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Differential distribution of total phenolics and flavonoids in *Kaempferia parviflora* Wall. ex Baker (black ginger)

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ABSTRACT

Kaempferia parviflora, commonly known as black ginger (Thai ginseng), belongs to the Zingiberaceae family and is native to northern and northeastern Thailand. This medicinal plant has gained significant attention for its potential health benefits, attributed to its phenolic and flavonoid constituents. While the rhizomes of *K. parviflora* are widely exploited for their pharmacological properties, the leaves and roots are typically discarded as agricultural waste. However, limited comparative studies have examined the distribution of total phenolic and flavonoid contents across the different plant organs of *K. parviflora*. Hence, this study aimed to investigate the variations in total phenolic (TPC) and total flavonoid (TFC) contents in different parts of *K. parviflora*. It involved the analysis of TPC and TFC in three parts of *K. parviflora*, namely rhizomes, leaves, and roots, using appropriate extraction and quantification methods. The leaves contained the highest concentration of total phenolics (4.921.92 ± 0.002 mg GAE/ g DW), while rhizomes scored the highest concentration of total flavonoids (0.111 ± 0.0001 mg QCE/g DW). There were significant differences in both TPC and TFC between the different parts of *K. parviflora*. Overall, this study provides baseline data that can support future phytochemical characterization, biological validation, and the rational utilization of specific *K. parviflora* plant parts in therapeutic applications.

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1. INTRODUCTION

Kaempferia parviflora is a member of the Zingiberaceae family, which is categorized as a medicinal plant. It has a wide distribution throughout the tropics, particularly in Southeast Asia. *K. parviflora*, also known as Thai black ginger or Thai ginseng, is a herbaceous plant from Thailand that is originally found in the North and Northeast of Thailand as well as in other tropical areas such as Malaysia, Sumatra, and Borneo Island (Chen et al., 2018). Black to purple rhizomes of *K. parviflora* are used as a flavouring agent in local food and have historically been used extensively to cure a variety of common illnesses. This is due to the highest quality of secondary metabolites that can be found in the black rhizome as compared to other types of *Kaempferia*, such as white, yellow, and red (Patanasethanont et al., 2007).

Previous research has demonstrated that the phytochemical analysis of the rhizome of *K. parviflora* revealed numerous secondary metabolites, including alkaloids, glycosides, flavonoids, phenols, polyphenols, lipophenols, saponins, tannins, and terpenes, but no reducing sugar (Yee & Lwin, 2019). There have been studies focusing on the determination and isolation of phytochemical substances in *K. parviflora,* particularly in its rhizomes. Compounds such as flavonoids, terpenoids, and phenolic acids have been found in the rhizomes, and recently, it was proven that leaves could also be used as a source for extracting phytochemical compounds (Park et al., 2021). Besides, another recent study stated that *K. parviflora* contains 25 methoxyflavones, and their glycosides were found and quantified as the predominant flavonoids from rhizome extracts (Asamenew et al., 2019). The presence of these compounds contributes to a wider range of medicinal properties, including antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, and anticancer (Suthhanut et al., 2007; Khalid et al., 2019). *Kaempferia* plants were also found to contain phenolic glycosides, cyclohexane oxide derivatives, diarylheptanoids, and essential oil with a variety of biological properties that provide valuable health benefits due to their phytochemical constituents. Hence, *K. parviflora* has attracted manufacturers to develop products containing rhizome extracts in powder and liquid formulations due to their high methoxyflavone content (Yeasmin et al., 2021). Despite the extensive commercial and pharmacological applications of black ginger rhizomes, other plant parts remain significantly underutilized, while the comparative distribution of bioactive secondary metabolites, particularly phenolics and flavonoids, across different plant organs remains underexplored.

In general, multiple factors, including the type of solvent, solvent concentration, time, temperature, pH, number of stages, liquid-to-solid ratio, and particle size of the plant material, influence the extraction efficiency. Hence, it is difficult to compare the phytochemical contents obtained from the extractions as they were discussed in the literature according to the different extraction conditions (Chaisuwan et al., 2022). *K. parviflora* has been reported to influence multiple biological pathways associated with the improvement of health and energy, which accounts for its widespread use in the pharmaceutical sector. Therefore, the evolution of analytical techniques has yielded a vast amount of information regarding the phytochemical composition and biological activities of *K. parviflora*. Following extraction, evaluating the total phenolic content (TPC) and total flavonoid content (TFC) serves as one of the important indicators of the plant's biological value. Phenolics govern the plant's general antioxidant capacity and oxidative defense, while flavonoids are responsible for its targeted pharmacological effects. Therefore, the aim of this study is to evaluate and compare the TPC and TFC across the leaves, rhizomes, and roots of *K. parviflora* to elucidate tissue-specific differences in phenolic and flavonoid distribution and highlight the potential of utilizing agricultural waste.

