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Identification of flavonoid compounds in clove leaves (*Syzygium aromaticum (L.) L.M. Perry*) using thin layer chromatography method

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ABSTRACT

Clove (*Syzygium aromaticum (L.) L.M. Perry*) is a plant that has been utilised for centuries in traditional medicine to treat various diseases. Clove leaves contain flavonoids, which are phenolic compounds that undergo a colour change when added to a base or ammonia. The objective of this research is to identify the flavonoid group present in clove leaves. The method employed for the identification of flavonoids involved thin-layer chromatography with a mobile phase consisting of acetic acid: concentrated HCl: water (30:3:1). The sample preparation procedure entailed subjecting the sample to heating with 2N HCl at a temperature of 100°C, followed by extraction with ethyl acetate. The flavonoid screening exhibited favourable outcomes, as evidenced by the manifestation of an orange hue within the amyl alcohol layer. The organoleptic characteristics of the clove leaf extract manifested as a reddish-brown hue, a viscous texture, and an absence of olfactory attributes. TLC test results indicated the presence of flavonoids belonging to the flavon group in clove leaves, as evidenced by a faint brown colour and Rf values of 0.66 and 0.70.

Keywords: clove leaves; flavonoids; thin layer chromatography

INTRODUCTION

Cloves (*Syzygium aromaticum*) have been used as a spice and traditional medicine in Indonesia for centuries. In traditional medicine, cloves are often used to treat respiratory ailments due to their antiviral and anti-inflammatory properties. Furthermore, cloves also have various other properties, such as anticoagulant, immune system booster, and antibacterial properties. The most important component in cloves, approximately 70-80%, is eugenol. This compound functions as a stimulant, local anesthetic, carminative (for flatulence), antiemetic (to relieve nausea), antiseptic, and antispasmodic (to relax smooth muscles) (Batiha et al., 2020).

Flavonoids represent the most abundant category of natural phenolic compounds that occur in nature. Liu et., al (2021) stated that the red, purple, blue, and blue pigments in plants are largely determined by the presence of flavonoids and anthocyanins. Flavonoids found in medicinal plants are known to exhibit a wide range of therapeutic properties, including antioxidant, antibacterial, antiviral, anti-inflammatory, antiallergic, anticancer effects (Nurbaety, Haeroni, 2018; Roy et al., 2022), and antimalarial (Taher et al.,2018).

Due to their potent antioxidant capabilities, flavonoids are recognized

as a highly beneficial compound in foods. Antioxidants, whether natural or synthetic, function by preventing or delaying the cellular damage initiated by oxidation. The detrimental effects of free radicals, including superoxide and hydroxyl, are known to worsen numerous diseases. Fortunately, research has shown that flavonoids are effective at neutralizing these reactive oxidizing species. As a result, consuming foods rich in flavo noids is considered a beneficial strategy for the prevention and treatment of conditions such as cancer and heart disease (Al-Khayri et al., 2022).

Flavonoids are water-soluble compounds, which allows for their effective extraction using a 70% ethanol solvent. This method ensures the flavonoids are retained within the aqueous phase. As phenolic compounds, flavonoids can be visually identified using Thin Layer Chromatography (TLC), where their detection is based on the characteristic color change that occurs when they react with a base or ammonia (Dias, Pinto, & Silva, 2021).

Among chromatographic techniques, Thin Layer Chromatography (TLC) is the most fundamental and frequently applied method (Kusumawati, Primaharinastiti, & Prasetyawan, 2024). The TLC method is a qualitative testing procedure that utilises the differential distribution of compounds between two phases, namely a stationary phase and a mobile phase, to perform the separation of flavonoids (Wahyulianingsih, Handayani, & Malik, 2016).

Based on the above explanation, the purpose of this study is to identify flavonoid compounds found in clove leaves (*Syzygium aromaticum (L.) L.M. Perry*) using thin layer chromatography (TLC).

