RESEARCH ARTICLE

EVALUATING NUTRITIONAL, ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF OVEN-DRIED RAMA AND KRISHNA TULSI MIXTURES WITH CONSIDERATION FOR STORAGE STABILITY

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ABSTRACT

The present study evaluates the nutritional, antimicrobial, and antioxidant properties of raw and oven-dried mixtures of Rama Tulsi (Ocimum sanctum) and Krishna Tulsi (Ocimum tenuiflorum), with particular emphasis on the effect of oven drying at 100°C and subsequent storage stability. Proximate and mineral analyses were performed to assess changes in the nutritional composition following drying. Qualitative and quantitative phytochemical analyses were conducted to determine the retention of bioactive constituents, while antioxidant activity was evaluated using DPPH and FRAP assays. The antimicrobial activity of the extracts was assessed against selected bacterial strains, and HPLC analysis was carried out to identify and characterize phenolic compounds. In addition, storage stability was evaluated by monitoring microbial counts over a three-month storage period. Oven drying resulted in a marked reduction in moisture content while influencing the carbohydrate and fat composition, with mineral contents remaining relatively stable. The oven-dried samples demonstrated appreciable phytochemical content along with enhanced antioxidant and antimicrobial activities compared with the raw mixtures. HPLC analysis further confirmed the presence of phenolic compounds, supporting the observed bioactivity of the dried samples. During storage, the oven-dried samples maintained comparatively lower microbial counts, indicating improved storage stability. Overall, the findings demonstrate that oven drying at 100°C can influence the nutritional composition, phytochemical retention, antioxidant and antimicrobial properties, and storage stability of Rama and Krishna Tulsi mixtures, highlighting their potential application as functional food ingredients and nutraceutical products.

KEYWORDS: Rama Tulsi; Krishna Tulsi; Oven Drying; Nutritional Properties; Storage Stability; Functional Food

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INTRODUCTION

Tulsi is widely known for its rich nutritional and therapeutic profile and it is abundant in vitamins, minerals, dietary fibre, and a diverse range of bioactive compounds. These constituents contribute to its antioxidant, antimicrobial and adaptogenic properties.[1] Tulsi has been consumed in both fresh and dried forms for medicinal as well as culinary purposes. In recent years, processed forms such as oven-dried Tulsi have gained increasing attention due to their enhanced stability. Oven drying, particularly at controlled temperatures, has been reported to preserve the therapeutic efficacy of herbs and also in improving their shelf-life.[2]

Despite many research on the medicinal properties of both the varieties of Tulsi, there remains a significant gap

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in understanding the effects of combining these two varieties, especially under different processing conditions. Previous studies, mostly, have focused on single-species evaluation, thereby overlooking the potential positive effects that may arise from blending these two types of Tulsi. Also, the influence of drying methods, particularly oven drying, on such combined formulations has not been thoroughly explored.[3] Processing methods like drying can alter the physicochemical and functional properties of plant materials by affecting their moisture content, enzyme activity and the stability of their bioactive compounds.

The present study tries to address this gap by systematically evaluating and comparing the nutritional composition, antioxidant potential, and antimicrobial efficacy of raw and oven-dried mixtures of Rama and Krishna Tulsi leaves. The study aims to assess whether processing enhances or reduces the bioactivity of the combined samples. Particular emphasis is given on knowing how drying affects the availability and stability of phytochemicals, which are primarily responsible for the health-promoting properties of Tulsi.

This research also considers the wider implications of processing on the application of Tulsi in modern health systems. With the growing demand for functional foods and natural health products, it is important to identify processing methods that not only preserve but also enhance the beneficial properties of plant-based ingredients. The findings of this study would be beneficial in providing valuable insights into the development of nutritionally enhanced and functionally effective Tulsi-based products. By bridging the gap between traditional knowledge and scientific evaluation, this study contributes to a better understanding of how processing techniques like oven drying can optimize the health benefits of Tulsi blends.

METHODOLOGY

Collection of samples: The two major samples Krishna Tulsi and Rama Tulsi were collected from the local market of Kolkata, West Bengal, India. They were kept in sterilized containers for further analysis.

Materials: The study utilized various reagents and chemicals for the analysis of proximate composition, mineral content, qualitative and quantitative phytochemicals analysis, antioxidant analysis using DPPH and FRAP assay methods, antimicrobial analysis and total phenolic content determination.

Tulsi leaves powder preparation: Both the type of leaves were washed properly to remove impurities and then dried (Adams et al., 2017). Oven-drying was employed for each of them and one sample for each of the types of Tulsi leaves were kept as raw. Oven-drying, done using the oven (LG MS4295CIS) set to 100°C, allowed for the controlled application of heat to dry the leaves.[4] The dried leaves were formed in the powder form using mortar and pestle, which was completely free of moisture.[5] Samples of each variety were stored in the airtight containers to maintain the quality.

Inclusion Criteria:

a. Two specific varieties of Tulsi leaves, namely Rama Tulsi (*Ocimum sanctum*) and Krishna Tulsi (*Ocimum tenuiflorum*) were selected for the study, both collected from a local market in Kolkata.

b. Only the leaves of the selected two species were used.

c. Fresh, clean and undamaged leaves were included for processing.

