Jurnal Jamu Indonesia (2026) 11(4): 298–306
https://jamu-journal.ipb.ac.id/index.php/JJI/index

ISSN 2407-7178
eISSN 2407-7763

Research Article
Doi: https://doi.org/10.29244/jji.v11i4.454

Optimization of a Three-Extract Polyherbal Antioxidant Formulation Using Simplex Lattice Design

Optimasi Formulasi Antioksidan Poliherbal Tiga Ekstrak Menggunakan *Simplex Lattice Design*

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ARTICLE INFO

Article history
Received on: 2025-10-20
Revised on: 2026-01-07
Accepted on: 2026-07-17

Keyword:
Antioxidant activity;
Caesalpinia sappan;
Mixture design;
Polyherbal formulation;
Simplex lattice design

ABSTRACT

Polyherbal formulations have attracted increasing interest because combining plant extracts may improve phytochemical composition and antioxidant performance. This study aimed to optimize the proportions of ethanolic extracts of *Cinnamomum burmannii* bark, *Gyrinops versteegii* leaves, and *Caesalpinia sappan* wood using a simplex lattice design. Ten formulations were generated and evaluated for total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and DPPH radical-scavenging activity (IC_{50}). The optimized formulation contained 10.00% *C. burmannii*, 21.51% *G. versteegii*, and 68.49% *C. sappan*, with experimental values of 79.27 mg GAE/g TPC, 197.64 mg QE/g TFC, 102.08 mg TAE/g TTC, and an IC_{50} of 24.78 µg/mL. Experimental verification closely agreed with model predictions, with an average relative deviation of 2.95%. These findings demonstrate that simplex lattice design is an effective approach for optimizing polyherbal antioxidant formulations based on integrated phytochemical characteristics and antioxidant activity.

Kata kunci:

Aktivitas antioksidan;
Caesalpinia sappan;
Desain campuran;
Formulasi poliherbal;
Simplex lattice design

ABSTRAK

Formulasi poliherbal semakin banyak dikembangkan karena penggabungan beberapa ekstrak tumbuhan berpotensi meningkatkan komposisi fitokimia dan aktivitas antioksidan. Penelitian ini bertujuan mengoptimalkan proporsi ekstrak etanol kulit batang *Cinnamomum burmannii*, daun *Gyrinops versteegii*, dan kayu *Caesalpinia sappan* menggunakan metode *simplex lattice design*. Sepuluh formulasi dievaluasi berdasarkan kadar fenolik total (TPC), flavonoid total (TFC), tanin total (TTC), dan aktivitas penangkapan radikal DPPH (IC_{50}). Formulasi optimum terdiri atas 10,00% *C. burmannii*, 21,51% *G. versteegii*, dan 68,49% *C. sappan*, dengan nilai TPC 79,27 mg GAE/g, TFC 197,64 mg QE/g, TTC 102,08 mg TAE/g, dan IC_{50} sebesar 24,78 µg/mL. Hasil verifikasi menunjukkan kesesuaian yang baik antara nilai prediksi dan hasil eksperimen dengan rerata deviasi relatif sebesar 2,95%. Temuan ini menunjukkan bahwa *simplex lattice design* merupakan pendekatan yang efektif untuk mengoptimalkan formulasi antioksidan poliherbal berdasarkan karakteristik fitokimia dan aktivitas antioksidan.

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Citation: Widhiartini, I. A. A., Ugrasena, P. Y., Widianti, I. G. A., Parwata, I. M. O. A., & Wirasuta, I. M. A. G. (2026). Optimization of a Three-Extract Polyherbal Antioxidant Formulation Using Simplex Lattice Design. *Jurnal Jamu Indonesia, 11*(4), 298–306. https://doi.org/10.29244/jji.v11i4.454

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1. INTRODUCTION

Oxidative stress arises when the production of reactive oxygen species exceeds endogenous antioxidant capacity, leading to molecular and cellular damage. Persistent oxidative stress is associated with the development and progression of metabolic, cardiovascular, neurodegenerative, and other chronic disorders. This has encouraged continued investigation of plant-derived antioxidants, particularly phenolic compounds that can donate electrons or hydrogen atoms and stabilise reactive species. However, the antioxidant performance of botanical preparations depends not only on the presence of individual constituents but also on their concentration, relative proportion, and interactions within complex extracts (Nikolaidis et al., 2012; Olszowy-Tomczyk, 2020; Yamauchi et al., 2024).

