ISSN: 3116-3475
International Journal
of Education, Research, and Innovation Perspectives
Volume 2 Issue 7 (July 2026)

Bioactive Compounds from *Garcinia binucao* Fruit Pulp: Evaluation of Antioxidant and Anti-inflammatory Properties

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Date Submitted:
March 20, 2026

Date Accepted:
May 19, 2026

Date Published:
July 22, 2026

DOI:
10.5281/zenodo.21503080

ABSTRACT

This experimental laboratory study evaluated the phytochemical constituents, antioxidant radical-scavenging activity, and anti-inflammatory activity of Garcinia binucao fruit pulp extract. Fresh fruit pulp was air-dried, macerated in 95% methanol for 48 hours, filtered, and concentrated by rotary evaporation. Qualitative phytochemical screening was conducted for flavonoids, phenols and tannins, saponins, alkaloids, and carbohydrates. Antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay at three extract concentrations (100%, 50%, and 25%), with pineapple juice and methanol as positive and negative controls, respectively. Anti-inflammatory activity was assessed through inhibition of heat-induced egg-albumin denaturation, using diclofenac as the positive control. Absorbance was measured by UV-Vis spectrophotometry in triplicate, and treatment differences were examined through one-way analysis of variance. Garcinia binucao tested positive for all screened phytochemical groups, whereas pineapple juice was positive only for saponins and alkaloids and methanol was negative for all groups. Reported DPPH inhibition increased from 70.3% at 100% extract to 76.9% at 50% and 83.3% at 25%, approaching the 85.4% inhibition of pineapple juice; treatment differences were significant ($p = .01$). Protein-denaturation inhibition was 9.30%, 2.51%, and 11.22% for the 100%, 50%, and 25% treatments, respectively, compared with 18.27% for diclofenac, with a significant treatment effect ($p = .001$). The findings indicate strong in vitro antioxidant activity but comparatively modest anti-inflammatory activity under the tested conditions. Garcinia binucao fruit pulp is a promising indigenous source of bioactive compounds, although standardized dose-response studies, compound characterization, and complementary biological assays are required before therapeutic claims can be established.

Keywords: *Garcinia binucao, phytochemical screening, DPPH assay, antioxidant activity, protein denaturation assay, anti-inflammatory activity*

INTRODUCTION

Oxidative stress develops when the production of reactive oxygen species exceeds the capacity of endogenous antioxidant defenses. Prolonged imbalance can damage lipids, proteins, and nucleic acids and can interact with inflammatory pathways involved in chronic disease development (Jomova et al., 2023; Zhang & Yang, 2024). Although synthetic antioxidants and anti-inflammatory drugs are widely used, concerns about

adverse effects and long-term exposure have encouraged the investigation of plant-derived compounds as alternative sources of protective bioactivity.

Bioactive compounds are naturally occurring constituents that can influence physiological processes beyond basic nutrition. Plant phenolics, flavonoids, tannins, saponins, and alkaloids have been associated with free-radical scavenging, membrane interactions, and modulation of biological pathways (Kurek, 2019; Makhaik et al., 2021; Martirosyan & Miller, 2018). Indigenous fruits are especially relevant because they may contain underutilized phytochemicals while also supporting local food systems and value-added product development.

Garcinia binucao, locally known as batuan or binukaw, is an indigenous sour fruit used in Philippine cuisine. Studies of Garcinia species have described medicinally relevant xanthones, benzophenones, biflavonoids, and other secondary metabolites with antioxidant and anti-inflammatory potential (Mariod, 2019; Santo et al., 2020). Screening of Philippine indigenous fruits has also identified *Garcinia binucao* as a notable source of phenolic compounds and antioxidant activity (Recuenco et al., 2020).

Despite these indications, the fruit pulp remains less studied than other Garcinia species and plant parts. More localized evidence is needed regarding which phytochemical groups are detectable in the pulp and how its extract performs in accessible in vitro assays. The DPPH method provides a rapid estimate of free-radical scavenging, while protein-denaturation assays offer a preliminary model for anti-inflammatory activity. Together, these procedures can establish an initial bioactivity profile for subsequent analytical and pharmacological work.

