RESEARCH PAPER

A COMPARATIVE ANALYSIS OF PHYTOCHEMICAL CONTENT AND FUNCTIONAL BIOACTIVITY OF MARKETED FORMULATIONS OF TRIPHALA

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ABSTRACT
Triphala, a traditional Ayurvedic polyherbal formulation comprising Terminalia chebula, Phyllanthus emblica, and Terminalia bellirica, is widely used for its antioxidant and metabolic health benefits. However, limited information is available regarding the phytochemical and functional consistency of commercially available formulations. Hence present study aimed to compare marketed Triphala brands for their total phenolic content (TPC), antioxidant activity, and anti-diabetic potential, and to assess batch-to-batch consistency within a selected brand. Six commercially available Triphala formulations were evaluated for TPC using the Folin-Ciocalteu method and antioxidant activity using the DPPH radical scavenging assay. Anti-diabetic potential was assessed through α-glucosidase and dipeptidyl peptidase-IV (DPP-IV) inhibition assays. Further, batch-to-batch consistency was examined across three production lots of one commercial brand. Correlation analysis was performed to explore relationships among phytochemical and biological parameters. Significant variability was observed among brands and batches. Commercial Triphala formulations exhibit considerable phytochemical variability with differential anti-oxidant and enzyme inhibitory activities, highlighting the need for robust quality assessment.

Keywords: Total Phenol content, Anti-oxidant, Anti-Diabetic, Enzyme inhibition.
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1: Introduction

Triphala is one of the most widely used polyherbal formulation in traditional and contemporary healthcare. Composed of an equal proportion of three dried fruits- *Terminaliachebula* (Haritaki), *Phyllanthusemblica* (Amalaki), and *Terminaliabellerica*(Bibhitaki), this formulation is known for its rasayana (rejuvenating) properties [1,2]. In recent years, it has received significant attention, globally, probably due to its increasing application in managing metabolic disorders, including type 2 diabetes, obesity, and hyperlipidemia, alongside its widespread use for gut health [2,3].

The therapeutic efficacy of Triphala is mainly attributed to its rich and complex phytochemistry, which is characterized by high concentrations of polyphenols, particularly gallic acid, ellagic acid, and chebulinic acid, as well as flavonoids and vitamin C. These secondary metabolites act synergistically to exert potent antioxidant, anti-inflammatory, and cytoproteptive effects [4,5]. However, the translation of this formulation into marketed commercial formulations introduces substantial biochemical variability [6]. Since the formulationis based on natural plant matrices, its final phytochemical profile is heavily influenced by upstream and downstream variables. Factors such as the geographical sourcing of raw materials, seasonal harvesting discrepancies, post-harvest drying processing, and distinct manufacturing methodologies (such as the production of raw powders versus concentrated aqueous-alcoholic extracts) can significantly alter the quantity and quality of active biomolecules [7,8].

Although numerous pharmaceutical and nutraceutical brands are using Triphala extensively, there are hardly any studies assessing product uniformity. Specifically, limited data exists regarding the inter-brand variability of active marker compounds and the batch-to-batch consistency of identical formulations over time [9-11]. Furthermore, it is poorly understood how these chemical discrepancies directly impact the functional bioactivity, like free radical scavenging potential and enzyme inhibition capacity.Since the variations in phytochemistry can lead to variations in clinical outcomes, a rigorous comparative analysis is essential to establish quality control benchmarks in the commercial herbal sector and thereby to deliver reproducible therapeutic outcomes [12,13].

To address these gaps, the present study was planned to evaluate and compare commercially available

Triphala formulations from six marketed brands with respect to their total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. Further, given the increasing clinical relevance of Triphala in managing metabolic syndromes, this study evaluates its anti-diabetic potential by quantifying their inhibitory activity against two key metabolic enzymes: alpha-glucosidase and dipeptidyl peptidase-IV (DPP-IV). Finally, to determine industrial manufacturing uniformity, this investigation assesses batch-to-batch consistency across three production lots of a selected commercial brand.