Cinnamomum burmannii (Nees & T. Nees) Blume, *Gyrinops versteegii* (Gilg) Domke, and *Caesalpinia sappan* L. were selected because they provide distinct but potentially complementary phytochemical profiles. *C. burmannii* bark contains phenolic compounds, flavonoids, tannins, catechins, and proanthocyanidins associated with antioxidant activity (Bahtiarsyah et al., 2023; Budiastuti et al., 2020). Phenolic and resin-associated constituents have also been reported in *G. versteegii* and related agarwood-producing species (Fadzil et al., 2024; Hashim et al., 2014; Wang et al., 2018), whereas *C. sappan* wood is a well-established source of phenolic compounds, particularly brazilin, brazilein, and related constituents (Pattananandecha et al., 2022; Vij et al., 2023; Wan et al., 2019). These compositional differences provide a reasonable basis for evaluating the three extracts as a combined formulation rather than as isolated botanical materials.

Although the antioxidant properties of these species have been investigated individually, the effect of combining their extracts at different proportions remains insufficiently characterised. A formulation with a high total phytochemical content may not necessarily exhibit the strongest radical-scavenging activity because the response can also be influenced by chemical structure, concentration, and composition-dependent interactions. Conventional one-factor-at-a-time experiments are therefore inadequate for optimising a multicomponent formulation in which the proportion of one extract necessarily affects the proportions of the others. Mixture-design methods provide a more appropriate approach because they evaluate the contribution of each component while maintaining a constant total mixture and allow nonlinear composition effects to be modelled (Akmal et al., 2023).

Accordingly, this study aimed to determine the effects of the relative proportions of *C. burmannii*, *G. versteegii*, and *C. sappan* ethanolic extracts on total phenolic, flavonoid, and tannin contents and DPPH radical-scavenging activity. A simplex lattice mixture design was used to model the responses, identify an optimised polyherbal formulation through numerical desirability, and verify the agreement between predicted and experimental values.

2. METHODS

2.1. Experimental Design

This laboratory study used a simplex lattice mixture design to investigate the effects of the relative proportions of three ethanolic extracts on phytochemical content and antioxidant activity. The mixture components were *C. burmannii* bark extract (X_1), *G. versteegii* leaf extract (X_2), and *C. sappan* wood extract (X_3). The measured responses were total phenolic content (TPC; Y_1), DPPH radical-scavenging activity expressed as IC_{50} (Y_2), total flavonoid content (TFC; Y_3), and total tannin content (TTC; Y_4). Formulation design, model fitting, numerical optimization, and response-surface generation were performed using Design-Expert® version 13.0.1 (Stat-Ease Inc., Minneapolis, MN, USA). The overall experimental procedure is summarized in **Figure 1**.

2.2. Plant Materials and Authentication

Dried *C. burmannii* bark and *C. sappan* wood were obtained from local markets in Denpasar, Bali, Indonesia, whereas *G. versteegii* leaves were obtained from a certified agarwood cultivation farm in Marga, Tabanan, Bali, Indonesia. The plant materials were taxonomically authenticated at UPT Laboratorium Herbal Materia Medica Batu, East Java, Indonesia. Authentication certificates were issued under Nos. 000.9.3/2462/102.20/2025 for *C. burmannii*, 000.9.3/2461/102.20/2025 for *C. sappan*, and 000.9.3/3317/102.20/2025 for *G. versteegii*.

2.3. Chemicals and Instruments

Ethanol 70% and chloroform were purchased from Bratachem (Jakarta, Indonesia). Methanol, ethanol, aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), ferric chloride ($FeCl_3$), and Folin–Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). Gallic acid, quercetin, tannic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade. Solvent evaporation was performed using a Büchi® R-100 rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland), and absorbance was measured using a Genesys™ 50 UV–visible spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.4. Preparation of Ethanolic Extracts

Each dried plant material was ground separately into a fine powder and macerated with 70% ethanol at a material-to-solvent ratio of 1:20 (w/v) for 48 h at 25 °C with occasional stirring. The macerates were filtered, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 60 °C. The concentrated extracts were subsequently dried in a dehydrator at 40 °C until constant weight was obtained. Extraction yield was calculated relative to the initial dry weight of the plant material (Marangyana et al., 2022; Sari et al., 2025).

