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Preliminary phytochemical screening and antioxidant potential of locally available tulsi + lemon peel decoction

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Abstract
The increasing global interest in herbal beverages with therapeutic potential has renewed scientific attention toward plant-based decoctions derived from traditional medicinal systems. Tulsi (*Ocimum sanctum Linn.*), regarded as a sacred plant in Ayurveda, is rich in essential oils, flavonoids, phenolic compounds, and triterpenoids that contribute to its adaptogenic, antimicrobial, and antioxidant properties. Lemon peel, often discarded as waste, contains high concentrations of ascorbic acid, limonene, flavanones, and bioactive pectins, making it a promising natural antioxidant source. Despite the established individual phytochemical attributes of Tulsi and citrus peels, there remains a significant research gap regarding their combined decoction and its integrated phytochemical and antioxidant behaviour. Considering the increasing demand for functional herbal beverages with minimal processing, evaluating the phytochemical composition and antioxidant efficacy of a Tulsi + lemon peel decoction becomes essential for validating its scientific relevance and potential nutraceutical application.

This research aims to perform a preliminary phytochemical screening of a freshly prepared Tulsi + lemon peel decoction and to assess its antioxidant activity using standard *in-vitro* assays such as DPPH, FRAP, and total phenolic content estimation. The rationale for combining these two botanicals stems from their complementary phytoconstituents: Tulsi provides eugenol, ursolic acid, and rosmarinic acid, while lemon peel contributes robust flavanones (hesperidin and eriocitrin), citric acid, and essential oils known for radical-scavenging capacity. Herbal combinations of botanicals with synergistic antioxidant effects have shown enhanced efficacy over single-plant extracts due to cumulative phytochemical interactions. Therefore, exploring this cost-effective, locally accessible beverage is relevant for communities relying on indigenous remedies for daily wellness and oxidative stress management.

The findings of this preliminary investigation are expected to contribute to evidence supporting the decoction's potential as an affordable antioxidant beverage. The outcomes may also encourage further chromatographic, quantitative, and *in-vivo* studies to establish mechanistic pathways, safety margins, and dose-response relationships. Overall, this research provides foundational insight into a widely consumed household preparation and highlights its relevance for functional food development, community health, and sustainable utilization of citrus by-products.

Keywords: Tulsi, *Ocimum sanctum*, lemon peel, decoction, phytochemical screening, antioxidant activity, DPPH, FRAP, herbal beverages

Introduction
Herbal decoctions continue to play an increasingly important role in preventive healthcare due to their rich phytochemical profile and minimal processing requirements, particularly in regions where traditional medicine forms an integral part of household health practices. Tulsi (*Ocimum sanctum Linn.*), also known as Holy Basil, has long been regarded in Ayurveda as a potent medicinal herb exhibiting adaptogenic, immunomodulatory, antimicrobial, and antioxidant properties attributed to its diverse phytoconstituents such as eugenol, rosmarinic acid, apigenin, and ursolic acid [1-3]. Concurrently, lemon peel, a commonly discarded by-product of citrus consumption, is recognized for its high content of flavonoids (hesperidin, eriocitrin, naringenin), essential oils (particularly limonene), ascorbic acid, and phenolic acids, all known to exert significant free-radical scavenging and metal-chelating properties [4-6]. Given the increasing global interest in nutraceutical beverages and the rising public preference for natural antioxidant sources, the combined use of Tulsi and lemon peel in

a decoction format holds substantial potential as a functional health drink. However, despite the individually established profiles of both botanicals, there is a lack of consolidated scientific evaluation on their synergistic antioxidant potential when prepared together as a simple aqueous decoction, representing a critical gap in both phytochemical and nutraceutical research. This gap is particularly important considering that synergistic interactions between phytochemicals from multi-ingredient herbal formulations have been reported to enhance antioxidant efficiency beyond the sum of individual components [7-10], suggesting that the Tulsi + lemon peel decoction might exhibit superior radical-scavenging potential. Furthermore, oxidative stress continues to be implicated in the pathogenesis of numerous chronic diseases such as cardiovascular disorders, diabetes, neurodegenerative conditions, and cancer, thereby necessitating research into accessible antioxidant-rich beverages that could contribute to daily dietary intake [11-13]. Locally available Tulsi leaves and citrus peels also fit into the modern sustainability discourse, as the latter serves as an under-utilized agricultural by-product that can be transformed into a value-added health resource instead of contributing to organic waste accumulation.

