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Preliminary Phytochemical Screening and In Vitro Antioxidant Activity of *Ocimum sanctum* (Tulsi) Leaf Extract: A Laboratory-Based Study

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ABSTRACT:

Background: Oxidative stress, driven by an imbalance between reactive oxygen species and endogenous antioxidant defences, contributes to the pathogenesis of numerous chronic and degenerative disorders. Medicinal plants remain an important, low-cost source of natural antioxidants, and *Ocimum sanctum* Linn. (Tulsi, holy basil) occupies a revered place in the traditional medicine of South India.

Objective: To perform a preliminary qualitative phytochemical screening and to evaluate the in vitro free-radical-scavenging capacity of a hydroalcoholic leaf extract of *Ocimum sanctum*, and to relate its antioxidant activity to its phenolic and flavonoid content.

Methods: This was an experimental in vitro laboratory study conducted on a hydroalcoholic (ethanol–water) leaf extract of *O. sanctum*. Fifteen extract batches were prepared and screened qualitatively for major phytochemical classes using standard tests. Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay across concentrations of 10–100 µg/mL in triplicate, with the median inhibitory concentration (IC50) derived by linear regression. Total phenolic content (TPC) and total flavonoid content (TFC) were determined by the Folin–Ciocalteu and aluminium chloride colorimetric methods, respectively. Data are summarised as mean ± standard deviation.

Results: The mean extraction yield was 15.25 ± 2.48% (range 11.14–18.57%). Flavonoids, phenolics and terpenoids were detected in all 15 batches (100%), with alkaloids and carbohydrates (73.3%), saponins (66.7%), tannins (60.0%), glycosides (53.3%) and steroids (46.7%) present at lower frequencies; proteins were trace or absent. DPPH scavenging was concentration-dependent, rising from 8.90% at 10 µg/mL to 79.89% at 100 µg/mL (linear fit $R^2 = 0.9985$), yielding an extract IC50 of approximately 62.0 µg/mL. TPC was 99.89 ± 16.58 mg gallic acid equivalents (GAE)/g and TFC was 51.40 ± 9.81 mg quercetin equivalents (QE)/g.

Conclusion: The hydroalcoholic leaf extract of *O. sanctum* is rich in phenolics, flavonoids and terpenoids and exhibits moderate, concentration-dependent antioxidant activity consistent with its high phenolic and flavonoid content. These findings provide laboratory support for the traditional South Indian medicinal use of Tulsi and illustrate a simple, low-cost screening approach.

1. INTRODUCTION

The Ayurvedic, Siddha and folk traditions have curated an extensive pharmacopoeia of medicinal plants, many of which remain in daily household use across South India. The renewed global interest in plant-based therapeutics is driven in part by concerns over the cost and adverse effects of synthetic agents and by the recognition that phytochemicals frequently possess pleiotropic biological activities, including antioxidant, anti-inflammatory, antimicrobial and immunomodulatory effects [1,2].

A central mechanism through which many medicinal plants are thought to act is the mitigation of oxidative stress. Oxidative stress arises when the generation of reactive oxygen species (ROS) and other free radicals—superoxide anion, hydroxyl radical, hydrogen peroxide and peroxyl radicals—exceeds the capacity of endogenous antioxidant defence systems. These reactive species can damage lipids, proteins and nucleic acids, and their sustained accumulation has been mechanistically linked to ageing and to the pathogenesis of cardiovascular disease, diabetes mellitus, neurodegenerative disorders and cancer [3,4]. Dietary and plant-derived antioxidants—particularly polyphenols and flavonoids—can neutralise free radicals by donating hydrogen atoms or electrons, chelating transition metals and interrupting radical chain reactions, thereby complementing the body's own enzymatic and non-enzymatic defenses [5].