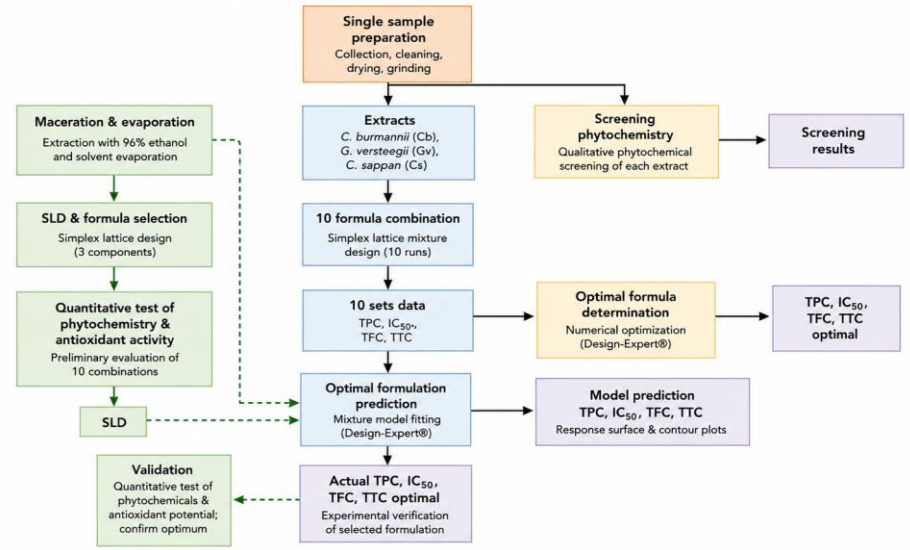

Figure 1. Schematic overview of the experimental workflow, including preparation and authentication of the three plant materials, ethanolic extraction, preliminary phytochemical screening, formulation using a simplex lattice mixture design, determination of total phenolic, flavonoid, and tannin contents, DPPH radical-scavenging assay, mixture-model fitting, numerical optimization, and experimental verification of the selected formulation.

2.5. Preliminary Phytochemical Screening

Each ethanolic extract was qualitatively screened for alkaloids, flavonoids, phenols, tannins, and saponins using colour and precipitation reactions based on the Indonesian Herbal Pharmacopoeia and previously reported procedures (Kementerian Kesehatan Republik Indonesia, 2022; Syahputra, 2024). The results were recorded as present (+) or not detected (−).

2.6. Simplex Lattice Design and Preparation of Polyherbal Formulations

The mixture consisted of *C. burmannii* extract (X_1), *G. versteegii* extract (X_2), and *C. sappan* extract (X_3), with the total proportion constrained to 100% and the proportion of each component restricted to 10%–80%. Design-Expert® generated 10 formulations covering the experimental mixture domain (**Table 1**). Each dried extract was accurately weighed and combined according to the weight-to-weight proportions (% w/w) specified in the mixture design. The resulting extract mixtures were then dissolved in ethanol to prepare stock solutions at a final concentration of 1,000 µg/mL for the determination of total phenolic content (TPC), DPPH radical-scavenging activity expressed as IC_{50}, total flavonoid content (TFC), and total tannin content (TTC).

2.7. Determination of Total Phenolic Content

TPC was determined using the Folin–Ciocalteu assay according to Kupina et al. (2019), with the conditions described by Amelia et al. (2025). Gallic acid standard solutions were prepared in methanol at concentrations of 40–120 µg/mL. One gram of each formulation was dissolved in distilled water and diluted as required. A 1-mL aliquot of the sample or standard solution was mixed with 1 mL of Folin–Ciocalteu reagent and allowed to react for 3 min at room temperature. Subsequently, 10 mL of 7% (w/v) sodium carbonate solution was added. The mixture was incubated in the dark for 90 min, and absorbance was measured at 765 nm. TPC was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Table 1. Simplex lattice mixture design for the three-extract formulations

Run	*C. burmannii* (X_1, %)	*G. versteegii* (X_2, %)	*C. sappan* (X_3, %)
1	80.00	10.00	10.00
2	21.67	21.67	56.67
3	10.00	80.00	10.00
4	10.00	10.00	80.00
5	56.67	21.67	21.67
6	10.00	45.00	45.00
7	45.00	10.00	45.00
8	45.00	45.00	10.00
9	21.67	56.67	21.67
10	33.33	33.33	33.33

Note: $X_1 + X_2 + X_3 = 100\%$.