Exclusion Criteria:

a. Other varieties of Tulsi such as Vana Tulsi (*Ocimum gratissimum*) and Kapoor Tulsi (*Ocimum kilimandscharicum*), were excluded.

b. Plant parts other than leaves, including stems, flowers and seeds, were not considered.

c. Leaves that were damaged, discolored or otherwise impure were excluded from the study.

Different prepared sample:

Sample A: Rama Tulsi raw leaves + Krishna Tulsi raw leaves (1:1 ratio)

Sample B: Rama Tulsi oven dried (100°C) + Krishna Tulsi oven dried (100°C) (1:1 ratio)

Extraction of the samples: For phytochemical analysis, 10 g of dried Tulsi leaf powder of each variety was soaked in 100 mL of 95% ethanol for 24 hours at room temperature. The mixture was filtered using Whatman Grade 1 filter paper to separate the extract.[6] The filtrate was then used for further analysis.

Proximate composition analysis: The proximate composition of the samples, including moisture and ash content was analyzed using standard biochemical methods with slight modifications.[7] Moisture was determined using a hot air oven at 100°C until constant weight. Ash was calculated after incineration in a muffle furnace (Infitek, FNC BX1700 Series). Fat was extracted by the Soxhlet method using hexane.[8] Protein content was analyzed using the standardized method.[9] Carbohydrate was determined using the standard method with slight modifications.[10]

Mineral content analysis: Calcium, magnesium, iron, and potassium content were estimated using a PerkinElmer Analyst 800 atomic absorption spectrophotometer and Systronics flame photometer (Model 128) by following the standard method with slight modifications.[11] Phosphorus content was

determined according to the standard procedure using colorimetric analysis techniques.[12]

Qualitative and quantitative phytochemical analysis: Phytochemical analysis of the extracts was conducted using standard methods with minor changes.[13] For quantitative estimation, a Shimadzu UV-Vis spectrophotometer (Model UV-1800) was used to measure absorbance at 400 nm. For the identification of aromatic and semi-aromatic compounds, Gas Chromatography-Mass Spectrometry (GC-MS) was performed using a Thermo Scientific TRACE 1300 system.

Antioxidant activity analysis using DPPH method: The antioxidant activity of the samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method with slight modifications.[14] Ascorbic acid (from Sigma-Aldrich) was used as a reference antioxidant (positive control) for comparative analysis. The IC_{50} value, representing the concentration required to neutralize 50% of free radicals, was calculated through regression analysis. A Shimadzu UV-Vis spectrophotometer (Model UV-1800) was used to measure absorbance at 400nm.

Antioxidant activity analysis using FRAP Assay: The Ferric Reducing Antioxidant Power (FRAP) assay was performed to determine the antioxidant potential of the samples, following the standard method with slight modifications.[15] The assay was conducted on a BioTek Epoch microplate reader (Model EPOCH2NSC) equipped with an in-built incubator maintained at 37°C. The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ (2,4,6-tri-2-pyridyl-1,3,5-triazine) solution (10 mM in 40 mM hydrochloric acid), and ferric chloride ($FeCl_3$) solution (20 mM) in a ratio of 10:1:1. All reagents were of analytical grade and procured from Sigma-Aldrich. The reduction of Fe^{3+} to Fe^{2+} was monitored by recording absorbance at 593 nm at regular intervals over a 30-minute incubation. The degree of colour change was directly proportional to the antioxidant capacity of the samples.

Antimicrobial activity analysis: The antimicrobial activity of the samples were assessed using the standard method with slight modifications.[16] Mueller-Hinton broth (Model BD Difco™) served as the growth medium in a 96-well microplate format. A BioTek Epoch microplate reader (Model EPOCH2NSC) with an integrated incubator set at 37°C was used to monitor bacterial growth kinetics. Optical density (OD) was recorded at predetermined time intervals over a 24-hour incubation period to evaluate bacterial growth inhibition caused by the sample extracts.

Phenolic compounds analysis: Phenolic compounds in the samples were analyzed using the standard method with slight modifications.[17]

a. **HPLC Equipment Setup:** Analysis was carried out on an Agilent 1260 Infinity II HPLC system comprising a quaternary pump (Model G7111B), autosampler (Model G7129A), column compartment (Model G7116A), diode array detector (DAD, Model G7117A) and fluorescence detector (FLD, Model G1321B). Chromatographic separation was performed using a C18 column (150 mm × 4.6 mm, 5 µm particle size).

b. **Preparation of Standard Solution:** Individual phenolic standards that were procured from Sigma Aldrich, such as rosmarinic acid, apigenin, orientin, luteolin, ursolic acid, eugenol, and caffeic acid (1 mg each) were weighed and dissolved in HPLC-grade methanol (Merck, Germany) to obtain a concentration of 0.1 mg/mL. These were combined to form a mixed stock solution containing 10 µg/mL of each compound. From this mixed stock, a series of calibration standards (1, 2, 4, 6, 8 and 10 µg/mL for each phenolic compound) was prepared by serial dilution using HPLC-grade methanol. Calibration curves were constructed by injecting 20 µL of each calibration standard into the HPLC system under the same chromatographic conditions used for the samples. These curves were later used to determine the concentration of phenolic compounds in the sample extracts with high accuracy.

c. HPLC Analysis of Phenolic Compounds: The mobile phase, a gradient elution of solvent A (0.1% formic acid in water, from Sigma-Aldrich) and solvent B (methanol, from Merck), flowed at a rate of 1 mL/min. Standard solutions (from Sigma-Aldrich) and sample extracts were injected into the HPLC system using the autosampler. Chromatographic separation was achieved on the C18 column under optimized conditions with the mobile phase gradient. The Diode Array Detector (DAD) – Agilent 1260 infinityII (Model G7117C) recorded absorbance spectra across multiple wavelengths to detect phenolic compounds, while the Fluorescence Detector (FLD) - Agilent 1260 infinity (Model G1321B) provided additional detection capability for specific compounds. Retention times and peak areas were used to identify and quantify phenolic compounds in the samples based on comparison with standard calibration curves. This rigorous analysis provided accurate quantification of phenolic compounds in the sample extracts.

