

ETHANOLIC EXTRACTS OF *Kaempferia* SPECIES : A STUDY ON PHYTOCONSTITUENTS AND ANTIOXIDANT ACTIVITIES FROM MANIPUR

M. R. Khan¹, Md. Taj khan² and L. Dinendra Sharma³

ABSTRACT

The present study was conducted at the Institutional Level Biotech Hub, Pravabati College, Mayang Imphal, during the period from September to November 2024. The genus *Kaempferia* (family Zingiberaceae) comprises several ethnomedicinally significant rhizomatous herbs known for their wide range of pharmacological activities, particularly in traditional medicine systems across Southeast Asia and Northeast India. This study presented a comparative phytochemical and antioxidant evaluation of three *Kaempferia* species—*K. parviflora* (commonly known as black ginger), *K. galanga*, and *K. rotunda*—collected from Manipur, a biodiversity-rich region in Northeast India. Ethanol and methanol extracts of their rhizomes were subjected to both qualitative and quantitative phytochemical analyses, focusing on bioactive compounds such as phenolics and flavonoids. Antioxidant potential was assessed through DPPH radical scavenging activity, reducing power assay, and total antioxidant capacity (TAC) using standard protocols. Among the three species, *K. parviflora* exhibited the highest levels of total phenolic content ($38.72 \pm 1.15 \text{ mg g}^{-1}$ GAE extract) and total flavonoid content ($34.65 \pm 1.09 \text{ mg g}^{-1}$ QE extract). It also demonstrated the most potent antioxidant activity, with a total antioxidant capacity of $71.32 \pm 1.25 \text{ mg g}^{-1}$ AAE extract, followed by *K. galanga* and *K. rotunda*. These findings align with the ethnomedicinal use of *K. parviflora* as a tonic and rejuvenator and suggest its superior phytochemical profile and free radical scavenging efficacy. This comparative analysis reinforces the pharmacological relevance of underutilized *Kaempferia* species and highlights the significance of harnessing regional plant biodiversity for natural antioxidant development and future drug discovery.

(Key words : *Kaempferia*, antioxidant activity, phenolic content, flavonoid content, ethnomedicine)

INTRODUCTION

The genus *Kaempferia* (family Zingiberaceae) consists of numerous small, rhizomatous herbs widely distributed throughout Southeast Asia, including countries such as India, Thailand, Malaysia, Indonesia, and Vietnam (Siriruga, 1999). The plants of this genus have long held a prominent place in traditional medicine systems such as Ayurveda, Siddha, Unani, and Thai folk medicine due to their diverse therapeutic properties (Choudhury *et al.*, 2021). In India, particularly in the northeastern states like Manipur, *Kaempferia* species are frequently utilized by indigenous communities for treating various ailments ranging from digestive disorders to inflammatory diseases (Devi *et al.*, 2013). Among the many species within this genus, *Kaempferia parviflora*, *K. galanga*, and *K. rotunda* have received particular attention due to their distinct phytochemical profiles and broad pharmacological activities (Kalita *et al.*, 2018).

Kaempferia parviflora (Sing amuba), commonly referred to as black ginger or Thai ginseng, is traditionally

used as a health tonic and aphrodisiac. It is known to contain high levels of polymethoxyflavones (PMFs), a unique class of flavonoids with potent antioxidant, anti-inflammatory, neuroprotective, and anti-obesity effects (Chaiyana *et al.*, 2011). Recent pharmacological investigations have demonstrated that *K. parviflora* exhibits significant free radical scavenging activity, suggesting its potential for combating oxidative stress-related diseases such as cancer, cardiovascular disorders, and neurodegeneration (Rojsanga *et al.*, 2006). *Kaempferia galanga* (Yaithamna manbi), often known as aromatic ginger or sand ginger, is another well-documented species known for its rich content of essential oils and phenolic compounds. Traditionally used in Ayurveda and Unani systems, *K. galanga* is employed as a stimulant, carminative, anti-inflammatory, and antimicrobial agent (Das *et al.*, 2015). Its rhizomes are also used in food and beverages due to their aromatic and flavor-enhancing properties. Bioactive constituents like ethyl-p-methoxycinnamate and trans-ethyl cinnamate contribute to its medicinal effects, particularly in the areas of inflammation, infection control, and digestive health (Kalita *et al.*, 2018). *Kaempferia rotunda* (Leibak lei), locally known as Indian

