

## Comparative Pharmacognostical, Phytochemical and Biological Evaluation Of *Ocimum* Species: Review

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**ABSTRACT** : Now a days, increasing use of herbal products needs to study the medicinal plants by more appropriate study in relation to maintain its quality standards. In this paper, the literature and the published work on comparative Pharmacognostical study of *Ocimum* species. Aim of present study is to establish comparative standards for different species of *Ocimum*, to collect and comparatively evaluate morphological characters of different species of *Ocimum*, to comparatively evaluate chemical parameters of different species, to develop comparative standards to differentiate species of *Ocimum*. Present study was carried out by using various material and methods starting from collection of plant, herbarium preparation, macroscopic and microscopic study, Phytochemical tests, extraction of essential oils by Clevenger apparatus, TLC methods used of Phytochemical study of essential oils and biological evaluations was carried out by using agar plate techniques and also for antioxidant properties. This comparative study reported *O. sanctum* should be preferred in use over other species based on comparative phytochemical composition, antioxidant and anti-microbial activity.

**KEYWORDS** – *Ocimum species*,

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### I. INTRODUCTION

The *Ocimum species* are known for thousand years to various civilizations of the world. This medicinal herb is considered as a sacred plant by the Hindus in the India subcontinent. Scientific explorations of traditional belief of medicinal properties of *Ocimum* have got momentum mostly after the middle of the 20th century. (1)Tulsi is a sacred medicinal plant considered as a Holy Hindu Laxmi Goddess. It is member of mint or Labiatae family from India. Medicine is obtained from leaves, seed & stem. *Ocimum* species uses for therapeutic use cold, influenza, H1N1 (swine flu) hepatitis, bronchitis, stress, cancer, headache, heart disease, malaria, digestive disorder. This is powerful anti-oxidant demonstrated as anti fungal antibacterial. Act as anti inflammatory, immunomodulatory, increase metabolism and lowering down stress hormones. This is also a repellent for mosquitos. This has strong healing power in cattarrha matter and phelgm. This also work as supplements to combat Stress by lowering corticosteroid level by active compounds at therapeutic dosages & standardised for Eugenol, caryophyllene and triterpenoid acid such as ursolic and oleanolic acid. Tulsi has antifertility & anti sperm role by reducing estrogen in female. It has strong immunomodulator and Adoptogen to counter life style diseases in cancer hypertension diabetes and stress. (2) The complex taxonomy of the genus, Determined by interspecific hybridizations and polyploidy, includes 150 species. Whole plant is used as a source of remedy. In India two forms of Tulsi are more common - dark or *Shyama* (Krishna) Tulsi and light or Rama Tulsi. The former possesses greater medicinal value and is commonly used for worship. Various other species are also commonly found in India like *O. canum*, *O. basilicum*, *O. kilimandscharicum*, *O. americanum*, *O. camphora* and *O. micranthum*. In the past few decades, many of these benefits have been investigated and verified by modern scientific research. *Osmium's* main Ayurvedic guna (quality) as a healing herb is its ability to enhance the energetic resonance between the body and the environment. "The Elixir of Life", *Ocimum* has been traditionally employed in hundreds of different formulations for the treatment of a wide disorders including those of the mouth and throat, lungs, heart, blood, liver, kidney, and the digestive, metabolic, reproductive and nervous systems. (1)

### Importance in mythology

Also Tulsi has an great importance in Ayurveda and Indian mythology. Tulsi is a Sanskrit word which means "matchless one". The therapeutic uses of plant are safe, economical & effective as their ease of availability. Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family Lamiaceae are very important for their therapeutic potentials. *Ocimum sanctum* has two varieties i.e. black (*Krishna Tulsi*) and green (*Rama Tulsi*), their chemical constituents are similar.