Determination of shelf-life of the samples: Storage stability of the samples was analysed through their microbial count as per the standard method.[18] Reagents included agar media suitable for microbial growth. The study utilized microbiological incubator (MBI-1000) sourced from Thermo Fisher Scientific with precise temperature control within a range of 20°C to 40°C and humidity control within a range of 50% to 80%, ensuring optimal conditions for microbial growth. A colony counter (CC-2000) equipped with adjustable LED lighting and 4x to 10x magnification, was employed

for accurate colony enumeration. These instruments enabled the reliable determination of microbial count in colony-forming units per gram (CFU/g) for each sample and storage duration, adhering to standard microbiological protocols.

Statistical Analysis: All experiments were done at least thrice (n=3) to enhance reproducibility, and standard deviation (±) was used to show variation of data among different repetitions. The results were analyzed using a one-way ANOVA (Analysis of Variance) test at $p \leq 0\ 05$ (5% significance level), which was followed by pairwise comparisons using a post hoc test of Tukey to determine any remarkable differences between any of the experimental groups.[19]

RESULTS

Proximate composition analysis: The proximate composition of the samples is presented in Table 1. Sample B showed a higher carbohydrate content (60.02%) compared to Sample A (55.01%). Protein content remained almost similar in both samples. Fat and ash contents were slightly higher in Sample B, while a significant reduction in moisture was observed in Sample B (5.00%) compared to Sample A (12.01%). Overall, Sample B demonstrated improved carbohydrate concentration and lower moisture content, indicating better stability, with minimal changes in other nutrients.

Table 1. Proximate Composition (%) of Rama Tulsi and Krishna Tulsi blended leaf powders:

Sample		A	B
Proximate composition	**Carbohydrate**	55.01 ± 0.50 [a]	60.02 ± 0.50 [a]
	Protein	25.01 ± 0.30[bd]	25.00 ± 0.30[b]
	Fat	3.03 ± 0.20[cd]	3.50 ± 0.20[cd]
	Moisture	12.01 ± 0.50 [dbc]	5.00 ± 0.50 [dce]
	Ash	5.00 ± 0.20 [ea]	6.00 ± 0.20 [eda]

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters ($P \leq 0.05$) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

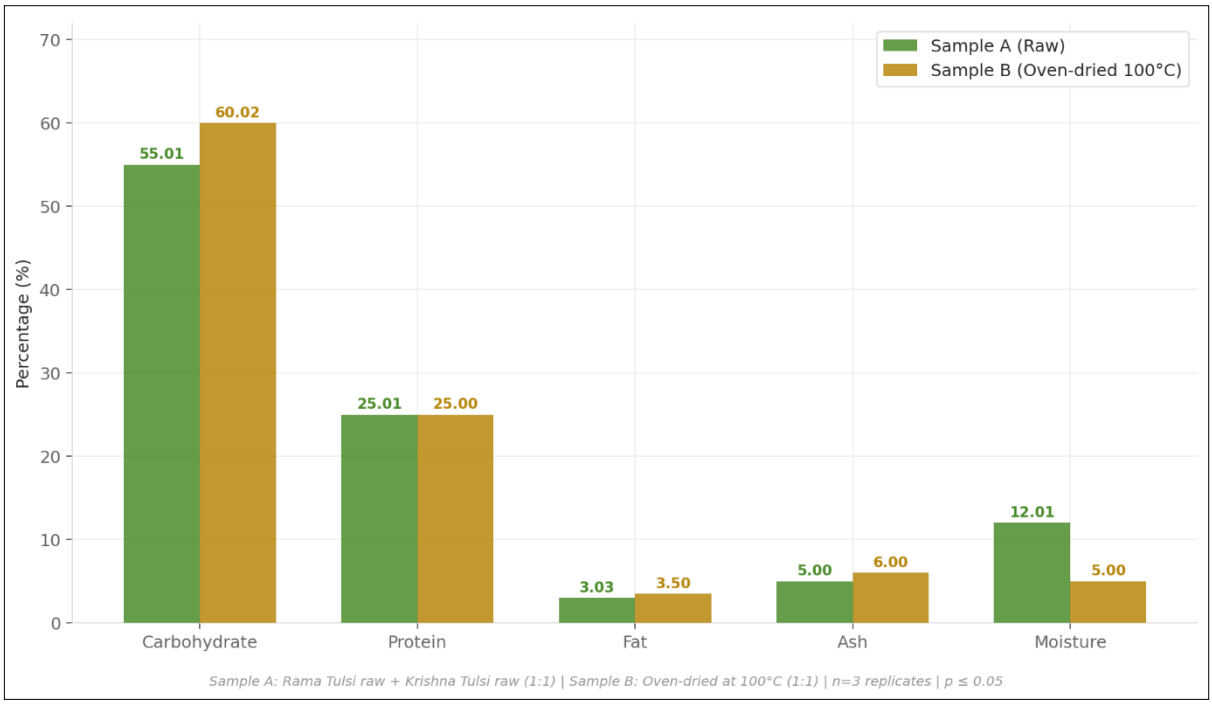


Figure 1. Proximate composition – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

Mineral content analysis: The mineral content of the samples is presented in Table 2. Sample B showed higher values of calcium (2.00%), magnesium (1.20%), phosphorus (0.80%), iron (2.50 mg/100g), and potassium (1.50%) compared to Sample A. Overall, Sample B demonstrated enhanced mineral concentration, suggesting that oven drying contributed to improved retention and concentration of essential minerals.