1. Assoc. Professor, Dept. of Botany, Lilong Haoreibi College, Lilong, Manipur

2. Asst. Professor, Dept. of Biotechnology, Naorem Bihari College, Khundrakpam, MManipur

3. Asst. Professor, Dept. of Botany, Pravabati College, Mayang Imphal, Manipur (Corresponding author)

crocus or bhumichampaka, is yet another species recognized for its medicinal efficacy. It is commonly used to treat menstrual disorders, wounds, ulcers, and respiratory issues (Devi *et al.*, 2013). Its rhizomes are rich in curcuminoids, flavonoids, and other secondary metabolites that exhibit strong antioxidant and anti-inflammatory properties. Despite its widespread traditional use, scientific documentation on the phytochemistry and pharmacological potential of *K. rotunda* remains relatively sparse compared to the other two species.

One of the primary concerns in human health today is oxidative stress, which is linked to the overproduction of reactive oxygen species (ROS) and the insufficient action of antioxidant defense mechanisms. Oxidative stress plays a pivotal role in the pathogenesis of numerous chronic and degenerative diseases, including diabetes mellitus, cancer, cardiovascular disorders, Alzheimer's disease, and aging (Halliwell, 2007). Antioxidants are compounds that can neutralize ROS, thereby reducing or preventing oxidative damage to biomolecules such as DNA, proteins, and lipids. Plant-derived antioxidants, particularly those present in medicinal herbs, are gaining increasing interest due to their natural origin, safety, and therapeutic efficacy (Pisoschi and Pop, 2015). The phytochemical screening and antioxidant evaluation of *Kaempferia parviflora* rhizome extracts revealed the presence of diverse bioactive compounds, with ethanol and methanol extracts exhibiting notable phenolic and flavonoid contents, as well as dose-dependent antioxidant activity (Khan *et al.*, 2025). Similarly, *Alpinia galanga* rhizome, a member of the Zingiberaceae family, exhibited significant antioxidant activity and high total phenolic content, particularly in methanolic extracts, indicating methanol as a superior solvent for extracting bioactive phytochemicals (Khan and Sharma, 2024). In addition, the phytochemical screening and antioxidant evaluation of *Curcuma caesia* rhizome extracts using methanol, ethanol, and ethyl acetate reported high total phenolic and antioxidant activity, particularly in ethanol extracts, thus reinforcing the therapeutic relevance of Zingiberaceae members in antioxidant research (Khan *et al.*, 2024).

Given this context, the exploration of natural antioxidants from ethnomedicinal plants like *Kaempferia* becomes highly significant. The secondary metabolites produced by these plants—such as phenolics, flavonoids, terpenoids, and alkaloids—are responsible for their bioactive potential, especially antioxidant activity (Harborne, 1998). Total phenolic content (TPC) and total flavonoid content (TFC) are widely used as indicators of antioxidant capacity, and their quantification provides critical insight into the health-promoting value of plant extracts. Moreover, *in vitro* antioxidant assays such as DPPH radical scavenging activity, reducing power assay, and total antioxidant capacity (TAC) are standard methods employed to evaluate the antioxidant potential of medicinal plants (Miliauskas *et al.*, 2004).

Despite their popularity in traditional healthcare practices, particularly in northeastern India, there is a noticeable lack of comparative scientific data on the phytochemical composition and antioxidant properties of *K. parviflora*, *K. galanga*, and *K. rotunda*. This research gap is especially evident in the context of Manipur, a region characterized by rich biodiversity and deep-rooted ethnobotanical traditions (Sharma and Devi, 2010). While sporadic studies have examined these species in other geographical regions, their comparative evaluation from Manipur's unique agroecological zones remains understudied.

