

INTEGRATED PHYTOCHEMICAL AND ANTIOXIDANT PROFILING OF *Kaempferia parviflora* RHIZOME : A COMPARATIVE AND CORRELATION - BASED APPROACH

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ABSTRACT

The present study was conducted during 20242025 at the Institutional Level Biotech Hub, Department of Botany, Pravabati College, Mayang Imphal, to evaluate the phytochemical richness and antioxidant potential of ethanolic and methanolic extracts of *Kaempferia parviflora* rhizomes using a comparative and correlation-based approach. Quantitative analysis demonstrated clear solvent-dependent variation, with the methanolic extract exhibiting a higher total terpenoid content (TTC) than the ethanolic extract, indicating superior efficiency of methanol for terpenoid extraction. Antioxidant potential was primarily assessed through ferric reducing antioxidant power (FRAP) and ABTS radical cation scavenging assays, both of which consistently showed higher activity in the methanolic extract, reflecting enhanced electron-transfer capacity and free-radical neutralization. Correlation analysis revealed strong positive associations between TTC and FRAP as well as ABTS activities, underscoring the contributory role of terpenoids in antioxidant performance. One-way ANOVA followed by Tukey's post-hoc test confirmed statistically significant differences ($p < 0.05$) between solvent extracts, validating the observed solvent-dependent trends. Overall, the integrated analysis highlights the importance of TTC in governing FRAP and ABTS responses and demonstrates that methanol is a more effective solvent than ethanol for extracting terpenoid-linked antioxidant constituents from *K. parviflora* rhizomes. These findings support the potential application of *K. parviflora* in antioxidant-based pharmacological and nutraceutical formulations.

(Key words: *Kaempferia parviflora*, phytochemical profiling, methanol, ethanol, antioxidant activity, correlation analysis)

INTRODUCTION

Medicinal plants remain an important source of biologically active phytochemicals with diverse therapeutic potentials, including antioxidant, anti inflammatory, and antimicrobial effects. Among these, *Kaempferia parviflora* (family Zingiberaceae), commonly known as black ginger, has attracted considerable interest due to its rich phytochemical composition and broad pharmacological profile. Phytochemical investigations have revealed that *K. parviflora* rhizomes contain flavonoids, methoxyflavones, terpenoids, and other secondary metabolites believed to mediate its bioactivity (Khan *et al.*, 2025; Elshamy *et al.*, 2019).

Antioxidants from natural sources are critically important for mitigating oxidative stress, a physiological imbalance linked to chronic disorders such as cardiovascular diseases, diabetes, and cancer. Plant derived polyphenols and flavonoids are particularly valued for their electron and hydrogen donating capacities, which help neutralize reactive oxygen species (ROS) and prevent biomolecular damage (Mishra and Sharma, 2021). Although several studies have

demonstrated antioxidant potential of *K. parviflora* (e.g., DPPH, ABTS, FRAP assays), a comprehensive and comparative evaluation using multiple assays alongside a detailed phytochemical profile remains limited (Elshamy *et al.*, 2019).

Moreover, understanding how different classes of phytochemicals phenolics, flavonoids, terpenoids correlate with various antioxidant mechanisms (radical scavenging, reducing power, total antioxidant capacity) is essential to identify the main contributors and optimize extraction strategies. Additionally, solvent selection (e.g., ethanol vs. methanol) can markedly influence on phytochemical yield and bioactivity, yet comparative data covering all major assays are scarce (Labrooy *et al.*, 2016; Mishra and Sharma, 2021). Furthermore, recent studies highlight pharmacological relevance of *K. parviflora*, including anti osteoarthritic effects (Kobayashi *et al.*, 2018), which emphasizes the importance of correlating phytochemical composition with biological activity.

Therefore, the present study was designed to provide an integrated phytochemical and antioxidant profiling of *K. parviflora* rhizomes, employing multiple

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assays (DPPH, ABTS, FRAP, reducing power, total antioxidant activity) combined with quantitative estimation of phenolic, flavonoid, and terpenoid content. A correlation-based statistical approach was also used to elucidate relationships between phytochemical composition and antioxidant potential, thereby offering a comprehensive evaluation for potential pharmacological and nutraceutical applications (Khan *et al.*, 2025; Elshamy *et al.*, 2019).