2: Materials and Methods

2.1 Sample Collection and Preparation

A total of six prominent commercial brands of Triphala (designated as Brand A–E) were procured from local pharmacies. To evaluate manufacturing reproducibility, three independent production batches (designated as B1, B2, and B3) were obtained for one specific brand. All samples were checked for seal integrity and stored under conditions as per manufacturer instructions. For the purpose of analysis, the formulations were standardized into aqueous extracts.

2.2 Phytochemical and Bioactivity Assays

2.2.1 Determination of Total Phenolic Content (TPC)

The TPC of the various Triphala formulations was determined using the Folin-Ciocalteu colorimetric method. Briefly, 5 ml of Folinciocalteau reagent (1:10 dilutedwith water) was added to 0.5 ml sample, 4 ml of 1 M Sodiumcarbonate added. Reaction mixture was incubated in dark at roomtemperature (15 min) and measuring absorbance at 765 nm. A Gallicacid standard curve was used to measure the phenolic content andwas expressed as mg/g of dry mass of Gallic acid equivalents (GAE) [14].

2.2.2 DPPH Free Radical Scavenging Assay

The antioxidant capacity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Various concentrations (100 to 1000µg/ml) of samples wereprepared using distilled water as a solvent. In 1 ml of sample 5 ml ofmethanolic DPPH (33 mg/ L) was added, mixture was incubated at37^0C for 30 min. Methanolic DPPH was kept as control.The radical scavenging activity of the test samples was expressed aspercentage inhibition [8].

2.2.3 Enzyme Inhibition Assays (alpha-glucosidase and DPP-IV)

The antidiabetic potential was evaluated through two primary pathways:alpha-Glucosidase Inhibition: This assay measured the ability of the extracts to prevent the hydrolysis of p-nitrophenyl-alpha-D-glucopyranoside (pNPG) [15]. The release of p-nitrophenol was monitored to determine the inhibitory effect on carbohydrate digestion.DPP-IV Inhibition: The capacity of the formulations to inhibit the Dipeptidyl peptidase-IV enzyme was evaluated using a fluorometric substrate [16].

2.3 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as Mean ± Standard Deviation (SD). To assess intra-brand consistency, three independent batches of a selected commercial Triphala formulation were analyzed, and the coefficient of variation (CV) was calculated for total phenolic content and antioxidant activity. The CV was used as a measure of batch-to-batch variability, with higher values indicating greater inconsistency among production lots.

Furthermore, Pearson's correlation analysis was performed to investigate the relationships between total phenolic content and the measured biological activities, including DPPH radical scavenging activity, α-glucosidase inhibition, and DPP-IV inhibition. This analysis was undertaken to determine the extent to whether phenolic content contribute to the observed antioxidant and anti-diabetic effects of the formulations. Lastly, cluster analysis and PCA was performed to understand the brands showing proximity with respect to the studied parameters.

Results

Table 1A: Raw data comparison of marketed formulations for phenol content and free radical scavenging activity

Brand	Phenol content mg/g of GAE	DPPH (%)						
		2.5(µg/ml)	5(µg/ml)	25(µg/ml)	50(µg/ml)	100(µg/ml)	250(µg/ml)	500(µg/ml)
A	110.36 ± 0.017	58.80 ± 0.011	43.25 ± 0.021	55.31 ± 0.021	69.07 ± 0.010	78.64 ± 0.001	78.77 ± 0.0010	79.16 ± 0.0010
B	188.76 ± 0.074	49.50 ± 0.016	54.86 ± 0.033	72.69 ± 0.008	75.81 ± 0.001	74.56 ± 0.004	75.31 ± 0.002	72.82 ± 0.003
C	90.54 ± 0.019	46.81 ± 0.005	40.30 ± 0.018	41.43 ± 0.005	69.64 ± 0.060	70.59 ± 0.005	79.10 ± 0.003	75.72 ± 0.007
D	111.04 ± 0.00	53.17 ± 0.010	54.08 ± 0.013	81.97 ±0.001	83.22 ±0.00006	83.44 ± 0.000	82.87 ± 0.0006	82.76 ± 0.0006
E	86.32 ± 0.008	25.19 ± 0.004	40.39 ± 0.013	48.21 ± 0.021	66.99 ± 0.014	80.78 ± 0.005	81.32 ± 0.002	79.04 ± 0.002
F	50.08 ± 0.011	43.53 ± 0.006	62.44 ± 0.013	78.36 ± 0.002	78.48 ± 0.003	75.50 ± 0.005	56.97 ± 0.005	61.82 ± 0.003

Data presented as Mean ± SD

The six commercial Triphala formulations exhibited considerable variation in total phenolic content (TPC), ranging from 50.08 to 188.76 mg GAE/g, indicating substantial inter-brand phytochemical variability. Brand B showed the highest TPC, while Brand F had the lowest.