Ocimum sanctum Linn. (synonym *Ocimum tenuiflorum*), known throughout India as Tulsi or holy basil, is among the most venerated plants in the subcontinent. It is cultivated in courtyards and temples across South India and is woven into daily religious and household practice, a cultural prominence that has historically been intertwined with its medicinal reputation [6]. Traditional systems have employed Tulsi for a remarkable breadth of indications, encompassing respiratory ailments, fever, digestive complaints, skin disorders and general vitality, earning it descriptions such as "the elixir of life" and "a herb for all reasons" [7]. Modern pharmacological investigation has substantiated many of these claims, documenting adaptogenic, anti-inflammatory, hepatoprotective, antimicrobial, anticancer, anthelmintic and radioprotective activities, several of which have been attributed to constituents such as eugenol, ursolic acid, rosmarinic acid and a range of flavonoids [8]. The pharmacological validation of the traditional claims for Tulsi has been an explicit aim of Indian experimental research for more than two decades [9].

Despite this substantial literature, there remains a continuing need for simple, reproducible, low-cost laboratory studies that characterise the phytochemical profile and antioxidant capacity of locally sourced Tulsi, both for teaching purposes and as a foundation for more detailed pharmacological work. Qualitative phytochemical screening establishes which broad classes of secondary metabolites are present, while quantitative assays of total phenolic and flavonoid content, coupled with a standardised radical-scavenging assay such as the DPPH method, provide a first estimate of antioxidant potency and allow the antioxidant activity to be correlated with the underlying chemistry [10-13].

The present study was therefore undertaken to characterise a hydroalcoholic leaf extract of *O. sanctum* obtained in a South Indian laboratory setting. The specific objectives were: (i) to determine the extraction yield; (ii) to screen the extract qualitatively

for major phytochemical constituents; (iii) to quantify total phenolic and total flavonoid content; and (iv) to evaluate in vitro antioxidant activity by the DPPH assay, deriving the IC50 and relating it to the phenolic and flavonoid content. In doing so, the study aims to provide laboratory evidence consistent with the traditional South Indian medicinal use of Tulsi.

2. MATERIALS AND METHODS

2.1 Study design and setting

This was an experimental in vitro laboratory study. Plant material collection, authentication, extraction and all analytical assays were carried out at the Department of Botany/Pharmacognosy, South India, during six months [14]. Because the study was confined to plant material and in vitro chemical assays, no human participants or animal subjects were involved, and formal ethics-committee approval was therefore not required.

2.2 Plant collection and authentication

Fresh, healthy and mature leaves of *Ocimum sanctum* Linn. were collected from locally cultivated plants in South India. The plant material was taxonomically identified and authenticated at the Department of Botany/Pharmacognosy, and a voucher specimen (voucher number [vno.2378] was deposited in the departmental herbarium for future reference.

2.3 Preparation of the extract

The collected leaves were washed under running water, shade-dried at ambient temperature and coarsely powdered using a mechanical grinder. The powdered material was subjected to hydroalcoholic extraction using an ethanol–water solvent system by Soxhlet extraction. The resulting extracts were filtered, concentrated under reduced pressure and dried to yield a semisolid residue [15]. Fifteen independent extract batches were prepared under identical conditions, and the percentage yield of each batch was calculated on a dry-weight basis. The dried extract was stored in an airtight container at 4 °C and reconstituted in the appropriate solvent immediately before analysis.

2.4 Qualitative phytochemical screening

Each of the 15 extract batches was screened for the presence of major classes of secondary metabolites using standard qualitative tests. Alkaloids were detected using Mayer's and Wagner's reagents; flavonoids by the Shinoda (magnesium–hydrochloric acid) test; phenolics and tannins by the ferric chloride test; saponins by the foam (froth) test; terpenoids and steroids by the Salkowski test; carbohydrates by Fehling's and Molisch's tests; glycosides by the Keller–Kiliani test; and proteins by the Biuret and Millon's tests. The outcome of each test was recorded as present or absent (with faint reactions recorded as trace), and the frequency of detection across the 15 batches was expressed as a percentage [16].

2.5 DPPH radical-scavenging assay

In vitro antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. Extract solutions were prepared at concentrations of 10, 20, 40, 60, 80 and 100 µg/mL. An equal volume of freshly prepared methanolic DPPH solution was added to each extract concentration, mixed and incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 517 nm against a blank. A DPPH solution without extract served as the control, and a reference antioxidant standard was processed in parallel according to the assay protocol. Each concentration was assayed in triplicate. The percentage inhibition of the DPPH radical was calculated as:

Percentage inhibition = [(A_control − A_sample) / A_control] × 100

where A_control is the absorbance of the control and A_sample is the absorbance in the presence of the extract. The concentration required to scavenge 50% of the DPPH radicals (IC50) was determined from the linear regression of percentage inhibition against extract concentration [10].