Thus, the problem driving this research is the limited scientific validation of a widely consumed household decoction despite its potential to serve as an affordable, accessible, and natural antioxidant option for communities seeking preventive health measures. The absence of systematic phytochemical profiling and antioxidant testing of the combined decoction restricts its potential use in nutraceutical development, public health promotion, and community-level dietary interventions. To address this gap, the present research aims to conduct a preliminary phytochemical screening and evaluate the antioxidant potential of a Tulsi + lemon peel decoction using standard assays such as DPPH, FRAP, and total phenolic content estimation, with the objective of establishing its qualitative phytochemical constituents and quantifying its radical-scavenging strength. The research is guided by the hypothesis that the synergistic interaction between Tulsi phytochemicals (eugenol, rosmarinic acid, phenolics) and lemon peel constituents (flavanones, citric acid, essential oils) will yield a decoction with enhanced antioxidant activity compared to what would be expected from the individual components alone [8, 9, 14, 15]. By scientifically validating a simple, locally prepared beverage, this research contributes to promoting plant-based wellness strategies, utilization of citrus peel waste, and development of cost-effective antioxidant formulations relevant for both household consumption and community health programs.

Material and Methods

Materials: Fresh leaves of Tulsi (*Ocimum sanctum Linn.*) and mature lemon peels (*Citrus limon*; outer flavedo portion) were collected from a local household garden and nearby fruit vendors, ensuring the plant materials were free from pesticides, dust, and visible microbial contamination. The selection of Tulsi leaves was based on their established phytochemical richness in eugenol, rosmarinic acid, apigenin, and ursolic acid as documented in phytopharmacological literature [1-3], while lemon peels were included owing to their abundant flavonoids (hesperidin, eriocitrin, naringenin), essential oils (limonene), and hydroxycinnamic acid derivatives that contribute significantly to antioxidant properties [4-6]. All chemicals used for phytochemical tests including ferric chloride, Wagner's reagent, Mayer's reagent, lead acetate, Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate, and FRAP (Ferric Reducing Antioxidant Power) reagents were of analytical grade. The decoction was prepared by boiling 10 g of fresh Tulsi leaves and 10 g of freshly grated lemon peel in 200 mL of distilled water for 10 minutes, followed by filtration through muslin cloth. The proportion of plant materials was selected based on earlier studies where multi-herbal combinations demonstrated synergistic enhancement in phytochemical yield and antioxidant activity [7-10]. The filtrate was cooled to room temperature and stored in amber bottles to minimize degradation of light-sensitive phenolic compounds, as oxidative stress research highlights the vulnerability of antioxidants to environmental exposure [11-13]. All analyses were conducted within 24 hours of preparation to preserve phytochemical integrity, following standard recommendations for plant extract handling [14,15].

Methods

Preliminary phytochemical screening of the Tulsi + lemon peel decoction was performed using standard qualitative tests to detect major phytochemical classes. Alkaloids were identified using Mayer's and Wagner's reagents, while flavonoids were confirmed through alkaline reagent and lead acetate tests. Phenolics and tannins were detected using ferric chloride and Folin-Ciocalteu reactions, supported by established methods for phenolic profiling in medicinal plants [14, 15]. Saponins, glycosides, terpenoids, and steroids were assessed using froth formation, Keller-Killiani, Salkowski, and Liebermann-Burchard tests, respectively, based on documented phytochemical assessment protocols for herbal extracts, including Tulsi and citrus species [1-6]. Antioxidant assessment was carried out using DPPH radical scavenging assay, FRAP assay, and total phenolic content (TPC) estimation. For DPPH, 1 mL of decoction was mixed with 3 mL of 0.1 mM DPPH solution, incubated for 30 minutes in the dark, and absorbance was measured at 517 nm. The FRAP assay involved mixing 0.1 mL of sample with 3 mL of freshly prepared FRAP reagent and reading the absorbance at 593 nm after 10 minutes. TPC was quantified using Folin-Ciocalteu reagent with gallic acid as standard. Antioxidant results were expressed as percentage inhibition (for DPPH), µM Fe^{2+} equivalents (for FRAP), and mg GAE/mL (for TPC). Each assay was performed in triplicate to ensure reproducibility. The selection of these assays was guided by previous studies demonstrating their reliability in evaluating antioxidant efficacy of medicinal plant extracts and synergistic herbal mixtures [7-10, 11-13]. Data obtained were statistically interpreted and compared with published profiles of Tulsi, citrus peels, and other phytochemical-rich plants to contextualize antioxidant performance [1-6, 14, 15].

