Trop J Nat Prod Res, December 2025; 9(12): 6026 - 6032
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Bidirectional Conversion Models to Harmonize Total Phenolic and Flavonoid Content Analysis in Medicinal Plant Extracts

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ARTICLE INFO

Article history:
Received 01 October 2025
Revised 18 November 2025
Accepted 28 November 2025
Published online 01 January 2026

ABSTRACT

Accurate quantification of total phenolic content (TPC) and total flavonoid content (TFC) is essential for evaluating the quality and biological potential of medicinal plant extracts. However, variations in reference standards and assay conditions often lead to inconsistent data across laboratories. Therefore, this study aimed to develop bidirectional conversion models to harmonize TPC and TFC values obtained using different calibration standards and compare with previous reports. A total of 15 Indonesian medicinal plant extracts were analyzed using UV–Visible spectrophotometry, with seven standards for TPC (gallic acid, catechin, quercetin, rutin, apigenin, kaempferol, and myricetin) and five TFC (quercetin, rutin, apigenin, kaempferol, and myricetin). Linear regression models (R^2 > 0.995) were established to interconvert values among these standards. The results showed that there was a strong positive correlation (r = 0.89) between TPC and TFC, while a weak negative relationship was observed with antioxidant activity (IC_{50}). This indicated that only total content did not reliably predict antioxidant capacity. The bidirectional conversion models enabled reliable transformation of data obtained using different reference compounds, facilitating data harmonization across laboratories and studies. This method provided a practical tool for standardizing spectrophotometric assays in natural products and supported the development of consistent phytochemical databases as well as quality control systems for herbal materials.

Keywords: Total Phenolic Content, Total Flavonoid Content, Calibration Standard, Conversion Model, Antioxidant Activity, Herbal Standardization.

Introduction

Quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) is essential for evaluating the biological potential and quality control of herbal extracts.[1, 2] These parameters are widely adopted in both academic and regulatory frameworks to assess antioxidant capacity and pharmacological relevance of medicinal plant. However, a significant barrier to inter-laboratory comparability is the lack of a harmonized calibration standard across studies.[3] The commonly used TPC and TFC assays, such as Folin-Ciocalteu and $AlCl_3$ colorimetric methods, are influenced concentration and chemical structure of reference standards used for calibration.[4, 5] For example, expressing TPC in gallic acid equivalents (GAE) versus catechin equivalents (CE) can produce quantitatively different values for the same sample due to varying molar absorptivities and reactivities. TFC values also vary significantly based on the use of quercetin, rutin, or kaempferol as calibration standards.[6, 7] This inconsistency complicates cross-study comparisons, meta-analyses, and regulatory assessments. Pharmacopoeias differ in the recommended standards. Although the European Pharmacopoeia frequently uses gallic acid and quercetin, Chinese and Indonesian Herbal Pharmacopoeias vary in references and methodological guidelines.

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Citation: Cahyono B, Anjani I, Oktaviana NA, Aminin ALN, Siahaan P, Widayat W, Suzery M. Bidirectional Conversion Models to Harmonize Total Phenolic and Flavonoid Content Analysis in Medicinal Plant Extracts. Trop J Nat Prod Res. 2025; 9(12):6026 – 6032 https://doi.org/10.26538/tjnpr/v9i12.16

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

This situation poses a significant challenge in countries with rich biodiversity like Indonesia, where more than 1,200 medicinal plants are used in traditional medicine, but comparative quantitative data remains fragmented and incompatible.[8] To address this challenge, there is a need for validated and statistically reliable conversion models that allow the transformation of quantitative results across various calibration standards. These models would facilitate harmonization of analytical data, improve the reliability of phytochemical comparisons, and support the standardization of herbal products in both study and industry.[9, 10] The novelty of this study lies in the development of comprehensive bidirectional conversion models that enable the interconversion of TPC and TFC values across multiple calibration standards used in UV–Visible spectrophotometry analysis. Compared to previous reports that proposed limited one-way conversions, this study systematically applies a unified linear regression method across 15 Indonesian medicinal plant extracts. The use of UV–Visible spectrophotometry is particularly relevant, representing a rapid, cost-effective, and widely accessible analytical method. This makes the proposed harmonization models practical for laboratories with limited resources. Based on the description above, this study aims to develop linear, bidirectional conversion models among seven commonly used TPC standards (gallic acid, catechin, quercetin, apigenin, kaempferol, myricetin, and rutin) and five TFC standards (quercetin, rutin, apigenin, kaempferol, and myricetin). Using spectrophotometry data from 15 Indonesian medicinal plant extracts, standardized conversion equations are established by investigating the interrelationship between TPC and TFC, as well as exploring their correlation with antioxidant activity. The results provide a novel, statistically validated framework for harmonizing phytochemical data and contribute to the enhancement of herbal medicine quality assurance systems globally.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Plant Materials and Reference Standards

A total of 15 medicinal plant samples were selected based on common use in Indonesian traditional herbal medicine and availability in local markets. The samples were collected between May and June 2024 from two main sources, namely *Toko Jamu DAMI* in Semarang (GPS: -6.978189° S, 110.431384° E) and local herbal gardens in Karangbener, Kudus, Central Java (GPS: -6.784273° S, 110.880117° E). The plant materials consisted primarily of dried leaves, except for *Oldenlandia corymbosa*, which included leaves, stems, and roots. The samples were authenticated by taxonomists at the Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro. The local and scientific names, collection sites, and plant parts used are listed in Table 1.

