

Prisma Sains: Jurnal Pengkajian Ilmu dan Pembelajaran Matematika dan IPA IKIP Mataram https://e-journal.undikma.ac.id/index.php/prismasains/index e-mail: prismasains.pkpsm@gmail.com July 2025. Vol. 13, No. 3 p-ISSN: 2338-4530 e-ISSN: 2540-7899 pp. 449-461

# Isolation and Identification of Terpenoids from Artocarpus camansi Leaves Based on Column Chromatography and UV-IR Spectroscopy

#### Ramadani

Chemistry Education Department, Faculty of Tarbiyah and Teacher Training, Institut Agama Islam Negeri Kerinci. Jl. Kapten Muradi, Sungai Penuh, Indonesia. Postal Code: 37112

Corresponding Author e-mail: ramadani.chem@gmail.com

Received: February 2025; Revised: May 2025; Published: July 2025

#### **Abstract**

This study aims to isolate and identify terpenoid compounds from kluwih leaves (Artocarpus camansi). Extraction was done using methanol solvent, followed by n-hexane fractionation, which produced a concentrated blackish green extract. Compounds were separated through column chromatography with silica gel as the stationary phase and n-hexane as the mobile phase, and a mixture of n-hexane: ethyl acetate, producing eight fractions. The third fraction, obtained as green crystals, exhibited a positive reaction to the Liebermann-Burchard reagent, a specific test for the presence of terpenoid compounds. The observed colour change indicated the presence of terpenoids, making this fraction a promising candidate for isolation and detailed analysis. After recrystallisation, white needle-shaped crystals were obtained with a melting point of 141–142.8°C. Purity testing using thin layer chromatography (TLC) showed a single spot in various eluents, indicating that the isolated compound was pure. Further characterisation using UV spectroscopy showed maximum absorption at 210.2 nm, while IR spectroscopy showed the presence of OH, methyl, and non-conjugated C=C double bonds. Based on these data, the isolated compounds can be identified as terpenoid compounds. This study provides a basis for further studies on the structure and biological activity of kluwih leaves.

Keywords: Artocarpus Camansi; Chromatography; Isolation; Spectroscopy; Terpenoid; Kluwih Leaves

**How to Cite:** Ramadani, R. (2025). Isolation and Identification of Terpenoids from Artocarpus camansi Leaves Based on Column Chromatography and UV-IR Spectroscopy. *Prisma Sains: Jurnal Pengkajian Ilmu dan Pembelajaran Matematika dan IPA IKIP Mataram*, 13(3), 449-461. doi:https://doi.org/10.33394/j-ps.y13i3.15526



Copyright© 2025, Ramadani.

This is an open-access article under the <u>CC-BY License</u>.



#### INTRODUCTION

Artocarpus communis, known as kluwih, has significant potential and diverse applications in various domains, including pharmacology, ethnobotany, and traditional medicine. The essential oil derived from Artocarpus communis exhibits various bioactivities such as antibacterial, antimicrobial, antidiabetic, antioxidant, and insecticidal properties, essential for maintaining health and treating chronic disorders. (Mali et al., 2024). In the context of traditional knowledge, Artocarpus communis is known for its medicinal and nutritional value, which contributes to food security and biodiversity conservation. This plant is part of the diversity of edible wild vegetables in Indonesia, although its use is still limited among local communities (Adnan et al., 2023; Navia et al., 2024). Plant extracts, especially methanol extracts, have shown significant antioxidant, anti-inflammatory, wound healing, and photoprotective effects, making them valuable in skin care and cosmetic applications. However, the low water solubility of these extracts is a limitation, which can be overcome through nanoparticle formulations that enhance skin penetration and efficacy (Yang et al., 2021).

Furthermore, Artocarpus communis is part of the Moraceae family, which is known for its therapeutic applications in treating various health conditions, including diabetes, due to its

bioactive compounds (Benjamin et al., 2024; Maqsood et al., 2022). The role of plants in traditional medicine is also highlighted in studies from regions such as Himalayan Kashmir, where medicinal plants are an integral part of the local health care system, underscoring the importance of preserving indigenous knowledge for sustainable use and conservation (Khoja et al., 2024). Overall, Artocarpus communis is a versatile plant with significant potential in pharmacological applications, traditional medicine, and as a nutritional source, which warrants further research and promotion to maximise its benefits (Mali et al., 2024; Navia et al., 2024; Yang et al., 2021).

One of the prominent groups of bioactive compounds found in various plant species, including possibly in Artocarpus communis, is terpenoid compounds. Terpenoids are secondary metabolites composed of isoprene units and are known to have complex chemical structures and broad biological activities, such as antioxidants, antimicrobials, anti-inflammatories, anticancer, and antivirals (Câmara et al., 2024; Zang et al., 2025). The pharmaceutical industry benefits greatly from terpenoids, as they are used in the treatment of various diseases, such as nonalcoholic fatty liver disease (NAFLD), which regulates lipid metabolism, insulin resistance, and inflammation through pathways such as AMPK and PPAR (Yao & Liu, 2022). In addition, terpenoids have shown potential as anticancer agents by inducing apoptosis, inhibiting angiogenesis, and modulating intracellular signalling pathways, which are crucial in cancer therapy (Kamran et al., 2022).