This study evaluated the phytochemical compounds present in *Garcinia binucao* fruit pulp extract, measured its antioxidant scavenging activity at different concentrations through the DPPH assay, assessed its anti-inflammatory activity through inhibition of protein denaturation, and tested whether treatment groups differed significantly. The study was intended to provide preliminary evidence for the potential development of the fruit as a natural functional-food or nutraceutical resource, while recognizing that in vitro activity alone does not establish clinical efficacy.

Literature Review

Oxidative Stress, Antioxidants, and Inflammation

Reactive oxygen species are generated through normal metabolism and environmental exposures. At controlled levels, they participate in signaling; at excessive levels, they contribute to oxidative injury and interact with inflammatory processes (Jomova et al., 2023). Antioxidants counter these reactions by donating electrons, chelating pro-oxidant metals, or interrupting radical-chain reactions. The biological value of an antioxidant source therefore depends on the nature, concentration, stability, and bioavailability of its active constituents.

Inflammation is likewise protective when acute but can become damaging when persistent. Oxidative stress and inflammation can reinforce each other through redox-sensitive signaling and cellular injury. Plant compounds with both radical-scavenging and anti-inflammatory properties are consequently of interest for food, pharmaceutical, and preventive-health research (Zhang & Yang, 2024). However, preliminary chemical assays must be interpreted as screening evidence rather than direct proof of therapeutic action.

Bioactive Constituents of Garcinia and Indigenous Fruits

Garcinia species contain diverse secondary metabolites, including xanthones, benzophenones, biflavonoids, and organic acids. These constituents have been examined for antioxidant, antimicrobial, anticancer, and anti-inflammatory actions (Mariod, 2019; Santo et al., 2020). The phytochemical profile varies according to species, plant part, maturity, geography, extraction solvent, and laboratory procedure, making species- and tissue-specific investigation necessary.

In Philippine indigenous fruits, Recuenco et al. (2020) documented meaningful phenolic content and antioxidant or antibacterial activity across several locally available species. Such findings support the scientific evaluation of native fruits that are traditionally consumed but remain underutilized in value-added products.

ISSN: 3116-3475
International Journal
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Garcinia binucao fruit pulp is relevant because it is locally accessible, culturally familiar, and potentially rich in compounds that can contribute to radical scavenging.

Individual phytochemical classes may contribute through different mechanisms. Phenols and tannins possess hydroxyl groups capable of electron or hydrogen donation; flavonoids comprise a broad group of polyphenols with redox and signaling effects; saponins have surfactant and membrane-interactive properties; and alkaloids include structurally diverse nitrogen-containing compounds with multiple pharmacological activities (Ciriminna et al., 2025; Kurek, 2019; Rai et al., 2021). Qualitative screening cannot quantify these compounds, but it establishes whether major classes are detectable and guides more precise analytical testing.

Phytochemical Screening and DPPH Radical-Scavenging Assay

Qualitative phytochemical screening relies on characteristic color changes, precipitates, rings, or foam formation following reaction with specific reagents. Although these tests are useful for rapid preliminary assessment, their interpretation depends on reagent quality, sample color, concentration, and observer judgment (Shakya, 2025). Consequently, positive findings should ideally be followed by quantitative total-phenolic or total-flavonoid assays and instrumental identification.

The DPPH assay measures the decrease in absorbance of a stable purple radical after reaction with an antioxidant. A reduction in absorbance indicates radical neutralization and is commonly expressed as percentage inhibition or, across a true concentration series, as an IC50 value (Gülçin & Alwasel, 2023; Martínez-Morales et al., 2020). Standardized reporting is essential because solvent systems, incubation time, wavelength, and concentration units can substantially affect comparisons across studies.

UV-Vis spectrophotometry is widely used in these assays because it enables reproducible measurement of light absorption at selected wavelengths (Hung, 2023). DPPH mixtures are commonly protected from light and incubated before measurement, as exposure and reaction time can influence the endpoint (Sharma, 2024). Replication and appropriate positive and negative controls are required to distinguish extract activity from solvent or matrix effects.