Table 1: List of Medicinal Plant Used in This Study

No	Local Name (Scientific Name)	Collection Site	Plant Part Used	Page
1.	Dewa *(Gynura divaricata)*	Toko Jamu DAMI, Semarang	Leaf	113
2.	Jati Blanda *(Guazuma ulmifolia)*	Toko Jamu DAMI, Semarang	Leaf	157
3.	Tempuyung *(Sonchus arvensis)*	Toko Jamu DAMI, Semarang	Leaf	480
4.	Kenikir (*Cosmos caudatus)*	Karangbener, Kudus	Leaf	234
5.	Sirih Hijau *Piper betle*	Karangbener, Kudus	Leaf	448
6.	Beluntas *Pluchea indica*	Toko Jamu DAMI, Semarang	Leaf	54
7.	Sembung *(Blumea balsamifera)*	Toko Jamu DAMI, Semarang	Leaf	413
8.	Tapak Liman *(Elephantopus scaber)*	Toko Jamu DAMI, Semarang	Leaf	468
9.	Jambu Biji *(Psidium guajava)*	Toko Jamu DAMI, Semarang	Leaf	149
10.	Kayu Putih *(Melaleuca cajuputi)*	Toko Jamu DAMI, Semarang	Leaf	192
11.	Daun Salam *(Syzygium polyanthum)*	Toko Jamu DAMI, Semarang	Leaf	377
12.	Meniran *(Phyllanthus niruri)*	Toko Jamu DAMI, Semarang	Leaf	318
13.	Rumput Mutiara *Oldenlandia corymbosa*	Toko Jamu DAMI, Semarang	Whole plant (leaf, stem, root)	373
14.	Bayam Duri *Amaranthus spinosus*	Karangbener, Kudus	Leaf	50
15.	Binahong *Anredera cordifolia*	Karangbener, Kudus	Leaf	71

To support botanical verification and standardization, each sample was cross-referenced with Indonesian Herbal Pharmacopoeia, Second Edition (FHI, 2017). For plant not included in FHI, identification was based on scientific literature and comparison with herbarium specimens available at the university. There were no voucher specimens deposited, although all identification records and documentation were retained by the department for reference.

The quantitative analysis of TPC and TFC used seven reference standards for calibration curves. These included gallic acid, catechin, quercetin, rutin, apigenin, kaempferol, and myricetin. All reference standards were of analytical grade (≥95% purity) and obtained from Sigma-Aldrich (Merck, Germany). Subsequently, stock solutions were freshly prepared in methanol and stored at 4°C before application. All reagents used, including methanol (p.a), ethanol (p.a), Folin–Ciocalteu reagent, sodium carbonate, aluminum chloride, sodium acetate, and DPPH (2,2-diphenyl-1-picrylhydrazyl), were analytical grade and sourced from Merck. Ultrapure water was used in the analytical process.

Sample Preparation and Extraction

Approximately 40 g of dried and powdered leaves from each medicinal plant sample were macerated in 96% ethanol for 3 × 24 hours at room temperature (27 ± 2°C) with occasional stirring. The pooled extracts were filtered using Whatman No. 1 filter paper (Cytiva, UK) and concentrated with a Rotary Evaporator (IKA RV 10 Digital, Germany) at 50°C. The extraction yield (%) was calculated as the ratio of dry extract mass to the initial plant powder weight. Each extraction was performed in triplicate (n = 3) to ensure reproducibility, following the procedure described by Ji-Eun Lee et al [11]. The crude extracts were preliminarily screened for major secondary metabolites, which included alkaloids, flavonoids, phenolics, saponins, steroids, and triterpenoids, using standard qualitative tests based on color reactions and foam formation.[12]

Determination of TPC

TPC was determined using the Folin–Ciocalteu method following the procedure described by Sánchez-Rangel et al. [13]. Initially, 0.5 mL of sample solution was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent, incubated for 5 minutes, and added with 2.0 mL of 7% sodium carbonate (Na_2CO_3). The solution was allowed to stand at room temperature in the dark for 30 minutes. Absorbance was measured at 765 nm using a UV–Visible Spectrophotometer (Shimadzu UV-1800, Japan). Gallic acid was used as the reference standard, and calibration curves were also constructed for catechin, quercetin, rutin, apigenin, kaempferol, and myricetin (10–50 µg/mL). All measurements were performed in triplicate (n = 3).

Determination of TFC

TFC was measured using the aluminum chloride colorimetric method adapted from Shraim et al. [14]. Initially, a 0.5 mL extract solution was mixed with 2.0 mL of 2% $AlCl_3$ in ethanol. After 30 minutes of incubation in the dark, absorbance was read at 415 nm using the same UV–Visible Spectrophotometer (Shimadzu UV-1800, Japan). Quercetin was used as the primary standard, and parallel curves were constructed for rutin, apigenin, kaempferol, and myricetin. All analyses were carried out in triplicate.

Calculation of Calibration Curves

Standard solutions of each reference compound were prepared in ethanol (10–50 µg/mL). Calibration curves were generated by plotting absorbance against concentration. Linear regression equations were derived and accepted only when $R^2 > 0.99$.

DPPH Radical Scavenging Assay

Antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. A 1.0 mL extract at different concentrations (20–100 µg/mL) was mixed with 1.0 mL of 0.1 mM DPPH in ethanol. After 30 minutes of incubation in the dark at room temperature, absorbance was measured at 517 nm, and percentage inhibition was calculated using Equation 1:

$$\%inhibition = \frac{A_0 - A_s}{A_0} x100\% \quad (1)$$

In this equation, A_0 is the absorbance of the control, and A_s is the absorbance of the sample. IC_{50} values were determined by plotting percent inhibition against concentration.[15]

Conversion Equation Modeling

Bidirectional linear regression models were constructed to enable conversion of TPC and TFC values between standards. TPC values in

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

GAE were regressed against corresponding values in CE, QE, RE, APE, KE, and ME. For TFC, conversions were made from QE to other flavonoid equivalents. Subsequently, models were validated using values obtained from all 15 plant extracts.

