# Elemental Composition and Physiological Profiling of Indian Tulsi Varieties: Insights from XRF, Chemical and Leaf Chamber Analysis

M. G. Shinde[1] and R. Saran[2]*

[1]156 Swamy Colony, Phase I, Katol Road, Nagpur, Maharashtra, India.

[2]* 82 Swamy Colony, Phase II, Katol Road Nagpur, Maharashtra, India.

Email- saranraghaw@gmail.com

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**Abstract**

Tulsi (Ocimum sanctum L.), a revered courtyard plant in India, is renowned for its antioxidant, antimicrobial, and medicinal properties. Tulsi, widely used in Ayurveda, is valued for its culinary, digestive, and restorative benefits, attributed to its diverse bioactive compounds such as eugenol, euginal in leaf volatile oil extract, fatty acid and sitosterol in seed volatile oil, sugars in seed gum and anthocyans in green leaves. Despite its extensive use, clinical practice data on dosage, constituents, and duration remain scarce. The variability in biologically active constituents among Tulsi strains, plants, harvesting methods, and processing conditions poses challenges for standardization. This attempt has been made to fill these knowledge gaps by investigating the elemental composition using X-ray fluorescence (XRF), chemical analysis, and physiological profiling such as gas exchange parameters including rate of respiration, $CO_2$ fixation and $O_2$ evolution by leaf chamber analysis, of different varieties of Indian Tulsi.

Key words: Tulsi, Elemental composition, Physiological profiling, XRF, Leaf chamber analysis

## 1.Introduction

Tulsi grows wild in the tropics and warm region. The plant is branched, fragrant, erect with aromatic entire or toothed margin, round shaped about 5 cm long leaves and attains height of about 75 to 90 cm when mature. Tulsi flowers are small, reddish -purple in color as small compact clusters or cylindrical spikes. The fruits are small and the seeds are reddish-yellow in color. Inflorescence of the plant is long spike or raceme with tiny purple flower

Tulsi (Ocimum sanctum L.), a revered courtyard plant in India, has been an integral part of traditional medicine, culinary practices, and spiritual rituals[1-4] for centuries. Its multifaceted

benefits, attributed to its diverse bioactive compounds, have made it a valuable herb in Ayurveda[5]. The antioxidant [6,7,10,11,18,21,29,31-33], antidiabetic[18,21], antimicrobial[12-15,29,37], antistress[16,18], antifatigue[17], anticariogenic[25-27] and medicinal properties[20,29,30,32,33,35] of the plant has been extensively utilized to treat various ailments, ranging from respiratory issues to cardiovascular diseases[20,23].

The therapeutic potential of Tulsi is ascribed to its rich composition of bioactive compounds, including eugenol, euginal, ursolic acid, carvacrol, linalool, fatty acids, and sitosterol[31]. These compounds have been identified in various parts of the plant, including leaves, seeds, seed gums, stems and roots. Moreover, phenolic actives like rosmarinic acid, propanoic acid, cirsimaritin, apigenin, vicenin besides saponins, flavonoids, tannins etc.[8-11,13,28,38-40], credited to show antioxidant and anti-inflammatory properties found to be present in Tulsi leaves. However, despite its widespread use and potential benefits, there is a notable lack of standardization and clinical data on Tulsi's efficacy, dosage, and constituent variability[29,30].

The variability in biologically active constituents among Tulsi strains, plants, harvesting methods, and processing conditions poses significant challenges for standardization and quality control. This knowledge gap hinders the integration of Tulsi into modern medicine and limits its potential applications[29].

This study aims to investigate the elemental composition and physiological profiling of different Indian Tulsi varieties using X-ray fluorescence spectrometry (XRF), chemical analysis, and leaf chamber analysis. By exploring the biochemical characteristics of Tulsi, the research seeks to provide valuable insights into its potential applications, quality control, and standardization.