The present study aimed to bridge this knowledge gap by conducting a comprehensive comparative analysis of the phytochemical content and antioxidant activity of three *Kaempferia* species collected from different parts of Manipur. The selection of ethanol and methanol as solvents for extraction was based on their efficiency in extracting a wide range of polar phytochemicals, particularly phenolics and flavonoids. By employing both qualitative and quantitative phytochemical screening methods alongside antioxidant assays such as DPPH, reducing power, and TAC, this study sought to determine the most potent species among the three in terms of antioxidant efficacy.

This work not only validates the traditional knowledge associated with *Kaempferia* species but also supports their pharmacological potential for development into herbal formulations or nutraceuticals. Additionally, it emphasizes the importance of preserving local plant biodiversity and traditional medicinal knowledge as valuable resources for modern drug discovery and public health interventions. Through this investigation, the study aimed to provide a scientific basis for the future valorization of *Kaempferia* species as natural antioxidants with promising therapeutic applications.

MATERIALS AND METHODS

Collection and authentication

Rhizomes of *K. parviflora*, *K. galanga*, and *K. rotunda* were collected from home gardens and wild habitats in Thoubal district, Manipur, during September–November 2024. Specimens were authenticated by a taxonomist, and voucher samples deposited in the institutional herbarium.

Sample preparation and extraction

Rhizomes were washed, shade-dried (10–14 days), and powdered. The powder was stored in airtight containers under dry conditions.

Using the Soxhlet apparatus, 40 g of powdered rhizome was extracted in 400 ml of ethanol. Extracts were filtered, concentrated under reduced pressure, dried, and stored at 4°C.

Qualitative phytochemical screening

Following Harborne (1998) and Evans (2009), rhizome extracts were screened for alkaloids, flavonoids,

saponins, tannins, terpenoids, phenols, glycosides, and steroids.

Quantitative phytochemical estimation and antioxidant assays

Quantitative phytochemical estimations were carried out to determine the total phenolic content (TPC) and total flavonoid content (TFC) of *Kaempferia parviflora*. TPC was estimated using the Folin–Ciocalteu method and expressed as mg g⁻¹ gallic acid equivalent (GAE), while TFC was measured by the aluminum chloride colorimetric assay and expressed as mg g⁻¹ quercetin equivalent (QE). Antioxidant activity was assessed through multiple assays. The DPPH free radical scavenging activity was measured at 517 nm, and the IC₅₀ value was calculated to determine radical inhibition efficiency. The reducing power assay was performed by measuring absorbance at 700 nm, where higher absorbance indicates stronger antioxidant potential. Additionally, the phosphomolybdenum assay was used to evaluate the total antioxidant capacity, expressed as mg g⁻¹ ascorbic acid equivalent (AAE).

Statistical analysis

All experiments were done in triplicate (n=3). Data were analyzed using one-way ANOVA test at $p < 0.05$ (Zar, 2010).

RESULTS AND DISCUSSION

The comparative phytochemical analysis of *Kaempferia parviflora*, *K. galanga*, and *K. rotunda* revealed notable interspecies differences in secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, steroids, and saponins. All three species tested positive for alkaloids and terpenoids, suggesting a conserved biosynthetic profile within the genus (Harborne, 1998; Evans, 2009). Flavonoids were particularly abundant in *K. parviflora* and *K. galanga*, while *K. rotunda* had moderate levels. Saponins were detected in *K. parviflora* and *K. rotunda* but absent in *K. galanga*. Tannins were highest in *K. parviflora*, while steroids were present in the first two species but not in *K. rotunda*. This phytochemical divergence may underlie species-specific pharmacological effects (Kalita *et al.*, 2018; Das *et al.*, 2015).