2. MATERIAL & METHODOLOGY

2.1 Sample preparation

The *K. parviflora* plant materials used in this study were cultivated in the UiTM Cawangan Selangor greenhouse under an established industrial collaboration with a company involved in the cultivation of *K. parviflora*. The plant material was provided by the company and originated from their established mother plants. The leaves, rhizomes, and roots of *K. parviflora* were dried in the drying oven at 50°C for two days, or until there were no changes in the samples' weight. The dried *K. parviflora* was ground in a food grinder until the samples turned into a powder form. Then, the powdered *K. parviflora* was packed in clean plastic bags and kept in bottles in a dark, dry, and cool storage at 2-8°C.

2.2 Extraction of samples

The sample extraction was conducted according to the methods described by Rahman et al. (Ab Rahman et al., 2018) with some modifications as detailed below. Distilled water was used to extract the phenolic and flavonoid compounds from *K. parviflora*. The extraction was performed at an agitation speed of 150 rpm at 90°C for 120 minutes in a shaking water bath. The leaf, rhizome, and root samples were weighed, and approximately 2 g of dried powdered samples from each plant part were dissolved in 50 mL of distilled water. Following filtration through nylon mesh, the extracts were centrifuged for 15 minutes, and the clear supernatant was collected.

2.3 Processing and storage of water extract

The clear supernatant was collected directly as the liquid aqueous extract without undergoing thermal concentration (e.g., rotary evaporation). This direct liquid handling approach was implemented to minimize potential heat-induced oxidation or degradation and to closely reflect traditional aqueous decoction preparations. The liquid extracts were stored at −18 °C until further analysis. Prior to spectrophotometric analysis, the stored extracts were thawed at room temperature, thoroughly homogenized using a vortex mixer, and analyzed directly on a raw plant dry weight basis (DW).

2.4 Total phenolic content (TPC) analysis

Total phenolic content in the extracts was determined using the Folin-Ciocalteu (FC) method described by McDonald et al. (2001). Standard gallic acid solutions were prepared at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL. Subsequently,10 µL of each gallic acid concentration was added to 200 µl of FC reagent (5-fold diluted with distilled water) and mixed using a vortex mixer for 3 minutes. Sodium carbonate (200 µL, 10% w/v) was added to the mixture. The resulting mixture was allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a UV-VIS spectrophotometer.

For the sample preparation, 10 µL of plant extract (4.0% w/v) was taken, and the procedure as described above for standard gallic acid was followed. The blank for the analysis contained all assay reagents and distilled water without the plant extract or standard. The absorbance readings were used to plot the calibration curve to determine the phenolic content in the extracts. TPC was expressed as milligram

gallic acid equivalent per gram of dry weight (mg GAE/g DW). The TPC in each part of *K. parviflora* was calculated by using the following formula (Osman et al., 2016):

$$C = c\ (v/m)$$

Where C = total phenolic content in mg GAE/g dry extract, c = concentration of gallic acid obtained from the calibration curve in mg/mL, v = volume of extract in mL, and m = mass of extract in grams.

2.5 Total flavonoid content (TFC) analysis

The TFC was determined using the method described by Yang et al. (2009), with quercetin used as the reference standard for calibration. Standard quercetin solution was prepared at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL. Subsequently, 250 µL of standard solution or plant extract (4.0% w/v) was mixed with 1.25 mL of distilled water and 75 µL of sodium nitrate solution (5% w/v). After 6 minutes, 150 µL of aluminum chloride (10% w/v) was added and left standing for 5 minutes prior to mixing with 0.5 mL of 1 M sodium hydroxide and 775 µL of distilled water. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer against a reagent blank consisting of distilled water in place of the sample. TFC was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalent per gram of dry plant weight (mg QCE/g DW).

2.6 Statistical analysis

All analyses were done at least in triplicate, and the results were expressed as means ± standard error of the mean (means ± SEM). The data collected were analysed using Microsoft Excel 2016 and statistically analysed using SPSS® version 17 by using ANOVA and Post Hoc tests, and p values < 0.05 were regarded as significant.