2.6 Total phenolic content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu colorimetric method. The extract was reacted with Folin–Ciocalteu reagent and sodium carbonate, and the absorbance of the developed blue colour was measured at 765 nm. A calibration curve was constructed using gallic acid as the standard, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Ten replicate determinations were performed.

2.7 Total flavonoid content (TFC)

Total flavonoid content was estimated by the aluminium chloride colorimetric method. The extract was reacted with aluminium chloride in the presence of sodium nitrite and sodium hydroxide, and the absorbance was measured at 415 nm. A calibration curve was prepared using quercetin as the standard, and results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). Ten replicate determinations were performed.

2.8 Statistical analysis

Quantitative data were summarised as mean ± standard deviation (SD) with ranges where appropriate. The relationship between DPPH percentage inhibition and extract concentration was modelled by linear regression, and the coefficient of determination (R^2) and the IC50 were derived from the fitted line. Analyses and figures were prepared using standard scientific computing tools. Given the descriptive, single-extract nature of the study, no inferential group comparisons were undertaken.

3. RESULTS

3.1 Extraction yield

The 15 hydroalcoholic extract batches gave a mean extraction yield of 15.25 ± 2.48%, ranging from 11.14% to 18.57% (Table 1). The consistency of the yield across independently prepared batches indicates a reproducible extraction process.

Table 1. Summary of hydroalcoholic extraction yield of *Ocimum sanctum* leaf (n = 15 batches).

Parameter	Value
Number of extract batches	15
Solvent system	Ethanol–water (hydroalcoholic)
Mean yield (%)	15.25
Standard deviation (%)	2.48
Minimum yield (%)	11.14
Maximum yield (%)	18.57

3.2 Qualitative phytochemical screening

Qualitative screening revealed a phytochemically rich extract (Table 2, Figure 2). Flavonoids, phenolics and terpenoids were detected in all 15 batches (100%). Alkaloids and carbohydrates were each present in 11 of 15 batches (73.3%), saponins in 10 of 15 (66.7%), tannins in 9 of 15 (60.0%), glycosides in 8 of 15 (53.3%) and steroids in 7 of 15 (46.7%). Proteins were not detected as a clear positive in any batch, being recorded as trace in 9 of 15 batches and otherwise absent; they are therefore reported as trace/absent. The uniform presence of flavonoids, phenolics and terpenoids is consistent with the known chemistry of *O. sanctum* and is of particular relevance to its antioxidant potential.

Table 2. Qualitative phytochemical screening of *Ocimum sanctum* hydroalcoholic leaf extract (n = 15 batches).

Constituent	Test used	Batches positive (n/15)	Frequency (%)	Interpretation
Flavonoids	Shinoda	15/15	100.0	Consistently present
Phenolics	Ferric chloride	15/15	100.0	Consistently present
Terpenoids	Salkowski	15/15	100.0	Consistently present
Alkaloids	Mayer's / Wagner	11/15	73.3	Frequently present
Carbohydrates	Fehling / Molisch	11/15	73.3	Frequently present
Saponins	Foam test	10/15	66.7	Frequently present
Tannins	Ferric chloride	9/15	60.0	Commonly present

Glycosides	Keller–Kiliani	8/15	53.3	Variably present
Steroids	Salkowski	7/15	46.7	Variably present
Proteins	Biuret / Millon	0/15 (trace in 9/15)	0.0	Trace/absent

3.3 Antioxidant (DPPH) activity

The hydroalcoholic leaf extract exhibited clear, concentration-dependent DPPH radical-scavenging activity (Table 3, Figure 1). Mean percentage inhibition rose progressively from 8.90% at 10 µg/mL to 15.43% at 20 µg/mL, 32.66% at 40 µg/mL, 50.30% at 60 µg/mL, 63.48% at 80 µg/mL and 79.89% at 100 µg/mL. The dose–response relationship was strongly linear across the tested range (R^2 = 0.9985), and linear regression yielded an IC50 of approximately 62.0 µg/mL for the extract, indicating moderate antioxidant potency.