The structural diversity of terpenoids, including sesquiterpenoids, diterpenoids, and triterpenoids, contributes to their broad spectrum of biological activities, making them promising candidates for drug development (Hu et al., 2021; Zang et al., 2025). Advances in genome sequencing and synthetic biology have increased our understanding of terpenoid biosynthesis, enabling the industrial production of these compounds through metabolic engineering (Hu et al., 2021; Q. Wang et al., 2023). Advances in genome sequencing and synthetic biology have increased our understanding of terpenoid biosynthesis, enabling the industrial production of these compounds through metabolic engineering (Q. Wang et al., 2023). In addition to their pharmaceutical applications, terpenoids are an integral part of the food and cosmetic industries, serving as flavoring agents and fragrance components due to their presence in essential oils (Das et al., 2024). Ongoing research and development in terpenoid biosynthesis and their diverse applications underscore their essential role in advancing the pharmaceutical and chemical industries, offering new avenues for therapeutic interventions and industrial applications. (Câmara et al., 2024; H.-Y. Chen et al., 2023).

Previous studies on Kluwih (Artocarpus camansi) leaves are limited, particularly in the comprehensive phytochemical profiling and isolation of specific bioactive compounds such as terpenoids. Most existing studies have focused on preliminary screening or general antioxidant activity, without proceeding to compound-level identification and structural characterisation. In addition, there is a lack of standardised extraction and purification protocols specific to Kluwih leaves, which may result in inconsistent yields and compound purity. These limitations highlight the need for detailed extraction, isolation, and spectral analysis tailored to Kluwih leaf constituents to ensure accurate identification and reproducibility of findings. In addition, the use of portable chlorophyll meters, which are often used in plant studies, can be limited by variations in leaf anatomy. These devices work well on laminar leaves but are less accurate for species with different anatomical structures, suggesting species-specific calibration is needed to improve measurement reliability (Lhotáková et al., 2025). Furthermore, the broader scientific context highlights issues such as the reproducibility of research findings and potential bias in the peer review process, which can impact the integrity and applicability of research results (Johnson et al., 2023). These methodological challenges underscore the need for more tailored approaches and empirical data collection specific to Kluwih leaves to improve the robustness and transferability of research findings in this field.

The urgency of isolating and characterising terpenoid compounds is underscored by their diverse biological activities and potential therapeutic applications. Terpenoids, a large family of secondary metabolites, exhibit a wide range of pharmacological effects, including antiproliferative, anti-inflammatory, antineuroinflammatory, and antiviral activities, making them essential candidates for drug development (Das et al., 2024; Kouno et al., 2024; Liu et al., 2023; Tousif et al., 2023; Zang et al., 2025) For example, isolation of terpenoids from Turraea delphinensis has revealed compounds with significant antiproliferative activity against human tumor cell lines, including those resistant to vinblastine (Kouno et al., 2024). Similarly, terpenoids from Artemisia vulgaris have demonstrated potent antineuroinflammatory effects, surpassing the efficacy of dexamethasone, and have shown promising results against HSV-1, including acyclovir-resistant strains (Liu et al., 2023). The potential of terpenoids as human neutrophil elastase inhibitors further highlights their role in treating inflammatory diseases such as COPD and emphysema (Tousif et al., 2023). The complexity of terpenoid structures requires advanced analytical techniques for their isolation and characterisation, essential for understanding their biochemical processes and optimising their therapeutic potential (Das et al., 2024). Recent advances have identified more than 500 terpenoids with interesting chemical architectures, emphasising the need for further research to explore their full potential as primary drugs (Zang et al., 2025). Thus, isolation and characterisation of terpenoids are essential to advancing pharmacological research and developing new therapeutic agents. This study was conducted to isolate and characterise terpenoid compounds from the leaves of the kluwih plant (Artocarpus communis) to uncover the potential of local bioactive compounds that have not been widely explored.

### **METHOD**

This research was conducted at the Chemistry Research Laboratory, Faculty of Mathematics and Natural Sciences, Padang State University. The research was conducted within a period adjusted to the extraction, isolation, and characterisation stages of terpenoid compounds from kluwih leaves (Artocarpus communis). The main material used in this study was fresh kluwih leaves, since fresh plant material is known to possess higher concentrations of biologically active compounds and essential phytochemicals compared to dried or aged samples, thereby ensuring the reliability and effectiveness of the chemical analysis (Kumar et al., 2022; Pinto et al., 2022). In addition, various solvents and chemical reagents were also used, such as methanol, ethyl acetate, n-hexane, concentrated H<sub>2</sub>SO<sub>4</sub>, acetic anhydride, silica gel, aquadest, and chloroform, as they assist in organic transformations and facilitate the extraction, separation, and identification of phytochemical compounds (Khandelia et al., 2024; Zhang et al., 2021). A thin layer chromatography (TLC) plate was used to monitor the compound separation process.

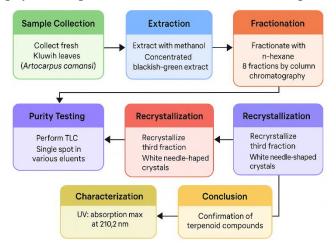


Figure 1. Research Procedure for the Isolation and Identification

The tools used include a set of rotary evaporators to concentrate the extract, a chromatography column as a medium for separating compounds, and general laboratory equipment such as glassware (beakers, Erlenmeyers, test tubes), separating funnels, porcelain mortars, droppers, dropper plates, tweezers, stirring rods, and capillary tubes. For the analysis and characterisation of the isolated compounds, a melting point determinant, a UV spectrophotometer, and an IR spectrophotometer were used to identify functional groups and estimate the chemical structure of the terpenoid compounds obtained (Balachandran et al., 2024; Hao & Cardin, 2023). The detailed steps of the research procedure can be seen in Figure.

### Preparation of Solvents, Samples, and Reagents Used

Before being used in the extraction and separation process, solvents such as technical methanol, technical n-hexane, and technical ethyl acetate are purified through a distillation process to obtain a purer solvent suitable for analysis. Meanwhile, the sample used was fresh leaves of the kluwih plant obtained from the Berok area, Siteba, Padang City. The leaves were cleaned of dirt and dust, then finely chopped to expand the contact surface and facilitate the extraction process of bioactive compounds in the next stage.