Protein Denaturation as a Preliminary Anti-inflammatory Model

Protein denaturation is associated with the loss of native protein structure and has been used as an in vitro screening model for anti-inflammatory activity. In the assay, a test substance is evaluated for its capacity to reduce heat-induced denaturation of albumin relative to a control. A standard anti-inflammatory drug provides a benchmark, while a negative control establishes the baseline response.

The assay is inexpensive and suitable for preliminary comparison of plant extracts, but it represents only one mechanism associated with inflammation. It does not directly measure cyclooxygenase inhibition, cytokine regulation, cellular toxicity, absorption, or metabolism. Therefore, promising results should be confirmed through complementary biochemical, cell-based, and eventually in vivo procedures using standardized extracts and ethical safeguards.

ISSN: 3116-3475
International Journal
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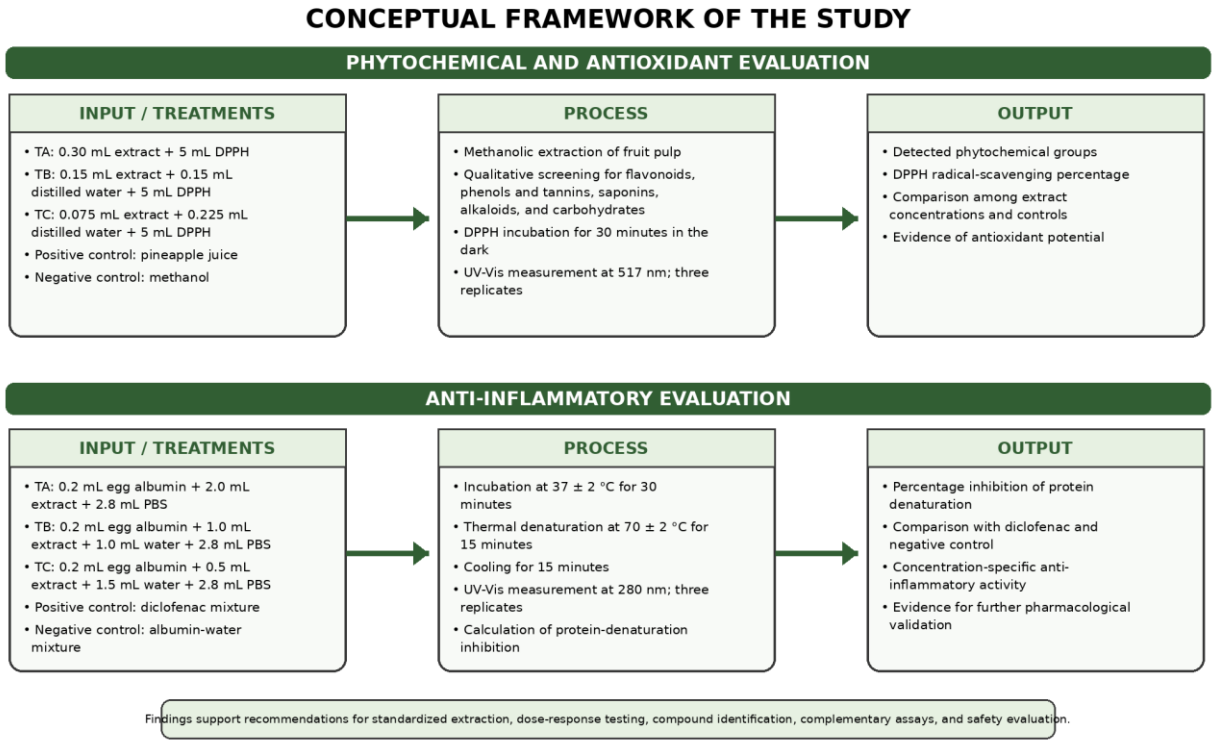

Figure 1. *Conceptual framework of the phytochemical, antioxidant, and anti-inflammatory evaluations*

METHODS

Research Design and Laboratory Setting

The study employed an experimental laboratory design to characterize the qualitative phytochemical profile and compare the in vitro antioxidant and anti-inflammatory activities of *Garcinia binucao* fruit pulp extract across three treatment concentrations and relevant controls. Fruit preparation and extraction were conducted in Passi City, Iloilo, while phytochemical screening and DPPH measurements were performed at the Gregor Mendel Laboratory of the University of San Agustin, Iloilo. The anti-inflammatory procedure used the same prepared extract and UV-Vis spectrophotometric analysis.