2.8. Determination of Total Flavonoid Content

TFC was measured using the aluminium chloride colorimetric method based on Lestari et al. (2022) and Matić et al. (2017). Quercetin standard solutions were prepared in ethanol at concentrations of 20–100 µg/mL. Sample solutions were prepared at 10 mg/mL. One millilitre of the sample or standard solution was mixed with 1 mL of 10% $AlCl_3$ and 8 mL of 5% acetic acid. After 30 minutes incubation for the predetermined operating time, absorbance was measured at 437 nm. TFC was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

2.9. Determination of Total Tannin Content

TTC was determined using the Folin–Ciocalteu method described by Haile and Kang (2019). Tannic acid standard solutions were prepared in distilled water at concentrations of 20–100 µg/mL. A 1-mL aliquot of the sample or standard solution was mixed with 1 mL of Folin–Ciocalteu reagent and allowed to stand for 5 min. Two millilitres of 15% (w/v) sodium carbonate solution was then added. The mixture was incubated in the dark for 40 min, and absorbance was measured at 765 nm. TTC was calculated from the tannic acid calibration curve and expressed as milligrams of tannic acid equivalents per gram of extract (mg TAE/g).

2.10. DPPH Radical-Scavenging Assay

DPPH radical-scavenging activity was evaluated according to Baliyan et al. (2022), with minor modifications. Each formulation was prepared at concentrations of 10, 25, 50, 100, and 200 µg/mL. A 1-mL aliquot of each sample solution was mixed with 2 mL of a 40 µg/mL DPPH solution prepared in methanol. The reaction mixture was incubated in the dark at room temperature for 45 min, after which the absorbance was measured at 516 nm.

The percentage of DPPH radical inhibition was calculated as:

$$DPPH\ inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the DPPH solution without sample and A_{sample} is the absorbance of the reaction mixture containing the formulation. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was estimated from the concentration–inhibition curve and expressed as µg/mL.

2.11. Mixture-Model Fitting and Statistical Analysis

The experimental responses were analysed using Design-Expert® version 13.0.1. TPC, IC_{50}, TFC, and TTC were fitted separately to polynomial mixture models. Model selection was based on statistical significance, lack of fit, the coefficient of determination, and overall model adequacy. The effects of the individual mixture components and their interaction terms were evaluated using the fitted regression coefficients and visualised through three-dimensional response-surface plots. Statistical significance was set at $p < 0.05$. All quantitative measurements were performed in triplicate using three independently prepared formulations, and the results are presented as mean values.

2.12. Numerical Optimization and Experimental Verification

Numerical optimization was performed using the desirability function in Design-Expert®. The optimization goals were set to maximize TPC, TFC, and TTC and minimize IC_{50}. The candidate formulation with the highest overall desirability was selected as the optimum mixture. The selected formulation was then prepared and analysed using the same procedures applied to the experimental runs. Experimental values were compared with the corresponding model predictions, and relative deviation was calculated as:

$$Relative\ deviation\ (\%) = \frac{|Experimental\ value\ -\ Predicted\ value|}{Predicted\ value}\ x\ 100$$

Agreement between the predicted and experimental responses was used to evaluate the predictive performance of the fitted models within the investigated formulation domain.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Profile of the Individual Extracts

Qualitative screening showed that phenols, flavonoids, and tannins were present in all three extracts (**Table 2**). Alkaloids were detected in *G. versteegii* and *C. sappan* but not in *C. burmannii*, whereas saponins were detected in *C. burmannii* and *G. versteegii* but not in *C. sappan*. The shared occurrence of phenolic compounds, flavonoids, and tannins supports the selection of these extracts for further evaluation in an antioxidant polyherbal formulation.

