Sand Ginger versus Real Ginger: Investigating the composition of *Kaempferia Galanga*

Gingembre de sable vs gingembre véritable : étude de la composition de *Kaempferia galanga*

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Abstract | Résumé

The aromatic rhizome *Kaempferia Galanga*, also known as sand ginger, has traditionally been used to relieve cough, inflammation and high blood pressure. This study aims to determine an optimal extraction method and ascertain the composition of commercially available *Kaempferia Galanga* powder. The extraction efficiencies for acetonitrile maceration extraction, microwave hydrodistillation, and supercritical-CO_2 (sc-CO_2) extraction are compared, with a focus on the target bioactive compounds ethyl cinnamate and ethyl p-methoxycinnamate. The results showed that the main essential oil components ethyl cinnamate, n-pentadecane, and ethyl p-methoxy cinnamate are present in the extracts of store-bought sand ginger powder. Acetonitrile maceration and sc-CO_2 extractions have similar composition, with the three main compounds extracted alongside long-chain fatty acids and terpenoids such as cyperene and germacrene–D. On the other hand, microwave hydrodistillation, followed by solid-phase extraction, produced a profile containing mainly ethyl cinnamate and ethyl p-methoxycinnamate with minimal co-extracted impurities. Processing 500 ml of hydrosol through solid-phase extraction yielded 48.3 mg of extract containing the two main bioactive compounds. Final derived concentrations for ethyl cinnamate, n-pentadecane, and ethyl p-methoxy cinnamate were 0.43-0.45, 0.24-0.26, and 8.7-8.9 mg/g (acetonitrile extract); 7.1, nil, and 16.3–16.4 mg/L (hydrosol); and 2.9%, 1.5%, and 92.9% (sc-CO_2).

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Le rhizome aromatique *Kaempferia galanga*, également connu sous le nom de gingembre de sable, est traditionnellement utilisé pour soulager la toux, l'inflammation et l'hypertension. Cette étude vise à déterminer une méthode d'extraction optimale et à établir la composition de la poudre de *Kaempferia galanga* disponible dans le commerce. Les efficacités d'extraction par macération à l'acétonitrile, hydrodistillation assistée par micro-ondes et au CO_2 supercritique (sc-CO_2) sont comparées, en mettant l'accent sur les composés bioactifs cibles : le cinnamate d'éthyle et le p-méthoxycinnamate d'éthyle. Les résultats montrent que les principaux constituants de l'huile essentielle — le cinnamate d'éthyle, le n-pentadécane et le p-méthoxycinnamate d'éthyle — sont présents dans les extraits de poudre de gingembre de sable achetée en magasin. Les extractions par macération à l'acétonitrile et par sc-CO_2 présentent une composition similaire, avec ces trois composés principaux extraits en plus d'acides gras à longue chaîne et de terpènoïdes tels que le cyperène et le germacrène-D.

Keywords: Kaempferia galanga; sand ginger; GC-MS; ethyl cinnamate; ethyl p-methoxycinnamate; supercritical CO_2 extraction; microwave hydrodistillation; phytochemical analysis; bioactive compounds; medicinal plants

Introduction

Kaempferia Galanga, commonly known as sand ginger (沙姜), is a frequently used spice for traditional cuisines and for medicinal purposes to treat ailments such as inflammation, cough, and high blood pressure in South-East Asia. However, *K. Galanga* is not botanically related to common ginger, *Zingiber officinale*. Although they belong in the same family, Zingiberaceae, they differ in genus and sand ginger lacks the distinct volatile compounds geraniol, borneol, terpineol, and zingiberene (1). Morphologically, sand ginger has a small, rounded appearance in reddish-brown colour, while common ginger has a long-branched structure with a yellowish-brown colour as observed in Figure 1.

Literature has demonstrated that *K. Galanga* contains 19 identified compounds as determined by gas chromatography-mass spectrometry (GC-MS), (2) with ethyl cinnamate, ethyl p-methoxycinnamate, and n-pentadecane making up the highest abundance. Ethyl cinnamate is commonly used as a fragrance and food additive. A recent study on ethyl cinnamate proves its ability to block tumor growth by directly interfering with VEGF (Vascular Endothelial Growth Factor)/VEGFR2 signaling (3). On the other hand, ethyl p-methoxycinnamate is shown to have the ability to inhibit the activity of the cancer survival protein, transcription factor NF-κB (4). Thus, when used in combination with paclitaxel, which itself induces NFκB activation, it restores and enhances the chemotherapy compound's interference with the growth and division of cancer cells (4).