## 2. Experimental

### 2.1 Sample Collection

Three types of Indian variety of phytochemically distinct Tulsi (Lamiaceae family), Rama with green leaves also known as Shri and Shyama or Krishna (Ocimum sanctum L)[30,31] and Vana or jungle Tulsi (Ocimum gratissimum)[32,33] are mostly found. With this in view, three Tulsi plants (Photographs 1-3) of each variety and one Sadafuli plant (for Leaf Chamber analysis) were planted for a period of four months. After completion of the time, the plants were cut down above the ground, dried, weighed and subjected to various analysis and three

other plants of Kadipatta, Neem and Peepal were collected from elsewhere for treating as comparative samples. Sadafuli (shrub plant) and Neem and Peepal (tree) were chosen for the sake of comparison in leaf chamber analysis to examine the variation in gas exchange parameters between shrubs; and a shrub and tree.

**1** 2

**Photograph 1 and 2 Rama Tulsi & Krishna Tulsi (Court yard)**

**Photograph 3 Vana Tulsi**

### 2.2 Sample preparation

The plants were thoroughly cleaned, washed, and dried in sunlight till complete dryness and ashed in a China dish in muffle furnace at 450-500°C for 7-8 hrs. The ash was ground to fine powder in ball mill using ceramic balls. The powder obtained was further ground in agate pestle and mortar to prepare the sample (-250 mesh) for major and trace element analysis.

### 2.3 Instrument used

WDXRF (Philips Panalytical PW 1480), ICP-OES (GBC-Integra XM sequential) and flame photometer (Elico CL 378) were used for determining elemental composition and trace elements. Leaf chamber analysis (LCA- 4), Analytical Development Company Ltd., England was utilized for physiological profiling such as gas exchange parameters including rate of respiration, $CO_2$ fixation and $O_2$ evolution.

### 2.4 Procedure

WD XRF analysis: 1.0 g sample was taken for palletization with boric acid backing by applying a pressure of 20 tons using hydraulic press for WD XRF analysis.

ICP-OES analysis: 1.0 g sample was digested with $HClO_4$. The digestion was repeated four or five times until evaporation of perchloric acid ceases. The final solution was made up to 25 ml volume with 0.5 M HCl. A process blank was similarly prepared. The final solution was used for ICP-OES analysis.

LCA - 4 (Leaf Chamber Analyzer) analysis: The Leaf Chamber Analyzer (LCA-4) procedure involves measuring $CO_2$ uptake and water transpiration rates in plants.

A leaf, leaf segment, or other plant sample is carefully selected and positioned within the leaf chamber. The LCA-4 is calibrated to ensure accurate measurements, often using known $CO_2$ and water vapor concentrations. Air is passed through the leaf chamber, and the changes in $CO_2$ and water vapor concentrations are measured using an Infra-Red Gas Analyzer (IRGA). The IRGA data, along with known air flow rates, are used to calculate the leaf's $CO_2$ assimilation rate and transpiration rate.

The system also measures leaf temperature, chamber air temperature, Photosynthetically Active Radiation (PAR), and atmospheric pressure. These values are then used to calculate other parameters like leaf radiant energy balance and stomatal conductance. Measured and calculated data are displayed on the LCA-4's LCD and can be logged on an SD card or transmitted to a computer. The PAR sensor provides a measure of photosynthetic photon flux density in the range 400-700 nm wavelength range.