3. RESULTS

3.1 Quantification of TPC

The TPC of different parts of *K. parviflora* was measured using Folin-Ciocalteu reagent, and the results are presented as mg GAE/g dry extract, as shown in Table 1. The values of TPC were obtained from a calibration curve of gallic acid standard, $y = 0.0046x + 0.0617$, with $R^2 = 0.9944$.

3.2 Quantification of TFC

Total flavonoid contents were summarized in Table 1. TFC values were obtained from a calibration curve of quercetin standard, $y = 0.12x - 0.019$, with $R^2 = 0.9905$.

Table 1. TPC and TFC of different parts of *K. parviflora*

Extract	TPC (mg GAE*/g DW)	TFC (mg QCE**/g DW)
Rhizome	4.157 ± 0.020^{a}	0.111 ± 0.0001^{a}
Leaf	4.921 ± 0.002^{b}	0.018 ± 0.0005^{b}
Root	2.693 ± 0.005^{c}	0.022 ± 0.0002^{c}

*GAE = gallic acid equivalent, **QCE = quercetin equivalent. Values are expressed as mean ± standard error mean (SEM), (n=3). Values in the same column followed by a different letter (a-b) are significantly different ($p < 0.05$).

4. DISCUSSION

Plants contain a diverse range of phytochemicals, including phenolics, flavonoids, alkaloids, and terpenoids. These bioactive constituents are believed to be responsible for the potential therapeutic effects of plants and their role in promoting human health and preventing various diseases (Tungmunnithum et al., 2018). In the present study, the TPC and TFC of *K. parviflora* rhizome, leaf, and root extracts were determined. Although the leaves, rhizomes, and roots of black ginger possess potential functional value, the rhizome remains the most extensively utilized plant part due to its established medicinal and commercial applications (Chew & Nyam, 2025).

The extraction protocol in this study utilized distilled water to simulate traditional preparation methods, such as aqueous decoctions, herbal teas, and infusions. While organic solvents are frequently used in laboratory settings to maximize extraction yield, aqueous extraction provides direct insight into the profile of phenolics and flavonoids ingested through traditional consumption. The characteristic flavonoids in *K. parviflora* consist predominantly of polymethoxyflavones (PMFs), which possess highly lipophilic structures with low water solubility (Mohammad Aidiel et al., 2024). As a highly polar solvent, distilled water selectively dissolves hydrophilic phenolics, such as free phenolic acids and glycosides. Thus, the chemical nature of water explains the observed phytochemical yields in this study, which particularly resulted in lower TFC.

Typically, polar flavonoids are extracted using a combination of water and organic solvents, with methanol being the preferred choice due to its high solubility and effectiveness in extracting a broad spectrum of flavonoids (Chaves et al., 2020). In contrast, in this study, distilled water was used as the extraction solvent. The high electronegativity of oxygen indicates a highly polar molecule that may form hydrogen bonds with another polar compound such as a phenolic compound (Nonglang et al., 2022). Water is known as a widely available solvent due to its affordability and its ability to dissolve a diverse range of substances. It is widely considered an environmentally friendly solvent due to its non-toxic, non-flammable, and non-corrosive nature, as well as its abundant availability and minimal impact on the environment (Plaza & Marina, 2019).

Evaluating the TPC and TFC values is essential because these secondary metabolites are the primary bioactive drivers in black ginger. Phenolic compounds are widely recognized for their strong antioxidant and free-radical scavenging capabilities, which mitigate cellular oxidative stress. Meanwhile, flavonoids, particularly the PMFs characteristic of *K. parviflora*, are responsible for the plant's specific pharmacological properties (Gan et al., 2024). Quantifying these chemical classes across different plant parts provides a critical foundation for understanding their functional and commercial potentials.

In this study, the phytochemical analysis showed positive results of *K. parviflora* for the presence of phenolics and flavonoids in different parts of *K. parviflora* (Table 1). The rhizomes show a substantial number of phenolic compounds, although slightly lower than the leaves. According to Krongrawa et al. (2020), their results have major differences compared to this research, as they used gamma radiation and a different solvent in their analysis. The levels of total phenolics and flavonoids in *K. parvilora* rhizomes reported after gamma radiation were 17.88-19.07 mg GAE/g and 15.90-16.68 mg QCE/g, respectively. The differences could be inferred to be due to the radiolytic free radicals generated following ionizing radiation that break covalent and glycosidic linkages binding polyphenols to cell wall polysaccharides. Consequently, it enhances matrix permeability and liberates bound phenolics and flavonoids into the extraction solvent, resulting in higher detectable TPC and TFC compared to non-irradiated control samples (Ahmed & Hassan, 2023).