Phenolic compounds can contribute to antioxidant activity through electron or hydrogen donation and radical stabilisation, although qualitative screening does not indicate their concentration or relative contribution to the overall activity (Baliyan et al., 2022; Yamauchi et al., 2024). The profile of *C. burmannii* was broadly consistent with previous reports of phenolic, flavonoid, and tannin constituents in its bark extract (Bahtiarsyah et al., 2023; Budiastuti et al., 2020). However, alkaloids were not detected in the present sample. Differences among studies may reflect variation in plant origin, processing, extraction conditions, extract concentration, or the sensitivity of the qualitative tests.

The phenolic reaction observed in *G. versteegii* agrees with reports describing phenolic and resin-associated constituents in agarwood-producing species (Fadzil et al., 2024; Wang et al., 2018). *C. sappan* is also well documented as a source of phenolic compounds, particularly brazilin, brazilein, and related constituents (Asevedo et al., 2025; Vij et al., 2023). The negative reactions for alkaloids or saponins should therefore be interpreted as not detected under the conditions used rather than as definitive evidence of absence from the extracts.

3.2. Phytochemical Content and Antioxidant Activity of the Polyherbal Formulations

The phytochemical contents and DPPH radical-scavenging activity of the ten formulations are shown in **Table 3**. Total phenolic content (TPC) ranged from 23.06 to 107.26 mg GAE/g. Run 4, containing 80% *C. sappan*, produced the highest TPC, whereas the equal-proportion mixture in Run 10 gave the lowest value. The high TPC of Run 4 is consistent with the abundant phenolic constituents reported in *C. sappan*, including brazilin, brazilein, and related compounds (Asevedo et al., 2025; Vij et al., 2023).

DPPH radical-scavenging activity varied from 14.84 to 26.97 µg/mL. Run 9, comprising 21.67% *C. burmannii*, 56.67% *G. versteegii*, and 21.67% *C. sappan*, showed the lowest IC_{50} and therefore the strongest activity. By contrast, Run 3, which contained 80% *G. versteegii*, had the highest IC_{50}. The formulation with the strongest antioxidant activity was not the one with the highest TPC. This discrepancy suggests that the DPPH response was influenced by the composition and reactivity of the constituent compounds rather than by total phenolic concentration alone. Differences in chemical structure, degree of hydroxylation, and interactions among antioxidant compounds may affect their radical-scavenging capacity (Baliyan et al., 2022; Yamauchi et al., 2024).

Total flavonoid content (TFC) ranged from 53.29 to 218.92 mg QE/g. The highest value was recorded for Run 2, which contained 56.67% *C. sappan*, whereas Run 1, dominated by *C. burmannii*, had the lowest TFC. Total tannin content (TTC) ranged from 15.27 to 124.33 mg TAE/g. Run 7, containing equal proportions of *C. burmannii* and *C. sappan*, produced the highest TTC, while Run 1 again showed the lowest value.

The formulation associated with the maximum response differed among TPC, TFC, TTC, and IC_{50}. Accordingly, selection of an optimum mixture required simultaneous consideration of all four responses rather than reliance on a single phytochemical parameter.

3.3. Effects of Extract Composition on Phytochemical Contents and Antioxidant Activity

The response surfaces showed that the four measured responses were influenced differently by changes in extract composition (**Figure 2**). TPC increased towards the region containing a high proportion of *C. sappan*, consistent with Run 4, in which 80% *C. sappan* produced the highest TPC of 107.26 mg GAE/g. The fitted equation likewise assigned the largest individual-component coefficient to *C. sappan* (**Table 4**). This result agrees with reports that *C. sappan* contains abundant phenolic constituents, including brazilin, brazilein, and related compounds (Asevedo et al., 2025; Vij et al., 2023).

Table 2. Qualitative phytochemical profile of the individual 70% ethanolic extracts

Phytochemical group	*C. burmannii*	*G. versteegii*	*C. sappan*
Alkaloids	−	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	−
Phenols	+	+	+

Notes: (+), detected; (−), not detected under the qualitative screening conditions used in this study.