As part of the chemical content analysis process in kluwih leaves, the Liebermann-Burchard reagent was prepared, which was used to identify the presence of terpenoid and steroid compounds. This reagent is made by mixing 1 mL of concentrated  $\rm H_2SO_4$  with 20 mL of acetic acid anhydride, then adding 50 mL of chloroform. The mixture is stored in a brown bottle to maintain the stability of the reagent. Furthermore, a series of preliminary tests was carried out to determine the chemical compounds in the sample.

### **Preliminary Test for Chemical Content**

The preliminary test began with testing for alkaloid content. A total of 4 grams of leaf samples were finely chopped, ground with fine sand, and chloroform was added. After grinding was continued, 10 mL of 0.05 N ammonia-chloroform solution (1:20) was added, and the mixture was filtered into a test tube. The filtrate obtained was added with 10 drops of 2 N sulfuric acid, shaken, then left to form two layers. The upper layer (sulfuric acid) was transferred to three test tubes to be tested with Mayer, Dragendorff, and Wagner reagents. The results showed no formation of white, red-orange, or brown deposits, so it can be concluded that the sample does not contain alkaloids. Meanwhile, the lower layer (chloroform) was used to test for terpenoid and steroid content.

Flavonoid tests were carried out by mixing 0.5 grams of the fine sample with 5 mL of methanol, heating it in a water bath for 5 minutes, and then filtering. The filtrate obtained was added to a few drops of concentrated hydrochloric acid and magnesium powder. The absence of a colour change to red or pink indicates that flavonoids were not detected in the sample. Furthermore, the steroid and terpenoid content tests were carried out using a chloroform layer obtained from the alkaloid test. A few drops of the solution were dropped onto a dropper plate and allowed to dry, then five drops of acetic anhydride and three drops of concentrated H<sub>2</sub>SO<sub>4</sub> were added. The appearance of a red-orange colour indicates a positive result for terpenoids, while a blue colour indicates the presence of steroids (Aliunir, 2000, p. 25). Finally, 0.5 grams of sample was put into a test tube to detect saponins, distilled water was added until submerged, then boiled for 2–3 minutes and cooled. After cooling, the solution was shaken vigorously. The absence of stable foam for 15–20 minutes indicates that the sample does not contain saponins.

#### Work procedures

The working procedure for isolating terpenoids is carried out through a series of stages, starting from extraction, fractionation, separation, and compound purification. The first stage is extraction and fractionation. A total of 5 kg of samples were finely chopped and then macerated using methanol solvent for five days, which was carried out three times. After that, the mixture was filtered, and the methanol extract obtained was evaporated using a rotary evaporator until a thick extract of 400 mL was obtained. This extract was then fractionated

using n-hexane solvent eight times. The resulting n-hexane fraction was then evaporated again using a rotary evaporator, and a concentrated n-hexane extract of 26 grams was obtained. The next step is a thin layer chromatography (TLC) test to determine the number of components contained in the extract and the optimal conditions for separation at the column chromatography stage. The concentrated extract sample was spotted on the TLC plate using a capillary tube, then eluted using a mobile phase of n-hexane: ethyl acetate (8:2) in a previously saturated chamber. After elution, the plate was dried and sprayed with Liebermann-Burchard reagent. The TLC test results showed three colored spots, indicating three different components in the extract.

Column chromatography was then carried out using the step gradient polarity elution method using silica gel as the stationary phase and a mixture of n-hexane: ethyl acetate as the mobile phase. Before use, the column was prepared by carefully inserting silica gel slurry in n-hexane into a vertical column coated with cotton at the bottom. 8.3 grams of concentrated n-hexane extract was inserted into the column and eluted successively with the following solvents: pure n-hexane, n-hexane: ethyl acetate (9: 1), (8: 2), (7: 3), (6: 4), and (5: 5), each according to the specified volume. The eluate was collected in 50 vials (each  $\pm$  15 ml), then monitored using TLC. Eluates with the same Rf value were combined into eight fractions. From the third fraction (vials 15–19), crystals were obtained and then purified. The purification process was carried out through recrystallisation using n-hexane solvent. The crystals formed were dissolved in hot n-hexane, then the solution was filtered and cooled until the crystals were reformed. This process was repeated several times until clean white crystals were obtained. The final stage of this procedure was testing the purity of the recrystallised crystals.

## **Purity Test of Isolated Compounds**

The purity of the isolated compounds was assessed using thin layer chromatography (TLC) and melting point determination. During the TLC analysis, the isolated compounds were tested with various eluents, including n-hexane, ethyl acetate, chloroform, and mixtures of n-hexane and ethyl acetate in ratios of 8:2 and 6:4. The results indicated that each eluent produced a single spot, which suggests that the tested compounds were chromatographically pure. The purity assessment continued with melting point determination using the Gallen-Kamp melting point apparatus. A small amount of the crystals was placed in a capillary tube and heated at 5°C per minute. The melting point was recorded from the temperature at which the crystals began to melt until they were completely liquefied. The observations revealed that melting commenced at 141°C and was complete at 142.8°C. This narrow range of melting points further corroborates the high purity of the isolated compound.

### **Characterisation of Isolated Compounds**

Characterisation of isolated compounds is carried out to determine the chemical structure in more depth, especially the presence of double bonds and functional groups. Two spectroscopic techniques are used in this stage: ultraviolet and infrared. In ultraviolet spectroscopy analysis, the main objective is to detect the presence of double bonds in the compound. A total of 1 mg of isolated crystals is dissolved in 100 mL of methanol, then this solution is put into a special cuvette compatible with an ultraviolet spectrophotometer. Absorption measurements are carried out at wavelengths between 200 nm and 400 nm to identify the presence of electron transitions related to the conjugation system or double bonds.

