Perceiving Cineole in Cardamoms for Chromatography Practical Procedures

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ABSTRACT

The cardamom or *Elettaria cardamomum* (L.) Maton, is a highly valued spice, worldwide. However, insufficient sources of these *E. cardamomum* as crude drug, involving work on various organic solvents, have limited its potential to be utilized as an active ingredient in sustainable pharmaceutical research education and industry. Therefore, the purpose of this study is to extract *E. cardamomum* using the maceration method and to screen the presence of phytochemicals using thin layer chromatography (TLC) for practical class. Dichloromethane was utilized as the solvent extraction to macerate the cardamom's fruits and tea samples. Meanwhile, a mixture of hexane: ethyl acetate (8:2 v/v) and hexane: diethyl ether (8:2 v/v) were prepared as the mobile phase. The standard chemical reference used was 1,8-cineole, also known as eucalyptol. It was found to have the retention factor (Rf) value of 0.82 using a mobile phase, comprising of hexane: ethyl acetate (8:2, v/v). The Rf values of the fruits' compounds ranging from 0.79-0.82, with purple-coloured spots, were identified as 1,8-cineole, indicating a consistent presence of this compound across the samples. 1,8-cineole is documented for its roles in providing characteristic fragrance and potential health benefits of the cardamoms. In summary, this laboratory protocol was deemed suitable for the chromatography practical procedures, in designing new curriculum for the pharmacy undergraduates.

Keywords: Cardamomum, Chromatography, Curriculum, Practical, Sustainability

INTRODUCTION

Elettaria cardamomum (*E. cardamomum*) is also known as the "Queen of Spices" (Mohamed Irshad et al. 2026). Originating in the ancient Indian language, it was referred to as "Ella" and is now commonly known as cardamom. This spice originates from the montane monsoon forests of the Western Ghats in Southern India, known as the Cardamom Hills (The Indian Culture Portal, 2024). Although it is native to India, it also grows in other Asian countries and is successfully introduced to Guatemala and Mexico which leads to significant production and export activities (Nair, 2020). Cardamom, treasured in many cuisines, is renowned for its diverse qualities. It offers a refreshing minty taste reminiscing of eucalyptus or camphor, providing a pleasant cooling effect that makes it a prized ingredient in both cooking and traditional uses (Jadav et al. 2018).

E. cardamomum is a perennial herb belonging to the ginger family of Zingiberaceae with stems that can grow up to 5 meters. The identification process of a fruit drug could be performed via microscopy. The biological source of the crude cardamom drug (*Elettaria* species) would consist of the dried, ripe capsule of cardamom. The fruits (Fig. 1) are elongated and three-sided, with a dry outer layer that's 0.5 to 1 mm thick, featuring a rough and woody texture (Nair, 2020). Inside the fruit, three locules are separated by membranous septa and the placentation occurs axially. Each section contains five to eight seeds, clustered together. The size of cardamom pods can vary depending on the type and growing conditions. Their physical properties are also depending on the moisture (Gebreselassie, 2012). Typically, they range from about 1 to 2.5 centimetres, or slightly larger. The dimensions, including the major and minor diameters of a cardamom capsule, could be measured by a digital

vernier calliper. The geometrical mean diameter for a large cardamom was recorded to be 1.8 cm (Khura et al. 2013). The grading of cardamom on a commercial scale is carried out using manual and mechanical methods. However, this step lacks adequate accuracy. Therefore, the electronic nose and computer vision are among the approaches for developing reliable, fast, and non-destructive system to grade quality cardamom capsules and seeds (Godini et al. 2025; 2026). The mechanistic insights of the spice's encapsulated oleoresins were also studied to enhance its stability and functionality (Abishek et al. 2025).



Figure 1 A digital microscope was used at the Imaging Centre, Faculty of Pharmacy, UiTM, to observe *E. cardamomum*'s dried whole fruit specimen (source: authors' collection).