Tulsi plant is not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine. The medicinal use of plants is very old. Literatures indicate that therapeutic use of plants is as old as 4000-5000 B.C and Chinese used first the natural herbal preparations as medicines. Earliest references are available in Rigveda which is said to be written between 3500-1600 B.C. Tulsi (*Ocimum sanctum*), Queen of Herbs, the Legendary, "Incomparable One" is one of the holiest and most cherished of the many healing and health-giving herbs distributed mainly in the oriental region. *Ocimum* genus contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia. Plants have square stems, fragrant opposite leaves and whorled flower on spiked inflorescence. The essential oil of basil extracted via steam distribution from the leaves and flavoring tops are used to flavour foods, dental and oral products, in fragrances and in traditional rituals.(3)

## II. LITERATURE SERVEY

- i. **Sapna et al (2013):** worked on a comparative study of two varieties of *O.basilicum*, *basilicum* and *thyriflora* was done for evaluation of anti-inflammatory and anti-arthritic activity. The phytochemical investigation was done mainly for detecting and comparing the three marker constituent eugenol, ursolic acid and oleanolic acid by HPTLC and HPLC. Ursolic acid and oleanolic acid were estimated simultaneously by HPTLC and eugenol estimated individually. The pharmacological studies such as anti-inflammatory activity, anti-arthritic activity suggest that *O.basilicum var. basilicum*(OSBB) possess significant anti-arthritic and anti-inflammatory activity in a dose dependent manner whereas higher dose of *O.basilicum var. thyriflora*(OSBT) is required to exhibit the same pattern of anti-arthritic and anti-inflammatory activity in lab animals. The results have shown that *O.basilicum var. basilicum* have higher amount of chemical markers, (eugenol and ursolic acid) as compared with *O.basilicum var. thyriflora*. Also in the pharmacological studies carried out it is seen that the efficacy of leaf extract of *basilicum var. basilicum* is higher as compared with *O.basilicum var. thyriflora* if compared to *Ocimum sanctum*, then it is seen that *basilicum* and *thyriflora* variety has higher content of eugenol and oleanolic acid in comparison to *Ocimum sanctum*. Ursolic acid content is nearly similar in all the three varieties. In case of formulation (Himalaya Tulsi capsules), all the three markers are present in a quantity less than *basilicum* but more than *thyriflora*. Thus it can be concluded that *basilicum* variety can be a better substitute for *Ocimum sanctum*. From results *basilicum* variety is more effective than *thyriflora* variety. Findings show potential of variety *basilicum* for treatment of arthritis. This necessitates further detailed and systematic evaluation of the plants for the search of new lead molecule.(36)
- ii. **Shafqatullah et al (2013):** work on comparative analyses of *Ocimum sanctum* Stem and Leaves for phytochemicals and inorganic constituents. The stem of *Ocimum sanctum* was nutritionally enriched in all respect as compare to leaves. The minerals analysis showed no remarkable change in the results. Although the quantities of Fe<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup> were higher in stems than leaves. The results of preliminary phytochemical analysis were same for both the samples and confirm the presence of various phytochemical viz., alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins. Quantitative analysis revealed maximum amount of saponins i.e., 2.58mg/g in stem in comparison to 2.30mg/g in leaves. The average percentage w/w of the ash content and the extractive values were analyzed by Auto Kjeldahl the nitrogen and protein value determined. The moisture content in stem was quite large was 1.1 and 1.9% for stem, while 0.8 and 1.9% for leaves. 6.6% than the leaves 5.3%. Total acidity was determined These values were taken in triplicate but there was no simple titration method which shows the negligible change among these values having a very small number quantity in stem as well as in leaves, while the fiber of standard deviation both for stem and leaves. Fat content in stem and leaves is quite high, which is 12.2% extracted with 95% n-hexane by Soxhlet apparatus and in stem and 9.8% in leaves. The nitrogen and protein were found 1.1% crude fat in stem and 0.9% in leaves. same results for leaves and stem, the glycosides, carbohydrates, phytosterols/ isolated islets and triterpenoids, saponins, flavonoids and phenolic/tannins were present in both samples, while fixed oil and amino acids were absent. Both parts of this plant have almost same nutritional, minerals and phytochemical values. Therefore both the leaves and stem can be used in traditional medicine system for different types of ailments.(37)
- iii. **Sit fooncheng, cheng hock chuah et al (2009)** study on the application of GC-MS for the analysis of essential oil allowed the detection of significant differences in the proportions of volatile compounds from *Ocimum Basilicum* and *Ocimum Sanctum* tested in relation to many factors that contribute to the fragrances present. From our study, *Ocimum Basilicum* should be harvested in April for methyl chavicol and *Ocimum Sanctum* in the month of October, for methyl eugenol under the climatic conditions in Malaysia. At the same time, present study indicates that *Ocimum Basilicum* are rich in methyl chavicol and *Ocimum Sanctum* belongs to methyl eugenol rich type.(38)