All formulations demonstrated antioxidant activity in the DPPH assay, with scavenging activity generally increasing with concentration. Brand D exhibited the strongest and most consistent antioxidant activity across concentrations, whereas Brand E showed the lowest activity at lower concentrations. Notably, antioxidant activity did not directly correlate with TPC. For instance, Brand B possessed the highest phenolic content but did not consistently show the highest DPPH inhibition, while Brand F exhibited appreciable antioxidant activity despite having the lowest TPC (Table 1A).

Table 1B: Raw data comparison of marketed formulations for enzyme inhibition activity

Brand	Alpha glucosidase (%)		DPP-IV (%)	
	250(µg/ml)	500(µg/ml)	250(µg/ml)	500(µg/ml)
A	35.90 ± 0.051	38.27 ± 0.008	2.59 ± 0.56	6.99 ± 3.04
B	47.84 ± 0.049	64.29 ± 0.018	0.8 ± 0.3	4.4 ± 1.6
C	33.22 ± 0.021	67.42 ± 0.019	3.5 ± 0.3	-3.4± 0.4
D	32.40 ± 0.058	43.03 ± 0.033	3 ± 0.7	11 ± 1.9
E	51.93 ± 0.030	59.05 ± 0.030	29.3 ± 0.7	10.2± 0.7
F	53.23 ± 0.023	66.90 ± 0.016	23.24 ± 0.4	27.37 ± 0.5

Data presented as Mean ± SD

The six commercial Triphala formulations demonstrated varying degrees of α-glucosidase and DPP-IV inhibitory activity, indicating differences in their potential anti-diabetic efficacy.For α-glucosidase inhibition, all brands showed increased inhibition at 500 µg/mL compared to 250 µg/mL, suggesting a concentration-dependent effect. Brands F (66.90%), C (67.42%), and B (64.29%) exhibited the highest inhibitory activity at 500 µg/mL, whereas Brands A (38.27%) and D (43.03%) showed comparatively lower activity. These findings indicate that certain formulations may be more effective in delaying carbohydrate digestion and reducing postprandial glucose absorption.In contrast, DPP-IV inhibition was generally lower than α-glucosidase inhibition and exhibited greater variability among brands. Brands F (27.37%) and E (29.30% at 250 µg/mL; 10.2% at 500 µg/mL) demonstrated the highest inhibitory activity, while Brands A–D showed minimal inhibition. Notably, Brand C exhibited a negative value at 500 µg/mL, indicating a lack of inhibitory activity under the assay conditions (Table 1B).