Statistical Analysis

All measurements were conducted in triplicate and expressed as mean ± standard deviation (SD). Relative standard deviation (%RSD) was used to assess repeatability, while correlations between TPC, TFC, and IC_{50} values were analyzed through Pearson correlation. Significance was set at $p < 0.05$, and all analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA).

Results and Discussion

Bidirectional Conversion Models for TPC and TFC

The quantification of TPC and TFC in medicinal plant extracts often depends on different reference standards, making direct comparisons between studies difficult. Therefore, this study was carried out to develop bidirectional linear conversion models to harmonize TPC and TFC values obtained using different calibration standards and compare with previous reports. The proposed models focused on facilitating inter-study comparison and data integration on phytochemical analysis. Table 2 provided the linear regression equations and determination coefficients (R^2) for calibration curves constructed using various phenolic and flavonoid standards. For TPC analysis, seven standards were evaluated, comprising gallic acid, catechin, quercetin, rutin, apigenin, kaempferol, and myricetin. All calibration curves showed excellent linearity with R^2 values greater than 0.995, confirming the validity of using each compound as a standard for TPC measurement. A total of five standards, including quercetin, rutin, apigenin, kaempferol, and myricetin, were used for TFC, which showed strong linearity, with R^2 values ranging from 0.9948 to 0.9953.

Table 2: Linear regression equations and determination coefficients (R^2) for calibration curves of seven reference standards used in TPC and TFC analysis

Reference Standard	Linear Regression Equation	R^2	Description
Reference standards used in TPC			
Gallic Acid	y = 0.0121x – 0.0093	0.9973	Valid, Linear
Catechin	y = 0.0105x + 0.0276	0.9926	Valid, Linear
Quercetin	y = 0.0123x + 0.1674	0.9928	Valid, Linear
Rutin	y = 0.0052x + 0.0782	0.9988	Valid, Linear
Apigenin	y = 0.0462x + 0.149	0.9959	Valid, Linear
Kaempferol	y = 0.0048x + 0.0757	0.9961	Valid, Linear
Myricetin	y = 0.0167x + 0.0544	0.9994	Valid, Linear
Reference standards used in TFC			
Quercetin	y = 0.0068x + 0.0261	0.9948	Valid, Linear
Rutin	y = 0.0034x + 0.0093	0.9949	Valid, Linear
Apigenin	y = 0.0577x + 0.0629	0.9917	Valid, Linear
Kaempferol	y = 0.0068x + 0.0272	0.9934	Valid, Linear
Myricetin	y = 0.0713x + 0.0921	0.9953	Valid, Linear

To ensure analytical reliability, all determinations of TPC, TFC, and antioxidant activity were performed in triplicate (n = 3). The results showed high repeatability, with relative standard deviation (%RSD) values ranging from 1.2% to 3.5% across all measurements. These values fall in the acceptable range (<5%) for spectrophotometry assays as recommended by Sánchez-Rangel et al. [13] and Shraim et al. [14] The data confirmed that the spectrophotometry procedures used in this study are reproducible and precise. The low RSD values showed consistent performance of the Folin–Ciocalteu and $AlCl_3$ colorimetric methods under the described experimental conditions, validating the reliability of data used for the bidirectional conversion models. Furthermore, the precision supported the robustness of the regression models ($R^2 > 0.995$), as the observed variability arises primarily from inherent sample differences rather than analytical error. In practical terms, this high repeatability ensures that the developed conversion equations can be confidently applied across laboratories, provided similar spectrophotometric and calibration conditions are maintained. The consistency enhances data comparability and supports the applicability of the harmonization framework proposed in this study.

Due to the variation in molar absorptivity and reactivity among compounds, direct comparisons of TPC or TFC values across different studies using separate standards can cause significant inconsistencies.[16] To address this issue, Figures 1 and 2 introduce bidirectional conversion equations for TPC and TFC, respectively, allowing the conversion of values from one standard to another with high accuracy. Equations on each arrow represented linear regression models used to convert between standards.

In Figure 1, bidirectional equations were established using gallic acid as the primary standard for TPC. For example, the conversion from gallic acid equivalent (TPCGAE) to catechin equivalent (TPCCE) was given by Equation 1:

$$TPCCE = (1.1524 \times \text{TPCGAE}) - 3.1543 \quad (1)$$

with the reverse conversion being (Equation 2):

$$TPCGAE = \frac{(\text{TPCCE} + 3.1543)}{1.1524} \quad (2)$$

This showed a consistent linear relationship and allowed mutual translation between standards. Specifically, quercetin and rutin showed strong correlations with gallic acid, using conversion Equations 3 and 4.

$$\text{TPCQE} = (0.9837 \times \text{TPCGAE}) + 14.3666 \quad (3)$$

$$\text{TPCRE} = (2.3269 \times \text{TPCGAE}) - 16.827 \quad (4)$$

The negative slope in gallic acid to rutin conversion showed an inverse relationship in response characteristics, which was crucial for accurate conversion.[17] The inclusion of apigenin, kaempferol, and myricetin in models broadened the applicability of the method, considering widespread application in flavonoid-rich extracts. For instance, the conversion from TPCGAE to myricetin equivalent (TPCME) follows Equation 5:

$$\text{TPCME} = (0.7246 \times \text{TPCGAE}) - 3.8144 \quad (5)$$

The results showed the flexibility of this harmonization method. Figure 2 shows conversion equations for TFC using quercetin as the base standard. For example, the conversion between quercetin and rutin follows (Equation 6):

