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POLARITY - DRIVEN PARTITIONING OF MAJOR PHYTOCHEMICALS IN *Kaempferia* SPECIES FROM MANIPUR

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ABSTRACT

The effect of solvent polarity on phytochemical distribution in three *Kaempferia* species (*K. parviflora, K. galanga, and K. rotunda*) was assessed during 2024 2025. Sequential extraction was performed using solvents of increasing polaritypetr oleum ether, benzene, ethyl acetate, ethanol, and methanoland all extracts were standardized to a concentration of 5 mg ml^{-1}. Quantitative estimation of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Alkaloid Content (TAC), and Total Terpenoid Content (TTC) revealed a strong solvent polaritydependent partitioning of phytochemicals, with statistically significant differences among solvent extracts ($p < 0.05$). Polar solvents, particularly methanol and ethanol, yielded significantly higher levels of TPC, TFC, and TAC across all three species, indicating their effectiveness in extracting hydroxyl-rich phenolics, flavonoids, and alkaloids. Among the species investigated, *K. galanga* consistently exhibited the highest concentrations of these bioactive compounds, followed by *K. rotunda* and *K. parviflora*. In contrast, non-polar solvents showed significantly higher TTC values, reflecting the preferential extraction of lipophilic terpenoids and essential oils, with petroleum ether extracts recording the maximum terpenoid content across all species. Pearsons correlation analysis demonstrated a strong positive correlation between solvent polarity and TPC, TFC, and TAC, while TTC showed a significant negative correlation with solvent polarity. These findings confirm solvent polarity as a critical determinant of phytochemical recovery and validate the effectiveness of polarity-based sequential extraction. Furthermore, the enrichment of phenolic- and flavonoid-rich fractions in polar extracts suggests their potential contribution to antioxidant activity, highlighting *Kaempferia* species as promising sources of natural bioactive compounds.

(Key words: *Kaempferia* species, sequential extraction, phytoconstituents, solvent polarity, quantitative analysis)

INTRODUCTION

Medicinal plants remain vital sources of bioactive compounds for traditional therapies and modern drug discovery (Evans, 2009; Harborne, 1998). Among them, *Kaempferia* species (Zingiberaceae) are widely used in ethnomedicine across Southeast Asia, including Manipur, India (Singh *et al*., 2025a; Singh *et al*., 2025b). These species are traditionally employed to treat digestive, inflammatory, and infectious disorders, attributed to their rich secondary metabolite content such as phenolics, flavonoids, alkaloids, and terpenoids. Phytochemical studies have identified diverse bioactive constituents, including flavonoids, phenolic acids, and diterpenoids, which contribute to their antioxidant, antimicrobial, and anti-inflammatory activities (Sharifi-Rad *et al*., 2017; Khan *et al*., 2025).

Efficient extraction is essential for accurate phytochemical profiling and bioactivity assessment, and largely depends on solvent selection, which governs compound solubility and yield (Sasidharan *et al*., 2011). Solvent polarity plays a critical role, with polar solvents (methanol, ethanol) favoring the extraction of hydroxyl-rich phytochemicals such as phenolics and flavonoids, non-polar solvents (petroleum ether, benzene) preferentially extracting lipophilic constituents including terpenoids and sterols, and semi-polar solvents like ethyl acetate yielding intermediate fractions with a broader phytochemical spectrum (Harborne, 1998; Evans, 2009).

Sequential extraction using solvents of increasing polarity enables systematic fractionation of plant material based on solubility differences, facilitating targeted qualitative and quantitative phytochemical analyses (Sasidharan *et al*., 2011). Quantitative assays such as total phenolic, flavonoid, alkaloid, and terpenoid contents are widely used to assess extraction efficiency and compare phytochemical abundance across solvent fractions (Swain and Hillis, 1959; Gao *et al*., 2008). These parameters also provide a scientific basis for correlating phytochemical composition with antioxidant and other biological activities (Halliwell and Gutteridge, 2015).

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Although phytochemical profiles and antioxidant potential of *Kaempferia* species have been reported using individual solvents (Khan *et al*., 2025), systematic evaluations of solvent polarity effects on quantitative phytochemical distribution remain limited, particularly for species from north-eastern India. Such analyses are essential for optimizing extraction strategies, improving reproducibility, and supporting bioactivity-guided fractionation.

The present study (20242025) aimed to quantitatively assess major phytoconstituents in *Kaempferia* species from Manipur using a polarity-based sequential extraction approach. All extracts were standardized at 5 mg ml^{-1} to evaluate the effect of solvent polarity on the distribution of phenolics, flavonoids, alkaloids, and terpenoids.

MATERIALS AND METHODS

Study design and objective

The present study was designed to quantitatively evaluate major phytoconstituents in *Kaempferia* species collected from Manipur using solvents of varying polarity. The investigation focused on the estimation of total phenolics, flavonoids, alkaloids, and terpenoids to understand solvent-dependent variation in phytochemical abundance.