Figure 1:Heat map for comparison of marketed formulations

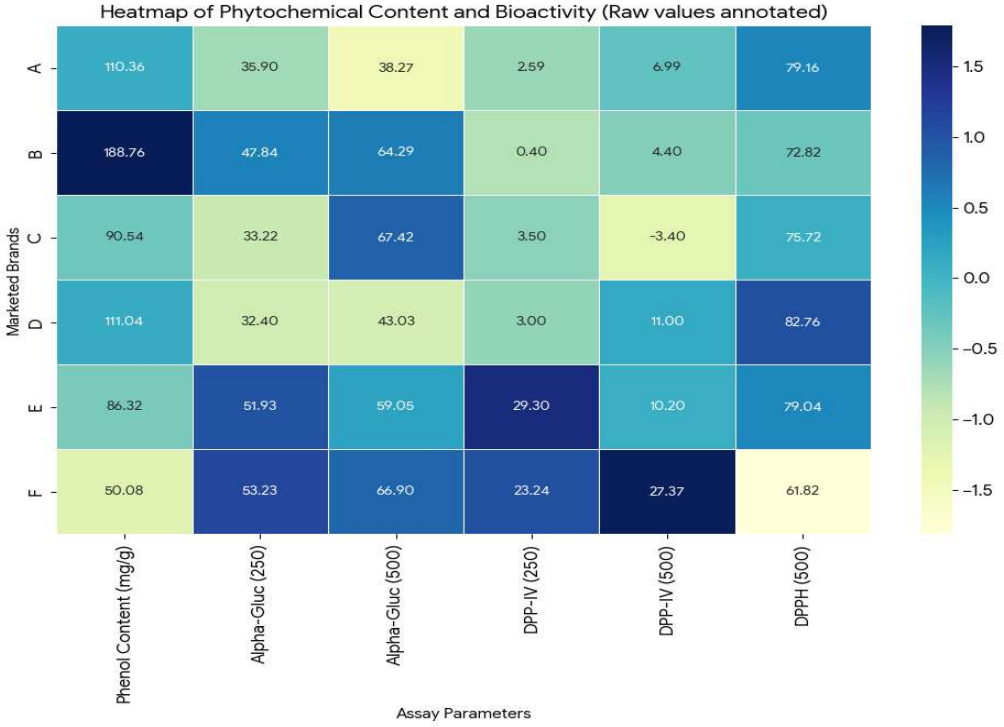
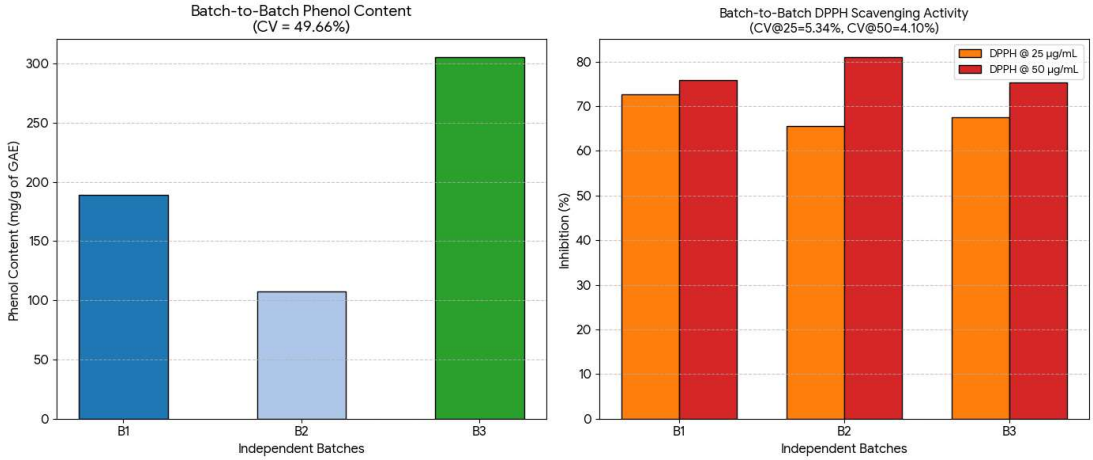
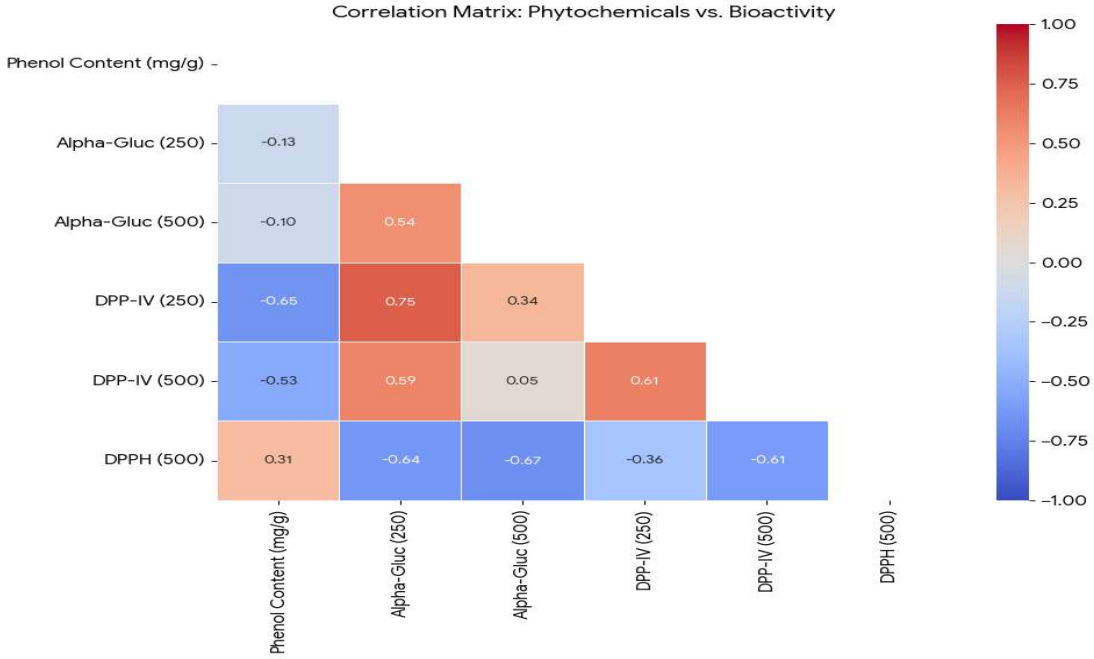