**Table – 1 Elemental analysis of Plant ash by WDXRF method (ppm, average of the three determination with %, RSD*)**

| Description | Dry wt. of plant gm | Wt. of ash gm | % of weight | Ti | Al | Fe (Total) | Mg | Ca | Na | K | P | Cu | Zn | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rama Tulsi (T-1) | 16.37 | 1.67 | 0.11 | 112 (2.1) | 77 (1.5) | 792 (0.1) | 357 (0.1) | 7423 (0.1) | 3300 (0.3) | 5140 (0.1) | 506 (0.2) | 36 (0.1) | 6 (3.1) | 310 (0.3) |
| Krisna Tulsi (T-2) | 18.85 | 1.52 | 0.08 | 83 (1.8) | 85 (0.2) | 566 (0.1) | 290 (0.2) | 6603 (0.1) | 3004 (0.3) | 5387 (0.1) | 352 (0.4 ) | 38 (0.2) | 7 (3.8) | 387 (0.3) |
| Vana Tulsi (T-3) | 18.50 | 1.48 | 0.08 | 62 (2.2) | 267 (0.2) | 468 (0.1) | 487 (0.10) | 8028 (0.1) | 3226 (0.3) | 5569 (0.1) | 405 (0.4) | 28 (0.3) | 6 (3.5) | 233 (0.3) |
| Neem | 24.30 | 2.60 | 0.11 | 28 (2.6) | 254 (0.9) | 309 (0.2) | 413 (0.2) | 6797 (0.1) | 3071 (0.3 ) | 5869 ( 0.1) | 246 (0.2) | 8 (0.2) | 8 (4.1) | 148 (0.4) |
| Kadipatta | 14.40 | 1.63 | 0.12 | 41 (2.7) | 215 (0.9) | 324 (0.2) | 347 (0.2) | 5962 (0.2) | 4152 (0.1) | 5711 (0.1) | 127 (0.3) | 26 (0.1) | 7 (3.6) | 144 (0.4) |
| Peepal | 15.64 | 2.52 | 0.06 | 46 (2.9) | 127 ( 1.2) | 136 (0.2) | 557 (0.1) | 4983 (0.2) | 4234 (0.2) | 4440 (0.2) | 273 (0.1 ) | 9 (0.2) | 4 (4.1) | 151 (0.4) |

In Parenthesis*

**Table – 2 Elemental analysis of Plant ash by ICP-OES and Flame Photometry (ppm, average of the three determination with %, RSD*)**

| Description | Dry wt. of plant gm | Wt. of ash gm | % of weight | Ti | Al | Fe (Total) | Mg | Ca | Na | K | P | Cu | Zn | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rama Tulsi (T-1) | 16.37 | 1.67 | 0.11 | 90 (1.9) | 85 (0.8) | 785 (0.1) | 468 (0.1) | 7337 (0.1) | 3552 (0.3) | 5395 (0.2) | 748 (0.3 ) | 36 (2.0) | 6 ( 3.1) | 232 ( 0.3) |
| Krisna Tulsi (T-2) | 18.85 | 1.52 | 0.08 | 75 ( 2.1) | 90 ( 1.8) | 525 (01) | 264 (0.1) | 6494 (0.1 ) | 4351 (0.1) | 5312 (0.1) | 238 (0.3) | 38 ( 1.6) | 7 (3.0) | 310 ( 0.2) |
| Vana Tulsi (T-3) | 18.50 | 1.48 | 0.08 | 60 (1.9) | 249 (1.8) | 489 (0.2) | 546 (0.1) | 6588 (0.1 ) | 3271 (0.2) | 5644 (0.1) | 339 ( 0.3) | 28 (1.7 ) | 6 (3.1) | 232 ( 0.2) |
| Neem | 24.30 | 2.60 | 0.11 | 30 (1.3 ) | 239 (0.9 ) | 295 (0.3) | 312 (0.18) | 5761 0.15 | 3471 (1.1) | 6059 (0.1) | 172 (0.4) | 8 ( 2.1 ) | 8 ( 3.1) | 145 (0.4) |
| Kadipatta | 14.40 | 1.63 | 0.12 | 38 (1.7) | 175 ( 0.2) | 295 (0.2) | 324 (0.3) | 5882 0.12 | 4063 (0.1 ) | 5486 (01) | 238 (0.3) | 26 (1.3) | 7 ( 3.4) | 144 (0.4) |
| Peepal | 15.64 | 2.52 | 0.06 | 30 (2.6) | 101 (1.3) | 115 (0.3) | 606 (0.1 ) | 4810 (0.1) | 4351 (0.1) | 4814 (0.1) | 145 (0.5) | 9 (2.1) | 4 (4.0 ) | 139 ( 0.1) |

In Parenthesis*

## 3. Results and Discussion

Elemental composition (major and trace constituents of the samples) is listed in Table-1 and Table-2 by XRF and Chemical analysis (employing ICP- OES and flame photometer) respectively.