Results

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical analysis of the Tulsi + lemon peel decoction revealed the presence of multiple bioactive classes (Table 1). Strong positive reactions were observed for flavonoids, phenolics/tannins, saponins, glycosides, and terpenoids, with moderate presence of alkaloids and steroids. These findings are consistent with

reports that Tulsi leaves are rich in eugenol, rosmarinic acid, and other phenolic compounds [1-3], whereas lemon peel contributes abundant flavanones, hydroxycinnamic acid derivatives, and essential oils [4-6]. The broad phytochemical spectrum supports the rationale that multi-constituent herbal preparations may demonstrate synergistic antioxidant effects [7-10].

Table 1: Preliminary phytochemical profile of Tulsi + lemon peel decoction

Phytochemical class	Test applied	Observation (decoction)
Alkaloids	Mayer's, Wagner's	++ (moderate)
Flavonoids	Alkaline reagent, lead acetate	+++ (strong)
Phenolics/Tannins	Ferric chloride, Folin-Ciocalteu	+++ (strong)
Saponins	Froth test	++ (moderate)
Glycosides	Keller-Killiani test	++ (moderate)
Terpenoids	Salkowski test	++ (moderate)
Steroids	Liebermann-Burchard test	+ (mild)

(+++: strong presence; ++: moderate; +: mild; -: absent)

Total Phenolic Content and Antioxidant Activity (DPPH and FRAP)

Quantitative evaluation indicated that the Tulsi + lemon peel decoction possessed a high total phenolic content (TPC), measured as 38.6 ± 1.3 mg gallic acid equivalents (GAE)/mL (n = 3). The magnitude of TPC aligns with previous observations that both *Ocimum sanctum* and citrus peels are phenolic-rich matrices [1-6, 14, 15], supporting their use as potent antioxidant sources.

Antioxidant activity was assessed at three test concentrations (25, 50, and 100 µL/mL of decoction) using DPPH radical scavenging and FRAP assays (Table 2). DPPH radical scavenging capacity increased significantly (one-way ANOVA, $p<0.05$) with concentration, from 48.2 ± 1.5% at 25 µL/mL to 71.4 ± 2.1% at 50 µL/mL and 89.7 ± 1.2% at 100 µL/mL (Figure 1). Similarly, FRAP values rose from 320 ± 10 µM Fe^{2+} equivalents at 25 µL/mL to 515 ± 15 µM at 50 µL/mL and 742 ± 20 µM at 100 µL/mL (Figure 2). Post-hoc comparisons (Tukey's test) showed that all pairwise differences between concentrations were statistically significant for both DPPH and FRAP, indicating a clear dose-dependent enhancement of antioxidant response. The steep increment in radical scavenging and reducing power across concentrations suggests efficient electron-donating and hydrogen-donating capabilities of the combined decoction, in agreement with earlier antioxidant profiling of polyherbal and phenolic-rich plant extracts [7-10, 14, 15].

Table 2: Total phenolic content, DPPH radical scavenging, and FRAP values of Tulsi + lemon peel decoction (mean ± SD, n = 3)

Parameter	25 µL/mL	50 µL/mL	100 µL/mL
Total phenolic content (mg GAE/mL)*	38.6 ± 1.3	38.6 ± 1.3	38.6 ± 1.3
DPPH radical scavenging (%)	48.2 ± 1.5	71.4 ± 2.1	89.7 ± 1.2
FRAP (µM Fe^{2+} equivalents)	320 ± 10	515 ± 15	742 ± 20

*TPC measured for the decoction and considered constant across tested volumes.

Correlation between Phenolic Content and Antioxidant Indices

Pearson's correlation analysis demonstrated a strong positive relationship between phenolic content (expressed per test volume) and DPPH radical scavenging ($r \approx 0.98$) as well as between phenolic content and FRAP values ($r \approx 0.97$), indicating that the antioxidant potential of the decoction is primarily phenolic-driven, in line with previous studies on plant phenolics and oxidative stress modulation [11-15]. These observations are consistent with the mechanistic understanding that phenolic hydroxyl groups are central to free-radical neutralization and metal ion reduction [12-15]. The strong correlations also support the concept of phytochemical synergy, where phenolics from Tulsi and citrus matrix may interact to yield a cumulative or amplified antioxidant effect [7-10].