Figure 2: Batch-to-batch variation of selected brands



The Total Phenolic Content (TPC) varies between batches, ranging from 107.36 mg/g in B2 up to 305.47mg/g in B3. This creates a remarkably high Coefficient of Variation (CV) of 49.66%. Despite these variations in phenol content, the actual biological antioxidant activity remains almost similar. The CV for DPPH inhibition is only 5.34% (at 25 µg/mL) and 4.10% (at 50 µg/mL).

Figure 3: Correlation matrix depicting Chemistry vs. Activity



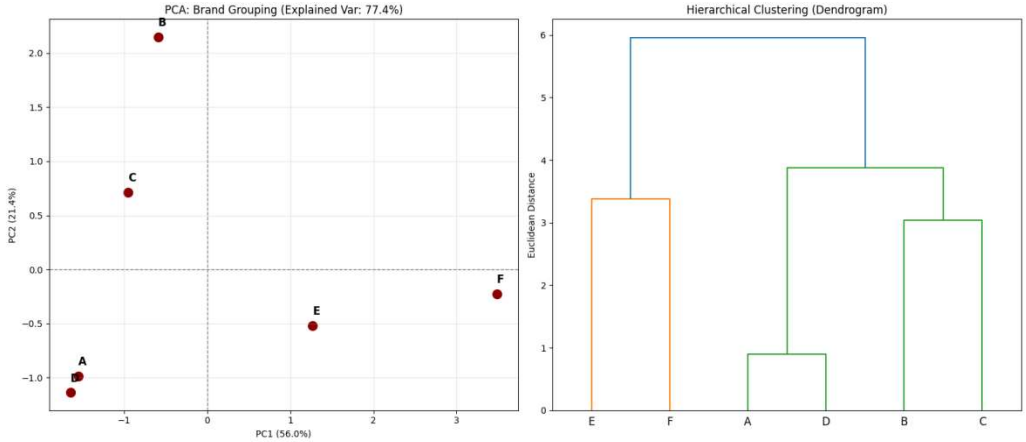
Alpha-Glucosidase and DPP-IV (Strong Positive Correlation,r = 0.75):This indicates that the commercial brands, which demonstrated high inhibition of alpha-glucosidase (carbohydrate digestion) also tended to show higher inhibition of DPP-IV (glucose regulation).

Phenols and Enzyme inhibition:Phenol Content shows a moderate negative correlation with DPP-IV inhibition (r = -0.65) and a very weak relationship with alpha-glucosidase. This indicates that the anti-diabetic effects of these Triphala brands may not be driven primarily by polyphenols, but likely by other constituents like saponins, specific tannins, or organic acids that vary independently of the total phenolic count.

DPPH and Phenols (r = 0.31):While positive, this correlation is lower than expected for herbal extracts. This implies that the antioxidant capacity in these marketed brands is a composite effect—likely involving Vitamin C (from *Phyllanthusemblica*) or other non-phenolic antioxidants.