METHODOLOGY

Identification of Clove Plants
The clove (*Syzygium aromaticum (L.) L.M. Perry*) plants used in this study were collected from Cukuh Balak in the Tanggamus Regency. The identification process involved botanical determination at the Botany Laboratory at the University of Lampung to validate the authenticity of the clove specimens. Identification was achieved by observing various characteristics of the plant, including leaf shape, venation pattern, flower colour and stem morphology.

Preparation of Clove Leaf Simplicia
Fresh clove leaves were collected and washed in running water. Subsequently, a wet sorting process was conducted in order to select the most suitable leaves. The leaves were then divided into smaller pieces and dried in the sunlight, with the pieces being covered with a black cloth to prevent direct exposure. Following the n drying process, a dry sorting procedure was conducted to eliminate any residual impurities. The dried material was then subjected to a grinding process, resulting in a fine powder. This powder was subsequently sieved through a sieve with a mesh size of 40.

Phytochemical Screening of Flavonoids

1. A quantity of one gram of cloves leaf simplicia powder was mixed with 10 millilitres of hot water.
2. Five milliliters of the filtrate were added with 0.1 grams of magnesium powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol, shaken, and allowed to separate.
3. A positive flavonoid result is indicated by the appearance of red, yellow, or orange coloration in the amyl alcohol layer.

Screening of Flavonoid Groups Using Thin Layer Chromatography (TLC)

Preparation of Forestal Eluent
To prepare the eluent, 22 mL of acetic acid, 2.3 mL of concentrated HCl, and 0.7 mL of water (30:3:1 ratio) were mixed in an Erlenmeyer flask until homogeneous, then stored in a container.

Preparation for TLC Testing
Phytochemical testing for flavonoid identification was conducted using the Harborne 1996 literature, which does not require a blank and involves pre- and post-extraction treatment. After extraction, clove leaves (*Syzygium aromaticum (L.) L.M. Perry*) were analyzed for their flavonoid group content.

1. 0.5 grams of clove leafs simplicia powder was weighed.
2. The powder was soaked in 20 mL of 2N HCl in a beaker glass.
3. The mixture was heated at 100°C for 30-40 minutes.
4. After cooling, it was filtered using a glass funnel and filter paper.
5. The filtrate was extracted three times with 10 mL of ethyl acetate using a separatory funnel.
6. The extract was evaporated to dryness and 1-2 drops of ethanol were added.
7. The sample was spotted onto a silica gel F254 plate.
8. Chromatography was performed using the forestal (acetic acid : concentrated HCl, and water) in a ratio of 30:3:1.

Chromatography Procedure

1. Equipment and materials were prepared.
2. Silica gel F254 plates were cut to 10 cm x 3 cm, with the following margins: 1 cm from the top and bottom edges, and 1.5 cm from the left and right sides for the spotting area, illustrated as follows.
3. The chromatography chamber was saturated with the forestal eluent.
4. The silica gel plate was activated by heating at 100°C for 30 minutes.
5. 2-10 µL of clove leaf extract was spotted onto the marked silica gel plate using a capillary tube.
6. The plate was developed in the chromatography chamber at room temperature.
7. After development, the plate was removed and dried at room temperature.

8. The silica gel plate was then examined under a UV lamp at 366 nm wavelength.
9. The Rf values were calculated, and spots were observed under UV light at 366 nm.
10. Flavonoid group identification was confirmed by analyzing the spots and their Rf values on the chromatogram.

RESULTS AND DISCUSSION

Organoleptic Properties of Clove Leaf Simplicia Powder

Clove leaves simplicia powder was prepared by drying the leaves under indirect sunlight for six days. A thin black cloth was used to cover the leaves during the drying process to prevent contamination by dirt and dust and to preserve the active compounds in the clove leaves. The organoleptic properties of the clove leaf simplicia are presented in Table 1.