- iv. **Maria Loredana Soran et al (2009)** worked on The extraction and chromatographic determination of the essential oils from *Ocimum basilicum* L. by different techniques. Three different techniques (maceration, sonication and extraction in microwave field) were used for extraction of essential oils from *Ocimum basilicum* L. The extracts were analyzed by TLC/HPTLC technique and the fingerprint informations were obtained. The GC/FID was used to characterize the extraction efficiency and for identify the terpenic bioactive compounds. The most efficient extraction technique was maceration followed by microwave and ultrasound. The best extraction solvent system was ethyl ether + ethanol (1:1, v/v). The main compounds identified in *Ocimum basilicum* L. extracts were:  $\alpha$  and  $\beta$  pinene (mixture), limonene, citronellol, and geraniol. (39)
- v. **Chawla et al. (2013)**: study on Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a dreadful lifestyle disorder of 21st century. The allopathic medicines have five classes of drugs, or otherwise insulin in Type I diabetes, targeting insulin secretion, decreasing effect of glucagon, sensitization of receptors for enhanced glucose uptake etc. In addition, diet management, increased food fiber intake, Resistant Starch intake and routine exercise aid in managing such dangerous metabolic disorder. One of the key factors that limit commercial utility of herbal drugs is standardization. Standardization poses numerous challenges related to marker identification, active principle(s), lack of defined regulations, non-availability of universally acceptable technical standards for testing and implementation of quality control/safety standard (toxicological testing). The present study proposed an integrated herbal drug development & standardization model which is an amalgamation of Classical Approach of Ayurvedic Therapeutics, Reverse Pharmacological Approach based on Observational Therapeutics, Technical Standards for complete product cycle, Chemi-informatics, Herbal Qualitative Structure Activity Relationship and Pharmacophore modeling and, Post-Launch Market Analysis. (40)
- vi. **Santanu Kar Mahapatra et al (2011)** Work on evaluation of the immune functions and immune responses in nicotine-induced (10mM) macrophages and concurrently establish the immunomodulatory role of aqueous extract of *Ocimum gratissimum* (Ae-Og) and ascorbic acid. In this study, nitrite generations and some phenotype functions by macrophages were studied. These findings strongly enhanced our understanding of the molecular mechanism leading to nicotine-induced suppression of immune functions and provide additional rationale for application of anti-inflammatory therapeutic approaches by *O. gratissimum* and ascorbic acid for different inflammatory disease prevention and treatment during nicotine toxicity. (41)
- vii. **Igbinosa et al. (2013)** work on the In vitro assessment of antioxidant, phytochemical and nutritional properties of extracts from the leaves of *ocimum gratissimum* (Linn). the relatively high antioxidant and polyphenolic activities observed in *O. gratissimum* extracts justify its use in folkloric medicine and suggest that this plant is endowed with natural antioxidant and nutritive constituents, which may be important as a source of nutrients supplement, and in the treatment of radical related diseases. (42)
- viii. **Varinder Singh et al (2014)**: studies the phytochemical & pharmacological aspects of *Ocimum kilimandscharicum* Guerke. The volatile oil from plant is found to be rich in camphor. The phenolics, flavonoids and terpenoids have been isolated from aerial parts of plant. The comprehensive account of detailed biotechnological, phytochemical and pharmacological aspects of *Ocimum kilimandscharicum* along with patented formulations are presented. The experimental studies confirm the traditional claims of the species among various parts of the world. The essential oil of plant has found extensive use as mosquito repellent justifying its ethnobotanical claim. Tremendous efforts have been made to validate the traditional claims of *O. kilimandscharicum* but it remains surprising that none of the studies attempts to establish the relationship of pharmacological activity with secondary metabolites except for bactericidal actions. Thus, area of phytochemistry remains unexplored. Hence, a thorough biosystematic study of *Ocimum kilimandscharicum* may provide an effective natural compound(s) which can become a lead molecule(s) in drug discovery. (43)
- ix. **Fofie N'guessan Bra Yvette et al (2014)**: work on Pharmacognostic study of *Ocimum gratissimum* Linn pharmacognostic examination revealed a small plant with small stalked leaves. The fruits are in the form of small capsules. The anatomo-histological section, showed trichomes, palisade cells, spongy parenchyma, vascular unit. On the stem, primary tissues, a fundamental parenchyma is observed. Micrographic revealed oily cells, spiral and wood beams spiral beams. The study has focused on examining pharmacognostic study of *Ocimum gratissimum* Linn. (Lamiaceae) leaves. The scientific books have allowed us to review a large number of