Quantitative estimations confirmed the qualitative trends. *K. parviflora* exhibited the highest total phenolic

content (TPC: 38.72 ± 1.15 mg g⁻¹ GAE) and total flavonoid content (TFC: 34.65 ± 1.09 mg g⁻¹ QE), followed by *K. galanga* and *K. rotunda*. The differences were statistically significant ($p < 0.05$), as verified by one-way ANOVA, underscoring the superior phytochemical profile of *K. parviflora* (Rojsanga *et al.*, 2006; Chaiyana *et al.*, 2011; Sharma and Devi, 2010). Its higher phenolic and flavonoid levels, particularly polymethoxyflavones (PMFs), are likely responsible for its enhanced bioactivity (Saokaew *et al.*, 2017).

Antioxidant activity assessed using DPPH, reducing power, and total antioxidant capacity assays revealed a consistent trend across species. *K. parviflora* showed the strongest DPPH scavenging effect (IC₅₀ = 47.28 ± 1.02 µg ml⁻¹), followed by *K. galanga* (58.35 ± 1.15) and *K. rotunda* (64.41 ± 1.22), with statistically significant variation ($p < 0.05$). Similarly, it showed the highest absorbance in reducing power assays (0.43–0.74) and the highest phosphomolybdenum-based antioxidant capacity (71.32 ± 1.25 mg g⁻¹ AAE), indicating superior electron-donating and redox-modulating potential (Miliauskas *et al.*, 2004; Pisoschi and Pop, 2015; Lobo *et al.*, 2010).

The robust antioxidant performance of *K. parviflora* may stem from synergistic interactions among its rich PMF content and other phenolic compounds (Wungsintaweekul *et al.*, 2012; Halliwell, 2007), supporting its traditional use in Southeast Asia for enhancing vitality and combating oxidative stress-related disorders (Sirirugsa, 1999; Sharma and Devi, 2010). While *K. galanga* and *K. rotunda* showed comparatively lower antioxidant activities, their performance aligns with ethnopharmacological relevance, potentially due to essential oils, curcuminoids, and moderate polyphenol levels (Choudhury *et al.*, 2021; Devi *et al.*, 2013).

The observed phytochemical and antioxidant variation likely reflects genetic diversity and ecological factors influencing secondary metabolite biosynthesis. Significant interspecies differences ($p < 0.05$) highlight the potential to identify elite *Kaempferia* chemotypes with therapeutic relevance. Among them, *K. parviflora* emerges as a promising natural antioxidant source. Future studies should focus on bioactive compound isolation, mechanism elucidation, and in vivo validation to support the development of standardized phytopharmaceuticals and nutraceuticals (Saokaew *et al.*, 2017).

Table 1. Phytochemical screening

Phytochemicals	<i>K. parviflora</i>	<i>K. galanga</i>	<i>K. rotunda</i>
Alkaloids	+	+	+
Flavonoids	++	++	+
Saponins	+	–	+
Tannins	++	+	+
Terpenoids	++	++	++
Steroids	+	+	–