LITERATURE REVIEW

The large or jumbo cardamoms are celebrated for its aroma and high medicinal value, possessing diverse physiological and pharmacological properties (Abdullah & Parvin, 2024). The plant's leaves have a distinct arrangement, with petioles reaching up to 2.5 centimetres long (Nair, 2020). These petioles sheathe at the base, creating a pseudo stem when combined with other sheaths, and a small additional part called ligule remains attached, measuring up to 1 centimetre. It grows in a thick cluster and branched rhizomes emerging from 10 to 20 leafy shoots and several decumbent flowering shoots that lie along the ground (AHPA, 2018; GlobinMed, 2022). The cardamom is a key component of garam masala and holds significant importance in Ayurvedic medicine and other traditional practices (Abdullah & Parvin, 2024). Although it is widely used in culinary and aromatherapy, its exact chemical makeup continues to be studied. The key compounds like 1,8-cineole (also known as eucalyptol, Fig. 2), alpha-terpinyl (α-terpinyl) acetate, sabinene, linalool and linalool acetate have been identified (Castillo et al. 2023). Eucalyptol and α-terpinyl acetate become the most noticeable compound in cardamom fruits (Al-Zereini et al. 2022), compared to others, such as linalool, sabinene, α-terpineol, geraniol, myrcene and limonene (Ashokkumar et al. 2020). There are likely many more yet to be discovered due to the complexity of cardamom's chemical profile. Unlike other spices, such as cinnamon, which boast cinnamaldehyde (Fig. 2) and ginger with gingerol, the cardamom's compound remains elusive. Cardamomin (also known as cardamonin or 2′,4′-dihydroxy-6′-methoxy-chalcone, Fig. 2) was named (Gonçalves et al. 2014), from the fact that it can be isolated from the cardamom spice (Zhu et al. 2025; Zhang et al. 2026).

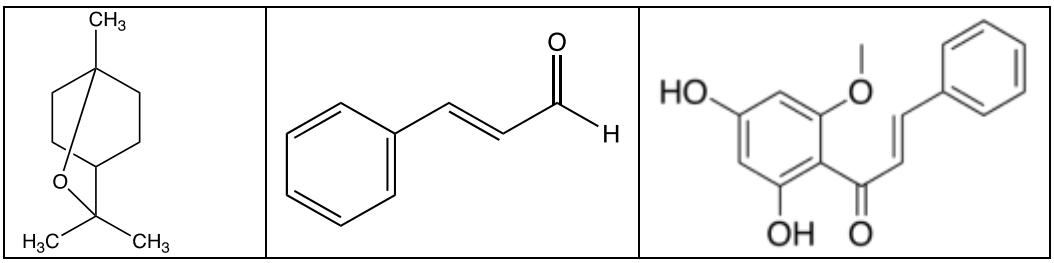
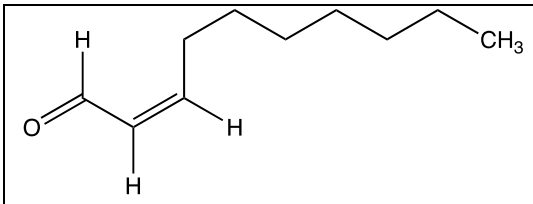


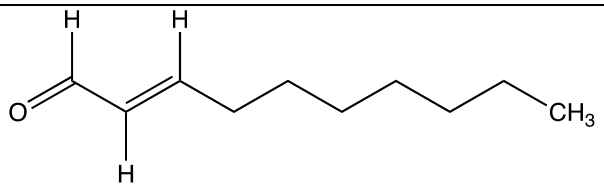
Figure 2 The chemical structure of 1,8-cineole (formula: $C_{10}H_{18}O$, left), cinnamaldehyde or (2E)-3-phenylprop-2-enal (formula: C_9H_8O, middle) and cardamomin (formula: $C_{16}H_{14}O_4$, right).