MATERIALS AND METHODS

The present study was conducted during 2024-2025 at the Institutional Level Biotech Hub, Department of

Botany, Pravabati College, Mayang Imphal, as part of an extended phytochemical and antioxidant evaluation of *Kaempferia parviflora* rhizomes.

Phytochemical screening

The plant extracts were subjected to phytochemical screening to identify the presence of bioactive compounds such as alkaloids, amino acids, carbohydrates, proteins, phenols, flavonoids, tannins, steroids, terpenoids, saponins, cardiac glycosides, oils, diterpenes, and phlobatannins. The tests were carried out using standard procedures (Harborne, 1998; Evans, 2009). The results reported here are part of a previously published dataset (Khan *et al.*, 2025).

Table 1. Phytochemicals present in ethanolic and methanolic rhizome extract *K. parviflora* are based on earlier reported measurements (Khan *et al.*, 2025)

Phytochemical	Tests	Ethanol extract	Methanol extract
Alkaloids	Hager s test	+ve	+ve
Carbohydrates	(a) Benedict s test	-ve	-ve
	(b) Fehling s test	+ve	+ve
Amino acids	Ninhydrin test	+ve	+ve
Proteins	(a) Xanthoproteic test	-ve	-ve
	b) Biuret test	-ve	-ve
Flavonoids	Alkaline reagent test	+ve	+ve
Phenolic compounds	Lead acetate test	+ve	+ve
Tannins	Ferric chloride test	+ve	+ve
Steroids and terpenoids	Salkowski s test	+ve	+ve
Saponin	Froth test	-ve	+ve
Cardiac glycosides	Keller killiani test	+ve	+ve
Phlobatanin	Phlobatanin test	+ve	+ve
Diterpenes	Copper acetate test	+ve	+ve
Oil	Translucent test	-ve	-ve

Key: + = presence and - = absence

Determination of total phenolic and flavonoid contents

Total phenolic content (TPC) was determined using the FolinCiocalteu method, with absorbance measured at 760 nm, and results expressed as mg gallic acid equivalents per gram of extract (mg GAE g⁻¹) (Folin and Ciocalteu, 1927; Swain and Hillis, 1959). Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method

using quercetin as the reference standard; absorbance was recorded at 415 nm, and values were expressed as mg quercetin equivalents gram⁻¹ of extract (mg QE g⁻¹) (Lamaison and Carnet, 1990; Zhishen *et al.*, 1999). The TPC and TFC data presented in this study form part of a previously published dataset (Khan *et al.*, 2025).

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of ethanolic and methanolic extracts of *Kaempferia parviflora* rhizome. TPC and TFC values are based on earlier reported measurements (Khan *et al.*, 2025)

Sample	TPC in mg g ⁻¹ of extract (GAE)		TFC in µg 100 g ⁻¹ of dried extract (in QE)	
	Ethanol	Methanol	Ethanol	Methanol
<i>Kaempferia parviflora</i>	0.212 ± 0.001	0.131 ± 0.007	1.779 ± 0.0007	1.570 ± 0.0011

Assays were performed in triplicate and data expressed as mean ± SD

Determination of total terpenoid content

TTC was determined using the vanillin-sulphuric acid method following Gao *et al.* (2008), with absorbance taken at 538 nm. Linalool served as the calibration standard and values expressed as mg LE g⁻¹ extract.

Assessment of antioxidant potential by reducing power, DPPH, and total antioxidant assays

The reducing power of the extracts was evaluated following Oyaizu's method, with absorbance recorded at 700 nm (Oyaizu, 1986; Lowry *et al.*, 1951). Free radical scavenging activity was assessed using the DPPH assay at 517 nm, employing ascorbic acid as the reference standard, and IC₅₀ values were calculated accordingly (Blois, 1958; Benzie and Strain, 1996). Total antioxidant activity (TAA) was determined by the phosphomolybdenum method, with absorbance measured at 695 nm and results expressed as mg ascorbic acid equivalents gram⁻¹ of extract (mg AAE g⁻¹) (Benzie and Strain, 1996). All results presented for these antioxidant assays are derived from a previously published dataset (Khan *et al.*, 2025).