Studies reveal higher absorption (mean of XRF and chemical values, ppm) of iron varying in Tulsi plant and in others (Neem, Kadipatta & Peepal) from 792 to 115 ppm and manganese 387 to144 ppm respectively. The higher relative values in Tulsi to other plants attribute Tulsi antimicrobial (due to synthesis of phenolics and flavonoids), antiviral, and antioxidant (by scavenging reactive oxygen species, ROS) properties besides different enzymatic reactions, photosynthesis, respiration, nitrogen metabolism and protection against pathogens. The elements bind siderophores produced by microbes helping plants take up essential nutrients. Further, they are synergistically chelated by plant phenolics reducing toxicity of these metals and enhance their antioxidant activity. Iron oxide may also be synthesized with plant extracts to form nanoparticles of iron oxide possessing antioxidant and antimicrobial properties.

Table -3 and Table- 4 Ratio of Fe to Mn in plant samples analyzed show their mean ratio (XRF, ICP OES respectively) in the range of 1.57 to 2.77 and 1.87 to 3.67 (higher) although the plants (Tulsi) were healthy and fully grown against reported in case of higher ratio in literature elsewhere [35,36]. Besides, Tulsi plants were also observed to contain Zn, Mg and higher amount of Cu as trace element (28-38 ppm) assisting metabolic reactions such as chlorophyll II formation.

**Table 3 Iron and Manganese ratio by XRF**

| Plant | Fe | Mn | Ratio |
|---|---|---|---|
| Rama tulsi | 858 | 310 | 2.77 |
| Krisna tulsi | 608 | 387 | 1.57 |
| Ran tulsi | 507 | 233 | 2.18 |
| Neem tree | 413 | 144 | 2.87 |
| Kadipatta | 351 | 144 | 2.44 |
| Peepal tree | 148 | 144 | 1.03 |

**Table 4 Iron and Manganese ratio by ICP-OES**

| Plant | Fe | Mn | Ratio |
|---|---|---|---|
| Rama tulsi | 850 | 232 | 3.67 |
| Krisna tulsi | 569 | 310 | 1.84 |
| Ran tulsi | 530 | 232 | 2.28 |
| Neem tree | 319 | 144 | 2.22 |
| Kadipatta | 319 | 144 | 2.22 |
| Peepal tree | 124 | 144 | 0.87 |

### 3.1 Leaf Chamber Analysis

Leaf chamber analysis was carried out of Rama tulsi, Krishna tulsi, Vana (Jungli) tulsi, Sadafuli and other plants for the sake of comparison like Neem, Peepal and Kadipatta. Results pertaining to respiration parameters are tabulated in Table -5. Table- 4 gives amount of $CO_2$ absorbed (mole minute$^{-1}$) by dried courtyard plants detailed above. Fig.1 gives photosynthesis rate and absorption of $CO_{.2}$. Fig. 2 shows the time required for fixation of one mole of $CO_2$ and release of one mole of $O_2$.

Experiments for measuring the respiratory rate and photosynthesis process of plants suggested that Rama Tulsi absorb more $CO_2$ (and release equal amount of $O_{2)}$ as compared other plants. The photosynthesis rate of Rama tulsi is also higher (7.42 μ mole m$^{-2}$ s$^{-1}$) and $CO_2$ absorption 6.77% $_{absorption}$ and improves air quality by releasing $O_2$. It was also observed that the photosynthetic rate of Neem tree (Azadirachta indica) is (45.54 μ mole m$^{-2}$ and $CO_2$ absorption is 6.266% comparatively higher than other plants (but lower than Rama Tulsi) and is useful as medical and air purifier plant. Table-5 also lists parameters such as $H_2O$ absorption, $CO_2$ sub- stomatal concentration, Stromal conductance, stomatal resistance, and molar flow per unit leaf area as these parameters are important for photosynthetic process playing an important role in preparing food for plants.