The cloves leaf simplicia powder intended for flavonoid group identification was first extracted using the method described by Harborne (1996). The cloves leaf extract (*Syzygium aromaticum (L.) L.M. Perry*) was obtained by heating the powder with 2N HCl for 30 minutes at 100°C. The mixture was then filtered using filter paper and extracted three times with 10 mL of ethyl acetate using a separatory funnel. The resulting extract was evaporated to dryness on a water bath, after which 1-2 drops of 96% ethanol were added. The extract was then subjected to thin layer chromatography. The organoleptic properties of the clove leaf extract are shown in Table 2.

Table 1.
Organoleptic properties of clove leaf simplisia

No	Organoleptic Characteristic	Description
1	Color	Greenish brown
2	Form	Slightly fine powder
3	Odor	Distinct, strong aroma
4	Taste	Bitter

Table 2.
Organoleptic properties of clove leaf extract

No	Organoleptic Characteristic	Description
1	Consistency	Thick and adheres to the porcelain dish
2	Color	Reddish brown
3	Odor	odorless

Screening of Flavonoids in Clove Leaves

Prior to the identification of flavonoid groups, clove leaf simplicia powder underwent acid hydrolysis with 2N HCl at 100°C for 30 minutes to break glycosidic bonds, thereby releasing flavonoid aglycones (such as flavones, flavonols, biflavon, and glycoflavons). These aglycones are

more readily extracted by non-polar solvents. Following the process of hydrolysis, a 3x10-mL ethyl acetate extraction was carried out using a separating funnel with the objective of isolating the free flavonoid aglycones. The resulting extract was subsequently subjected to desiccation via a water bath, which resulted in a concentrated extract that was found to be replete with flavonoid compounds (Harborne, 1996). Flavonoid screening was performed in duplicate. The results are presented in Table 3.

The results of the screening of ethyl acetate extracts from clove leaves indicated the presence of flavonoids. The detection process involved the use of magnesium powder (Mg) as a reducing agent and concentrated hydrochloric acid (HCl) as a strong acid catalyst. These two elements were instrumental in facilitating the reduction reaction and the formation of colored flavinium salts. This positive indication was marked by the appearance of a red color on the amyl alcohol layer (top layer) (Marjoni, 2023).

Table 3.
Phytochemical screening results of flavonoids in clove leaves

No Replication	Observation Result	Test Result
1	Formation of a red colour in the amyl alcohol layer.	+
2	Formation of a red colour in the amyl alcohol layer.	+

Identification Results of Clove Leaf Flavonoid Groups Using Thin Layer Chromatography (TLC)

Thin-layer chromatography is one of the most commonly used phytochemical screening methods due to its simplicity and affordability (Kowalska & Sajewicz, 2022). This method has been demonstrated to possess the capability of identifying compounds, monitoring reaction progress, and determining purity (Marsila et al., 2025). This capability is achieved through the utilization of various parameters, including but not limited to retention factor values and Rf numbers (Hasanah, Narsih, & Azis, 2024).

A thorough investigation was conducted on clove leaf extract (*Syzygium aromaticum (L.) L.M. Perry*) utilising the TLC (thin-layer chromatography) method, yielding a favourable outcome for the presence of flavones. This was evidenced by a faint brown colouration accompanied by Rf values of 0.66 and 0.7 (Figure 1). However, the extract was found to be negative for flavonols, glycoflavones and biflavones, as evidenced by the non-matching of Rf values and spot colours with the Harborne (1996) reference values.

The elution process was carried out utilising a forestal eluent.

The results of Thin Layer Chromatography (TLC) analysis of clove leaf extract indicate the presence of flavonoid compounds. This prediction is based on the similarity of the Rf values obtained (0.66 and 0.70) with the Rf value range for flavones published by Harborne (1996:69), which is 0.66 to 0.83. This hypothesis is further substantiated by the visual characteristics of the observed spots, which manifest as pale brown under UV light, aligning with Harborne's (1996:69) characterization of flavonoids.