Table 2. Mineral content analysis of Rama Tulsi and Krishna Tulsi blended leaf powders:

Sample		A	B
Mineral Content	**Calcium (%)**	1.50 ± 0.10^{a}	2.00 ± 0.20^{a}
	Magnesium (%)	0.80 ± 0.05^{ba}	1.20 ± 0.10^{b}
	Phosphorus (%)	0.60 ± 0.05^{cd}	0.80 ± 0.10^{cbe}
	Iron (mg/100g)	2.00 ± 0.20^{dae}	2.50 ± 0.30^{da}
	Potassium (%)	1.20 ± 0.10^{e}	1.50 ± 0.15^{ea}

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters (P≤ 0.05) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

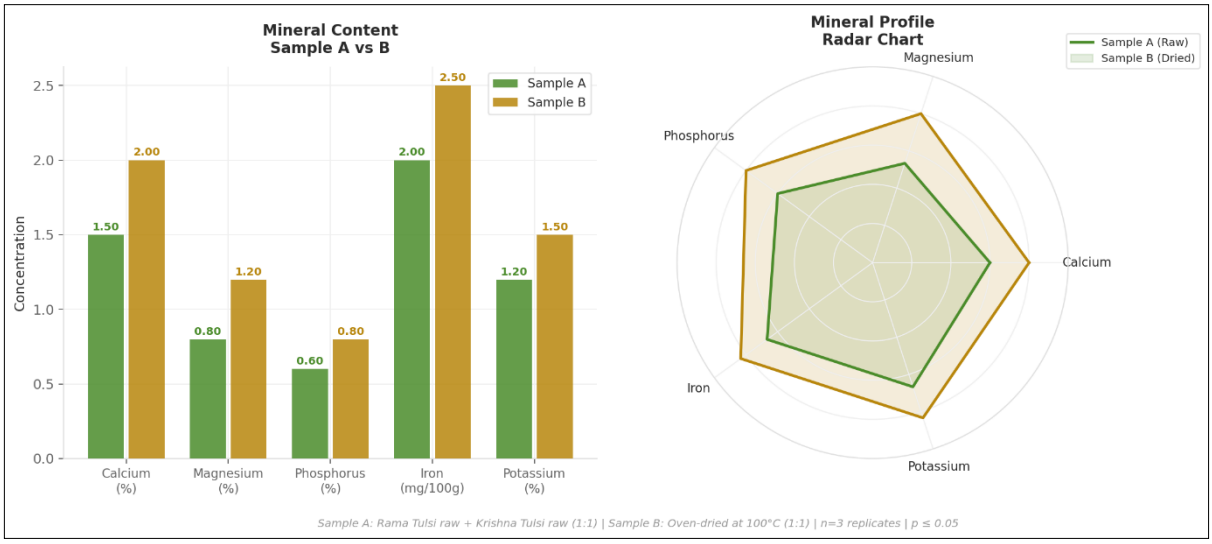


Figure 2. Mineral content analysis – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

Qualitative phytochemical analysis: The qualitative Phytochemical Analysis is presented in Table 3. Both Sample A and Sample B showed positive results for alkaloids, glycosides, tannins, flavonoids, and oxalates. Overall, the presence of similar phytochemicals in both samples indicates that the oven drying process did not significantly affect the retention of these bioactive compounds.

Table 3. Qualitative Phytochemical Analysis of Rama Tulsi and Krishna Tulsi blended leaf powders extract:

Sample		A	B
Qualitative phytochemical tests	**Alkaloid**	Positive (+)	Positive (+)
	Glycoside	Positive (+)	Positive (+)
	Tannin	Positive (+)	Positive (+)
	Flavonoid	Positive (+)	Positive (+)
	Oxalate	Positive (+)	Positive (+)

Note. + = presence confirmed by standard colour/precipitation reactions.

[Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

Quantitative phytochemical analysis: The quantitative phytochemical analysis is presented in Table 4. Both Sample A and Sample B showed comparable levels of alkaloids, glycosides, tannins, flavonoids, and oxalates. Sample A had slightly higher alkaloid and oxalate content, while Sample B showed marginal variations in other components. Overall, both samples exhibited similar phytochemical profiles, indicating that oven drying had minimal impact on the quantitative retention of these bioactive compounds.