**Table 5 Analysis Results for respiration of plants**

| Sr. No | Plant | Botanical name | Family | US | A | E | $W_{abs}$ | $C_{abs}$ | $O_{eva}$ | Ci | gs | Can |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Rama tulsi | *Ocimum Sanctum* | Lamiaceae | 402.17 | 5.79 | 0.13 | 0.679 | 6.254 | 6.254 | 395.14 | 0.060 | 367.60 |
| 2. | Krishna tulsi | *Ocimum Sanctum* | Lamiaceae | 396.6 | 5.45 | 0.19 | 0.376 | 6.332 | 6.332 | 531.27 | 0.034 | 372.44 |
| 3. | Van tulsi | *Hyptis suavealeus* | Lamiaceae | 400.57 | 2.17 | 0.06 | 0.871 | 7.100 | 7.100 | 202.7 | 0.067 | 369.73 |
| 4. | Neem | *Azadirachta indica* | Maliaceae | 403.10 | **45.54** | 0. 20 | 0.542 | 6.268 | 6.268 | 839.93 | 0.027 | 375.37 |
| 5. | Peepal | *Ficus religiosa* | Moraceae | 400.90 | 3.58 | 0.11 | 0.506 | 6.132 | 6.132 | 363.34 | 0.010 | 372.34 |
| 6. | Kadipatta | *Murraya Koenigii* | Rutaceae | 399.3 | 3.02 | 0.11 | 0.609 | 6.407 | 6.407 | 393.23 | 0.034 | 403.17 |
| 7. | Sadafuli | *Catharanthus roseus Linn.* | Apocyna-ceae | 401.80 | 3.86 | 0.13 | 0.643 | 6.466 | 6.466 | 381.54 | 0.040 | 374.6 |

US - Airflow per $m^2$ leaf, m mole $m^{-2}$ $s^{-1}$

A - Photosynthetic rate, µ mol $m^{-2}$ $s^{-1}$

E - Transpiration rate, m mole $m^{-2}$ $s^{-1}$

$W_{abs}$ – $H_2O$ absorption % abs (W abs)

$C_{abs}$ – $CO_2$ absorption % abs (C abs)

Ci - $CO_2$ sub stomatal ($CO_2$ concentration) in vpm

gs- Stomatal conductance, mole $m^{-2}$ $s^{-1}$

$C_{an}$ - $CO_2$ analysis in vpm

*$O_{abs}$ – $O_2$ evolution % abs (O abs) a.

*The amount of $CO_2$ consumed is equal to the amount of $O_2$ produced. Thus, the rate of respiration of a plant oxidizing carbohydrate could be determined by measuring either the rate of $O_2$ consumption or the rate of $CO_2$ production.*

**Table -6 Comparison of $CO_2$ production and time required for fixation of one $O_2$ molecule in plant**

| Name of plant | $CO_2$ production mole per minute | Time in sec. |
|---|---|---|
| Rama tulsi | 4.72 | 12.71 |
| Krishna tulsi | 3.23 | 18.57 |
| Vana tulsi | 2.83 | 21.20 |
| Sadafuli | 2.83 | 21.20 |