In addition, there is another study of different *Kaempferia* species that compares the TPC and TFC between the leaf and rhizome extracts, in which this was done on *K. galanga* (Khuntia et al., 2022). It was revealed that extracts from the leaves of *K. galanga* have a higher TPC value than those from the rhizomes, which is consistent with the current findings. Meanwhile, the roots have the lowest TPC value, indicating a comparatively lower presence of phenolic compounds. The higher TPC observed in *K. parviflora* leaves compared to its rhizomes in this study also aligns with *Zingiber officinale* (ginger) varieties, where leaf tissues yielded higher total phenolics than young rhizomes (Ghasemzadeh et al., 2010b).

The high TPC in leaves is primarily driven by light exposure. Direct solar UV radiation activates key enzymes in the phenylpropanoid pathway such as L-phenylalanine ammonia-lyase (PAL), which further induces the synthesis of simple phenolic acids (Ghasemzadeh et al., 2010a). These compounds act as UV screens and oxidative stress mitigators, hence resulting in a high TPC accumulated in leaves, which is primarily composed of non-flavonoid compounds (Chowdhary et al., 2021). Conversely, the 6-fold higher TFC in rhizomes than in leaves reflects the specialized role of subterranean storage organs, which serve as long-term metabolic sinks in Zingiberaceae species (Ghasemzadeh et al., 2010a). They express specific enzymes (O-methyltransferases) that convert basic flavonoid precursors into polymethoxyflavones (PMFs) which accumulate in internal storage cells to protect against pathogenic bacteria and fungi (Mierziak et al., 2014), accounting for the higher flavonoid concentration in rhizome tissue. Meanwhile, the roots exhibited the lowest TPC and TFC. This might be due to their primary function in water uptake rather than storage or plant defense.

Previous studies revealed that *K. parviflora* rhizome extract had 10 methoxyflavones, which are known as flavonoid compounds that belong to the flavone subclass. According to AI Elshamy et al. (2019), the rhizomes of *K. parviflora* contain flavonoid nuclei that are characterized by the presence of methoxy groups in specific positions. These results highlight organ-specific differences in secondary metabolism, with *K. parviflora* leaves, rhizomes, and roots each showing distinct patterns of phenolic and flavonoid accumulation. This may affect the antioxidant activities and other biological properties of the plant extract (Nonglang et al., 2022). In addition, statistical analysis conducted in this study indicated that there is a significant difference in TPC and TFC values between all different parts of *K. parviflora* (p <0.05). This means that different parts of *K. parviflora* have different values for TPC and TFC, which can be beneficial for further analysis related to therapeutic uses.

5. CONCLUSION

Significant differences in TPC and TFC were observed among all plant parts, highlighting the heterogeneous distribution of bioactive compounds within *K. parviflora*. These findings provide insights into the differential accumulation of phenolic and flavonoid compounds in different plant parts. This variation may be attributed to differences in the distribution of specific compound classes, whereby leaves typically accumulate phenolic acids, whereas subterranean parts may preferentially accumulate polymethoxyflavone (PMF) flavonoids. Further studies are recommended to isolate and characterize the specific compounds responsible for these differences and to investigate their pharmacological potential. Such investigations could contribute to the development of novel pharmaceutical and nutraceutical products. Overall, this study provides a valuable foundation for future research aimed at optimizing the therapeutic applications of *K. parviflora* through the strategic selection of plant parts enriched in bioactive compounds.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest

AUTHORS' CONTRIBUTIONS

Hanis Athirah Hilmi: Methodology, formal analysis, investigation, and writing of the original draft. **Nurul Izzati Osman:** Conceptualisation, supervision, review, and validation.

DECLARATION OF GENERATIVE AI IN THE WRITING PROCESS

The authors used ChatGPT (OpenAI) and Grammarly solely for language refinement and improvement of manuscript readability The AI tools did not generate novel ideas or data. The authors thoroughly reviewed, verified, and revised the content, and take full responsibility for the originality and integrity of the article.

ETHICS STATEMENT

Not applicable. The authors declare that this research did not involve human or animal subjects.

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