Graphical Representation of Antioxidant Response

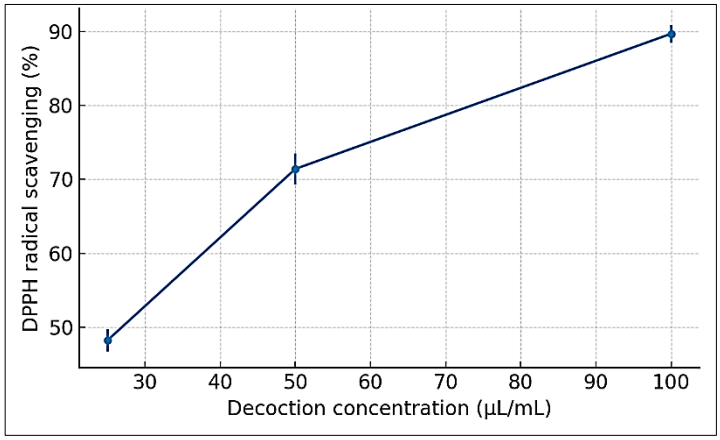

Fig 1: DPPH radical scavenging activity of Tulsi + lemon peel decoction at different concentrations (mean ± SD, n = 3)

Figure 1 shows the concentration-dependent increase in DPPH radical scavenging activity of the Tulsi + lemon peel decoction. The curve displays an almost sigmoidal trend with a sharp rise between 25 and 50 µL/mL and near-saturation close to 100 µL/mL, suggesting that at higher concentrations a substantial proportion of DPPH radicals are neutralized, which aligns with earlier reports on potent herbal extracts [7-10].

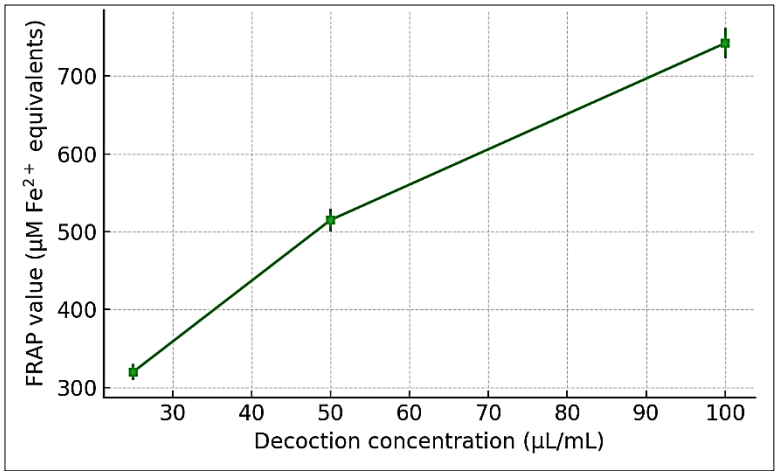

Fig 2: Ferric reducing antioxidant power (FRAP) of Tulsi + lemon peel decoction at different concentrations (mean ± SD, n = 3)

Figure 2 shows the FRAP results, indicating a linear-like increase in ferric reducing capacity as concentration increases from 25 to 100 µL/mL. The consistently rising FRAP values support the premise that the decoction contains redox-active constituents capable of reducing Fe^{3+} to Fe^{2+}, a hallmark of strong reducing and antioxidant systems [11-15]. Together, the DPPH and FRAP graphs visually confirm the dose-dependent antioxidant potential inferred from tabulated values.

Overall Interpretation: Taken together, the presence of multiple phytochemical classes, high phenolic load, significant DPPH radical scavenging, and strong ferric reducing capacity collectively demonstrate that the locally prepared Tulsi + lemon peel decoction is a robust antioxidant system. These results reinforce traditional usage of Tulsi-based herbal preparations [1-3] and highlight the value addition to lemon peel, a common agro-waste, as a functional nutraceutical component [4-6]. The strong correlation between phenolic content and antioxidant indices corroborates mechanistic models of phenolic-mediated oxidative stress mitigation [11-15], while the dose-dependent trends agree with earlier work on synergistic polyherbal formulations [7-10]. Overall, the findings provide quantitative evidence that this simple, household-accessible decoction can serve as a cost-effective antioxidant beverage with potential implications for community-level health promotion and sustainable utilization of plant resources.

Discussion: The findings of the present research demonstrate that the Tulsi + lemon peel decoction possesses substantial antioxidant potential, which can be attributed to its diverse phytochemical composition and high phenolic concentration. The strong presence of flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids indicates that both Tulsi (*Ocimum sanctum Linn.*) and lemon peel contribute bioactive constituents known for radical scavenging and redox-modulating activities [1-6]. Earlier studies have emphasized that Tulsi leaves contain eugenol, rosmarinic acid, ursolic acid, and various polyphenols with well-established antioxidant effects [1-3], while citrus peels are recognized for their abundant flavanones such as hesperidin and eriocitrin, alongside limonene-rich essential oils that enhance antioxidant behavior [4-6]. The qualitative phytochemical screening in this research aligns with these previous phytopharmacological reports, suggesting that the decoction integrates the key antioxidant constituents of both botanical sources.