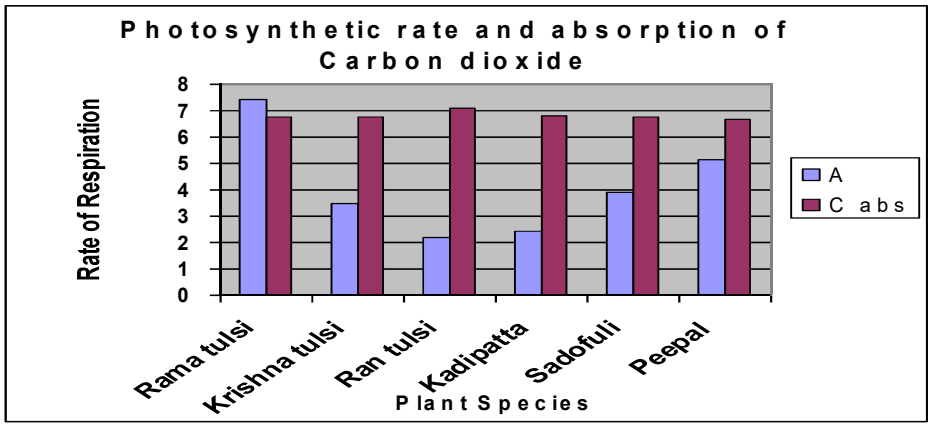


**Fig.1** Photosynthetic rate and adsorption of carbon dioxide

A- Photosynthetic rate & C abc- Absorption of $CO_2$

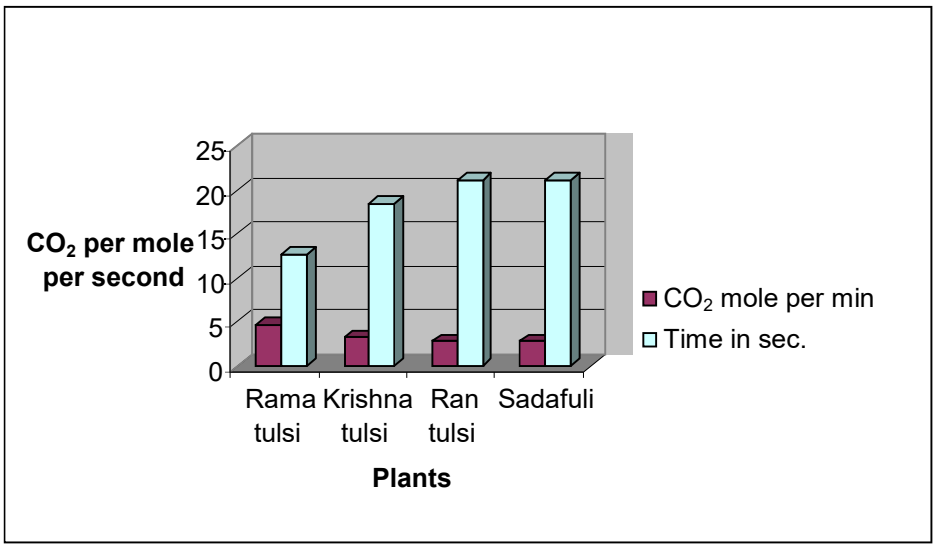


**Fig. 2** Time required for fixation of one CO2 molecule and release of one O2 molecule

## Conclusion

Studies carried out on the sacred plant (Indian varieties of Tulsi) and other studied plants; revealed that Tulsi absorbs heavy elements like iron, manganese from soil. Manganese being synergistic with iron need each other to work together during biological process. Oxidation potential of Fe is lower than Mn.

The respiratory rate and photosynthesis process of plants suggested that Rama Tulsi absorb more $CO_2$ (and release equal amount of $O_{2)}$ as compared to other plants besides higher photosynthesis rate and $CO_2$ absorption and improves air quality by releasing $O_2$. It was also observed that the photosynthetic rate of Neem tree (Azadirachta indica) and $CO_2$ absorption is comparatively higher than other plants (but lower than Rama Tulsi) and is useful as medical and air purifier plant.

It was also observed that Tulsi grows fast and remain healthy for more than two years on maintaining the normal vegetation of Tulsi plant cutting the flowering part of plant periodically.

## Authors Contribution

Author[1] carried out experimental part and prepared the manuscript under guidance of Author [2].

## Conflict of interest

There is no conflict of interest.

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