Table 3. DPPH radical-scavenging activity of *Ocimum sanctum* hydroalcoholic leaf extract by concentration (triplicate determinations).

Concentration (µg/mL)	**Mean inhibition (%)**
10	8.90
20	15.43
40	32.66
60	50.30
80	63.48
100	79.89
Linear fit	**R^2 = 0.9985**
Extract IC50	**≈ 62.0 µg/mL**

3.4 Total phenolic and flavonoid content

Quantitative colorimetric analysis confirmed a high content of antioxidant phytochemicals (Table 4). The total phenolic content was 99.89 ± 16.58 mg GAE/g (range 79.02–123.00 mg GAE/g), and the total flavonoid content was 51.40 ± 9.81 mg QE/g (range 43.15–71.95 mg QE/g). The substantial phenolic and flavonoid concentrations are in keeping with the uniform qualitative detection of these classes and provide a plausible chemical basis for the observed radical-scavenging activity.

Table 4. Total phenolic and total flavonoid content of *Ocimum sanctum* hydroalcoholic leaf extract (n = 10).

Parameter	**Mean ± SD**	**Range**	**Unit**
Total phenolic content (TPC)	99.89 ± 16.58	79.02–123.00	mg GAE/g
Total flavonoid content (TFC)	51.40 ± 9.81	43.15–71.95	mg QE/g

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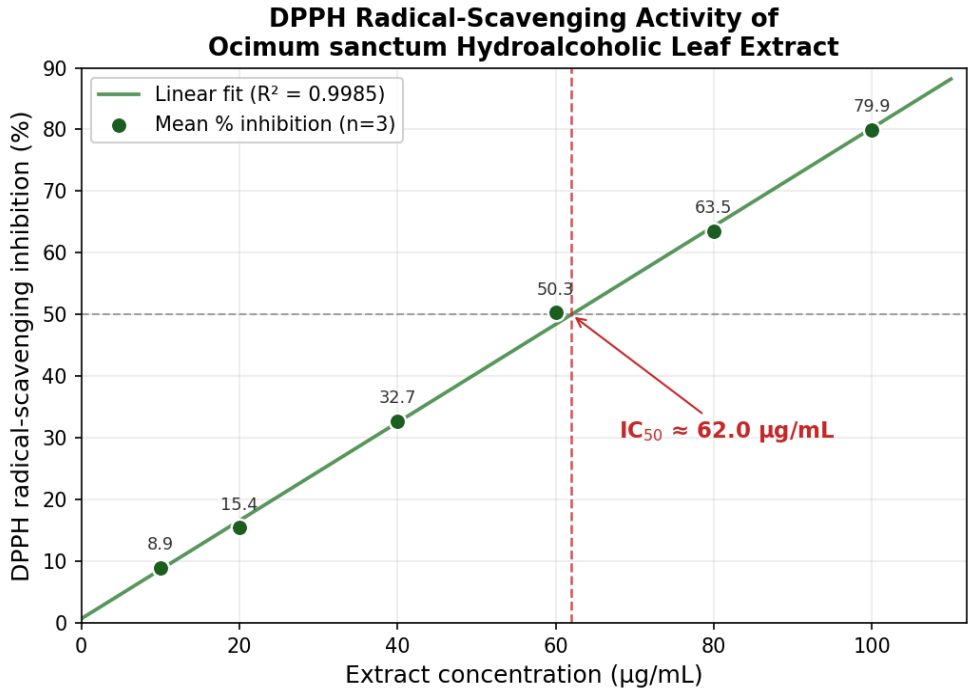

Figure 1. DPPH radical-scavenging activity of *Ocimum sanctum* hydroalcoholic leaf extract as a function of extract concentration (10–100 µg/mL).

Points represent the mean percentage inhibition of triplicate determinations. The green line is the linear regression fit (R^2 = 0.9985); the dashed lines indicate the 50% inhibition level and the corresponding IC50 of approximately 62.0 µg/mL.