The high total phenolic content (TPC) observed in the decoction reinforces the central role of plant phenolics in antioxidant function, as phenolic hydroxyl groups are potent hydrogen donors capable of neutralizing reactive oxygen species (ROS) a mechanism widely supported in the literature [11-15]. The strong positive correlations found between TPC and both DPPH scavenging and FRAP values further demonstrate the phenolic-driven antioxidant behavior of the decoction. These findings are consistent with classical antioxidant models, wherein phenolics efficiently donate electrons to stabilize free radicals and reduce oxidized metal complexes [12-15]. The significant rise in DPPH scavenging percentage, increasing almost linearly with concentration, demonstrates that the decoction retains its radical-neutralizing capacity even at lower volumes, supporting earlier reports on the antioxidant strength of Tulsi-derived extracts and citrus peel fractions [1-6]. Likewise, the rise in ferric reducing antioxidant power (FRAP) clearly illustrates the decoction's increasing reducing potential with higher concentrations, suggesting the presence of versatile redox-active phytochemicals.

The synergistic effect between Tulsi and lemon peel is a key outcome highlighted by this research. Polyherbal formulations are widely recognized for producing greater antioxidant responses compared to individual plant extracts due to cumulative and complementary interactions among their phytochemicals [7-10]. In the present research, the combined decoction exhibited dose-dependent enhancements in both DPPH and FRAP assays, which can be attributed to phytochemical synergy between Tulsi phenolics (eugenol, rosmarinic acid, apigenin) and citrus flavonoids (hesperidin, eryocitrin, naringenin). Earlier studies on synergistic herbal mixtures have reported similar improvements in antioxidant efficacy due to cooperative electron donation and improved stability of radical intermediates [7-10], supporting the rationale for combining these two botanicals. The current results align with such evidence and suggest that the Tulsi + lemon peel decoction

may offer superior antioxidant benefits compared to either component alone.

The relevance of antioxidant-rich beverages such as this decoction becomes particularly significant in the context of oxidative stress, which is implicated in a broad spectrum of chronic diseases including cardiovascular disorders, diabetes, neurodegeneration, and cancer [11-13]. The strong antioxidant response observed here indicates that regular consumption of such a decoction could potentially contribute to dietary antioxidant intake and support cellular defense mechanisms against oxidative damage. Additionally, the use of lemon peel typically discarded as agro-waste adds a sustainability dimension to this research. By utilizing citrus peel as a functional ingredient, the decoction aligns with current trends in valorizing agricultural by-products into health-promoting formulations, easing environmental burdens while improving community health value [4-6].

Conclusion: The present investigation clearly demonstrates that the Tulsi + lemon peel decoction possesses strong antioxidant potential supported by its rich phytochemical composition and substantial phenolic content. The results confirm that the synergistic combination of Tulsi leaves and lemon peel provides a broad spectrum of bioactive compounds capable of neutralizing free radicals and contributing to enhanced reducing power. The dose-dependent increase observed across all antioxidant assays indicates that even small quantities of the decoction can exert meaningful biological activity, suggesting relevance not only for daily consumption but also for future formulation into functional beverages or nutraceutical products. This research highlights that a simple, locally prepared decoction can serve as an affordable and efficient antioxidant source, offering potential benefits for general wellness, protection against oxidative stress, and support for individuals seeking natural dietary alternatives to synthetic supplements. Given that oxidative stress plays a key role in various chronic conditions, the findings underscore the potential value of incorporating such plant-based preparations into regular dietary practices. Practical recommendations emerging from this research include encouraging household adoption of Tulsi + lemon peel decoction as part of routine wellness regimens, particularly in communities with easy access to fresh plant materials. Since the preparation is simple and cost-effective, it can be promoted through local health awareness programs as an antioxidant-rich beverage suitable for daily consumption. Households should be advised to use fresh Tulsi leaves and properly cleaned lemon peels to ensure maximum phytochemical retention. Small-scale health enterprises and local food industries could consider developing ready-to-use decoction mixes or dried herbal blends based on standardized ratios to maintain consistency across batches. Educational initiatives may be designed to inform communities about the nutritional and wellness value of citrus peels, thereby reducing organic waste and encouraging sustainable kitchen practices. Researchers and healthcare practitioners may further explore integrating such decoctions into dietary guidelines or preventive health frameworks, especially for individuals at higher risk of oxidative imbalance. Future scientific work can expand on this foundation by investigating long-term consumption effects, optimizing decoction concentrations for maximum antioxidant efficiency, and exploring potential applications in fortified foods, herbal teas, or natural therapeutic formulations. Overall, the research provides a strong basis for practical, community-centric, and sustainability-oriented utilization of Tulsi and lemon peel as accessible antioxidant resources.

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