Table 3. Phytochemical contents and DPPH radical-scavenging activity of the polyherbal formulations

Run	Ratio of *C. burmannii* (X_1) : *G. versteegii* (X_2) : *C. sappan* (X_3)	TPC (mg GAE/g) (Y_1)	IC_{50} (µg/mL) (Y_2)	TFC (mg QE/g) (Y_3)	TTC (mg TAE/g) (Y_4)
1	80.00:10.00:10.00	35.77	22.74	53.29	15.27
2	21.67:21.67:56.67	47.47	23.48	218.92	82.46
3	10.00:80.00:10.00	23.78	26.97	117.35	47.46
4	10.00:10.00:80.00	107.26	26.25	177.77	108.08
5	56.67:21.67:21.67	48.69	21.76	138.71	67.15
6	10.00:45.00:45.00	43.36	22.02	197.98	70.27
7	45.00:10.00:45.00	43.86	21.32	194.13	124.33
8	45.00:45.00:10.00	25.01	21.46	111.63	45.58
9	21.67:56.67:21.67	26.08	14.84	184.13	113.71
10	33.33:33.33:33.33	23.06	23.48	114.13	44.65

Notes: X_1, *C. burmannii* extract; X_2, *G. versteegii* extract; X_3, *C. sappan* extract; TPC, total phenolic content; TFC, total flavonoid content; TTC, total tannin content; IC_{50}, concentration required to inhibit 50% of DPPH radicals. Extract ratios are expressed as % (w/w).

TFC and TTC showed a broadly similar dependence on *C. sappan*. In both linear mixture models, the coefficient for *C. sappan* was greater than those for *G. versteegii* and *C. burmannii*. Nevertheless, the maximum experimental values were obtained from different formulations. Run 2, containing 56.67% *C. sappan*, produced the highest TFC, whereas the highest TTC was recorded for Run 7, which contained equal proportions of *C. burmannii* and *C. sappan*. Thus, increasing the proportion of *C. sappan* generally favoured flavonoid and tannin contents, although the observed responses remained dependent on the complete mixture composition.

The DPPH response followed a different pattern. Run 9, comprising 21.67% *C. burmannii*, 56.67% *G. versteegii*, and 21.67% *C. sappan*, had the lowest IC_{50} value and therefore the strongest radical-scavenging activity. This formulation did not have the highest TPC, TFC, or TTC. The antioxidant response was therefore not explained solely by the total concentration of any single phytochemical group. Differences in chemical structure, hydroxyl substitution, redox potential, and interactions among constituents may influence their reactivity towards DPPH radicals (Baliyan et al., 2022; Yamauchi et al., 2024).

The fitted models further distinguished the response patterns (**Table 4**). TFC and TTC were described by linear mixture equations, indicating that their predicted values were primarily governed by the proportional contribution of each extract. In contrast, the TPC and IC_{50} equations contained higher-order interaction terms, reflecting nonlinear changes across the formulation space. For IC_{50}, the negative interaction term indicates that specific combinations of the three extracts lowered the predicted response. This statistical interaction should not be interpreted as definitive evidence of pharmacological synergism, which would require additional combination-index or isobolographic analysis.

Taken together, *C. sappan* contributed most strongly to the predicted TPC, TFC, and TTC, whereas the strongest DPPH radical-scavenging activity occurred in a formulation containing a higher proportion of *G. versteegii*. As the four responses did not converge on the same formulation, simultaneous optimization was required to identify a mixture that balanced phytochemical content and antioxidant activity.

Table 4. Fitted mixture models for phytochemical contents and DPPH radical-scavenging activity

Response	Model form	Fitted equation
TPC	Higher-order mixture	$Y_1 = 36.009X_1 + 24.105X_2 + 107.595X_3 - 106.643X_1X_3 - 1678.57X_1^2X_2X_3 - 1686.67X_1X_2X_3^2$
IC_{50}	Higher-order mixture	$Y_2 = 22.5975X_1 + 26.8275X_2 + 26.1075X_3 - 611.007X_1X_2^2X_3$
TFC	Linear mixture	$Y_3 = 78.1357X_1 + 139.240X_2 + 211.032X_3$
TTC	Linear mixture	$Y_4 = 32.944X_1 + 57.1936X_2 + 115.194X_3$

Notes: X_1, proportion of *C. burmannii* extract; X_2, proportion of *G. versteegii* extract; X_3, proportion of *C. sappan* extract; TPC, total phenolic content; TFC, total flavonoid content; TTC, total tannin content; IC_{50}, concentration required to inhibit 50% of DPPH radicals.