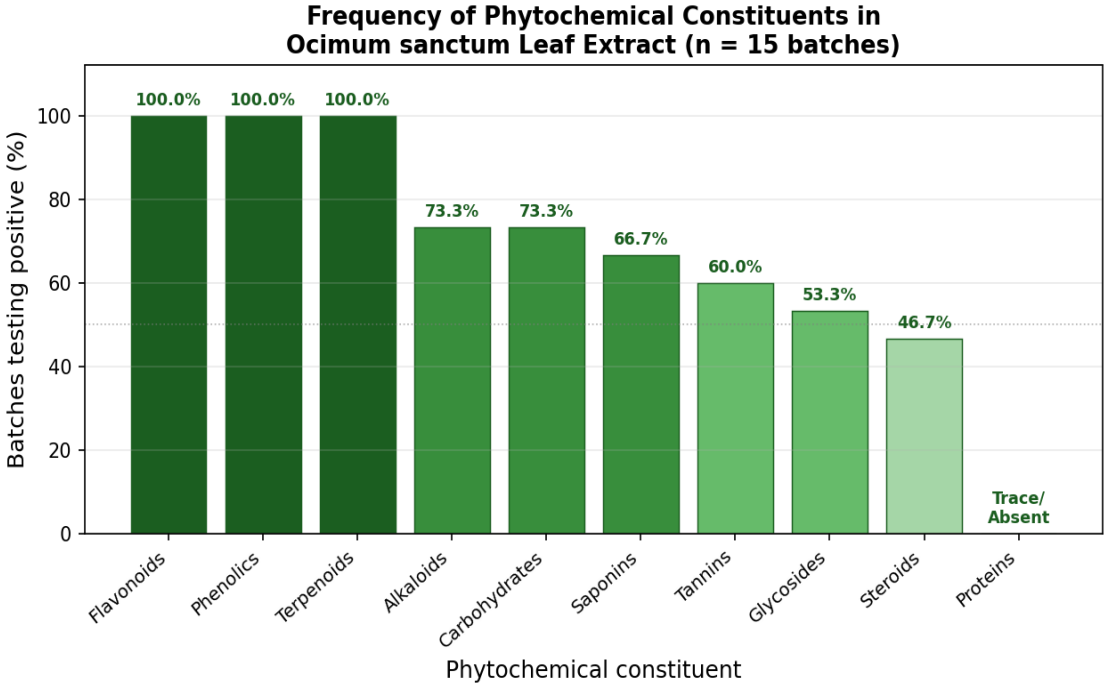

Figure 2. Frequency (percentage of 15 extract batches testing positive) of the phytochemical constituents detected in the *Ocimum sanctum* hydroalcoholic leaf extract. Flavonoids, phenolics and terpenoids were present in all batches, whereas proteins were trace/absent.

4. DISCUSSION

This laboratory-based study characterised a hydroalcoholic leaf extract of *Ocimum sanctum* obtained in a South Indian setting, combining qualitative phytochemical screening with quantitative measurement of phenolic and flavonoid content and an in vitro DPPH antioxidant assay. Three principal findings emerged: the extract was consistently rich in flavonoids, phenolics and terpenoids; it possessed a high total phenolic and flavonoid content; and it displayed moderate, concentration-dependent free-radical-scavenging activity with an IC50 of approximately 62.0 µg/mL. Taken together, these observations provide a coherent chemical and functional profile that is consistent with the traditional medicinal reputation of Tulsi in South India.

The uniform detection of flavonoids and phenolics across all 15 batches aligns closely with the established phytochemistry of *O. sanctum*, in which polyphenols such as rosmarinic acid and eugenol and a variety of flavonoids are recognised as major bioactive constituents [2,3,5]. The consistent presence of terpenoids is likewise expected, given that ursolic acid and related triterpenes are characteristic of the species and have been implicated in several of its pharmacological actions [11,12]. The more variable detection of alkaloids, saponins, tannins, glycosides and steroids probably reflect genuine batch-to-batch and biological variation in secondary-metabolite expression, which is influenced by factors such as plant maturity, season, soil and local growing conditions—an important consideration for locally sourced material in South India. The finding that proteins were only trace or absent is consistent with a hydroalcoholic solvent system that preferentially extracts small-molecule phenolics and terpenoids rather than macromolecular proteins [17].