$$\text{TFCRE} = (0.17 \times \text{TFCQE}) - 4.9412 \quad (6)$$

and the reverse equation (Equation 7):

$$\text{TFCQE} = \frac{(\text{TFCRE} + 4.9412)}{0.17}. \quad (7)$$

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

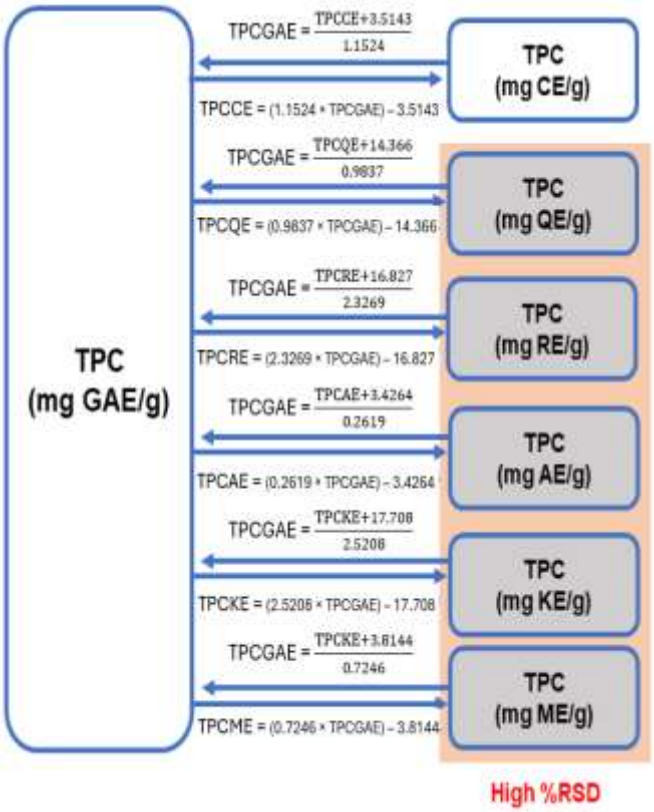

Figure 1: Conversion pathways for TPC standards. Gallic acid serves as the central reference

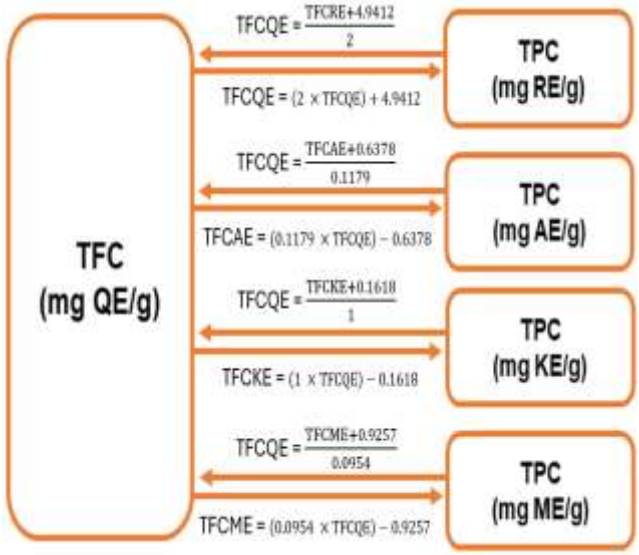

Figure 2: Conversion pathways for TFC standards. Quercetin acts as the intermediary standard for indirect conversions among flavonoids

Other flavonoid pairs, such as quercetin–apigenin, quercetin–kaempferol, and quercetin-myricetin, were modeled. The results showed strong linear relationships with slopes and intercepts, indicating the characteristic response of each compound under spectrophotometry measurement. The bidirectional models allowed the conversion of TPC and TFC values using different reference compounds, which enabled standardized reporting and meta-analysis of phytochemical data.[18] Additionally, the models permitted indirect conversions through intermediary standards. For instance, converting from kaempferol to myricetin equivalents can be performed through quercetin as an intermediary, increasing the versatility of the framework.[19]

Practical Implications and Limitations

The bidirectional conversion models developed in this study offered significant practical benefits for studies, analytical laboratories, and the phytopharmaceutical industry, particularly where there is limited access to a broad range of reference standards. By enabling the conversion of TPC and TFC values between commonly used standards, these models promote standardized data reporting, ensuring consistency in phytochemical analysis across different laboratories and studies.[20] The harmonization is essential to facilitate comparative studies, regulatory compliance, and the development of quality assurance frameworks, particularly in regions where only a limited number of reference compounds are available due to cost, supply chain, or import restrictions.

The models support the retrospective harmonization of historical datasets, eliminating the need for reanalysis of archived extracts. This enables broader meta-analyses, thereby enhancing the comparability of ethnopharmacological studies, which contributes to the construction of centralized herbal composition databases. For industries in botanical supplements and traditional medicines, the models serve as a valuable tool to fulfill regulatory labeling requirements, particularly when different reference compounds are mandated across markets or regulatory authorities.[21]

Despite the significant contribution, there are inherent chemical and methodological limitations associated with the models. The variability in conversion accuracy is influenced by the chemical structure of compounds used as standards. Phenolic acids like gallic and catechin, which lack complex conjugated systems, tend to produce consistent and linear conversions.[22] In comparison, flavonoid standards, particularly those with extended conjugated π-systems and carbonyl functionalities, show non-proportional colorimetric responses due to differences in electron delocalization and redox behavior.[23] These structural differences reduce the precision of inter-standard conversions and contribute to high variation, particularly when depending on reagents like Folin-Ciocalteu or $AlCl_3$ sensitive to electron-donating and complexation behaviors.