Table 4. Quantitative Phytochemical Analysis of Rama Tulsi and Krishna Tulsi blended leaf powders extract:

Sample		A	B
Quantitative phytochemical tests	**Alkaloid**	10.50 ± 1.00 [a]	9.80 ± 0.80 [a]
	Glycoside	5.00 ± 0.50 [bce]	5.20 ± 0.60 [b]
	Tannin	8.20 ± 0.70 [cd]	8.50 ± 0.60 [ca]
	Flavonoid	3.10 ± 0.40 [db]	3.00 ± 0.30 [d]
	Oxalate	2.00 ± 0.30 [e]	1.80 ± 0.20 [eab]

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters (P≤ 0.05) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

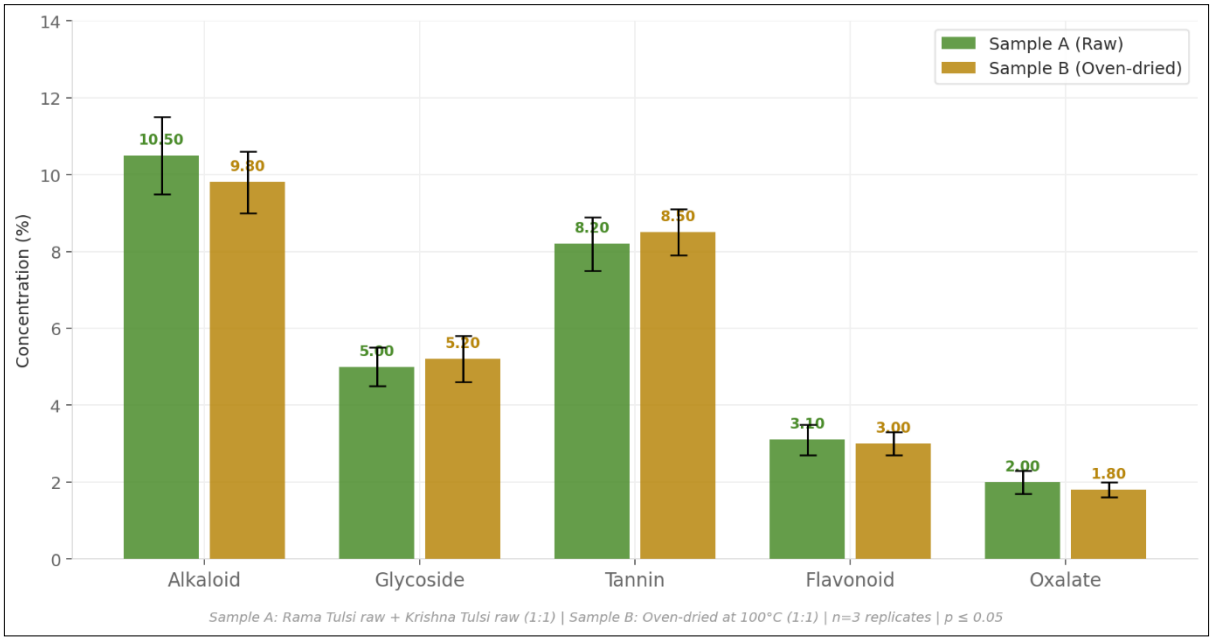


Figure 3. Quantitative phytochemical analysis – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

Antioxidant activity analysis using DPPH method: Among the two formulated samples, Sample B exhibited higher antioxidant activity (75.02%) as compared to Sample A (70.00%). This indicates a slight enhancement in antioxidant potential in Sample B. The increase is due to the oven drying process at 100°C, which has contributed to better retention or release of antioxidant compounds in the Tulsi leaves. The detailed results of antioxidant activity using the DPPH method are presented in Table 5.

Table 5. Antioxidant Activity Analysis of Rama Tulsi and Krishna Tulsi blended leaf powders using the DPPH Radical Scavenging method:

Sample	Anti-oxidant activity (% inhibition)
A	70.00 ± 0.80 [a]
B	75.02 ± 1.20 [b]

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters (P≤ 0.05) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi

raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

Antioxidant activity analysis using FRAP method: Among the two formulated samples, Sample B exhibited higher FRAP activity (468.8 µM Fe^{2+}/g) compared to Sample A (436.3 µM Fe^{2+}/g), indicating greater antioxidant potential. The increase is due to the oven drying process at 100°C, which has contributed to better retention or release of antioxidant compounds in the Tulsi leaves. The detailed results of antioxidant activity using the FRAP assay are presented in Table 6.

Table 6. Antioxidant Activity Analysis of Rama Tulsi and Krishna Tulsi blended leaf powders using FRAP Assay:

Sample	FRAP Activity (µM Fe^{2+}/g)
A	436.3 ± 0.05 [a]
B	468.8 ± 0.03 [b]

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters (P≤ 0.05) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