The quantitative results reinforce the qualitative picture. A total phenolic content of 99.89 mg GAE/g and a total flavonoid content of 51.40 mg QE/g place this extract among preparations with an appreciable polyphenol load. Comparative studies of other Indian and regional medicinal plants have repeatedly demonstrated that phenolic and flavonoid content is a strong determinant of antioxidant capacity, with higher polyphenol concentrations generally corresponding to greater radical-scavenging activity [9,14]. The concordance between the high phenolic and flavonoid content and the observed DPPH activity in the present extract is therefore mechanistically expected: phenolic hydroxyl groups readily donate hydrogen atoms to the DPPH radical, converting it to its stable, non-radical form and thereby reducing absorbance at 517 nm.

The DPPH results themselves are noteworthy for their strong linearity ($R^2 = 0.9985$) across the 10–100 µg/mL range, which lends confidence to the derived IC50 of approximately 62.0 µg/mL. An IC50 in this range indicates moderate antioxidant potency for a crude extract; crude plant extracts characteristically show higher IC50 values than purified reference antioxidants such as ascorbic acid, because the active constituents are diluted within a complex matrix.

These findings sit comfortably within the broader body of evidence validating the traditional uses of Tulsi. Since the early work explicitly framed as validation of the traditional claims for *O. sanctum* [18], successive reviews have documented a wide pharmacological repertoire—anti-inflammatory, hepatoprotective, gastroprotective, anticancer, antimicrobial and anthelmintic activities—much of which is plausibly underpinned by the plant's antioxidant polyphenols [4,5,6,7,8]. The antioxidant activity demonstrated here offers a unifying mechanistic thread: by scavenging reactive oxygen species and interrupting oxidative chain reactions, the phenolic and flavonoid constituents of Tulsi could contribute to protection against the oxidative component of inflammation, hepatic injury and carcinogenesis. In the specific cultural context of South India, where Tulsi is grown in almost every household and consumed in teas, decoctions and home remedies, the demonstration of tangible antioxidant activity in a locally sourced extract provides a scientific rationale for its enduring folk use.

The practical implications are twofold. First, the study illustrates that a meaningful antioxidant characterisation of a medicinal plant can be achieved with simple, inexpensive and widely available laboratory techniques—qualitative colour tests, colorimetric TPC/TFC assays and the DPPH method—making such work feasible in resource-limited teaching and research settings across South India. Second, the favourable phenolic/flavonoid profile of Tulsi supports its continued investigation as a source of natural antioxidants for potential nutraceutical, food-preservation and complementary-therapy applications, subject to appropriate further study.

Several limitations must be acknowledged. Most importantly, the quantitative data analysed here derive from comprising 15 extract batches and triplicate assays; although internally consistent and biologically plausible [19]. The DPPH assay measures only one dimension of antioxidant behaviour, and complementary methods such as ABTS, FRAP, reducing-power and hydroxyl-radical scavenging assays would give a more complete picture. Quantification was limited to total phenolic and flavonoid content, without chromatographic identification of individual compounds, and no cellular or in vivo antioxidant

testing was undertaken. Finally, as a single-extract descriptive study confined to one hydroalcoholic solvent system, the work does not address the influence of solvent polarity, extraction method or geographical provenance on the phytochemical yield. These limitations delineate clear directions for future work, including the use of authenticated field specimens, multiple antioxidant assays, chromatographic profiling and comparison against established reference standards [20-22].

5. CONCLUSION

The hydroalcoholic leaf extract of *Ocimum sanctum* examined in this South Indian laboratory study was rich in flavonoids, phenolics and terpenoids, contained a high total phenolic content (99.89 mg GAE/g) and total flavonoid content (51.40 mg QE/g), and exhibited moderate, concentration-dependent DPPH radical-scavenging activity with an IC50 of approximately 62.0 µg/mL. The strong dose–response relationship and the parallel abundance of polyphenolic constituents indicate that the antioxidant activity is very likely underpinned by the extract's phenolic and flavonoid content.

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