The models assume uniformity in reaction conditions and analytical methods, specifically those based on UV-Vis spectrophotometry measurements using Folin-Ciocalteu reagent for TPC and aluminum chloride complexation for TFC.[24] Any deviations in reaction time, pH, temperature, or solvent composition can affect the chromogenic response and limit the reliability of the conversion equations.[25] Despite the robustness in the confines of standardized spectrophotometry protocols, the models are not directly applicable to other analytical methods. These include HPLC, LC-MS, or electrochemical assays, which detect phenolics and flavonoids based on different physicochemical properties.[26, 27]

As a recommendation, future work should focus on validating and expanding the models under diverse assay conditions and across alternative analytical platforms. Extending the conversion framework to include other classes of bioactive secondary metabolites, such as tannins, anthocyanins, and stilbenes, would further enhance relevance for the comprehensive profiling of medicinal plant extracts.[28] Collaboration between regulatory agencies, industry stakeholders, and academic studies is essential in translating these models into international standards for phytochemical analysis and quality control.

Correlation of TPC and TFC with Antioxidant Activity

The quantification of TPC, TFC, and antioxidant activity (as measured by IC_{50}) across 15 medicinal plant extracts showed significant trends and complexities in phytochemical-antioxidant relationships.[29] As shown in Table 3, TPC values ranged from 66.20 to 98.75 mg GAE/g, while TFC varied between 48.33 and 74.20 mg QE/g. Antioxidant activity, expressed as IC_{50}, varied widely from 31.69 to 143.74 µg/mL. These variations showed the diversity in secondary metabolite composition among medicinal plant. Furthermore, statistical analysis showed that samples with higher TPC and TFC values, such as *Piper betle* and *Syzygium polyanthum*, had lower IC_{50} values, suggesting stronger antioxidant activity ($p < 0.05$). The correlation coefficients ($r = –0.36$ for TPC–IC_{50} and $r = –0.24$ for TFC–IC_{50}) indicated that only total content values were insufficient to predict antioxidant potential. This result, supported by Table 3, showed the importance of considering

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

compound structure and synergistic effects rather than relying only on TPC or TFC. As shown in Figure 3, there was a weak negative correlation between TPC and IC_{50} values (r = –0.36), indicating that higher TPC is generally associated with stronger antioxidant activity.[30] However, the correlation was not strong enough to establish TPC as a reliable predictor of antioxidant capacity. Based on Figure 4, there was a weaker negative correlation between TFC and IC_{50} (r = –0.24), suggesting that only TFC was insufficient to accurately reflect antioxidant activity.

Table 3: Total Phenolic, Flavonoid, and Antioxidant Activity Content of 15 Medicinal Plants

No	Scientific Name	TPC (mg GAE/g)	TFC (mg QE/g)	IC_{50} (µg/mL)
1.	*Gynura divaricata*	94.60	71.62	143.74
2.	*Guazuma ulmifolia*	75.30	60.10	59.18
3.	*Sonchus arvensis*	66.20	48.33	89.29
4.	*Cosmos caudatus*	92.50	69.40	35.62
5.	*Sirih Hijau Piper betle*	98.75	74.20	69.24
6.	*Beluntas Pluchea indica*	81.45	61.77	73.99
7.	*Blumea balsamifera*	79.20	58.41	55.68
8.	*Elephantopus scaber*	88.60	66.22	37.34
9.	*Psidium guajava*	85.10	64.55	70.48
10.	*Melaleuca cajuputi*	72.75	53.14	68.53
11.	*Syzygium polyanthum*	90.33	67.40	31.69
12.	*Phyllanthus niruri*	77.25	59.31	73.41
13.	*Oldenlandia corymbosa*	86.50	65.10	110.22
14.	*Amaranthus spinosus*	70.80	52.90	61.79
15.	*Anredera cordifolia*	93.80	72.00	96.23

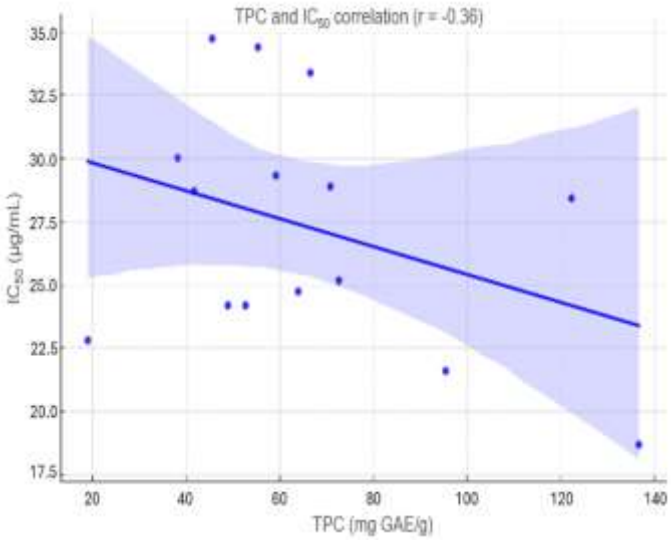

Figure 3: Correlation between TPC and antioxidant activity (IC_{50}) in 15 medicinal plant extracts

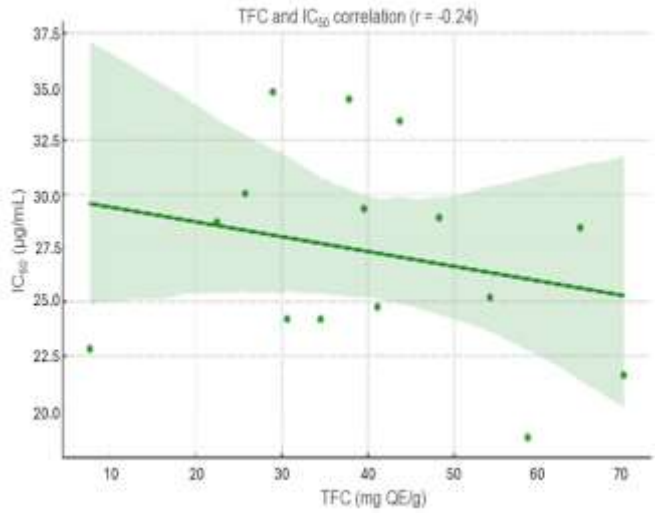

Figure 4: Correlation between TFC and antioxidant activity (IC_{50}) in 15 medicinal plant extracts