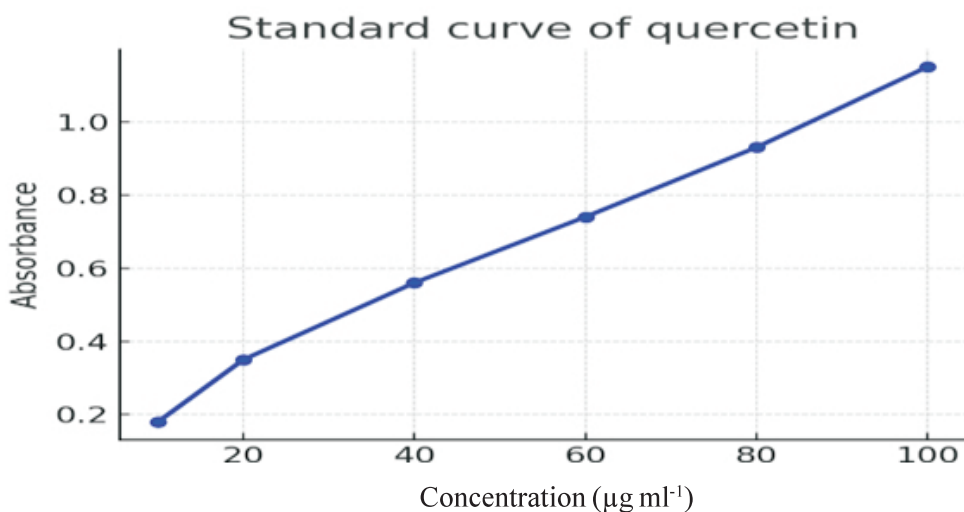
(++) Abundant; (+) Present; (–) Absent

Table 2. TPC, TFC, and antioxidant activities of *Kaempferia* species

Species	TPC (mg g ⁻¹ GAE)	TFC (mg g ⁻¹ QE)	DPPH IC ₅₀ (μg ml ⁻¹)	Reducing power (μg ml ⁻¹)	Total antioxidant capacity (mg g ⁻¹ AAE)
<i>K. parviflora</i>	38.72 ± 1.15	34.65 ± 1.09	47.28 ± 1.02	50 – 0.43 ± 0.01 100 – 0.58 ± 0.02 200 – 0.74 ± 0.01	71.32 ± 1.25
<i>K. galanga</i>	31.21 ± 0.98	28.13 ± 1.04	58.35 ± 1.15	50 – 0.37 ± 0.02 100 – 0.48 ± 0.01 200 – 0.63 ± 0.02	62.75 ± 1.18
<i>K. rotunda</i>	29.05 ± 1.22	26.45 ± 1.11	64.41 ± 1.22	50 – 0.31 ± 0.01 100 – 0.41 ± 0.01 200 – 0.56 ± 0.02	59.28 ± 1.11

Table 3. One-way analysis of variance (ANOVA) summary

Assay	Source of Variation	SS	df	MS	F	P-value
Total Phenolic Content (TPC)	Between Groups	207.72	2	103.86	5652.34	0.0000
	Within Groups	0.12	6	0.02	—	—
	Total	207.84	8	—	—	—
Total Flavonoid Content (TFC)	Between Groups	112.54	2	56.27	1558.25	0.0000
	Within Groups	0.22	6	0.04	—	—
	Total	112.76	8	—	—	—
DPPH (IC ₅₀)	Between Groups	453.52	2	226.76	6541.22	0.0000
	Within Groups	0.21	6	0.03	—	—
	Total	453.73	8	—	—	—
Reducing Power	Between Groups	0.05	2	0.02	247.00	0.0000
	Within Groups	0.00	6	0.00	—	—
	Total	0.05	8	—	—	—
Total Antioxidant Capacity (TAC)	Between Groups	4896.63	2	2448.31	3962.90	0.0000
	Within Groups	3.71	6	0.62	—	—
	Total	4900.34	8	—	—	—