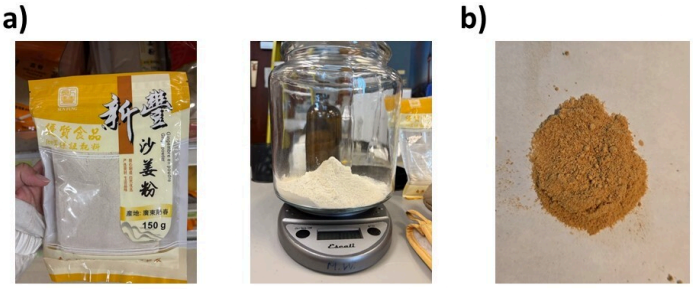

Figure 1. a) *Kaempferia Galanga* (sand ginger) vs b) *Zingiber officinale* (ginger).

This study seeks to ascertain the volatile composition of store-bought *K. Galanga* powder. Additionally, maceration extraction, microwave hydrodistillation, and supercritical-CO_2 (sc-CO_2) extraction will be utilized to identify the optimal extraction method for the ethyl cinnamate and ethyl p-methoxy cinnamate for future applications.

Methods

Acetonitrile Maceration Extract

Commercially acquired *K. Galanga* powder was sourced from Guangdong, China. Approximately one g of the sample was extracted with acetonitrile using the cold maceration method. After 24 hours, the solution was filtered and used for GC-MS analysis.

Supercritical-CO_2 Extraction

Approximately 10 g of *K. Galanga* powder was placed in the sample holder of an SFT-250 SFE System from Supercritical Fluid Technologies Inc. operating at 300 bar and 45 °C. It was subjected to a five-minute soak, followed by five minutes of extraction and collection. This cycle was repeated at least six times for a total of one hour.

Microwave Hydrodistillation

Approximately 108 g of *K. Galanga* powder was evenly placed along the inner walls of a large microwave-safe jar with a collection beaker positioned in the center. The sample was then hydrated with 300 ml of boiling water, and the opening of the jar was covered with an ice cone of distilled water. This setup was then placed into the 1,000-Watt microwave for seven minutes, and the heating cycle was repeated six times. Rising vapour in the container condenses on the ice cone and subsequently drips into the collection beaker. The collected hydrosol was passed over a C18 solid-phase extraction (SPE) cartridge, and the organics were eluted with one:one (v/v) acetonitrile: methanol for GC-MS analysis.

Instrument Method

One µl of sample was injected into the Agilent 6890 GC with mass spectrometer detector (MSD). The column was a 30 m Agilent 19091J-433 HP-5 column, with an internal diameter of 250 mm, a stationary phase thickness of 0.25 mm, He carrier gas with a flow rate of 30 cm/s. The oven temperature was programmed from 80 °C for two minutes, then increased by 15 °C/min to 300 °C. Chemical identification was carried out by comparing the mass spectra of each chromatographic peak with the National Institute of Standards and Technology (NIST) database (5). The hydrosol and acetonitrile maceration extract results were quantified using an external calibration curve of cinnamaldehyde standard, Figure 2.

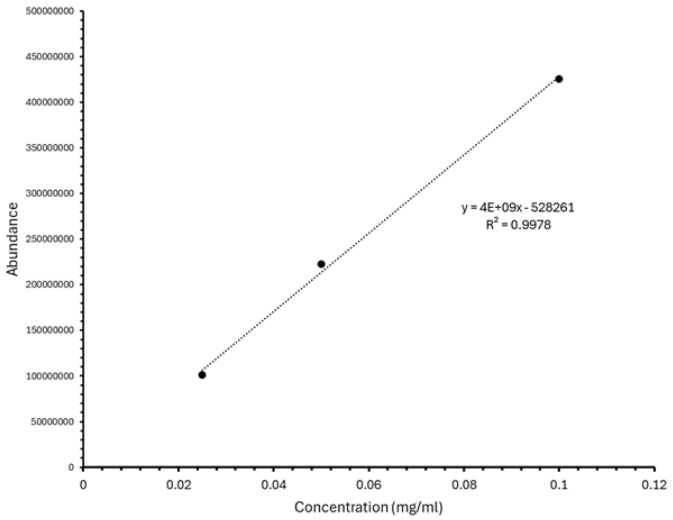

Figure 2. External calibration curve of cinnamaldehyde standard.

Results and Discussion

The three major compounds of *K. Galanga* essential oil: ethyl cinnamate, n-pentadecane, and ethyl p-methoxy cinnamate, were present in the samples as expected; however, other terpenoids and terpenes were present only in trace amounts and thus were not quantified, Figures 3 and 4 (2). The low abundance could be explained by the unknown length of time the commercially acquired powder was dried and exposed to the atmosphere, potentially losing many compounds due to oxidation. The compounds in the *K. Galanga* hydrosol and acetonitrile extracts were quantified using an external calibrant of cinnamaldehyde, whereas only a relative percent composition was established for the sc-CO_2 extract. The calculated retention indices (RI) for ethyl cinnamate (RI =1476), n-pentadecane (RI = 1499), and ethyl p-methoxy cinnamate (RI = 1762) and mass spectra were consistent with NIST values, validating their identity (5). The derived quantities are shown in Table 1.