Enzyme Inhibition vs. DPPH (Negative Correlation):Interestingly, as DPPH scavenging increases (antioxidant capacity), enzyme inhibition tends to decrease (approx. -0.6). This suggests that some brands can demonstrate antioxidant protection, while others can show pronounced metabolic enzyme inhibition.

Figure 4: Cluster analysis / PCA for brand similarity



Distinct Brand Clusters (The Dendrogram):

PCA Mapping (The Scatter Plot):

- PC1 (46.8%) represents the "Bioactivity Axis." Brands further to the right (E and F) are more potent in enzyme inhibition.
- PC2 (30.1%) represents the "Phytochemical Density Axis." Brands higher up on the plot are richer in the specific polyphenols you measured.

The dendrogram (right) reveals that the brands fall into three primary groups based on Euclidean distance:

- Group 1 (E and F): High enzyme inhibition values, even though their phenol content is lower.
- Group 2 (A and D): These brands show high similarity, specifically regarding their antioxidant (DPPH) and phenolic profiles.
- Group 3 (B and C): These are the "Outliers." Brand B is a significant outlier because of its exceptionally high phenolic content, while Brand C is unique due to its specific enzyme inhibition patterns (including that negative DPP-IV result at high concentration).

The fact that B (high phenol) and E/F (high bioactivity) are in opposite quadrants confirms our correlation analysis.

Discussion

The comparative analysis of six marketed Triphala formulations reveals lack of pharmaceutical equivalency across the commercial brands [17]. Our findings, validated through multivariate statistical modeling, demonstrate that products sold under the same classical nomenclature exhibit heterogeneity in both their chemical composition and their bioactivities [18]. The hierarchical clustering and Principal Component Analysis (PCA) demonstrate that the market is fractured into distinct quality archetypes rather than a uniform product e.g. a distinct clustering was observed for Brands A and D, reflecting high antioxidant capacity, whereas Brands E and F grouped together based on their capacity for enzyme inhibition. This chemical and functional divergence is dependent on upstream and downstream dynamics along the commercial supply chain, including variations in raw material sourcing, the precise ratio of the three constituent fruits, extraction efficiencies, and proprietary manufacturing methodologies [19].

Beyond the expected quantitative differences in total phenolic content, a notable side finding emerged regarding the disconnect between bulk chemical markers and biological potency [20]. Interestingly, the highest phenolic concentration observed in Brand B did not automatically translate into superior functional activity across all assays; instead, brands with lower total phenolic, such as E and F, demonstrated significantly higher inhibition against alpha-glucosidase and DPP-IV. This inverse relationship, reinforced by a negative correlation coefficient, suggests that Triphala's metabolic enzyme inhibition may be driven by non-phenolic sub-fractions or specific rare tannins that vary independently of the overall phenolic count. This nuance highlights the complexity of polyherbal matrices, where synergistic interactions or variations in processing parameters can selectively optimize certain therapeutic pathways over others [21].

This pronounced variability among marketed formulations carries critical real-world implications for multiple stakeholders within the healthcare ecosystem. For consumers and clinicians, the lack of product uniformity introduces a layer of unpredictability, as the therapeutic efficacy or physiological outcomes of a treatment regimen may fluctuate significantly depending on the specific commercial brand procured [22]. Most crucially, this heterogeneity provides a plausible explanation for the historical inconsistencies frequently observed across independent experimental and clinical studies evaluating Triphala, as a trial's reproducibility may inadvertently hinge upon the chemical fingerprint of the manufacturer's batch selected for study.

In sharp contrast to this high inter-brand discrepancy, the selected brand evaluated for a lot-to-lot reproducibility demonstrated remarkably low internal variation. This high level of batch consistency proves that manufacturing uniformity is entirely achievable within the herbal sector when stringent processing controls and quality assurance benchmarks are actively maintained [23]. Consequently, these findings underscore an urgent need to transition the commercial Ayurvedic industry from basic raw-material identity testing toward mandatory, bioactivity-linked phytochemical standardization, ensuring that every marketed formulation delivers a predictable, reproducible, and standardized therapeutic response.

Conclusion

Commercial Triphala formulations demonstrated substantial variation in phytochemical content and functional bioactivity. These findings underscore the importance of quality standardization and routine

bioactivity-based assessment to ensure consistency of herbal formulations used in research and clinical practice.

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