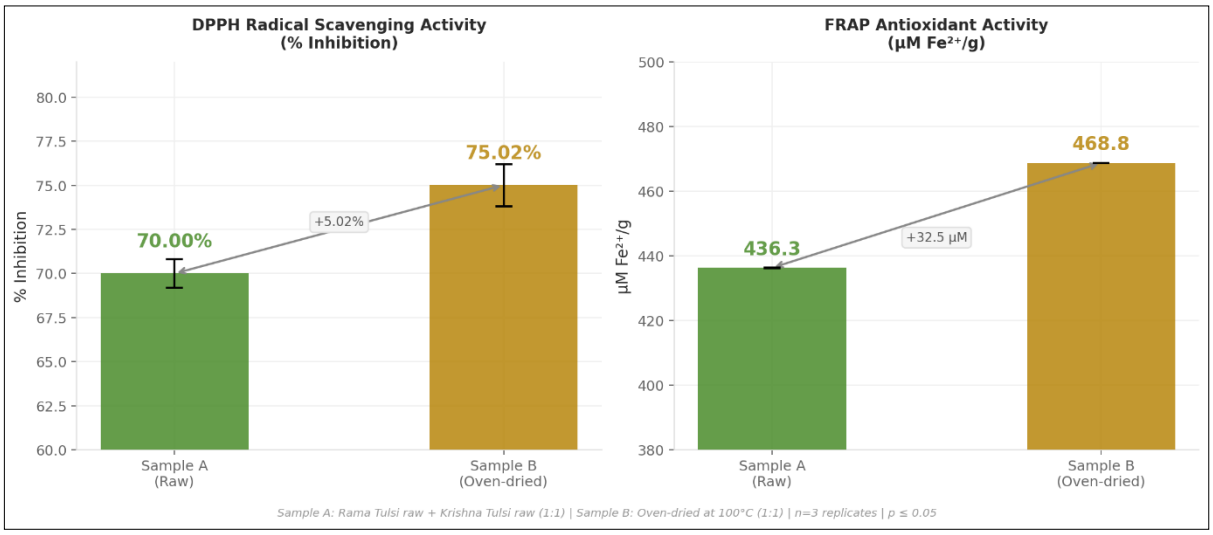


Figure 4. Antioxidant activity analysis using DPPH method and FRAP assay – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

Antimicrobial activity analysis: Among the two samples, Sample B exhibited higher antimicrobial activity (16.00 mm) compared to Sample A (15.02 mm), indicating better inhibitory effect against microbial growth. This enhancement may be due to the oven drying process at 100°C, which could have improved the availability of bioactive compounds in the Tulsi samples. Sample A, prepared using raw Rama Tulsi and Krishna Tulsi leaves (1:1), showed slightly lower inhibition. In contrast, Sample B, consisting of oven-dried (100°C) Rama Tulsi and Krishna Tulsi in the same ratio, demonstrated stronger antimicrobial activity. The detailed results are presented in Table 7.

Table 7. Evaluation of Antimicrobial Activity of Rama Tulsi and Krishna Tulsi blended leaf powders:

Sample	Anti-microbial activity (Inhibition Zone Diameter in mm)
A	15.02 ± 0.70 [a]
B	16.00 ± 1.10 [b]

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters (P≤ 0.05) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

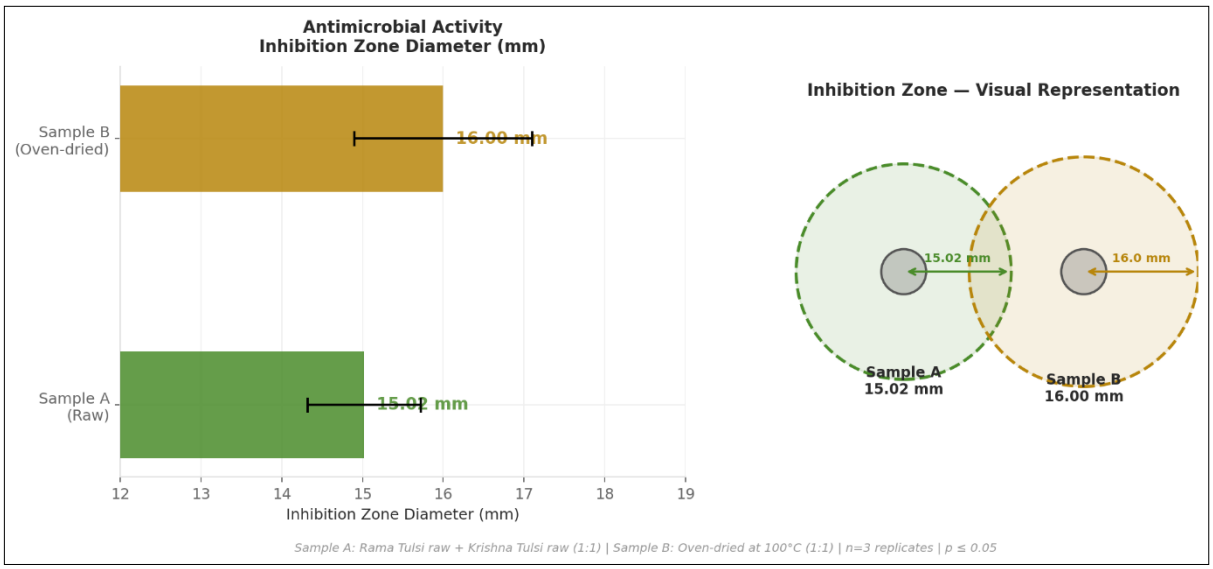


Figure 5. Antimicrobial activity analysis – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

Phenolic components analysis: The HPLC analysis of both samples revealed the presence of several important bioactive compounds. In Sample A, retention times ranged from 10.07 to 14.03 minutes, with rosmarinic acid (10.20 min) and orientin (14.03 min) showing relatively higher presence among the identified compounds. The area percentages were generally low, ranging from 0.30% to 0.90%, indicating comparatively lower concentration of phytochemicals. In Sample B, retention times ranged from 10.09 to 14.09 minutes. Rosmarinic acid (11.01 min) and luteolin (13.02 min) were prominently observed. The area percentages varied between 0.20% and 0.70%, showing slight variation in compound concentration as compared to Sample A. Both samples exhibited a similar profile of bioactive compounds; however, slight differences in retention time and area percentage suggest that the oven drying process have influenced the composition and concentration of phytochemicals. The detailed results are presented in Table 8.