The correlation between TPC and TFC (Figure 5) was very strong and positive (r = 0.89), suggesting a high concentration of flavonoid in most of the tested extracts.[31] This is attributed to the chemical nature of flavonoid, which are a subclass of polyphenols. Therefore, in phytochemical analysis, TFC often contributes significantly to overall TPC values, particularly in flavonoid-rich species such as *Guazuma ulmifolia*, Piper betle, and *Syzygium polyanthum*. The weak correlations between TPC/TFC and antioxidant activity (IC_{50}) emphasize a key point often reported in the literature. This suggests that the quantity of TPC and TFC does not always show antioxidant effectiveness.[32] The variation in results is attributed to the specific chemical structures of phenolic and flavonoid content present in each extract. As reported by Rice-Evans et al. [4], structural features such as the number and position of hydroxyl groups, the presence of double bonds, and conjugated π-systems significantly influenced the electron-donating ability of these compounds. Therefore, two extracts with similar TPC values can show significantly different antioxidant potentials based on the bioactivity of dominant compounds.

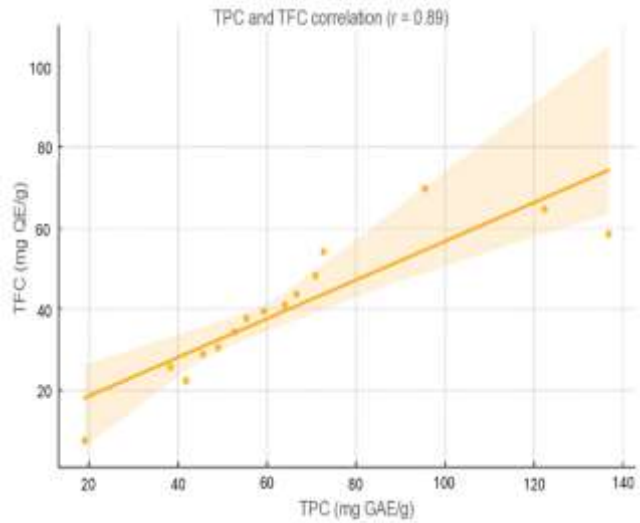

Figure 5: Correlation between TPC and TFC in 15 medicinal plant extracts

Floegel et al. [33] stated that correlations between TPC and antioxidant capacity varied based on plant matrix and the antioxidant assay used, such as DPPH, ORAC, and ABTS. The use of DPPH-based IC_{50} method in this study showed variation between phytochemical content and antioxidant activity. *Guazuma ulmifolia* had a moderate TPC (75.30 mg

GAE/g) but showed one of the lowest IC_{50} values (59.18 µg/mL), indicating strong antioxidant performance. In comparison, *Guazuma duvaucaia*, with the highest TPC (94.60 mg GAE/g), showed poor antioxidant activity (IC_{50} = 143.74 µg/mL). This shows the lack of a linear relationship between TPC and antioxidant strength.

Belew et al. [8] and Janiak et al. [6] reported strong correlations between TPC and antioxidant activity in specific species ($r > 0.78$). However, the results were limited to certain plant types or extract conditions. Tang et al. [7] and Ran et al. [9] emphasized that structure–activity relationships were more meaningful than total quantities when evaluating antioxidant function. This suggests that the quality and nature of phenolic compounds are essential compared to cumulative concentration.[34]

Another study by Blainski et al. [1] stated that other bioactive compounds, such as ascorbic acid (vitamin C), alkaloids, terpenoids, and carotenoids, contributed significantly to antioxidant capacity with phenolic constituents. The phenomenon could explain some of the inconsistencies observed in this study, particularly *Blumea balsamifera* and *Melaleuca cajuputi*, containing essential oils and terpenoids that impacted antioxidant assays. Therefore, the complex phytochemical composition of medicinal plant enhanced the contributions of specific compound classes.

Kurek-Górecka et al.[10] suggested that in simpler systems, such as propolis, high correlations could be observed due to dominance by one or two phenolics. However, in diverse and complex extracts, the multitude of minor compounds and their interactions limited the prediction of antioxidant activity based on only total content values.[35] In line with the analysis, TPC and TFC remain valuable as supportive indicators for the screening and quality evaluation of medicinal plant, but should not be used alone for antioxidant potential. Therefore, a more accurate assessment requires the identification and quantification of individual active compounds, consideration of extract matrix complexity, and the use of complementary bioactivity assays. This study shows the need for integrative methods combining quantitative and qualitative phytochemical analyses to better understand the functional bioactivity of plant-derived extracts.

Conclusion

In conclusion, this study establishes bidirectional conversion models enabling comparison of TPC and TFC across various calibration standards. The models provide a practical and novel framework for harmonizing quantitative data in phytochemical analysis, addressing inconsistencies among laboratories and studies. A strong correlation between TPC and TFC shows the interrelated nature of phenolic and flavonoid compounds. The weak relationship with antioxidant activity emphasizes that compound structure and interactions play greater roles than total quantity. TPC and TFC can serve as supportive indicators rather than sole predictors of biological efficacy. The developed conversion framework enhances analytical efficiency, supports data standardization, and strengthens quality control of herbal products. Moreover, future expansion to other bioactive classes and advanced analytical methods is recommended to promote global data interoperability and integration in phytochemical studies and pharmacope databases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This study was funded by the Directorate of Research and Community Service (DRPM), Ministry of Education and Culture, Republic of Indonesia, under Contract No. 601-50/UN7.D2/PP/VI/2024 (2024).