There are two versions of the cardamom, the green one and the black or dark brown cardamom, which is 3 times bigger than the green cardamom. The black cardamom is known as large or false cardamom, scientifically named

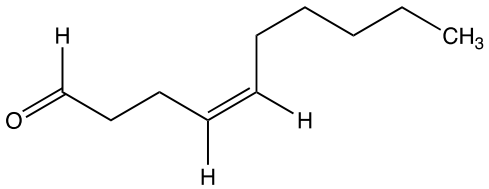
as *Amomum subulatum* Roxb. (Abdullah et al. 2024; Hamdy et al. 2025; Rai et al. 2026) or *Amomum costatum*. It is mainly grown in the Himalayan regions, comprising Nepal and Bhutan. The composition of black cardamom (*Amomum* tsao-ko) (Li et al. 2022) includes aldehydes. Here, a hypothetical compound, called cardamom aldehydes or cardamomaldehydes (Fig. 3), would exist. It is believed that cardamom aldehydes are synonymous with either 2- or 4-decenals. Li et al. (2022) reported that 1,8-cineole (Figure 2) was the most abundant component of black cardamom (28.9–36.6%), followed by (E)-dec-2-enal (8.5–12.0%). While some research highlighted the cardamom's antibacterial properties and many other potentials (Moulai-Hacene et al. 2020; Hoch et al. 2023), however, this chemical aspect is not as well-studied or recognized as it should be. Thus, the research study aimed to extract *E. cardamomum* samples using the maceration method and to screen the presence of phytochemicals, via Thin Layer Chromatography (TLC). Hence, this research would increase the awareness and elucidate the advantageous qualities of *E. cardamomum* fruits, positioning them as a valuable resource for potential health benefits (Singletary, 2022), and any related purpose (Jadav et al. 2018).



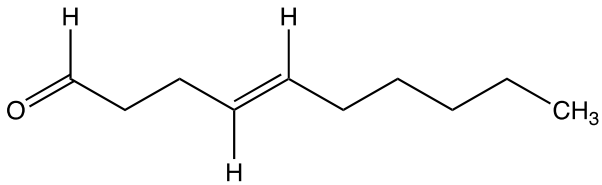
cis-2-decenal
Synonyms: (Z)-2-decenal, (Z)-dec-2-enal
Linear Formula: $OCHCH{=}CH(CH_2)_6CH_3$



trans-2-decenal
Synonyms: (E)-2-decenal, (E)-dec-2-enal
Linear Formula: $OCHCH{=}CH(CH_2)_6CH_3$



cis-4-decenal
Synonyms: (Z)-4-decenal, (Z)-dec-4-enal
Linear Formula: $OCH(CH_2)_2CH{=}CH(CH_2)_4CH_3$



trans-4-decenal
Synonyms: (E)-4-decenal, (E)-dec-4-enal
Linear Formula: $OCH(CH_2)_2CH{=}CH(CH_2)_4CH_3$

Figure 3 The chemical structures of the hypothetical compounds, called cardamom aldehyde or cardamomaldehydes ($C_{10}H_{18}O$).

METHODOLOGY

In the methods, there were chromatographic experiments to determine the similarities and differences of the cardamom fruit and powder extracts, and to determine the prime solvent for extraction and the best mobile phase to separate the compounds.

Chemical, materials and equipment

The fruit samples were purchased from five different retail stores around Selangor Darul Ehsan, Malaysia, and Saudi Arabia in June 2023. The powder sample and two different brands of cardamom tea samples were purchased from retail stores around Selangor Darul Ehsan, Malaysia. All chemical and reagents were analytical grade solvents including methanol, dichloromethane, chloroform, hexane, ethyl acetate, diethyl ether, ethanol, sulphuric-anisaldehyde reagent and distilled water. Pre-coated silica gel 60 F245 sheets (Merck, Darmstadt, Germany), TLC development glass chamber, Bosch GHG 630 DCE Heat gun, analytical balance, UVGL-58 handled ultraviolet (UV) lamp, Cole-Parmer UV viewing cabinet were utilized.