Furthermore, infrared spectroscopy (IR) analysis is carried out to determine the functional groups in the compound structure. The isolated crystals are ground with potassium bromide (KBr) in a ratio of 1:100, then pressed into thin, transparent pellets. The pellet is then mounted on a sample of an IR spectrophotometer, and the absorption intensity is recorded over a frequency range between 4000 cm<sup>-1</sup> and 650 cm<sup>-1</sup>. The resulting spectrum allows the identification of various functional groups based on their characteristic absorption patterns.

Thus, the combination of these two methods provides a fairly comprehensive initial picture of the chemical structure of the isolated compound.

### RESULTS AND DISCUSSION

The results of preliminary tests on the chemical content in the leaves of the kluwih plant (Artocarpus camansi) showed the presence of secondary metabolite compounds that are suspected to play a role in the biological activity of the plant. The test was carried out using a colour reaction method with specific reagents for each group of compounds, and the results are presented in Table 1.

**Table 1.** Preliminary Test Results of Chemical Content of Kluwih Leaves (Artocarpus camansi)

No	<b>Chemical Content</b>	Reagents	Test Result
1	Alkaloids	Mayer	-
2	Flavonoids	Magnesium/HCl (reaksi Shinoda)	-
3	Steroids	Liebermann-Burchard	+
4	Terpenoids	Liebermann-Burchard	+
5	Saponins	Air (uji buih)	-

Alkaloid tests were conducted using Mayer's reagent, which should produce a white precipitate if alkaloid compounds are present. However, the test results showed no precipitate formation, indicating that alkaloid compounds were not detected in the sample. Flavonoid tests were conducted through the Shinoda reaction using magnesium powder and hydrochloric acid (HCl); the absence of a pink or orange colour change indicates the absence of flavonoid compounds.

On the other hand, positive results were obtained in the steroid and terpenoid compound tests using the Liebermann-Burchard reagent, which was marked by the formation of a bluish green colour typical of the reaction. This indicates that kluwih leaves contain steroid and terpenoid compounds. Meanwhile, the saponin test carried out using the water shaking method to observe the formation of stable foam did not show any persistent foam, so it was concluded that saponins were not detected. Based on these results, it can be concluded that Artocarpus camansi leaves contain terpenoid and steroid compounds, which are the basis for this study's isolation and further characterisation.

**Table 2.** Column Chromatography Results of Concentrated n-Hexane Extract of Artocarpus camansi Leaves

No	Vial	Faction	Shape and Colour Fraction	Terpenoid Test
1	1–8	1	Red oil	_
2	9–14	2	Blackish green oil	+
3	15–19	3	Yellowish green crystals	+
4	20–25	4	Dense green sediment	_
5	26–29	5	Yellowish green sediment	+
6	30-40	6	Blackish green sediment	_
7	41–46	7	Greenish yellow sediment	_
8	47–50	8	Yellow sediment	+

The results of fractionating the methanol extract of Artocarpus camansi leaves using nhexane solvent produced 26 grams of concentrated n-hexane extract with physical characteristics in the form of a blackish green colour. Preliminary tests on the extract using the Liebermann-Burchard reagent showed positive results, indicating the presence of terpenoid compounds in the extract. Further separation was carried out through column chromatography using silica gel stationary phase, n-hexane solvent mobile phase, and a mixture of n-hexane: ethyl acetate with a graded composition. The elution process produced eight fractions collected in 50 vials, then each fraction was tested for the presence of terpenoids using the Liebermann-Burchard reagent. The results of the separation and initial characterisation of each fraction are presented in Table 2.

From the test results, it is known that fractions 2, 3, 5, and 8 gave a positive reaction to the terpenoid test. However, only fraction 3 showed the formation of yellowish-green crystals after the solvent was evaporated. The other fractions (2, 5, and 8) did not form crystals and were not continued to the next stage because their terpenoid content was relatively low or did not allow further isolation. Crystals from fraction three were then purified through a recrystallisation process using n-hexane solvent. This process produces pure white crystals with a needle-like morphology. Re-testing the recrystallised crystals using the Liebermann-Burchard reagent showed a red-orange colour change, indicating the presence of terpenoid compounds in pure form. This finding indicates the successful isolation of terpenoid compounds from Artocarpus camansi leaves in pure crystal form.

The initial identification of crystals isolated from fraction 3 using the Liebermann-Burchard reagent showed a colour change to red-orange, indicating the presence of terpenoid compounds. The purity test of the isolated crystals was carried out using thin-layer chromatography (TLC) with various types of eluents. This test showed only one spot in each eluent system used, indicating that the isolated compound was in a relatively pure state. The TLC data are presented in Table 3.

No	Eluen	Rf
1	n-Heksana	0
2	n-Heksana: Etil Asetat (8:2)	0,28
3	n-Heksana: Etil Asetat (6:4)	0,54
4	Kloroform	0,23
5	Etil Asetat	0,68

**Table 3.** Thin Layer Chromatography Results of Isolated Compounds

The melting point measurement results showed that the crystals melted at  $141^{\circ}$ C and finished melting at  $142.8^{\circ}$ C. This narrow melting point range supports the assumption that the compound obtained is pure. Further characterisation was carried out using ultraviolet (UV) and infrared (IR) spectroscopy. The UV spectrum showed a maximum absorption ( $\lambda$ max) at a wavelength of 210.2 nm, indicating a chromophore system in the compound structure.