References

1. Blainski, A.; Lopes, G.C.; De Mello, J.C.P. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. Molecules 2013; 18(6):6852-6865. https://doi.org/10.3390/molecules18066852
2. Prior, R.L.; Wu, X.; Schaich, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. J. Agric. Food Chem. 2005; 53(10):4290-4302. https://doi.org/10.1021/jf0502698
3. Frahm, E.; Wright, J. Evaluation of Inter-Laboratory Comparison Results: Representative Examples. Measurement 2023; 223:113723. https://doi.org/10.1016/j.measurement.2023.113723
4. Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 1996; 20(7):933-956. https://doi.org/10.1016/0891-5849(95)02227-9
5. George, J.; Edwards, D.; Pun, S.; Williams, D. Evaluation of Antioxidant Capacity (ABTS and CUPRAC) and Total Phenolic Content (Folin-Ciocalteu) Assays of Selected Fruit, Vegetables, and Spices. Int J Food Sci 2022; 2022(1):2581470. https://doi.org/10.1155/2022/2581470
6. Janiak, M.A.; Gryn-Rynko, A.; Sulewska, K.; Amarowicz, R.; Penkacik, K.; Graczyk, R.; Olszewska-Słonina, D.; Majewski, M.S. Phenolic profiles and antioxidant activity of *Morus alba* L. infusions prepared from commercially available products and naturally collected leaves. Sci Rep 2025; 15(1):13030. https://doi.org/10.1038/s41598-025-97223-9
7. Tang, S.; Wang, B.; Liu, X.; Xi, W.; Yue, Y.; Tan, X.; Bai, J.; Huang, L. Structural insights and biological activities of flavonoids: Implications for novel applications. Food Front 2025; 6(1):218-247. https://doi.org/10.1002/fft2.494
8. Belew, A.A.; Hanan, G.G.M.W.; Meshesha, D.S.; Akele, M.L. Evaluation of total phenolic, flavonoid contents, antioxidant and antibacterial activity of leaf extracts from *Rhus vulgaris*. Discov Plant 2025; 2(1):141. https://doi.org/10.1007/s44372-025-00222-3
9. Ran, Y.; Li, F.; Xu, Z.; Zeng, K.; Ming, J. Recent advances in dietary polyphenols (DPs): antioxidant activities, nutrient interactions, delivery systems, and potential applications. Food Funct 2024; 15(20):10213-10232. https://doi.org/10.1039/d4fo02111h
10. Kurek-Górecka, A.; Keskin, Ş.; Bobis, O.; Felitti, R.; Górecki, M.; Otręba, M.; Stojko, J.; Olczyk, P.; Kolayli, S.; Rzepecka-Stojko, A. Comparison of the Antioxidant Activity of Propolis Samples from Different Geographical Regions. Plants 2022; 11(9):1203. https://doi.org/10.3390/plants11091203
11. Lee, J.E.; Jayakody, J.T.M.; Kim, J.I.; Jeong, J.W.; Choi, K.M.; Kim, T.S.; Seo, C.; Azimi, I.; Hyun, J.M.; Ryu, B.M. The Influence of Solvent Choice on the Extraction of Bioactive Compounds from *Asteraceae*: A Comparative Review. Foods 2024; 13(19). https://doi.org/10.3390/foods13193151
12. Dubale, S.; Kebebe, D.; Zeynudin, A.; Abdissa, N.; Suleman, S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. J Exp Pharmacol 2023; 15:51-62. https://doi.org/10.2147/JEP.S379805
13. Sánchez-Rangel, J.C.; Benavides, J.; Heredia, J.B.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Anal. Methods 2013; 5(21):5990-5999. https://doi.org/10.1039/C3AY41125G
14. Shraim, A.M.; Ahmed, T.A.; Rahman, M.M.; Hijji, Y.M. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. LWT 2021; 150:111932. https://doi.org/10.1016/j.lwt.2021.111932
15. Evtyugin, D.D.; Evtuguin, D.V. A comprehensive DPPH study on the antioxidant activity of ellagic acid from *Eucalyptus* sulphite pulping liquor for food applications. Food Bioprod. Process. 2025; 154:350-358. https://doi.org/10.1016/j.fbp.2025.10.005
16. Mehmood, A.; Javid, S.; Khan, M.F.; Ahmad, K.S.; Mustafa, A. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chemistry 2022; 16(1):64. https://doi.org/10.1186/s13065-022-00858-2