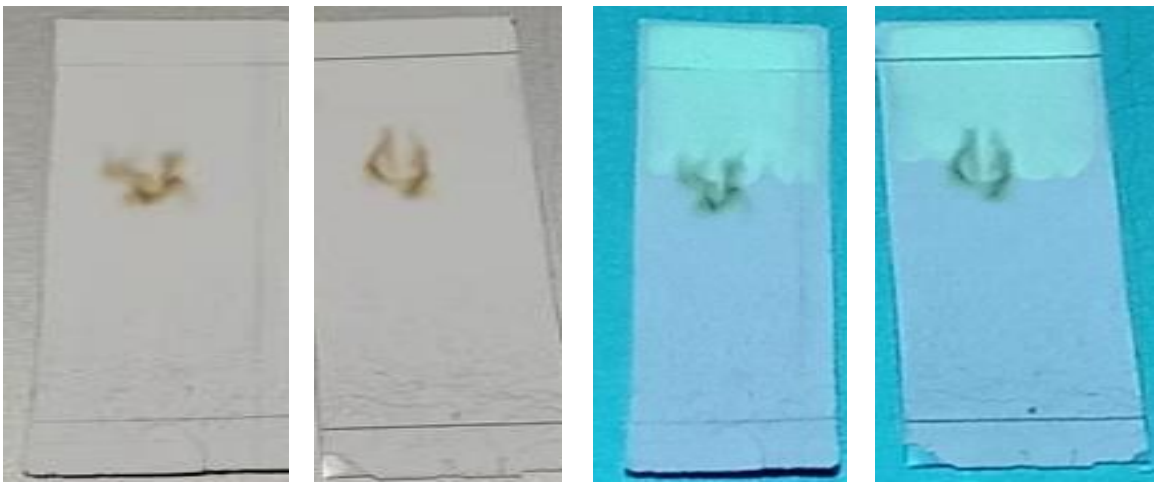

Figure 1. Chromatogram of 2x replication before (left) and after exposure to UV light (right). Rf values from left to right: 0.66, 0.70, 0.66, 0.70; all the chromatogram were faded brown

In contrast, the prediction results indicated negative extracts for flavonols, glycoflavones, and biflavonyls. This discrepancy can be attributed to the Rf values obtained, which did not align with the established Rf range for glycoflavones (>0.83) and biflavonyls (tending toward 1). Additionally, the Rf values and colors of flavonols exhibited significant variation, further supporting the conclusion that the Rf values obtained did not correspond to the expected values for these compounds (Harborne, 1996).

The Rf values obtained were notably elevated, with values of 0.66 and 0.70, suggesting that the compounds exhibited weak interactions with the stationary phase (TLC plate) but strong interactions with the mobile phase (eluent). Consequently, the flavone compounds demonstrated a propensity to migrate further along the plate (Marjoni, 2023).

Mappa et al. (2023), Flavonoids were identified from a methanol extract of clove flowers using Thin-Layer Chromatography (TLC). The results showed that the sample positively

contained flavonoids, which yielded an Rf value of 0.58 when an ethyl acetate (1:9) eluent was used. The study also noted that the obtained Rf value was influenced by the specific plant part and other eluents used in the research (Mappa et al., 2023)

The spots that were identified on the forestal eluent in this study exhibited an irregular pattern, which may be attributed to the presence of migration barriers. The phenomenon of migration barriers can be attributed to various factors, including molecular friction, electrostatic forces, adsorption, chemical bond solubility, and ion interactions (Rafi, Heryanto, & Septiningsih, 2017). Furthermore, the irregular shape of the spots can be attributed to the presence of cracks in the silica gel plate and the angle of the plate during elution. The presence of cracks in the silica gel plate was attributed to the presence of highly acidic eluent. This phenomenon can be attributed to the composition of the forestal eluent, which consists of acetic acid, concentrated HCl, and water in a ratio of 30:3:1 (Hujjatusnaini, 2021).