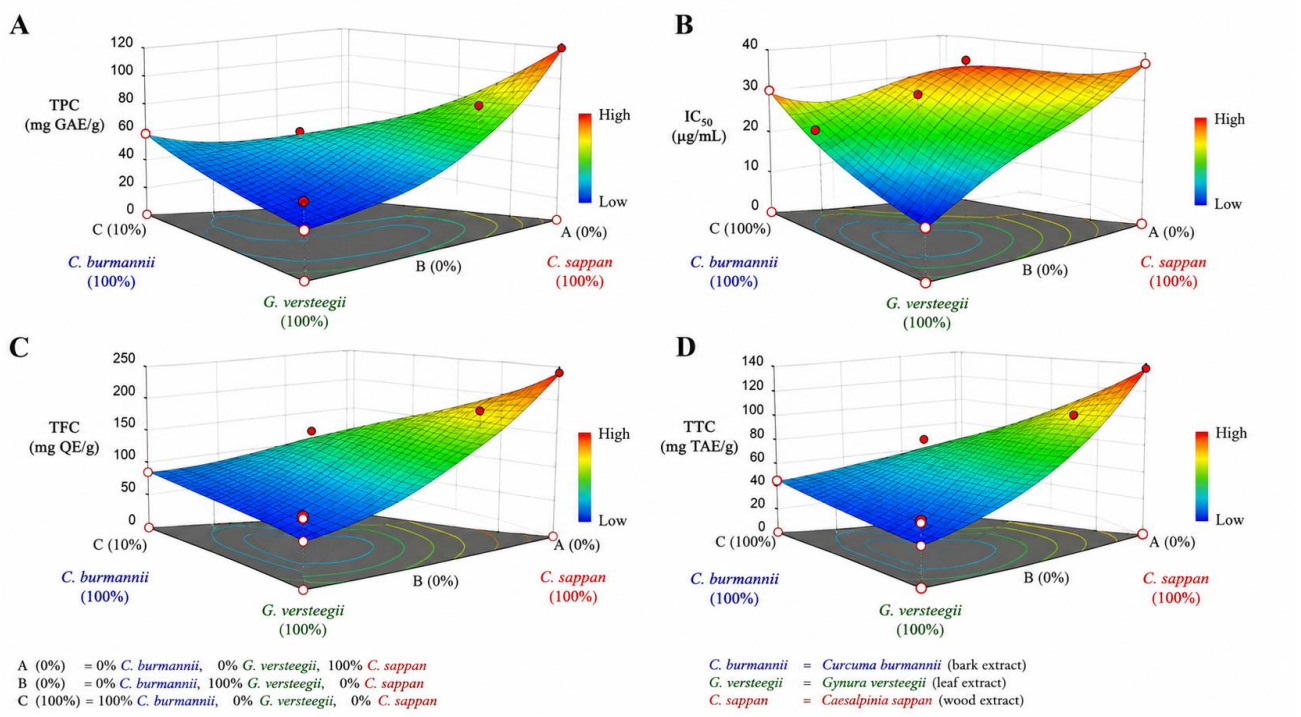

Figure 2. Three-dimensional response surface plots showing the effects of extract proportions on (A) total phenolic content, (B) DPPH radical-scavenging activity expressed as IC_{50}, (C) total flavonoid content, and (D) total tannin content.

3.4. Multiresponse Optimization and Experimental Verification

Numerical optimization was performed by maximizing TPC, TFC, and TTC while minimizing the IC_{50} value. This approach was used because the formulation yielding the highest phytochemical content did not necessarily produce the strongest DPPH radical-scavenging activity.

The desirability function therefore provided a practical means of identifying a compromise among the four responses rather than selecting a formulation based on a single outcome. Similar mixture-design approaches have been used to optimize multicomponent herbal formulations in which several response criteria must be considered simultaneously (Akmal et al., 2023).

The selected formulation contained 10.00%*C. burmannii*, 21.51% *G. versteegii*, and 68.49% *C. sappan*, with an overall desirability of 0.604. The model predicted a TPC of 82.25 mg GAE/g, an IC_{50} of 23.83 µg/mL, a TFC of 199.23 mg QE/g, and a TTC of 105.66 mg TAE/g. Experimental evaluation of the same formulation yielded 79.27 mg GAE/g, 24.78 µg/mL, 197.64 mg QE/g, and 102.08 mg TAE/g, respectively (**Table 5**). These values corresponded to relative deviations of 3.62% for TPC, 3.99% for IC_{50}, 0.80% for TFC, and 3.39% for TTC, with an average deviation of 2.95%.