Maceration of the whole cardamom fruits and powdered samples

The fruit samples were obtained in dried form; hence, the drying process was omitted. Each fruit samples were weighed (30 grams) by using an analytical balance and poured into a glass bottle. Each glass bottle was labelled, as seen in Table 1. Next, dichloromethane (60 ml) was added into each bottle, until the fruits were fully immersed. Each bottle was then wrapped with the aluminum foil. Afterward, the bottles were swirled for 2-3 minutes, and the immersed samples were kept for 48 hours in a dark area, at room temperature. Meanwhile, the drying and grinding processes were omitted since the cardamom fruits were readily available in powdered form. 6 grams of the powdered sample were weighed. The sample was then poured into four different amber glass bottles, with each bottle containing 6 grams of the sample. Next, 20 ml of methanol, dichloromethane, chloroform, and hexane were added to respective glass bottles until the fine powders were fully soaked. Each glass bottle was labelled accordingly (Table 2). Similarly, the immersed samples were swirled and kept in a dark area at room temperature.

FINDINGS AND DISCUSSION

The maceration of the fruits from those locations (A, B, C, D, and E, Table 1) was conducted using dichloromethane. Thin-layer chromatography (TLC) was employed with a binary mobile phase of hexane: diethyl ether (8:2 v/v). The TLC analysis utilized a sulphuric-anisaldehyde reagent for spraying, which visualized the well-separated spots developing towards the solvent front. The colour of these spots included violet-pink, grey and purple, with the purple spots being larger and more intense. The reference was 1,8-cineole, which was found to have an Rf value of 0.82. The analysis of the five fruit samples from different locations revealed similar spots, all in line with the reference spot. The Rf values for samples A, B, C, D, and E were 0.79, 0.82, 0.82, 0.82, and 0.79, respectively. The purple-coloured large spots with Rf values of 0.79 and 0.82 were identified as 1,8-cineole (Figure 2), indicating a consistent presence of this compound, across the samples. Alongside the identification of 1,8-cineole, the presence of other unknown compounds was observed, based on their distinct Rf values. While the reference spot for 1,8-cineole was confirmed at the Rf value of 0.82, additional spots with different Rf values emerged, indicating the presence of other constituents within the cardamom extracts. According to Al-Zereini et al. (2022) and Castillo et al. (2023), the possible compound that can be detected in cardamom's fruit are α-terpinyl acetate, linalool, eugenol, geraniol and nerolidol.

Table 1 The cardamom's fruit samples and their Rf values, were observed under UV (254 nm) and visible light.

Label	Retail Place	Sample Origin	Hexane: diethyl ether (8:2 v/v)
A	Puncak Bestari, Selangor Darul Ehsan	Unknown	0.08, 0.23, 0.27, 0.42, 0.53, 0.79, 0.97
B	Puncak Alam, Selangor Darul Ehsan	India	0.08, 0.23, 0.27, 0.42, 0.53, 0.58, 0.82, 0.97
C	Madinah, Kingdom of Saudi Arabia	Unknown	0.10, 0.23, 0.27, 0.40, 0.53, 0.82, 0.97
D	Mecca, Kingdom of Saudi Arabia	Unknown	0.10, 0.23, 0.27, 0.40, 0.53, 0.58, 0.82, 0.95
E	Shah Alam, Selangor Darul Ehsan	India	0.20, 0.27, 0.40, 0.55, 0.58, 0.79, 0.95

Table 2 The summary of extraction and the mobile phases for optimal TLC development of the research specimen.

Powder sample	Extract	Hexane: ethyl acetate (8:2 v/v)	Hexane: diethyl ether (8:2 v/v)	CH_3OH: $CHCl_3$ (8:2 v/v)	Ethanol: $CHCl_3$ (2:8 v/v)
F	CH_3OH	Good separation and distinct	Good separations and distinct	No separation, smearing upwards	Distinct but closely aligned
G	CH_2Cl_2	Good separation and distinct	Good separations and distinct	No separation, smearing upwards	Distinct but closely aligned

H	$CHCl_3$	Good separation and distinct	Good separations and distinct	No separation, smearing upwards	Distinct but closely aligned
I	Hexane	Separate but unclear	Separate but unclear	No separation, smearing upwards	Distinct but closely aligned