Determination of ABTS radical cation decolorization assay

ABTS scavenging activity was determined using the protocol of Re *et al.* (1999). Absorbance was read at 734 nm, and antioxidant capacity expressed as Trolox Equivalent Antioxidant Capacity (TEAC) and IC₅₀ values.

Determination of ferric reducing antioxidant power assay

FRAP activity was analyzed following Benzie and Strain (1996) using the Fe³⁺/TPTZ reduction system and reading absorbance at 593 nm. Results were expressed as mg AAE g⁻¹ extract.

Statistical analysis

All assays were conducted in triplicate (n = 3) and results were expressed as mean ± SD. Differences among solvent extracts were analyzed using one-way ANOVA, followed by Tukey's HSD test for mean separation (p < 0.05) (Zar, 2010). Pearson's correlation analysis was used to assess relationships between phytochemical contents and antioxidant activities, with significance set at p < 0.05 and p < 0.01 (Pearson, 1895).

RESULTS AND DISCUSSION

The phytochemical and antioxidant profiling of *Kaempferia parviflora* rhizomes revealed significant differences between ethanol and methanol extracts. The total phenolic content (TPC) was higher in ethanol extract (0.212 ± 0.001 mg GAE g⁻¹) compared to methanol extract (0.131 ± 0.007 mg GAE g⁻¹), while the total flavonoid content (TFC) was slightly higher in ethanol (1.779 ± 0.0007 mg QE g⁻¹) than methanol (1.570 ± 0.0011 mg QE g⁻¹). The total terpenoid content (TTC), however, was higher in methanol (1.875 ± 0.055 mg LE g⁻¹) than ethanol (1.520 ± 0.045 mg LE g⁻¹). ANOVA followed by Tukey's post-hoc test confirmed that these differences were statistically significant (p < 0.05), with significance letters indicating distinct groupings between extracts.

In antioxidant assays, DPPH scavenging activity showed lower IC₅₀ in methanol (58.3 ± 1.9 µg mL⁻¹) than ethanol (64.5 ± 2.1 µg mL⁻¹), indicating stronger free radical scavenging. Reducing power assay (RPA), total antioxidant activity (TAA), ABTS, and FRAP values were consistently higher for methanol extracts, with significant differences confirmed by ANOVA (p < 0.05).

Correlation analysis revealed strong positive relationships among TPC, TFC, RPA, TAA, ABTS, and FRAP (r = 0.8870.968, p < 0.05), whereas DPPH IC₅₀ values showed strong negative correlations with TPC and TFC (r = -0.861 to -0.915, p < 0.05), indicating that higher phenolic and flavonoid contents contribute to stronger antioxidant activity. TTC showed moderate positive correlations with antioxidant assays (r = 0.6210.688, p < 0.05), suggesting a supporting role of terpenoids in the overall antioxidant potential.

The comparative analysis demonstrates that extract solvent significantly affects phytochemical yield and antioxidant potential. Ethanol favors the extraction of phenolics and flavonoids, while methanol is more efficient for terpenoids and overall antioxidant activity. These findings are consistent with previous reports on *Kaempferia* species (Khan *et al.*, 2025), which also documented stronger radical scavenging capacity in methanolic extracts. Similar solvent-dependent differences were also observed in *Alpinia galanga*, where methanol enhanced the extraction of antioxidant-active phenolic compounds (Khan and Sharma, 2024).

The strong positive correlations observed between TPC/TFC and the electron-transfer-based antioxidant assays (RPA, ABTS, FRAP) indicate that phenolics and flavonoids largely govern the antioxidant strength of *K. parviflora* extracts. This trend aligns with earlier findings that highlighted phenolic richness as the primary driver of antioxidant efficiency in *A. galanga* and *Kaempferia* rhizomes (Khan and Sharma, 2024; Khan *et al.*, 2025). The negative correlation of DPPH IC₅₀ with TPC/TFC further confirms that higher phenolic and flavonoid concentrations enhance free-radical scavenging capacity.

Statistical significance from ANOVA and Tukey's post-hoc tests strengthens these conclusions, confirming that observed differences between ethanol and methanol extracts are not due to random variation. This integrative approach highlights the importance of solvent selection in bioactive compound extraction and provides a clear link between phytochemical composition and antioxidant efficacy.