Table 1. Quantified Target Constituents of *K. Galanga*

	Compounds	Acetonitrile (mg/g)	Hydrosol (mg/L)	sc-CO_2 (% of Total)
1	ethyl cinnamate	0.43-0.45	7.1	2.9%
2	n-pentadecane	0.24-0.26	-	1.5%
3	ethyl p-methoxy cinnamate	8.7-8.9	16.3–16.4	92.9%

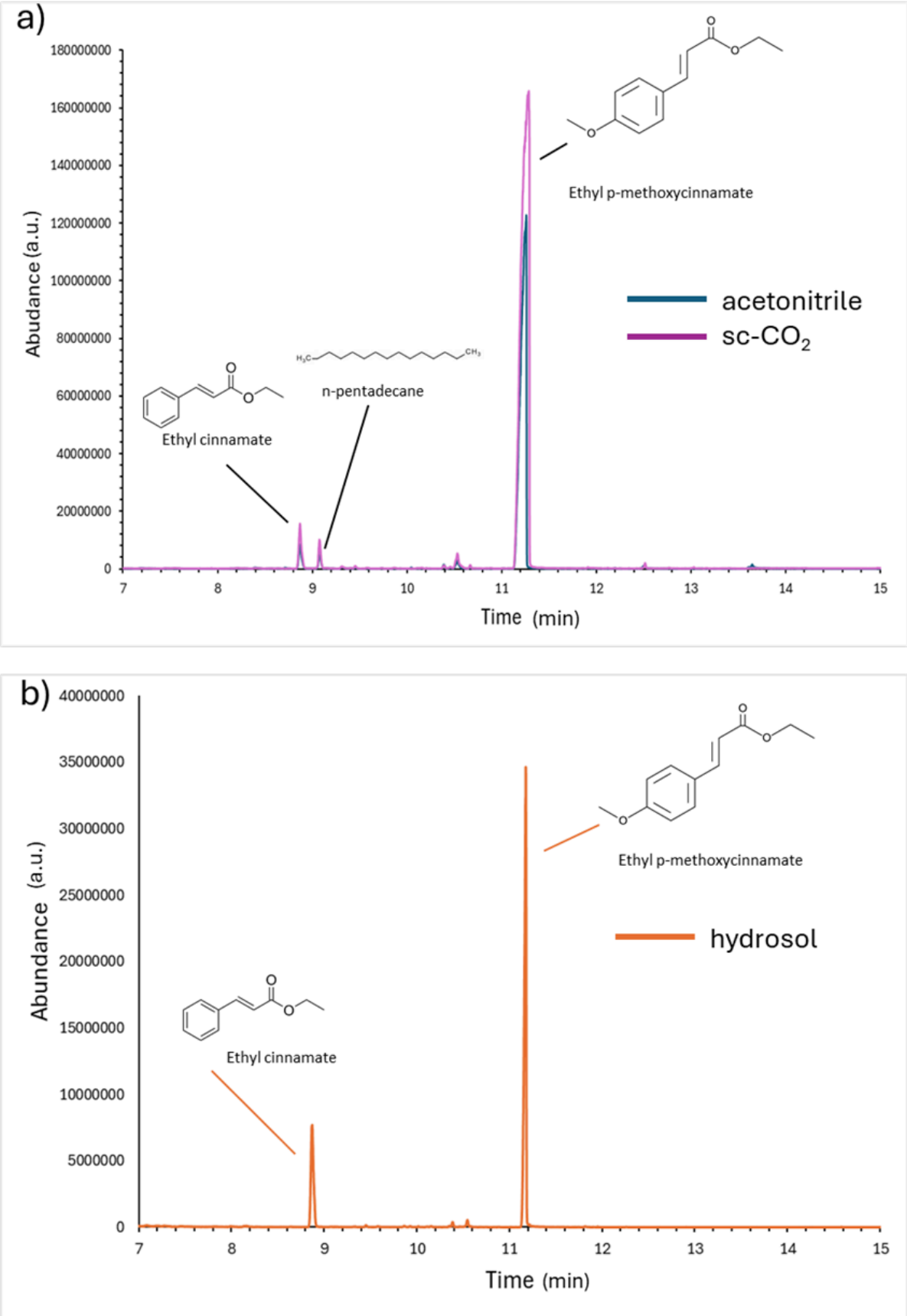

Figure 3. Chromatograms and compound identification for a) acetonitrile and sc-CO_2 extracts, and b) hydrosol.