The use of *p*-anisaldehyde-sulfuric acid reagent in Thin Layer Chromatography (TLC) is a powerful technique for detecting and visualizing a broad range of organic compounds such as phenols and terpenes. This method involves spraying a developed TLC plate with a mixture of *p*-anisaldehyde and sulfuric acid, followed by heating the plate to approximately 105°C. The heating step accelerates the chemical reactions between the reagent and the compounds on the TLC plate, resulting in the formation of distinct coloured spots. These colour changes occur because the reagent interacts with specific functional groups in the compounds, leading to the formation of chromophores. The violet colours (Fig. 4 & 5) indicate phenolics, while terpenes appear as purple, blue or grey spots (Agatonovic-Kustrin et al. 2019).

The presence of polyphenols, such as phenolic acid and flavonoid, could be proven from the TLC, observed under UV light at a wavelength of 254 nm. The spots appear as dark spots due to their strong absorbance in this region of the UV spectrum. Polyphenols contain conjugated double bond systems and aromatic rings, which can resonate and absorb UV light, effectively. Each of the fruit samples obtained from different locations showed almost similar spots under UV light at 254 nm. The possible flavonoids that could be present in cardamom fruits are luteolin, a basic flavone consisting of two benzene rings and myricetin. Myricetin which is classified as flavonol, consists of three hydroxyl groups attached to the flavanol backbone, which is a three-ring structure made up of two benzene rings (Ramadan et al. 2022). *E. cardamomum* also contains various flavonoid glycosides, including kaempferol-3-O-hexoside, luteolin-7-O-hexoside, and quercetin-3-O-hexoside. Phenolic acids such as gallic acid and chlorogenic acid were detected in trace amounts (Guruvaurappan et al. 2022).

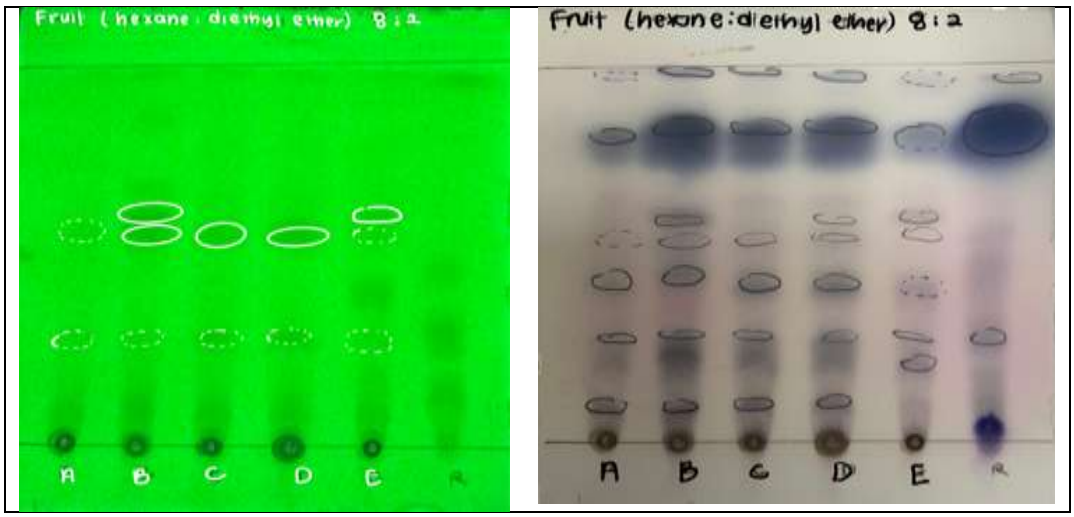
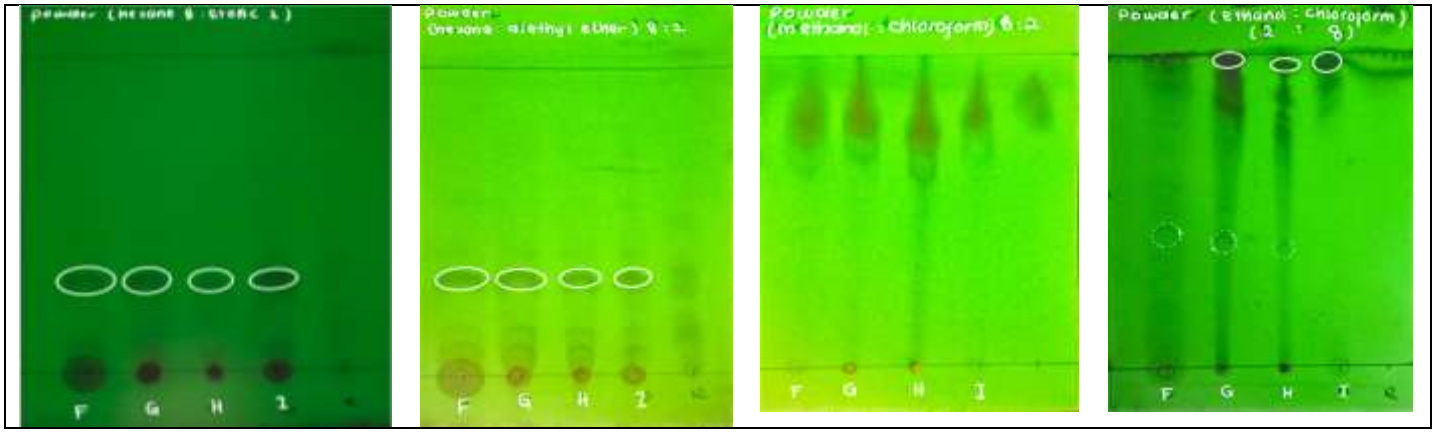