Table 8. Determination of Total Phenolic Component in Rama Tulsi and Krishna Tulsi blended leaf powders:

Samples		Compounds						
		Rosmarinic acid	Apigenin	Orientin	Luteolin	Ursolic acid	Eugenol	Caffeic acid
A	Retention time (min)	10.20	12.00	14.03	12.30	13.70	10.07	12.80
	Area percentage (%)	0.60	0.90	0.70	0.60	0.50	0.40	0.30
B	Retention time (min)	11.01	12.10	14.09	13.02	13.90	10.09	13.10
	Area percentage (%)	0.70	0.30	0.50	0.70	0.50	0.50	0.20

Samples were analyzed in triplicates, and results are expressed. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

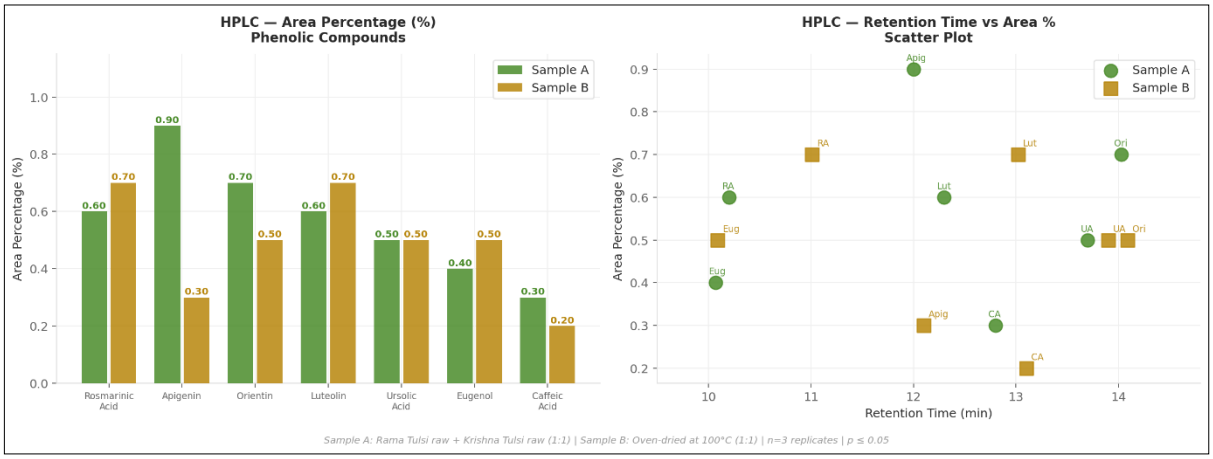


Figure 6. Total phenolic content, HPLC analysis – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

Shelf-life analysis: The storage stability of the samples was evaluated based on microbial count over a period of 3 months. Sample A showed a rise in microbial count from 1000.03 CFU/g at 0 days to 1500.02 CFU/g after 3 months. In comparison, Sample B exhibited a comparatively lower increase, from 950.00 CFU/g initially to 1100.01 CFU/g at the end of 3 months. This indicates that Sample B had better microbial stability over time. The detailed results are presented in Table 9.

Table 9. Determination of shelf-life of Rama Tulsi and Krishna Tulsi blended leaf powders:

Storage Duration	Microbial Count (CFU/g) - A	Microbial Count (CFU/g) - B
0 days	1000.03 ± 0.01^{a}	950.00 ± 0.02^{a}
30 days	1200.10 ± 4.27^{ba}	1050.02 ± 3.21^{bc}
90 days	1500.02 ± 10.03^{c}	1100.01 ± 9.01^{cb}

Samples were analysed in triplicates. Mean and ±SD were calculated. Significant differences were denoted in same column by different letters (P≤ 0.05) from each other. [Sample A: Rama tulsi raw leaves + Krishna tulsi raw leaves (1:1 ratio); Sample B: Rama tulsi oven dried (100°C) + Krishna tulsi oven dried (100°C) (1:1 ratio)]