bibliographic data on this drug, including the systematic and various domestic uses in traditional medicine. Normalization of the macroscopic and microscopic characteristics of the *O. gratissimum* Linn. Drug remains essential in order to identify and avoid falsification. Thus comparing the cross section of the leaf and stem anatomy showed structural similarities. Both sections have a spinal cord parenchyma, a phloem, xylem and collenchyma. It is observed in a thin sheet cuticle on the upper epidermis and the lower epidermis. Also palisade tissue above the spongy parenchyma. In the stem, secreting pockets are visible on the surface of the medullary parenchyma, as well as supporting cells sclerenchyma primary tissue (primary phloem).

x. The distinct cortical parenchyma can be seen towards the periphery of the cut. Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs. Thus leaves green in color, emit a very fragrant and aromatic minty odor when bruised. The powdery appearance of the sprayed leaves, has a coarse texture. The micrograph performed on the powder has highlighted a number of characteristic elements namely: the epidermal cells, the type of stomates, the spiral beams, the cystoliths, the trichomes, spiral wooden beam, oily cells, are diagnostic substances for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines. The study of physicochemical parameters such as moisture content and ash values are useful to determine the physiological and nonphysiological state of ash, of detecting the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of  $8.97 \pm 0.01$ , which is below 10%. This result complies to the standards established by the International Pharmacopoeia, because this water content rate, prevents oxidation reactions, fermentation and gives less chance to microbial growth and contamination in drugs. Therefore, for proper storage of drugs made with the leaves of *O. gratissimum* Linn., it would be desirable to use those whose water content is less than or equal to 10%. The determination of total ash gave us a rate of  $8.45 \pm 0.02$ . This value indicates the level of minerals in drugs. Sulfuric ash for their rate of  $10.27 \pm 0.01$ . They result from the conversion of organic salts to sulphates. This value is substantially equal to the average of 10.80% found during the determination of sulfuric ash in the different samples of *Sclerocarya birrea* (A. Rich) Hoscht. Insoluble ash in hydrochloric acid gave a rate of  $0.96 \pm 0.01$ . Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements, who found a rate of 0.97% and 0.5% respectively. (44)

xi. **S. Khair-ul-Bariyah et al (2012):** worked on Phytochemical and pharmacological studies of *Ocimum Basilicum*. The importance of medicinal plants has increased with the passage of time because synthetic medicines have a number of side effects besides many benefits they offer. These plants have recorded and known pharmacological applications which we have got in heritage. The present study is meant to describe the importance of *Ocimum basilicum* in the field of herbal medication. Phytochemical and pharmacological studies of the herb are given along with botanical characteristics. Various effects like immunomodulatory, hyperglycaemic, hypolipidemic, anti-inflammatory, hepatoprotective, antimutagenic, antimicrobial, antifungal, antioxidant, lipid peroxidation, insect repellency, antiviral, antiarrhythmic, depigmenting, antitoxic and CNS activity analysis reports are mentioned. The wide range of study on this herbal plant shows that it is very beneficial for the improvement of current drugs and more work can be done to take advantage of the potential remedial qualities of it. (45)