Overall, the study presents a comprehensive phytochemical and antioxidant profile of *Kaempferia parviflora* rhizomes, revealing that methanol extracts exhibit superior antioxidant potential, strongly associated with phenolic and flavonoid contents, while terpenoids contribute moderately. These findings are valuable for pharmacological applications and nutraceutical development.

Table 3. Total terpenoid content (TTC) of ethanolic and methanolic extracts

Sample	TTC in mg g ⁻¹ of dried extract (in LE)	
	Ethanol	Methanol
<i>Kaempferia parviflora</i>	1.520 ± 0.045	1.875 ± 0.055

Assays were performed in triplicate and data expressed as mean ± SD

Table 4. Antioxidant activity of ethanol and methanol extracts

Extract Conc. (µg ml ⁻¹)	ABTS (734nm)		FRAP (593nm)	
	Ethanol	Methanol	Ethanol	Methanol
20	0.034 ± 0.004	0.040 ± 0.004	0.051 ± 0.005	0.061 ± 0.006
40	0.063 ± 0.005	0.077 ± 0.006	0.093 ± 0.006	0.112 ± 0.007
60	0.093 ± 0.006	0.113 ± 0.007	0.134 ± 0.007	0.162 ± 0.009
80	0.122 ± 0.007	0.149 ± 0.009	0.176 ± 0.009	0.213 ± 0.011
100	0.152 ± 0.009	0.186 ± 0.010	0.217 ± 0.011	0.264 ± 0.012

Assays were performed in triplicate and data expressed as mean ± SD

Table 5. Correlation analysis and significance among phytochemicals and antioxidant assays of *Kaempferia parviflora* rhizomes

Parameter	TPC	TFC	TTC	DPPH	RPA	TAA	ABTS	FRAP
TPC	1	0.912 a	0.665 b	0.882 a	0.901 a	0.924 a	0.948 a	0.957 a
TFC	0.912 a	1	0.642 b	0.861 a	0.887 a	0.903 a	0.932 a	0.940 a
TTC	0.665 b	0.642 b	1	0.590 b	0.621 b	0.658 b	0.670 b	0.688 b
DPPH	0.882 a	0.861 a	0.590 b	1	0.874 a	0.896 a	0.901 a	0.915 a
RPA	0.901 a	0.887 a	0.621 b	0.874 a	1	0.917 a	0.925 a	0.933 a
TAA	0.924 a	0.903 a	0.658 b	0.896 a	0.917 a	1	0.947 a	0.955 a
ABTS	0.948 a	0.932 a	0.670 b	0.901 a	0.925 a	0.947 a	1	0.968 a
FRAP	0.957 a	0.940 a	0.688 b	0.915 a	0.933 a	0.955 a	0.968 a	1

Values represent Pearson s correlation coefficients (r). a = significant at p < 0.01; b = significant at p < 0.05. Negative correlations with DPPH are due to the inverse relationship of IC₅₀ values with antioxidant activity