Plant material collection and authentication

Fresh rhizomes of *Kaempferia* species were collected from selected locations in Manipur, thoroughly washed, shade-dried at room temperature, and ground into a fine powder. Botanical authentication was performed by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference (Bridson and Forman, 1999).

Preparation of extracts

Powdered plant material (2550 g) was subjected to sequential Soxhlet extraction using solvents of increasing polaritypetroleum ether , benzene, ethyl acetate, ethanol, and methanolto fractionate phytoconstituents based on solubility. After each extraction, the residue was dried before proceeding to the next solvent. The resulting extracts were re-dissolved in their respective solvents to obtain a final concentration of 5 mg ml^{-1} and stored at 4 °C until further analysis (Table 1) (Harborne, 1998).

Quantitative phytochemical analysis

The total phenolic content (TPC) of the extracts was quantified using the FolinCiocalteu reagent method as described by Singleton and Rossi (1965). Gallic acid served as the calibration standard, and the results were expressed as milligrams of gallic acid equivalents g^{-1} of extract (mg GAE g^{-1}). Total flavonoid content (TFC) was estimated by the aluminum chloride colorimetric assay following the protocol of Chang *et al*. (2002), using quercetin as the reference compound, and values were reported as milligrams of quercetin equivalents g^{-1} of extract (mg QE g^{-1}). Total alkaloid content (TAC) was determined using the bromocresol green method according to Harborne (1998), with atropine employed as the standard, and the results were expressed as milligrams of atropine equivalents g^{-1} of extract (mg AE g^{-1}). Total terpenoid content (TTC) was assessed using the vanillinsulfuric acid assay as outlined by Gao *et al*. (2008), using linalool as the standard, and the content was expressed as milligrams of standard equivalents g^{-1} of extract (mg SE g^{-1}).

Statistical analysis

All quantitative estimations were carried out in triplicate and expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate the effect of solvent polarity on phytochemical content. Tukey s multiple comparison test was applied to determine significant differences among solvent extracts. Differences were considered statistically significant at $p < 0.05$ (Steel and Torrie, 1980; Tukey, 1949).

RESULTS AND DISCUSSION

The quantitative profiling of phytoconstituents in three *Kaempferia* species (*K. parviflora, K. galanga, and K. rotunda*) at a standardized extract concentration of 5 mg ml^{-1} demonstrated a pronounced influence of solvent polarity on phytochemical distribution (Table 2). Sequential extraction using solvents of increasing polarity (Table 1) resulted in distinct partitioning of phytochemical classes, with statistically significant differences observed among solvent extracts ($p < 0.05$).

Across all three species, polar solvents (methanol and ethanol) yielded significantly higher Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Alkaloid Content (TAC) compared to semi-polar and non-polar solvents. In *K. parviflora*, TPC increased from 6.5 ± 0.4 mg GAE g^{-1} in petroleum ether to 41.9 ± 1.9 mg GAE g^{-1} in methanol, while TFC showed a comparable trend, reaching a maximum of 36.8 ± 1.6 mg QE g^{-1} in methanolic extracts. A similar polarity-dependent increase was recorded for TAC, with the highest value observed in methanol (28.7 ± 1.3 mg AE g^{-1}).

Among the species studied, *K. galanga* consistently exhibited the highest levels of TPC, TFC, and TAC in polar extracts, followed by *K. rotunda* and *K. parviflora*. Methanolic extracts of *K. galanga* recorded maximum values of 52.7 ± 2.2 mg GAE g^{-1} (TPC), 45.6 ± 2.0 mg QE g^{-1} (TFC), and 36.9 ± 1.6 mg AE g^{-1} (TAC), indicating a richer abundance of polar bioactive compounds. Ethanol extracts showed slightly lower but comparable values, confirming the efficiency of polar solvents in extracting hydroxyl-rich phenolics, flavonoids, and alkaloids (Harborne, 1998; Evans, 2009). Ethyl acetate extracts displayed intermediate phytochemical concentrations across all species, reflecting their semi-polar extraction capability.

In contrast, Total Terpenoid Content (TTC) exhibited an inverse relationship with solvent polarity. Non-polar solvents, particularly petroleum ether and benzene, showed significantly higher TTC values across all species ($p < 0.05$). Petroleum ether extracts recorded the highest TTC in *K. galanga* (58.7 ± 2.5 mg SE g^{-1}), followed by *K. rotunda* (52.4 ± 2.3 mg SE g^{-1}) and *K. parviflora* (48.6 ± 2.1 mg SE g^{-1}). This trend is consistent with the lipophilic nature of terpenoids and essential oils, which preferentially dissolve in non-polar solvents (Gao *et al*., 2008). Polar extracts contained comparatively lower TTC values, confirming selective enrichment of terpenoids in non-polar fractions.