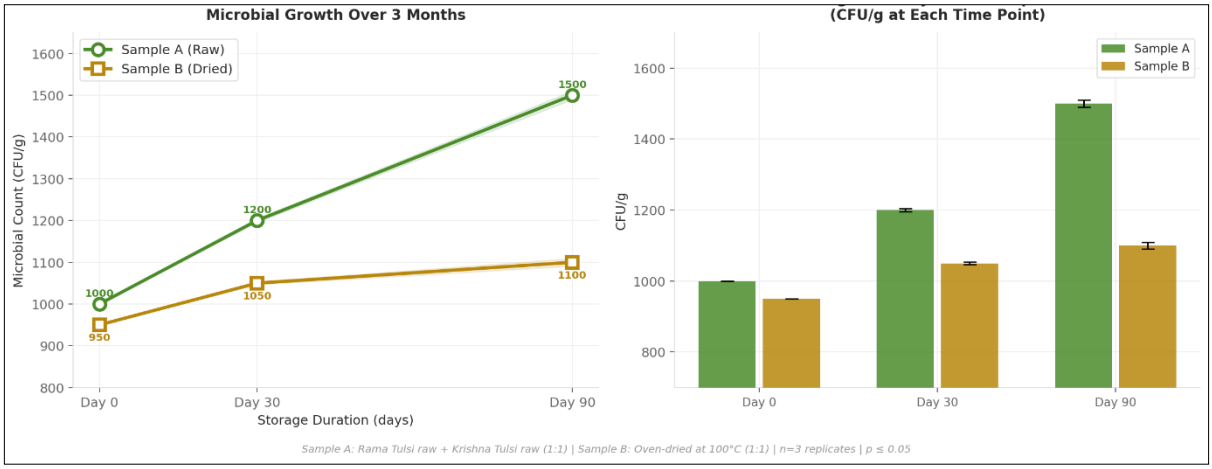


Figure 7. Determination of shelf-life – Sample A vs Sample B (%) for Rama and Krishna Tulsi blended leaf powders

DISCUSSION

The present study provides a comparative analysis of raw and oven-dried (100°C) mixtures of Rama Tulsi (*Ocimum sanctum*) and Krishna Tulsi (*Ocimum tenuiflorum*) which focused on their nutritional composition, phytochemical profile, antioxidant potential, antimicrobial activity, and shelf-life. In proximate composition analysis, moisture content in Sample B (oven-dried at 100°C) had shown significant reduction which contributes to improved shelf-life and stability. Lower moisture inhibits microbial growth and enzymatic degradation, helping in better preservation as shown in previous studies.[20] Increase in carbohydrate, fat and ash content in the dried sample is majorly due to concentration effects of moisture removal. Similar observations have been reported in earlier studies, where dehydration processes increased nutrient density without altering protein structure. Temperature-controlled drying at 100°C appears optimal, as excessive heat could degrade sensitive nutrients, whereas moderate heat preserves structural integrity.[21]

Mineral analysis revealed higher concentrations of calcium, magnesium, phosphorus, iron, and potassium in the oven-dried sample and this result aligns with findings from previous studies which suggested that drying enhances mineral availability due to reduced moisture dilution. Here, it is also important to note environmental factors such as soil composition, altitude and agro-climatic conditions, which influences mineral uptake in plants. Studies have shown that organic carbon of soil, microbial biomass and enzyme activity can directly affect mineral and phytochemical content in *Ocimum* species.[22]

Phytochemical analysis indicated that both samples retained key compounds such as alkaloids, flavonoids, tannins, glycosides and oxalates. The minimal variation between raw and dried samples suggests that oven drying does not easily degrade these compounds. But slight quantitative differences are shown to attribute to heat-induced changes. Environmental factors such as light intensity, altitude and temperature during cultivation also play a crucial role. Studies reported that controlled light spectra, particularly red-to-blue light ratios, can enhance phenolic accumulation in Tulsi, which explains variation in phytochemical concentration.[23]

Antioxidant activity, assessed using DPPH and FRAP assays, was higher in the oven-dried sample. This improvement may be due to enhanced availability of phenolic compounds such as rosmarinic acid, luteolin, and caffeic acid. Heat treatment can disrupt plant cell walls, causing the release of bound antioxidants. Similar findings have been reported which suggested that certain heat-stable compounds retain or even increase in concentration after drying.[24] Also, regional and environmental variations, such as altitude and temperature fluctuations, can affect antioxidant capacity by influencing secondary metabolite synthesis.

The antimicrobial activity results support the advancement of the oven-dried sample, which exhibited a larger inhibition zone. This may be attributed to higher concentrations of bioactive compounds such as eugenol and flavonoids, known for their antimicrobial properties. Studies lso reported that Krishna Tulsi tends to have stronger antimicrobial effects due to its essential oil composition.[25] Processing methods can influence these properties, as drying reduces moisture and enhances compound stability, thus improving antimicrobial efficacy.

HPLC analysis provided detailed insights into phenolic composition, confirming the presence of compounds such as rosmarinic acid, apigenin, orientin, luteolin, ursolic acid, eugenol, and caffeic acid in both samples. Although the overall profiles were similar, slight variations in retention time and area percentage indicate that oven drying influences compound concentration. These changes may result from reduced enzymatic degradation and increased compound stability. Additionally, genetic variation between plant species, geographical location, harvest time, and post-harvest handling can contribute to these differences.[26, 27]

Shelf-life analysis clearly demonstrated that the oven-dried sample had better microbial stability over three months. The lower increase in microbial count confirms that reduced moisture and enhanced antimicrobial compound availability contribute to extended shelf-life. This finding is consistent with general food preservation principles and previous research on dried herbal products.[28]

Statistical analysis using one-way ANOVA further validated that the differences observed between raw and oven-dried samples were significant, particularly in antioxidant and antimicrobial parameters, although similarity in phytochemical profiles suggests that drying primarily enhances availability rather than altering composition.

CONCLUSION

The utilization of oven-drying method in this study has proven to be useful in analysing the effect of controlled temperature's effect on Rama and Krishna Tulsi leaves. Oven drying efficiently reduced moisture content while

preserving the integrity of essential nutrients and bioactive compounds. This method enhanced the antioxidant activity of the leaves and also made the powder last longer during storage. Because it is easy, fast and effective, oven-drying is a good method for preparing Tulsi samples both in research and industry. In future, this method can help understand Tulsi's benefits even better and support its use in different health-improving products.

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DISCLOSURE OF INTEREST

The authors report there are no competing interests to declare.

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