Plant Material, Authentication, and Extraction

Fresh *Garcinia binucao* fruits were collected from Barangay Poblacion Ilawod, Passi City, Iloilo. Plant identity was verified through the City Agriculture Office in Barangay Sablogon, Passi City. The fruits were washed under running water for 20 minutes, and the pulp was separated from the seeds and cut into small pieces. The pulp was air-dried for six hours, transferred to a clean container, and soaked in 1 L of 95% methanol for 48 hours. The mixture was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 45 °C under reduced pressure to remove the solvent.

Qualitative Phytochemical Screening

Aliquots of the *Garcinia binucao* extract, pineapple juice, and methanol were screened for major phytochemical groups. Phenols and tannins were tested using ferric chloride; flavonoids using sodium hydroxide followed by dilute hydrochloric acid; saponins using the foam test; alkaloids using hydrochloric acid and Hager's reagent; and carbohydrates using Molisch's reagent and concentrated sulfuric acid. Color formation, precipitate,

ISSN: 3116-3475
International Journal
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turbidity, foam stability, or interface-ring formation was recorded and compared with the expected qualitative reactions (Shakya, 2025).

DPPH Radical-Scavenging Assay

Three extract treatments were prepared: TA contained 0.30 mL extract, TB contained 0.15 mL extract plus 0.15 mL distilled water, and TC contained 0.075 mL extract plus 0.225 mL distilled water. Each treatment was combined with 5 mL DPPH solution. Pineapple juice served as the positive control and methanol as the negative control. The mixtures were protected from light with aluminum foil and incubated at room temperature for 30 minutes. Three replicates per treatment were transferred to a microplate, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Percentage inhibition was calculated as [(A0 - A1)/A0] × 100, where A0 was the negative-control absorbance and A1 was the sample absorbance (Gülçin & Alwasel, 2023; Sharma, 2024).

Protein-Denaturation Assay

A 1% egg-albumin solution was prepared by dispersing 1 mL albumin in 99 mL cold distilled water. TA contained 0.2 mL albumin, 2.0 mL undiluted extract, and 2.8 mL phosphate-buffered saline (PBS). TB contained 0.2 mL albumin, 1.0 mL extract, 1.0 mL distilled water, and 2.8 mL PBS. TC contained 0.2 mL albumin, 0.5 mL extract, 1.5 mL distilled water, and 2.8 mL PBS. The positive control contained albumin, diclofenac solution, and PBS, while the negative control contained albumin, distilled water, and PBS. Each group was prepared in triplicate.

The mixtures were incubated at 37 ± 2 °C for 30 minutes and then heated at 70 ± 2 °C for 15 minutes to induce denaturation. After cooling at ambient temperature for 15 minutes, absorbance was measured at 280 nm, using distilled water as the blank. Percentage inhibition of protein denaturation was determined relative to the control response.

Data Analysis

Qualitative screening results were summarized as present or absent. Antioxidant and anti-inflammatory outcomes were reported using treatment-level absorbance summaries, percentage inhibition, and one-way analysis of variance. Statistical significance was evaluated at the .05 level. The source study also used letter-based treatment groupings to describe pairwise differences.

Safety and Ethical Considerations

The study did not involve human participants or live-animal experimentation. Plant identity was documented before extraction. Laboratory personnel used protective equipment and followed chemical-hygiene procedures. Residual plant material and chemical waste were segregated into labeled biodegradable and hazardous-waste containers, and glassware and equipment were cleaned to prevent cross-contamination. Further animal or clinical testing would require appropriate institutional ethics approval.