From the research that has been done, a compound was obtained in the form of a white, needle-shaped crystal. Identification of the compound obtained with the Liebermann-Burchard reagent produced a red-orange colour. The emergence of this red-orange colour indicates that the compound is a terpenoid. The purity test of the crystals obtained by thin-layer chromatography in various eluents showed a single spot. This means that the isolated terpenoid compound can be considered pure. This is also supported by the melting point obtained, which is 141-142.8 °C. The small melting point range of 5 °C indicates that the isolated terpenoid compound can be considered pure. This is evidenced by several previous studies that focused on lowering the melting point to determine the purity of a compound. (Singh et al., 2024; W. Wang et al., 2025).

Furthermore, Melting point determination is a fundamental technique in assessing the purity of organic compounds, including terpenoids. Pure compounds typically exhibit a sharp

melting point range (usually within 1–2°C), whereas the presence of impurities often leads to a broader melting range and a depression in the melting point. This principle is widely utilised in phytochemical studies to evaluate the purity of isolated compounds. For instance, in studying terpenoids extracted from Datura fastuosa roots, researchers employed melting point analysis to assess the purity of isolated compounds. They observed that one compound exhibited a sharp melting point range of 230–231°C, indicating high purity, while others displayed broader ranges, suggesting the presence of impurities. (Melese et al., 2024). A narrow melting point range is a reliable indicator of compound purity, as it reflects a uniform phase composition without interference from impurities that can change melting characteristics. Meanwhile, analysis with infrared spectroscopy showed typical absorption bands at the following wave numbers:

3350 cm<sup>1</sup>: indicates the presence of –OH (hydroxyl) groups,

2920 cm<sup>1</sup>: absorption from aliphatic C-H stretch,

1630 cm<sup>-1</sup>: possibly derived from C=C double bonds or carbonyl groups (C=O),

1385 cm<sup>-1</sup>: indicates the presence of methyl groups (–CH<sub>3</sub>),

1075 cm<sup>1</sup> indicates the presence of C–O or C groups in the ring system.

The combination of data from chemical tests, TLC, melting points, UV, and IR indicates that the isolated compound is a high-purity terpenoid compound.

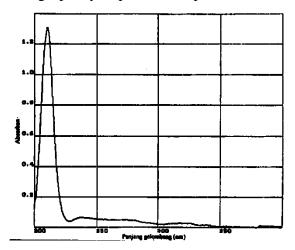


Figure 2. Ultraviolet (UV) Spectrum of the Isolated Compound in Methanol

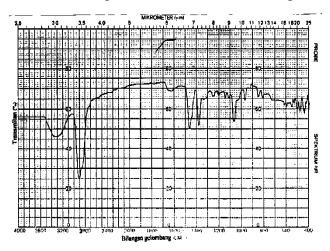


Figure 3. Infrared (IR) Spectrum of the Isolated Compound at 4 cm<sup>-1</sup> Resolution

From the ultraviolet spectrum, absorption at a wavelength of 210.2 nm indicates that the terpenoid compound contains unconjugated double bonds. Compounds such as geraniol and  $\beta$ -

caryophyllene, which are monoterpenes and sesquiterpenes, respectively, are examples of terpenoids with this structural feature (Tousif et al., 2023; Zielińska-Błajet & Feder-Kubis, 2020). These unconjugated double bonds are important because they affect the ability of terpenoids to participate in various biological activities, including their role as human neutrophil elastase inhibitors and their potential therapeutic applications in treating inflammatory conditions and cancer (Kamran et al., 2022; Tousif et al., 2023).

The presence of these double bonds also plays a crucial role in the terpenoid biosynthesis pathway, allowing the formation of complex structures through reactions such as cycloaddition, which is essential for the diversity of terpenoid compounds (R. Chen et al., 2024; Fu & Liu, 2024). Furthermore, the structural diversity of terpenoids, including those with unconjugated double bonds, is being expanded through synthetic biology approaches, aiming to enhance their potential applications in various industries (R. Chen et al., 2024). Overall, the presence of unconjugated double bonds in terpenoids supports their chemical diversity and biological properties, making them valuable in both natural and synthetic contexts.

An IR absorption band usually indicates the presence of C=C double bonds in the range of  $1680-1620~\text{cm}^{-1}$ , parallel to the absorption band at  $1630~\text{cm}^{-1}$ . This range is consistent with literature data describing weak absorption for C=C bonds, especially in conjugated systems. The absorption range of conjugated dienes at 215-230~nm further supports this, as conjugated systems often exhibit characteristic UV-Vis absorption due to their broadened  $\pi$ -electron system (Bryliakov, 2024; Stoyanov et al., 2023). Studies of vinyl carbocations, for example, highlight how the introduction of substituents such as Cl or O atoms can affect the electron density and charge distribution at the C=C bond, potentially affecting their spectroscopic properties (Stoyanov et al., 2023).

Additionally, oxidative cleavage of C=C bonds, as explored in the context of BODIPY photoframes, demonstrates the reactivity of these bonds under certain conditions, such as visible light irradiation, which can lead to the formation of new functional groups (Xu et al., 2021). This reactivity is critical in synthetic applications, including late-stage functionalisation of complex molecules, where selective activation and modification of C=C bonds are used to enhance molecular diversity and functionality (Bryliakov, 2024; Liang et al., 2023).

The formation and cleavage of C=C bonds is an integral part of the synthesis of conjugated polymers, which benefit from the stability and electronic properties conferred by these bonds (Wen et al., 2020). Overall, the spectroscopic and chemical behaviour of the C=C bond, as discussed throughout this study, underscores the importance of this bond in fundamental research and practical applications in organic synthesis and materials science.