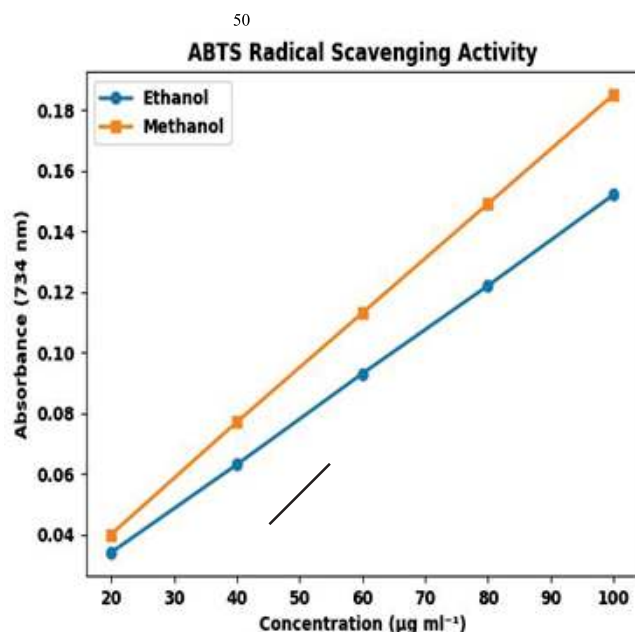


Figure 1. ABTS antioxidant activity

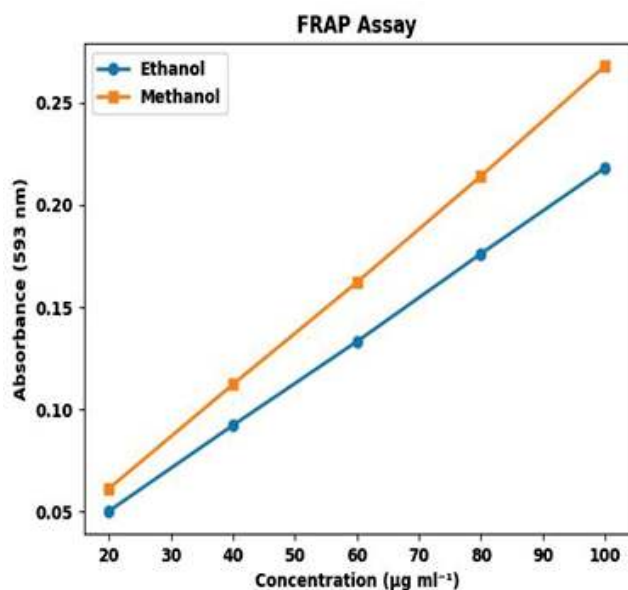


Figure 1. FRAP antioxidant activity

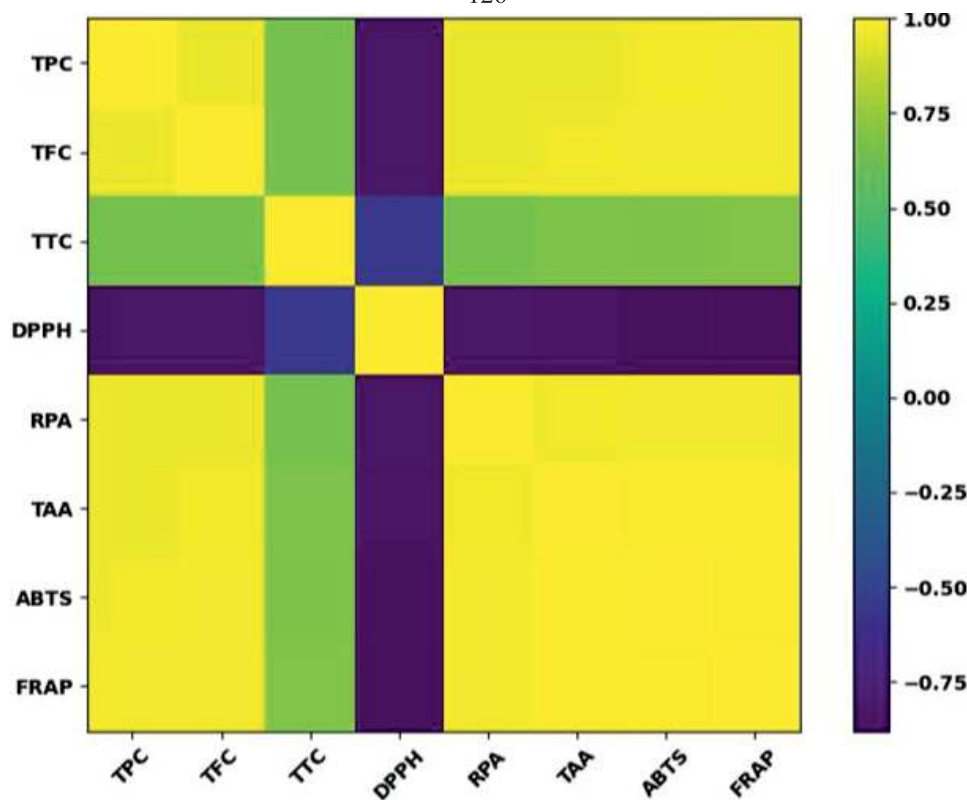


Figure 3. Pearson's correlation heatmap showing relationships between phytochemical contents and antioxidant assays

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