The close agreement between the predicted and experimental values indicates that the fitted models adequately represented the responses within the formulation region investigated. The smallest deviation was observed for TFC, whereas IC_{50} showed the largest, although all four experimental responses remained close to their predicted values. Because verification was conducted only for the selected optimum, the predictive performance of the models should be interpreted within the experimental domain examined rather than extrapolated beyond the tested component ranges.

The optimized formulation was dominated by *C. sappan*, reflecting its strong contribution to TPC, TFC, and TTC. However, the presence of 21.51% *G. versteegii* was also retained in the optimum, consistent with the distinct composition dependence observed for DPPH radical-scavenging activity. The selected ratio therefore represents a balance between phytochemical enrichment and antioxidant response rather than the maximum value of any single parameter. The use of a mixture design for this purpose is consistent with previous applications in which formulation performance was optimized by adjusting the relative proportions of multiple components (Akmal et al., 2023).

Table 5. Predicted and experimental responses of the optimized polyherbal formulation

Parameter	Predicted value	Experimental value	Relative deviation (%)
C. burmannii (%)	10.00	10.00	—
G. versteegii (%)	21.51	21.51	—
C. sappan (%)	68.49	68.49	—
TPC (mg GAE/g)	82.25	79.27	3.62
IC_{50} (µg/mL)	23.83	24.78	3.99
TFC (mg QE/g)	199.23	197.64	0.80
TTC (mg TAE/g)	105.66	102.08	3.39

Notes: Overall desirability = 0.604. Relative deviation was calculated as the absolute difference between the experimental and predicted values divided by the predicted value and multiplied by 100. TPC, total phenolic content; TFC, total flavonoid content; TTC, total tannin content; IC_{50}, concentration required to inhibit 50% of DPPH radicals.

4. CONCLUSION

The relative proportions of *C. burmannii*, *G. versteegii*, and *C. sappan* markedly influenced the phytochemical profile and DPPH radical-scavenging activity of the polyherbal formulations. *C. sappan* contributed most strongly to total phenolic, flavonoid, and tannin contents, whereas the strongest antioxidant activity was observed in a formulation containing a higher proportion of *G. versteegii*, indicating that antioxidant performance cannot be predicted from total phytochemical content alone. The simplex lattice design identified an optimized formulation containing 10.00% *C. burmannii*, 21.51% *G. versteegii*, and 68.49% *C. sappan*, and the close agreement between predicted and experimental values confirmed the suitability of the fitted models within the investigated formulation range. These findings provide a reproducible basis for selecting extract proportions in antioxidant polyherbal formulations and offer a useful reference for future studies on the mixture-based optimization of medicinal plants. Further chemical characterization and biological validation are needed to identify the compounds responsible for the observed activity and to establish the efficacy and safety of the optimized formulation.

AUTHOR CONTRIBUTIONS

Conceptualization, I.A.A.W.; methodology, I.A.A.W. and P.Y.U.; software, P.Y.U.; validation, I.A.A.W., P.Y.U., and I.M.O.A.P.; formal analysis, P.Y.U.; investigation, I.A.A.W.; resources, I.M.A.G.W.; data curation, P.Y.U.; writing—original draft preparation, I.A.A.W.; writing—review and editing, P.Y.U. and I.G.A.W.; visualization, P.Y.U.; supervision, I.M.O.A.P. and I.M.A.G.W.; project administration, I.G.A.W. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human participants or experimental animals.

INFORMED CONSENT STATEMENT

Not applicable. This study did not involve human participants.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

FUNDING

This research received no external funding.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Faculty of Medicine, Udayana University, for providing laboratory facilities and institutional support through the Integrated Biomedical Laboratory, which facilitated the completion of this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) and Gemini (Google) to improve the clarity, structure, and readability of the text. After using these tools, the authors thoroughly reviewed, edited, and verified the entire manuscript to ensure that it accurately reflects their own ideas, data, and interpretations. The authors take full responsibility for the integrity, originality, and accuracy of the published work.

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