Furthermore, the infrared spectrum data shows OH stretching vibrations in the 3350 cm<sup>-1</sup> region. Infrared spectral data indicating the presence of OH groups oscillating in the 3350 cm<sup>-1</sup> region can be understood through various studies on OH stretching vibrations. The OH stretching region is a critical area in infrared spectroscopy, which is often associated with hydrogen bonds and the presence of hydroxyl groups. In the study of  $\alpha$ -hydroxyethyl radicals, OH stretching vibrations were observed at 3654.6 cm<sup>-1</sup>, which is higher than 3350 cm<sup>-1</sup>, indicating a strong OH bond without significant hydrogen bonding effects (Zasimov et al., 2024). In contrast, studies of hydroxylapatite-chlorapatite solid solutions identified OH stretching peaks at 3574 cm<sup>-1</sup> and 3548 cm<sup>-1</sup>, which are closer to 3350 cm<sup>-1</sup> and are influenced by the presence of chlorine, which affects the OH bonding environment and causes a shift in the vibrational frequency (Tacker et al., 2024).

In addition, studies of amorphous water at low temperatures reveal hanging OH features at 3720 and 3696 cm<sup>-1</sup>, free OH stretches. At the same time, hydrogen-bonded OH groups usually appear at lower frequencies, closer to the 3350 cm<sup>-1</sup> region (Hasegawa et al., 2024). The presence of OH groups in coal, as studied by infrared spectroscopy, also suggests that hydrogen bonding can shift the OH stretching vibrations to lower frequencies, supporting the idea that the 3350 cm<sup>-1</sup> region may be indicative of such interactions (Dai et al., 2023).

Furthermore, herbal infusion studies using FT-IR spectroscopy identified the range of 3400-3200 cm<sup>-1</sup> as characteristic of hydroxyl groups and v. All these studies collectively indicate that the 3350 cm<sup>-1</sup> region is likely related to OH groups involved in hydrogen bonding or influenced by nearby atoms or molecules, which change their vibrational characteristics.

### **CONCLUSION**

Based on the results of the isolation and characterisation of terpenoid compounds from kluwih leaves (Artocarpus camansi), it can be concluded that 501 mg (0.01% of the fresh leaf weight) of terpenoid compounds were successfully isolated from 5 kg of fresh kluwih leaves. The isolated compound appeared as white needle-shaped crystals with a melting point of 141–142.8°C. Characterisation using UV spectrophotometry revealed strong absorption at wavelengths around 200–220 nm, indicating the presence of non-conjugated double bonds. Furthermore, IR spectrophotometry showed the presence of OH functional groups, methyl groups, and non-conjugated C=C double bonds, characteristic of terpenoid compounds. Although this study successfully confirmed the presence of terpenoids in the n-hexane fraction of kluwih leaves, the specific molecular structure of the compound has not yet been determined. Therefore, further studies are recommended to elucidate the complete molecular structure using advanced spectroscopic techniques such as NMR and MS. In addition, future research should explore the pharmacological activities of the isolated compound through bioactivity assays, which could provide valuable insights for its potential application in development or synthetic modifications.

#### RECOMMENDATION

This study has the strength in the selection of local plant samples of Artocarpus camansi, which are still rarely studied, as well as the application of systematic compound isolation and identification methods through fractionation, column chromatography, TLC, and valid and measurable UV-IR spectroscopy, so that pure terpenoid compounds in crystal form are obtained. However, the study's weakness lies in the limitations in confirming the structure of the compound, along with further bioactivity tests that can support the potential application of the isolated compounds. This has the potential in other fractions that indicate the possibility of active compounds that have not been explored further. Therefore, it is recommended that further research be carried out to complete structural characterisation using NMR and MS, test the biological activity of isolated compounds, and examine other plant fractions or parts to explore the chemical potential of Artocarpus camansi more widely.

#### REFERENCES

- Adnan, Navia, Z. I., Jamil, M., & Suwardi, A. B. (2023). The diversity and traditional knowledge of wild edible vegetables in Aceh, Indonesia. *Ethnobotany Research and Applications*, 26(2). https://doi.org/10.32859/era.26.49.1-16
- Balachandran, S., De Somer, T., Hogie, J., Roosen, M., Meers, E., & De Meester, S. (2024). An energy-efficient upconcentration of the bio-based carboxylic acids using multiple-effect evaporators. *Journal of Environmental Management*, 370, 122709. https://doi.org/10.1016/j.jenvman.2024.122709
- Benjamin, M. A. Z., Mohd Mokhtar, R. A., Iqbal, M., Abdullah, A., Azizah, R., Sulistyorini, L., Mahfudh, N., & Zakaria, Z. A. (2024). Medicinal plants of Southeast Asia with anti-α-glucosidase activity as potential source for type-2 diabetes mellitus treatment. *Journal of Ethnopharmacology*, *330*, 118239. https://doi.org/10.1016/j.jep.2024.118239
- Bryliakov, K. P. (2024). Mechanisms of C(sp3)–H and C=C selective oxidative heterofunctionalizations by non-heme Fe and Mn mimics of oxygenase enzymes. *Coordination Chemistry Reviews*, 508, 215793. https://doi.org/10.1016/j.ccr.2024.215793
- Câmara, J. S., Perestrelo, R., Ferreira, R., Berenguer, C. V., Pereira, J. A. M., & Castilho, P.