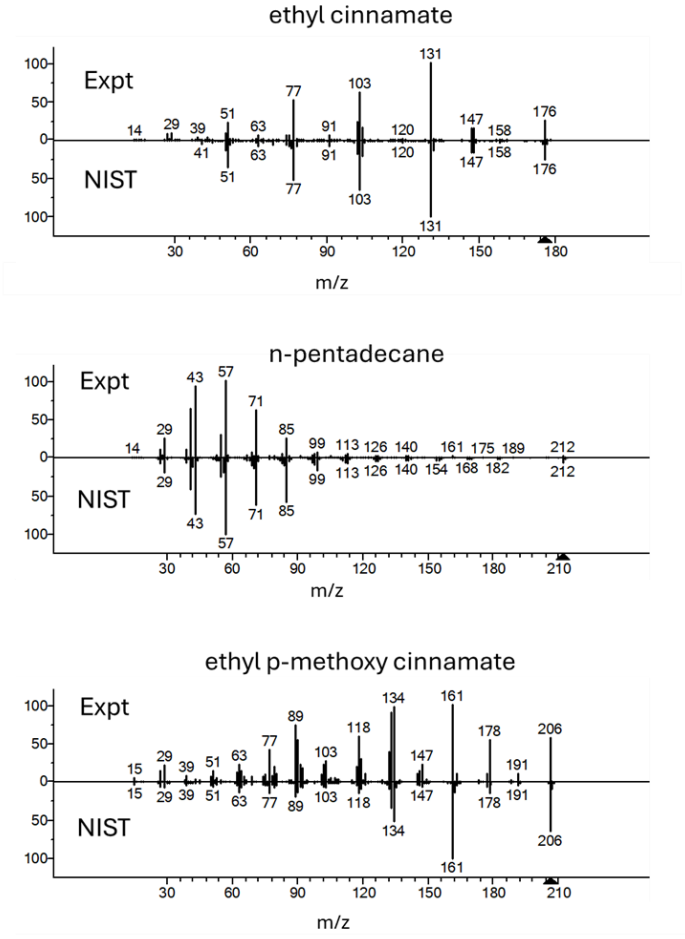

Figure 4. Observed mass spectra (Expt) of ethyl cinnamate, n-pentadecane, and ethyl p-methoxy cinnamate compared to the NIST database.

In the acetonitrile and sc-CO_2 extractions, multiple minor peaks could be observed in the chromatograms due to the low polarity solvent compared to water. This leads to the creation of extracts with unwanted impurities, which subsequently necessitate more purification, time and cost. However, these methods are selective in extracting ethyl p-methoxycinnamate in approximately the same abundance as ethyl cinnamate, and there is ten times more ethyl p-methoxycinnamate in these two extracts compared to the hydrosol, which may be due to its lower volatility. On the other hand, n-pentadecane was not present in the hydrosol extract due to the solvent's polarity, demonstrating selectivity for the two cinnamates. The hydrosol also does not contain significant amounts of the fatty acids present in the other two extracts, and thus, potentially, is more suitable for direct isolation of the target compounds.

It should also be noted that fresh *K. Galanga* material could not be obtained. This may limit the abundance of the target compounds. Additionally, SPE to remove the target compounds from water is not foolproof; the wastewater from preparing the SPE cartridges did have a similar smell to the sample, indicating that some volatile compounds were lost during processing and not caught by the SPE, decreasing their final concentration.

Due to high concentrations of both ethyl cinnamate and ethyl p-methoxycinnamate in this rhizome and their proven properties, this easily acquired, commercially dried plant material could be extracted in large amounts to develop new drugs. However, the optimal extraction method is dependent on the purpose. On one hand, natural product chemists would prefer a natural solvent for research, therapeutic and cosmetic purposes. The hydrosol extraction provides a water-based and cleaner extract, which is a more suitable choice for compound isolation. On the other hand, despite acetonitrile and sc-CO_2 extraction delivering a broader yield of volatiles, they have a larger total yield of the two cinnamates and may be more suited and efficient for industrial-scale operations.

Conclusions

While sc-CO_2 and acetonitrile extraction yielded a broader range of compounds and higher cinnamate abundance, they may demand more elaborate purification. Microwave hydrodistillation displayed a superior selectivity for the targeted volatile polar compounds. Distillation also produced a cleaner extract without the long-chain fatty acids, facilitating easier purification. The hydrosol component is often discarded in essential oil creation, but could now be repurposed, improving sustainability.

Acknowledgements

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