Figure 4 The TLC plate of the fruit's extracts, obtained from different locations, was visualized under UV light (254 nm) (left) and after being treated with sulphuric-anisaldehyde (right).



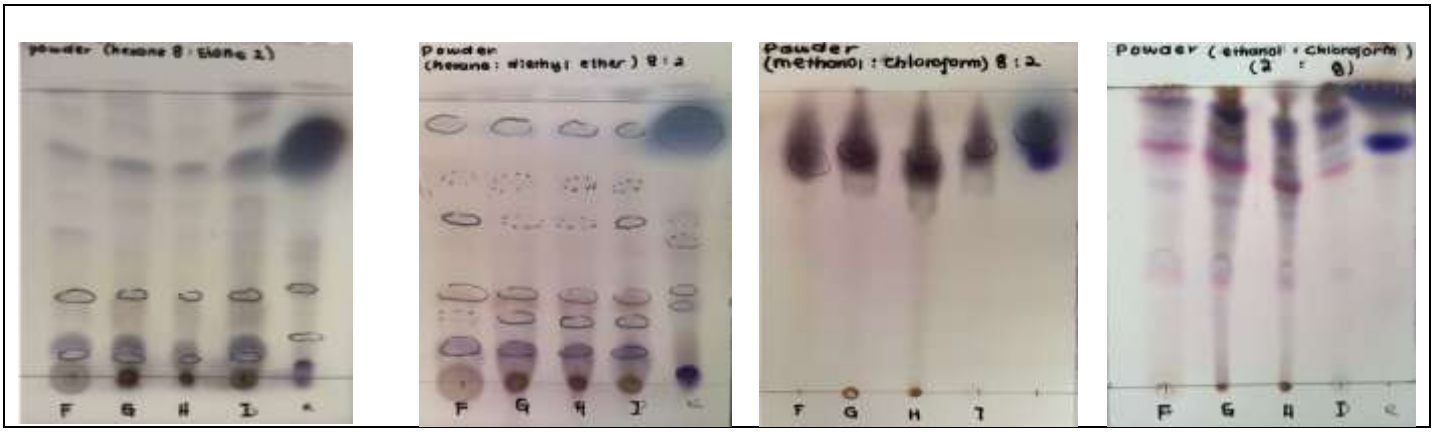


Figure 5 The TLC plates of powdered samples in different extraction solvents under UV light (254 nm, first row) were treated with sulphuric-anisaldehyde reagent (second row).