Table 1. *Experimental treatments and controls*

Assay	Group	Composition / concentration	Role
DPPH	TA	0.30 mL extract + 5 mL DPPH	100% extract treatment
DPPH	TB	0.15 mL extract + 0.15 mL water + 5 mL DPPH	50% extract treatment
DPPH	TC	0.075 mL extract + 0.225 mL water + 5 mL DPPH	25% extract treatment
DPPH	Positive control	Pineapple juice + DPPH	Reference antioxidant matrix
DPPH	Negative control	Methanol + DPPH	Baseline control
Protein denaturation	TA	0.2 mL albumin + 2.0 mL extract + 2.8 mL PBS	100% extract treatment

ISSN: 3116-3475
International Journal
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Protein denaturation	TB	0.2 mL albumin + 1.0 mL extract + 1.0 mL water + 2.8 mL PBS	50% extract treatment
Protein denaturation	TC	0.2 mL albumin + 0.5 mL extract + 1.5 mL water + 2.8 mL PBS	25% extract treatment
Protein denaturation	Positive control	0.2 mL albumin + 2.0 mL diclofenac + 2.8 mL PBS	Standard drug control
Protein denaturation	Negative control	0.2 mL albumin + 2.0 mL water + 2.8 mL PBS	Baseline control

RESULTS AND DISCUSSION

Qualitative Phytochemical Profile

Table 2. *Phytochemical compounds detected in the test samples*

Phytochemical group	*Garcinia binucao* extract	Pineapple juice	Methanol
Flavonoids	Positive (+)	Negative (-)	Negative (-)
Phenols and tannins	Positive (+)	Negative (-)	Negative (-)
Saponins	Positive (+)	Positive (+)	Negative (-)
Alkaloids	Positive (+)	Positive (+)	Negative (-)
Carbohydrates	Positive (+)	Negative (-)	Negative (-)

The *Garcinia binucao* extract tested positive for all five screened phytochemical groups: flavonoids, phenols and tannins, saponins, alkaloids, and carbohydrates. Pineapple juice was positive only for saponins and alkaloids, while methanol was negative across all tests. The negative solvent response supports the interpretation that the observed reactions arose from constituents of the biological samples rather than from the extraction solvent.

The broad qualitative profile is consistent with the medicinal potential reported for Garcinia species and with earlier screening of Philippine indigenous fruits (Recuenco et al., 2020; Santo et al., 2020). Phenolic and flavonoid constituents are especially relevant to antioxidant activity because their structures can facilitate electron or hydrogen donation. Saponins and alkaloids may also contribute to biological responses through membrane-related and signaling mechanisms (Kurek, 2019; Rai et al., 2021). Because the tests were qualitative, the results establish presence but not concentration, purity, or the contribution of each class to the measured bioactivity.

Antioxidant Radical-Scavenging Activity

Table 3. *Reported DPPH radical-scavenging activity*

Treatment	Extract level	DPPH inhibition (%)	Comparative result
TA	100%	70.3	Active
TB	50%	76.9	Active
TC	25%	83.3	Highest extract treatment
Positive control	Pineapple juice	85.4	Highest overall
Negative control	Methanol	Baseline	No expected scavenging activity

Note. Values are the treatment-level percentage inhibitions. The one-way ANOVA result was p = .01, indicating a significant difference among groups.

All three extract treatments exhibited substantial DPPH radical-scavenging activity. Inhibition increased from 70.3% in TA to 76.9% in TB and 83.3% in TC. The 25% treatment approached the pineapple-juice control at 85.4%. The one-way ANOVA indicated a significant treatment effect (p = .01), leading to rejection of the null hypothesis for antioxidant activity.

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International Journal
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The antioxidant result is compatible with the detection of phenols, tannins, and flavonoids and with previous reports of antioxidant constituents in Garcinia species and Philippine indigenous fruits (Recuenco et al., 2020; Santo et al., 2020; Tomer & Gupta, 2025). Nevertheless, the inverse pattern in which the more diluted treatment produced greater percentage inhibition warrants careful confirmation. Matrix color, solvent composition, reaction kinetics, pipetting, or concentration labeling can influence DPPH absorbance. Future dose-response work should express extract concentrations in mass-per-volume units and calculate IC50 only from a validated multi-point curve, consistent with standardized interpretation recommendations (Martínez-Morales et al., 2020).