- C. (2024). Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. *Molecules*, 29(16). https://doi.org/10.3390/molecules29163861
- Chen, H.-Y., Jin-Yu, L., Shu-Li, L., Li-Qiong, G., Jun-Fang, L., Guang-Hong, W., Jun, L., & and Ye, Z.-W. (2023). Progress in biological activities and biosynthesis of edible fungi terpenoids. *Critical Reviews in Food Science and Nutrition*, 63(25), 7288–7310. https://doi.org/10.1080/10408398.2022.2045559
- Chen, R., Wang, M., Keasling, J. D., Hu, T., & Yin, X. (2024). Expanding the structural diversity of terpenes by synthetic biology approaches. *Trends in Biotechnology*, 42(6), 699–713. https://doi.org/10.1016/j.tibtech.2023.12.006
- Dai, F., Zhuang, Q., Huang, G., Deng, H., & Zhang, X. (2023). Infrared Spectrum Characteristics and Quantification of OH Groups in Coal. *ACS Omega*, 8(19), 17064–17076. https://doi.org/10.1021/acsomega.3c01336
- Das, D. D., Nikita, S., Viney, C., & and Chawla, P. A. (2024). Current Trends of Analytical Techniques for Bioactive Terpenoids: A Review. *Critical Reviews in Analytical Chemistry*, 54(8), 2984–3000. https://doi.org/10.1080/10408347.2023.2219757
- Fu, S., & Liu, B. (2024). Naturally Occurring [4+2] Type Terpenoid Dimers Assembled through Unmatched-electron-demand Cycloaddition. *Chemistry A European Journal*, 30(69), e202402786. https://doi.org/10.1002/chem.202402786
- Hao, W., & Cardin, D. B. (2023). Full Evaporative Vacuum Extraction—A Quantitative and Green Approach for Analysis of Semivolatile Organic Compounds in Drinking Water and Surface Water Using GC-MS. *Analytical Chemistry*, *95*(8), 3959–3967. https://doi.org/10.1021/acs.analchem.2c03414
- Hasegawa, T., Yanagisawa, H., Nagasawa, T., Sato, R., Numadate, N., & Hama, T. (2024). Infrared Band Strengths of Dangling OH Features in Amorphous Water at 20 K. *The Astrophysical Journal*, 969(2), 134. https://doi.org/10.3847/1538-4357/ad5318
- Hu, Z., Liu, X., Tian, M., Ma, Y., Jin, B., Gao, W., Cui, G., Guo, J., & Huang, L. (2021). Recent progress and new perspectives for diterpenoid biosynthesis in medicinal plants. *Medicinal Research Reviews*, 41(6), 2971–2997. https://doi.org/10.1002/med.21816
- Johnson, B., Addolorato, G., Lesch, O., Liu, L., & Rodd, Z. A. (2023). A critical scientific evaluation of a purportedly negative data report response to Seneviratne et al. 2022. *Frontiers in Psychiatry*, *14*(October). https://doi.org/10.3389/fpsyt.2023.1271229
- Kamran, S., Sinniah, A., Abdulghani, M. A. M., & Alshawsh, M. A. (2022). Therapeutic Potential of Certain Terpenoids as Anticancer Agents: A Scoping Review. *Cancers*, *14*(5). https://doi.org/10.3390/cancers14051100
- Khandelia, T., Panigrahi, P., Ghosh, S., Mandal, R., Doley, B., & Patel, B. K. (2024). Solvent Dictated Organic Transformations. *Chemistry An Asian Journal*, 19(24), e202400603. https://doi.org/10.1002/asia.202400603
- Kouno, H., Amuti, S., Saito, Y., Fukuyoshi, S., Miyake, K., Goto, M., Newman, D. J., O'Keefe, B. R., Lee, K.-H., & Nakagawa-Goto, K. (2024). Isolation, Characterization, and Antiproliferative Activity of Terpenoids from the Tropical Plant Turraea delphinensis. *Journal of Natural Products*, 87(7), 1763–1777. https://doi.org/10.1021/acs.jnatprod.4c00313
- Kumar, S., Ahmad, R., Saeed, S., Azeem, M., Mozūraitis, R., Borg-Karlson, A. K., & Zhu, G. (2022). Chemical Composition of Fresh Leaves Headspace Aroma and Essential Oils of Four Coriander Cultivars. *Frontiers in Plant Science*, *13*(February), 1–9. https://doi.org/10.3389/fpls.2022.820644
- Lhotáková, Z., Neuwirthová, E., Potůčková, M., Červená, L., Hunt, L., Kupková, L., Lukeš, P., Campbell, P., & Albrechtová, J. (2025). Mind the leaf anatomy while taking ground truth with portable chlorophyll meters. *Scientific Reports*, *15*(1), 1855. https://doi.org/10.1038/s41598-024-84052-5