CONCLUSION

Organoleptic testing of cloves leaf extract showed a reddish-brown colour, sticky texture and no odour. Phytochemical screening revealed that the ethyl acetate extract of clove leaves tested positive for flavonoids, as indicated by the appearance of an orange colour on the amyl alcohol layer. The results of thin layer chromatography (TLC) analysis of ethyl acetate extract of clove leaves are suspected to be positive for the presence of flavonoids of the flavone group. This supposition is substantiated by the Rf value of 0.66 and 0.70, as well as the pigmentation of the pale brown spots.

REFERENCES

Al-Khayri, J., Sahana, G., Nagella, P., & Joseph, B. V. (2022). Flavonoids as Potential Anti-Inflammatory Molecules: A Review. *Molecules*, *2901*.

Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum l. (myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2).

Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules*, *26*(17), 1–16.

Harborne, J. (1987). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*.

Hasanah, R. M., Narsih, U., & Dimas Abdul Azis, F. (2024). Identifikasi Flavonoid Ekstrak Kulit Buah Naga Merah (Hylocereus polyrhizus) secara Kromatografi Lapis Tipis (KLT) dengan Pelarut Etanol 96% dan Metanol 96%. *JI-KES (Jurnal Ilmu Kesehatan)*, *8*(1), 30–37.

Hujjatusnaini, N. (2021). *Buku Referensi Ekstraksi*. Palangkaraya: Institut Agama Islam Negeri Palangkaraya.

Kowalska, T., & Sajewicz, M. (2022). Thin-Layer Chromatography (TLC) in the Screening of Botanicals–Its Versatile Potential and Selected Applications. *Molecules*.

Kusumawati, I., Primaharinastiti, R., & Rifqi Prasetyawan, H. (2024).

Teknik Aplikasi Sampel Pada Pengujian Kuantitatif Kromatografi Lapis Tipis: Tinjauan Terhadap Area dan Faktor Retensi. *Media Farmasi*, *20*(2), 143–150.

Liu, W., Feng, Y., Yu, S., Fan, Z., Li, X., Li, J., & Yin, H. (2021). The flavonoid biosynthesis network in plants. *International Journal of Molecular Sciences*, *22*(23), 1–18.

Mappa, M. R., Bahi, R. R. R., Nurfathin, R., & Istiqomah, H. (2023). Standardisasi Ekstrak Metanol Bunga Cengkeh (Syzygium aromaticum L.) sebagai Bahan Baku Obat Tradisional. *Jurnal Farmasi Tinctura*, *5*(1), 35–46.

Marjoni, R. (2023). *Buku Teks Fitokimia Seri Isolasi*. Jakarta: CV Trans Info Media.

Marsila, R., Febrianti, A., Vusvita Sari, A., Nur Afifah, S., Tri Naurah, N., & Wahab, S. (2025). Ekstraksi dan Identifikasi Senyawa Bioaktif Ekstrak Daun Kemangi (Ocimum basilicum L.). *Jurnal Lentera Ilmiah Kesehatan*, *3*(88), 1–8.

Nurbaety, B., Haeroni, A. S., & Hambat, D. (2018). "Uji Daya Hambat Ekstrak Daun Cengkeh (Syzygium aromaticum) Terhadap Bakteri Escherichia coli dengan Menggunakan Metode Sumuran." *Jurnal Insan Farmasi Indonesia*, *1*(2), 274–281.

Rafi, M., Heryanto, R., & Septiningsih, D. (2017). *Atlas Kromatografi Lapis Tipis Tumbuhan Obat Indonesia*. Bogor: IPB Press.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Islam, M. R. (2022). Review Article Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications.

Taher, D., Solihin, D., Cahyaningsih, U., & Sugita, P. (2018). Ekstrak Metanol Cengkeh (Syzygium Aromaticum (L.) Merry & Perry). *Acta Veterinaria Indonesiana*, *6*(2), 38–47.

Wahyulianingsih, W., Handayani, S., & Malik, A. (2016). Penetapan Kadar Flavonoid Total Ekstrak Daun Cengkeh (Syzygium aromaticum (L.) Merr & Perry). *Jurnal Fitofarmaka Indonesia*, *3*(2), 188–193.

