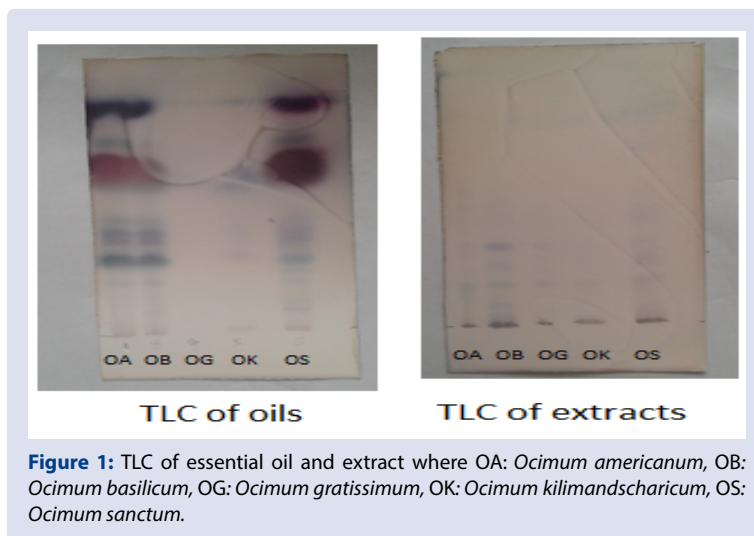


Table 8 more in *O. sanctum* while *O. basilicum* and *O. gratissimum* also showed good anti-oxidant activity.

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. americanum*, *O. basilicum*, *O. gratissimum*, *O. sanctum* while later species have more antibacterial activity.

CONCLUSION

Present work has provided useful information to identify, differentiate and evaluate most commonly used and confusing species of genus *Ocimum*-*O. americanum*, *O. basilicum*, *O. 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.

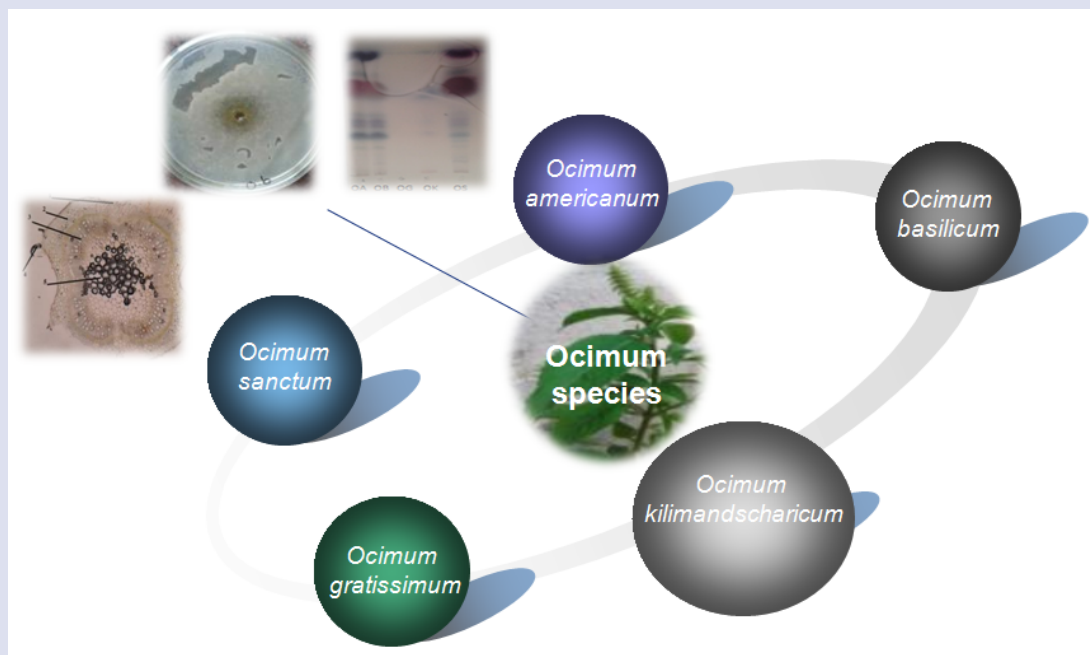
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Nil.

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GRAPHICAL ABSTRACT



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