The presence of similar spots across all samples implies that the primary phenolic acids, terpenes and flavonoid compounds and other UV-absorbing phytochemicals, are consistently present in cardamom fruits, regardless of their origin. On another note, the chemical composition of the cardamom tea is not as much as the fruits. This is due to some volatiles and certain flavonoids which are sensitive to heat. These compounds might be lost during the process of making tea, which involves exposing the cardamom to drying and grinding.

During the maceration of the powdered cardamom, it is crucial to keep the extraction samples away from excessive light and store them in a dark area. This is to preserve the integrity and efficacy of the extracted phytochemicals (Cacique et al. 2020). Light, particularly UV radiation, can cause photodegradation, which leads to the breakdown of sensitive chemical compounds within the plant material (Kosović et al. 2020). This degradation can alter the chemical structure and reduce the potency of the active constituents. Additionally, exposure to light can accelerate oxidation processes, further diminishing the quality and stability of the extracts (Lo Turco et al. 2023). Flavonoids and other phenolic compounds have a significant role in detoxifying reactive oxygen species (ROS) by acting as potent antioxidants that prevent lipid peroxidation and scavenge free radicals (Del Valle et al. 2020). Therefore, to maintain the stability and integrity of all extracted compounds, it is essential to protect the samples from light during maceration. This prevents photodegradation and ensures accurate, reproducible analysis of the plant's phytochemical profile.

CONCLUSION

In the selection of stationary phase for TLC analysis of cardamom, normal phase silica is generally more suitable than reversed phase silica. Cardamom contains a complex mixture of compounds, including 1,8-cineole (Fig. 2), α-terpinyl acetate, and other moderately polar to nonpolar constituents. The normal phase silica gel, with its polar nature, effectively separates these compounds based on their interactions with the polar stationary phase and the less polar mobile phase. This setup is well-suited for the diverse range of polarities found in cardamom constituents. Conversely, reversed phase silica, which involves a nonpolar stationary phase and a polar mobile phase, is typically used for separating highly polar compounds. Given the nature of cardamom's components, normal phase silica offers better separation efficiency and resolution for its characteristic compounds, making it the choice for TLC analysis.

This TLC work would remain invaluable for initial screenings and qualitative assessments in cardamom research. Researchers can quickly identify the key compounds and variations across different parts of the cardamom plants using TLC, laying the groundwork for more targeted investigations using higher-resolution techniques. Such approaches alike the High-Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC), could enhance the resolution and sensitivity, through advanced software. These instrumentations could enable more precise quantification and detailed analysis of compounds in cardamom extracts. Meanwhile, the isomeric cardamom aldehydes or cardamomaldehydes, either 2- or 4-

decenals (Fig. 3) might then be theoretically explored and practically investigated by gas chromatography (Castillo et al. 2023; Yang et al. 2025), as part of sustaining the research and development of this herbal species.

Heading to the future of the TLC analysis of the cardamom, it is recommended to optimize the mobile phase by conducting systematic studies to fine-tune the ratios of solvents in binary and tertiary systems. This approach will help identify the most efficient solvent combinations for separating the diverse range of compounds in cardamom, e.g. the cardamom aldehydes (Figure 4). Additionally, comparative studies between analytical and preparative TLC should be performed to evaluate their efficiency and practicality for various applications, such as routine quality control versus large-scale compound isolation. Developing standardized TLC protocols for cardamom will also be beneficial, ensuring consistent and reproducible results across different studies. Furthermore, expanding the scope of research to include different parts of the plant, not just the fruits, can uncover additional valuable compounds and provide a more comprehensive understanding of the plant's chemical profile. A chromatographic practitioner could then provide a qualified opinion about the sequence of elution and the underlying electronic signatures of the cardamom's constituents; to understand how different parameters may affect their relative retentions (Primdahl et al. 2022). In summary, this protocol was deemed suitable for the chromatography practical procedures, in designing new laboratory module for the pharmacy undergraduates.

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