- Liang, Y.-F., Bilal, M., Tang, L.-Y., Wang, T.-Z., Guan, Y.-Q., Cheng, Z., Zhu, M., Wei, J., & Jiao, N. (2023). Carbon–Carbon Bond Cleavage for Late-Stage Functionalization. *Chemical Reviews*, 123(22), 12313–12370. https://doi.org/10.1021/acs.chemrev.3c00219
- Liu, T., Dai, M., Zhu, H., Huang, Y., Chen, J., Li, M., Guo, Y., Huang, C., La, C., Wang, Z., Wang, Z., Ren, Z., Ye, C., Zheng, X., & Wang, Y. (2023). Activity-guided isolation and identification of antiherpesvirus and antineuroinflammatory active terpenoids from Artemisia vulgaris L. based on the LC-MS/MS molecular network. *Phytochemistry*, 216, 113863. https://doi.org/10.1016/j.phytochem.2023.113863
- Mali, S. N., Pandey, A., de Oliveira, M. S., & Jawarkar, R. D. (2024). A decade review of analysis of essential oils from genus Artocarpus: Its phytochemistry and pharmacology. *Pharmacological Research Natural Products*, 2, 100016. https://doi.org/10.1016/j.prenap.2024.100016
- Maqsood, M., Anam Saeed, R., Sahar, A., & Khan, M. I. (2022). Mulberry plant as a source of functional food with therapeutic and nutritional applications: A review. *Journal of Food Biochemistry*, 46(11), e14263. https://doi.org/10.1111/jfbc.14263
- Melese, G. M., Aychiluhim, T. B., Yessuf, A. M., & Eshete, M. (2024). Identification and characterization of phytochemicals in methanolic extract of roots of Datura fastuosa using various techniques. *Future Journal of Pharmaceutical Sciences*, 10(1). https://doi.org/10.1186/s43094-024-00682-6
- Navia, Z. I., Suwardi, A. B., Nuraini, Adnan, Baihaqi, Yakob, M., Lubis, P., & Chairul. (2024). Diversity and ethnobotany of useful plants in Bandar Pusaka, Aceh Tamiang District, Indonesia. *Ethnobotany Research and Applications*, 28(100), 1–26. https://doi.org/10.32859/era.28.35.1-26
- Pinto, M., Soares, C., Pereira, R., Rodrigues, J. A., Fidalgo, F., & Valente, I. M. (2022). Untargeted metabolomic profiling of fresh and dried leaf extracts of young and mature Eucalyptus globulus trees indicates differences in the presence of specialized metabolites. *Frontiers in Plant Science*, 13(November), 1–18. https://doi.org/10.3389/fpls.2022.986197
- Singh, S., Marayathungal, J. H., Das, D. K., Khan, A. A., Bakthavatsalam, R., Hathwar, V. R., & Kundu, J. (2024). Rational Design of Zero-dimensional Manganese(II) Halide Hybrids with Suppressed Melting Temperatures. *The Journal of Physical Chemistry C*, *128*(35), 14849–14859. https://doi.org/10.1021/acs.jpcc.4c04444
- Stoyanov, E. S., Bagryanskaya, I. Y., & Stoyanova, I. V. (2023). Substitution of H Atoms in Unsaturated (Vinyl-Type) Carbocations by Cl or O Atoms. *International Journal of Molecular Sciences*, 24(13), 1–9. https://doi.org/10.3390/ijms241310734
- Tacker, R. C., Rakovan, J., Harlov, D., Hughes, J. M., & Cichy, S. B. (2024). The OH-stretching region in infrared spectra of the apatite OH-Cl binary system. *American Mineralogist*, 109(12), 2086–2094. https://doi.org/10.2138/am-2023-9059
- Tousif, M. I., Nazir, M., Riaz, N., Saleem, M., Tauseef, S., Azam, S. M., Arfan Yawer, M., & Zengin, G. (2023). Terpenoids as Human Neutrophil Elastase (HNE) Inhibitors: A Comprehensive Review of Natural Anti-inflammatory Isoprenoids. *ChemBioChem*, 24(22), e202300346. https://doi.org/10.1002/cbic.202300346
- Wang, Q., Zhao, X., Jiang, Y., Jin, B., & Wang, L. (2023). Functions of Representative Terpenoids and Their Biosynthesis Mechanisms in Medicinal Plants. *Biomolecules*, 13(12). https://doi.org/10.3390/biom13121725
- Wang, W., Zhang, J.-M., Jin, M.-L., Jing, C.-Q., & Zhang, W. (2025). A 9R-like 2D hybrid metal halide with remarkably low melting temperature. *Chemical Communications*, 61(2), 362–365. https://doi.org/10.1039/D4CC05616G
- Wen, J., Zhu, L., & Li, M. (2020). C–C Coupling Reactions for the Synthesis of Two-Dimensional Conjugated Polymers. *ChemPlusChem*, 85(12), 2636–2651. https://doi.org/10.1002/cplu.202000643

- Xu, Y., Lin, S., He, R., Zhang, Y., Gao, Q., Ng, D. K. P., & Geng, J. (2021). C=C Bond Oxidative Cleavage of BODIPY Photocages by Visible Light. *Chemistry A European Journal*, 27(44), 11268–11272. https://doi.org/10.1002/chem.202101833
- Yang, C. Y., Huang, P. H., Tseng, C. H., & Yen, F. L. (2021). Topical artocarpus communis nanoparticles improved the water solubility and skin permeation of raw a. Communis extract, improving its photoprotective effect. *Pharmaceutics*, *13*(9). https://doi.org/10.3390/pharmaceutics13091372
- Yao, P., & Liu, Y. (2022). Terpenoids: Natural Compounds for Non-Alcoholic Fatty Liver. *Molecules*, 28(1), 1–37. https://doi.org/10.3390/molecules28010272
- Zang, Y., Sun, R., Feng, R., Zhu, H., & Li, X. (2025). Recent Advances of Terpenoids with Intriguing Chemical Skeletons and Biological Activities. *Chinese Journal of Chemistry*, 43(4), 443–469. https://doi.org/10.1002/cjoc.202400697
- Zasimov, P. V, Volosatova, A. D., Góbi, S., Keresztes, B., Tyurin, D. A., Feldman, V. I., & Tarczay, G. (2024). Infrared spectroscopy of the α-hydroxyethyl radical isolated in cryogenic solid media. *The Journal of Chemical Physics*, 160(2), 24308. https://doi.org/10.1063/5.0177189
- Zhang, M., Sui, H., Yang, H., Li, X., He, L., & Liu, J. (2021). Adsorption—Desorption Behaviors of Methanol and Ethyl Acetate on Silica Gel: Modeling and Experimental Tests. *Industrial & Engineering Chemistry Research*, 60(4), 1829–1838. https://doi.org/10.1021/acs.iecr.0c05205
- Zielińska-Błajet, M., & Feder-Kubis, J. (2020). Monoterpenes and their derivatives—recent development in biological and medical applications. *International Journal of Molecular Sciences*, 21(19), 1–38. https://doi.org/10.3390/ijms21197078