Pearson s correlation analysis further supported these observations, revealing a strong positive correlation between solvent polarity and TPC, TFC, and TAC, while TTC showed a significant negative correlation with solvent polarity (Pearson, 1895). The overall findings clearly demonstrate solvent polarity as a critical determinant of phytochemical recovery and validate the effectiveness of polarity-based sequential extraction for comprehensive phytochemical profiling of *Kaempferia* species.

Table 1. Sequential extraction of *Kaempferia* species using solvents of increasing polarity, showing major phytochemical classes targeted and final extract concentration

Sl. No.	Solvent	Polarity	Major phytochemical classes targeted	Final extract concentration
1	Petroleum ether	Non-polar	Lipids, sterols, waxes, non-polar terpenoids	5 mg ml^{-1}
2	Benzene	Non-polar	Essential oils, aromatic terpenoids	5 mg ml^{-1}
3	Ethyl acetate	Semi-polar	Flavonoid aglycones, phenolic acids, moderate terpenoids	5 mg ml^{-1}
4	Ethanol	Polar	Phenolics, flavonoids, glycosides, alkaloids	5 mg ml^{-1}
5	Methanol	Polar	Phenolics, flavonoids, tannins, alkaloids	5 mg ml^{-1}

Table 2. Quantitative phytoconstituent content of *Kaempferia* species at 5 mg ml^{-1} extract concentration

Species	Solvent	Polarity	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	TAC (mg AE g^{-1})	TTC (mg SE g^{-1})
K. parviflora	Petroleum ether	Non -polar	6.5 ± 0.4 ^{e}	4.2 ± 0.3 ^{e}	2.1 ± 0.2 ^{e}	48.6 ± 2.1 ^{a}
	Benzene	Non -polar	8.1 ± 0.5 ^{d}	6.0 ± 0.4 ^{d}	3.4 ± 0.3 ^{d}	42.3 ± 1.8 ^{b}
	Ethyl acetate	Semi -polar	22.4 ± 1.2 ^{c}	19.6 ± 1.0 ^{c}	14.2 ± 0.8 ^{c}	26.8 ± 1.3 ^{c}
	Ethanol	Polar	35.7 ± 1.6 ^{b}	31.2 ± 1.4 ^{b}	24.5 ± 1.1 ^{b}	14.3 ± 0.9 ^{d}
	Methanol	Polar	41.9 ± 1.9 ^{a}	36.8 ± 1.6 ^{a}	28.7 ± 1.3 ^{a}	11.6 ± 0.7 ^{e}
K. galanga	Petroleum ether	Non -polar	9.4 ± 0.6 ^{e}	6.8 ± 0.5 ^{e}	3.6 ± 0.3 ^{e}	58.7 ± 2.5 ^{a}
	Benzene	Non -polar	12.6 ± 0.7 ^{d}	9.2 ± 0.6 ^{d}	6.1 ± 0.4 ^{d}	51.3 ± 2.2 ^{b}
	Ethyl acetate	Semi -polar	31.8 ± 1.5 ^{c}	27.6 ± 1.3 ^{c}	21.4 ± 1.0 ^{c}	34.5 ± 1.6 ^{c}
	Ethanol	Polar	44.9 ± 1.9 ^{b}	39.2 ± 1.7 ^{b}	30.8 ± 1.3 ^{b}	20.6 ± 1.1 ^{d}
	Methanol	Polar	52.7 ± 2.2 ^{a}	45.6 ± 2.0 ^{a}	36.9 ± 1.6 ^{a}	16.8 ± 0.9 ^{e}
K. rotunda	Petroleum ether	Non -polar	7.8 ± 0.5 ^{e}	5.3 ± 0.4 ^{e}	2.8 ± 0.2 ^{e}	52.4 ± 2.3 ^{a}
	Benzene	Non -polar	10.2 ± 0.6 ^{d}	7.4 ± 0.5 ^{d}	4.6 ± 0.3 ^{d}	46.1 ± 2.0 ^{b}
	Ethyl acetate	Semi -polar	26.9 ± 1.3 ^{c}	23.4 ± 1.1 ^{c}	17.8 ± 0.9 ^{c}	30.2 ± 1.4 ^{c}
	Ethanol	Polar	39.6 ± 1.7 ^{b}	34.8 ± 1.5 ^{b}	27.1 ± 1.2 ^{b}	17.9 ± 1.0 ^{d}
	Methanol	Polar	46.3 ± 2.0 ^{a}	40.5 ± 1.8 ^{a}	32.6 ± 1.4 ^{a}	14.1 ± 0.8 ^{e}

Values are mean ± SD (n = 3). Different superscript letters within a column indicate significant differences ($p < 0.05$) as determined by one-way ANOVA followed by Tukey s multiple comparison test

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