Anti-inflammatory Activity through Protein-Denaturation Inhibition

Table 4. *Reported inhibition of heat-induced protein denaturation*

Treatment	Extract level	Inhibition (%)	Interpretation relative to control
TA	100%	9.30	Modest activity
TB	50%	2.51	Lowest extract activity
TC	25%	11.22	Highest extract activity
Positive control	Diclofenac	18.27	Highest overall

Note. The one-way ANOVA result was p = .001, indicating a significant difference among treatment groups.

The extract produced measurable but comparatively modest inhibition of protein denaturation. TC recorded the highest extract activity at 11.22%, followed by TA at 9.30% and TB at 2.51%. Diclofenac produced the greatest inhibition at 18.27%. The treatment effect was statistically significant (p = .001), indicating that the observed differences were unlikely to be attributable to sampling variation alone under the study conditions.

The anti-inflammatory pattern partially parallels the DPPH result because the 25% treatment again showed the highest activity among the extract groups. However, all extract values remained below the standard drug control and below the levels generally regarded as strong inhibition in preliminary screening. The findings should therefore be interpreted as evidence of possible anti-inflammatory constituents rather than proof of therapeutic efficacy. Compound quantification, assay replication, cell-viability testing, and complementary inflammatory targets are necessary to determine whether the observed effect is reproducible and biologically meaningful.

Integrated Interpretation and Study Contribution

Taken together, the results show that *Garcinia binucao* fruit pulp contains a broad range of detectable phytochemicals and exhibits stronger antioxidant than anti-inflammatory activity in the selected in vitro models. The antioxidant response supports the use of the fruit as a candidate for further functional-food and natural-product research. The anti-inflammatory response was weaker but still provides a basis for more rigorous testing because multiple phytochemical groups associated with bioactivity were present.

The study contributes preliminary laboratory evidence on an indigenous Philippine fruit that is more commonly valued as a culinary souring agent. It also demonstrates the feasibility of combining qualitative screening with spectrophotometric assays in a local research setting. Its limitations include qualitative rather than quantitative phytochemical analysis, a narrow concentration range, potential treatment-label or matrix effects, reliance on single antioxidant and anti-inflammatory assays, and the absence of toxicity and compound-identification procedures. These limitations define the next steps required before product-development or health claims are considered.

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CONCLUSION

Garcinia binucao fruit pulp extract contained detectable flavonoids, phenols and tannins, saponins, alkaloids, and carbohydrates. The extract demonstrated high DPPH radical-scavenging percentages at all tested concentrations, with the 25% treatment producing the strongest extract response and approaching the pineapple-juice control. Antioxidant activity differed significantly among treatments.

The extract also inhibited heat-induced protein denaturation, but the magnitude of inhibition was modest compared with diclofenac. The 25% treatment again produced the highest extract response, and treatment differences were statistically significant. The study therefore concludes that *Garcinia binucao* fruit pulp is a promising source of bioactive constituents with clear in vitro antioxidant potential and preliminary, weaker anti-inflammatory activity. The evidence supports further laboratory investigation but does not yet justify therapeutic or clinical claims.

Recommendation

Future studies should standardize the extract using dry-extract yield and concentration in mg/mL, use analytical-grade solvent and controlled extraction conditions, and increase the number of biological and technical replicates. A broader dose-response series should be tested with recognized antioxidant standards such as Trolox or ascorbic acid, and IC50 values should be calculated only from a properly fitted concentration-response curve.

The phytochemical findings should be extended through quantitative total-phenolic and total-flavonoid assays and through compound identification using chromatographic or mass-spectrometric methods. Antioxidant activity should be confirmed with complementary assays such as ABTS or FRAP, while anti-inflammatory potential should be evaluated using additional biochemical or cell-based models. Toxicity and safety testing must precede any food, nutraceutical, animal, or clinical application, and all in vivo work should undergo appropriate ethical review.

Local researchers, schools, agricultural offices, and higher education laboratories may collaborate to document the seasonal availability, cultivation, postharvest handling, and sustainable use of *Garcinia binucao*. Such collaboration can strengthen the scientific basis for conserving and adding value to indigenous fruit resources while avoiding premature medicinal claims.

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