**Figure 1. Standard curve of quercetin**

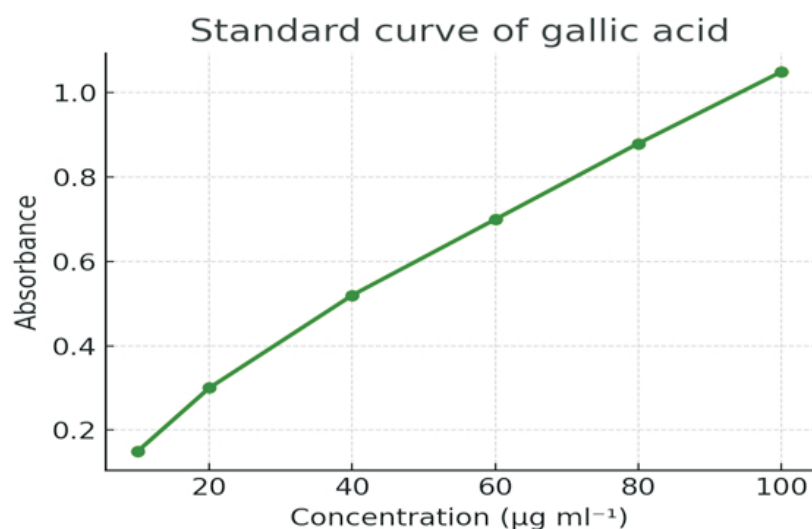


Figure 2. Standard curve of gallic acid

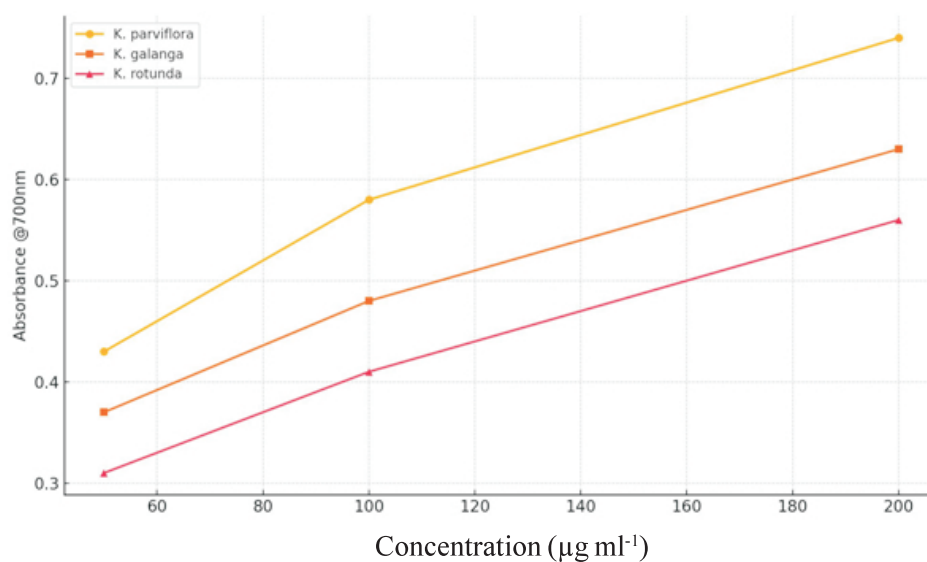


Figure 3. Reducing power of *K. parviflora*, *K. galanga*, and *K. rotunda*

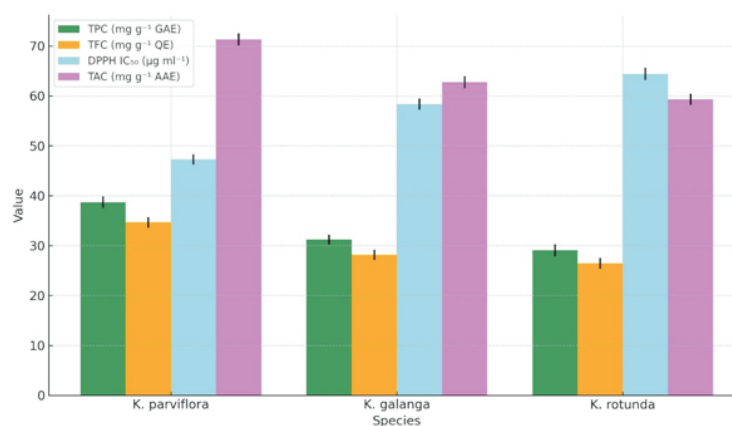


Figure 4. Total phenolic content (TPC), total flavonoid content (TFC), DPPH IC_{50} value, and total antioxidant capacity (TAC) of *K. parviflora*, *K. galanga*, and *K. rotunda*. Values are represented as mean \pm standard deviation (n = 3)

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