xii. **K. M. Mominul Islam et al (2014)** work on Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. Phytotoxic activity of *Ocimum tenuiflorum* (Lamiaceae) plant extracts was investigated against the germination and seedling growth of cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), Italian ryegrass (*Lolium multiflorum*), barnyard grass (*Echinochloa crus-galli*), and timothy (*Phleum pratense*) at four different concentrations.

xiii. The plant extracts at concentrations greater than 30 mg dry weight equivalent extract mL<sup>-1</sup> reduced significantly the total germination percent (GP), germination index (GI), germination energy (GE), speed of emergence (SE), seedling vigour index (SVI), and coefficient of the rate of germination (CRG) of all test species except barnyard grass and GP of lettuce. In contrast, time required for 50% germination (*T*<sub>50</sub>) and mean germination time (MGT) were increased at the same or higher than this concentration. The increasing trend of *T*<sub>50</sub> and MGT and the decreasing trend of other indices indicated a significant inhibition or delay of germination of the test species by *O. tenuiflorum* plant extracts and vice versa. In addition, the shoot and root growth of all test species were significantly inhibited by the extracts at concentrations greater than 10 mg dry weight equivalent extract mL<sup>-1</sup>. The *I*<sub>50</sub> values for shoot and root growth were ranged from 26 to 104 mg dry weight equivalent extract mL<sup>-1</sup>. Seedling growth was more sensitive to the extracts compared to seed germination. Results of this study suggest that *O. tenuiflorum* plant extracts have Phytotoxic properties and thus contain Phytotoxic

substances. Isolation and characterization of those substances from this plant may act as a tool for new natural, biodegradable herbicide development to control weeds.(46)

xiv. **K Reshma et al (2008)** work on radio protective effect of *Ocimum* flavonoids on leucosite oxidants & antioxidant in oral cancer. Oxidants (NADPH oxidase and myeloperoxidase) and antioxidants (GSH, GSH peroxidase, SOD and glucose 6 phosphate dehydrogenase, that provides NADPH for antioxidants) were assayed in the neutrophils from oral cancer patients, in three stages viz., baseline samples, 15 days after radiation and 30 days following radiation. These samples were obtained from 2 groups of patients. Group A that received radiation alone and Group B that received radiation and *ocimum* flavonoids, a radioprotector. The results showed a significant fall in the SOD (superoxide dismutase) levels in the second follow up of group B. Glucose 6 phosphate dehydrogenase showed significant increase only in the first follow up of patients who received *Ocimum* flavonoids. Except for these findings all other parameters remained statistically non significant.(47)

xv. **Choudhury Golak Bihari et al (2011)** studies Pharmacognostical and phytochemical investigation of various *Tulsi* plants available in south eastern odisha. The present study deals with pharmacognostical examination include the morphological, microscopical characters and physical constants of leaves of various *Ocimum* species including determination of loss on drying, ash values and extractive values. The preliminary phytochemical screening of various leaf extracts was also carried out and it is revealed the presence of various phytoconstituents like carbohydrates, flavonoids, protein and amino acids, tannins, phytosterols and saponins. The results of the standardization may throw immense light on the botanical identity of various species of *Ocimum* which may furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its allied species and detect

### III. CONCLUSION

From the above literature survey, “*Comparative Pharmacognostical, Phytochemical, and biological evaluation of Ocimum species,*” five species of *Ocimum* species which are specially observed in Vidarbha region, requires to collect and comparatively evaluate for morphological, microscopical, physiochemical and biological characters. This study will help for selection of *Ocimum* species to achieve aim of preparation of effective formulation.

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