17. Arab, Z.A.; Nouri, M.; Asadi, G. Optimization of microwave-assisted extraction for horseradish (*Armoracia rusticana*) roots: phytochemical components and bioavailability. Biomass COnv. Bioref. 2025. https://doi/org/10.1007/s13399-025-06847-4
18. Papaefthimiou, M.; Kontou, P.I.; Bagos, P.G.; Braliou, G.G. Integration of Antioxidant Activity Assays Data of *Stevia* Leaf Extracts: A Systematic Review and Meta-Analysis. Antioxidants 2024; 13(6):692. https://doi.org/10.3390/antiox13060692
19. Andriopoulos, V.; Kornaros, M. Microalgal Phenolics: Systematic Review with a Focus on Methodological Assessment and Meta-Analysis. Mar. Drugs 2024; 22(10):460. https://doi.org/10.3390/md22100460
20. Sultana, S.; Lawag, I.L.; Lim, L.Y.; Foster, K.J.; Locher, C. A Critical Exploration of the Total Flavonoid Content Assay for Honey. Methods Protoc. 2024; 7(6):95. https://doi.org/10.3390/mps7060095
21. Sarma, N.; Upton, R.; Rose, U.; Guo, D.-a.; Marles, R.; Khan, I.; Giancaspro, G. Pharmacopeial Standards for the Quality Control of Botanical Dietary Supplements in the United States. J. Diet. Suppl. 2023; 20(3):485-504. https://doi.org/10.1080/19390211.2021.1990171
22. Sahraeian, S.; Rashidinejad, A.; Golmakani, M.-T. Recent advances in the conjugation approaches for enhancing the bioavailability of polyphenols. Food. Hydrocoll. 2024; 146:109221. https://doi.org/10.1016/j.foodhyd.2023.109221
23. Rocchetti, G.; Gregorio, R.P.; Lorenzo, J.M.; Barba, F.J.; Oliveira, P.G.; Prieto, M.A.; Simal-Gandara, J.; Mosele, J.I.; Motilva, M.-J.; Tomas, M., et al. Functional implications of bound phenolic compounds and phenolics–food interaction: A review. Compr. Rev. Food Saf. 2022; 21(2):811-842. https://doi.org/10.1111/1541-4337.12921
24. Evary, Y.M.; Amir, M.N.; Raihan, M.; Laela, D.P. Effects of Solvent Type and Simplicia-to-Solvent Ratio on the Phenolics, Flavonoids, and Antioxidant Activity of Torch Ginger (*Etlingera elatior*) Fruit. Trop. J. Nat. Prod. Res. 2025; 9(2):527-533. https://doi.org/10.26538/tjnpr/v9i2.16
25. Sakiroff, L.-M.; Chennell, P.; Yessaad, M.; Pereira, B.; Bouattour, Y.; Sautou, V. Evaluation of color changes during stability studies using spectrophotometric chromaticity measurements versus visual examination. Sci. Rep. 2022; 12(1):8959. https://doi/org/10.1038/s41598-022-13025-3
26. Beaver, K.; Dantanarayana, A.; Minteer, S.D. Materials Approaches for Improving Electrochemical Sensor Performance. J. Phys. Chem. B 2021; 125(43):11820-11834. https://doi.org/10.1021/acs.jpcb.1c07063
27. Vidal-Casanella, O.; Núñez, O.; Granados, M.; Saurina, J.; Sentellas, S. Analytical Methods for Exploring Nutraceuticals Based on Phenolic Acids and Polyphenols. Appl. Sci. 2021; 11(18):8276. https://doi.org/10.3390/app11188276
28. Abidin, Z.; Razak, R.; Pratama, M. Phytochemical Constituents, Antioxidant and Anti-inflammatory Potentials of Dechlorophyllized Extract of *Kenikir* Leaves (*Cosmos caudatus Kunth*). Trop. J. Nat. Prod. Res. 2025; 9(5). https://doi.org/10.26538/tjnpr/v9i5.11
29. Lama-Muñoz, A.; Contreras, M.d.M. Extraction Systems and Analytical Techniques for Food Phenolic Compounds: A Review. Foods 2022; 11(22):3671. https://doi.org/10.3390/foods11223671
30. Grondalska, J.; Kolniak-Ostek, J. Evaluation of Anti-Inflammatory, Antidiabetic, Antioxidant, and Anticholinergic Activities, as Well as Chemical Composition and Polyphenolic Compounds in Novel SCOBY-Fermented Juices. Molecules 2025; 30(9):1940. https://doi.org/10.3390/molecules30091940
31. Bibi, N.; Shah, M.H.; Khan, N.; Al-Hashimi, A.; Elshikh, M.S.; Iqbal, A.; Ahmad, S.; Abbasi, A.M. Variations in Total Phenolic, Total Flavonoid Contents, and Free Radicals' Scavenging Potential of Onion Varieties Planted under Diverse Environmental Conditions. Plants 2022; 11(7):950. https://doi.org/10.3390/plants11070950
32. Okolie, N.P.; Falodun, A.; Davids, O. Evaluation of The Antioxidant Activity of Root Extract of *Pepper* Fruit (*Dennetia Tripetala*), and it's Potential for the Inhibition of Lipid Peroxidation. Afr. J. Tradit. Complement. Altern. Med. 2014; 11(3):221-227. https://doi.org/10.4314/ajtcam.v11i3.31
33. Floegel, A.; Kim, D.-O.; Chung, S.-J.; Koo, S.I.; Chun, O.K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compos Anal 2011; 24(7):1043-1048. https://doi.org/10.1016/j.jfca.2011.01.008
34. Iheanacho, C.M.; Akubuiro, P.C.; Oseghale, I.O.; Imieje, V.O.; Erharuyi, O.; Ogbeide, K.O.; Jideonwo, A.N.; Falodun, A. Evaluation of the Antioxidant Activity of the Stem Bark Extracts of *Anacardium occidentale* (Linn) Anacardiaceae. Trop. J. Phytochem. Pharm. Sci. 2023; 2(2):65-69. https://www.doi.org/10.26538/tjpps/v2i2.4
35. Egharevba, E.; Chukwuemeke-Nwani, P.; Eboh, U.; Okoye, E.; Bolanle, I.O.; Oseghale, I.O.; Imieje, V.O.; Erharuyi, O.; Falodun, A. Evaluation of the antioxidant and hypoglycaemic potentials of the leaf extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop. J. Nat. Prod. Res. 2019; 3:170-174. https://doi.org/10.26538/tjnpr/v3i5.4