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Isolation of Flavonoid Compound from Kareumbi Fruit (*Homalanthus populneus (Geiseler) Pax*)

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ABSTRACT

Flavonoid compounds were isolated from Kareumbi fruit (*Homalanthus populneus*) using a sequential extraction and purification process. Methanol-concentrated extract was partitioned with ethyl acetate and n-hexane. The concentrated methanol extract was separated by column chromatography using a gradient elution of chloroform and methanol, varying the ratio 90:10; 80:20; 70:30; and 60:40 (v/v). Subsequent column chromatography yielded a yellowish-orange paste (19.6 mg, Rf = 0.73) from the chloroform:methanol (70:30) fraction. UV-visible, FT-IR, and ^{1}H-NMR spectroscopic analysis confirmed that the isolated compounds belonged to the flavonol class of flavonoids.

Keywords: Flavonoid, Flavonol, *Homalanthus populneus (Geiseler) Pax*, Kareumbi

ABSTRAK

Senyawa flavonoid diisolasi dari buah Kareumbi (*Homalanthus populneus*) menggunakan proses ekstraksi dan pemurnian berurutan. Ekstrak metanol pekat dipartisi dengan etil asetat dan n-heksana. Ekstrak metanol pekat selanjutnya diseparasi melalui kromatografi kolom dengan eluen gradien kloroform dan metanol (90:10; 80:20; 70:30; dan 60:40 (v/v)). Kromatografi kolom selanjutnya menghasilkan pasta berwarna oranye kekuningan (19,6 mg, Rf = 0,73) dari fraksi kloroform:metanol (70:30). Analisis spektroskopi UV-visibel, FT-IR, dan 1H-NMR mengkonfirmasi bahwa senyawa hasil isolasi termasuk dalam kelas flavonoid flavonol.

Kata Kunci : Flavonoida, Flavonol, *Homalanthus populneus (Geiseler) Pax*, Kareumbi

1. Introduction

Flavonoids are polyphenolic compounds characterized by a C6-C3-C6 framework, comprising two benzene rings connected by a three-carbon chain. These compounds exhibit potent antioxidant properties, making them valuable constituents in food [1]. Flavonoids effectively scavenge reactive oxygen species, thereby reducing oxidative stress [2].

In addition, the potential of Kareumbi Extract (*Homalanthus populneus (Geiseler) Pax*) in Inhibition of T Cell Receptor Expression: HIV Infection Inhibition Study, reported that an ethanol extract of Homalanthus populneus stem significantly reduced the expression of gp41 and gp120, the envelope proteins of HIV, as determined by ELISA and talicytometry analysis [3]. Next, the results of the acute toxicity test showed that the ethanol extract of H.populneus (Geiseler) Pax stems was not toxic to the erythrocyte profile and histological structure of the lien and did not cause swelling of the mouse lymph nodes [4].

Kartika, N., 2015, in the journal Antiviral Activity of Ethanolic Extract of Stem Close Abang (H.populneus (Geiseler) Pax) Against Avian Influenza H5N1 Virus reported that based on the results of HA and PCR tests, the ethanol extract of H.populneus (Geiseler) Pax stems at a concentration of 20 µg/mL can inhibit the growth of the H5N1 virus with a dilution concentration of 104 [5].

2. Materials and Methods

2.1. Equipment

The tools used in this study include: glassware, extractor, rotaryevaporator, chromatography column, uv lamp, analytical balance, water bath, chamber, FT-IR spectrophotometer, ^{1}H-NMR spectrophotometer, UV-Vis spectrophotometer.

2.2. Materials

The materials used in this study include: Kareumbu fruit (*Homalanthus populneus (Geiseler) Pax*), methanol, n-hexane, Chloroform, ethyl acetate, aquadest, $FeCl_3$ 5%, Silica Gel 40.

2.3 Research Procedures

2.3.1 Kareumbi Fruit Extraction

The extraction process begins with soaking 1500 grams of Kareumbi fruit powder in 10 liters of methanol for 24 hours. Subsequent to the maceration process, the extract was concentrated using evaporation until the complete removal of the methanol solvent was achieved. The resulting concentrated residue was then dissolved in water. To separate the tannin compounds, ethyl acetate was used to extract the residue solution over and over again until the $FeCl_2$ reagent in the water layer stopped reacting positively. The ethyl acetate fraction containing tannin compounds was then evaporated and dissolved in methanol. Afterwards, n-hexane was used to extract the residue repeatedly to remove the remaining non-polar components. The final methanol fraction, which is expected to contain flavonoid compounds, evaporates until a pure methanol extract is obtained [6].

2.3.2 Separation by Column Chromatography

The column chromatography begins with the preparation of a column filled with ASTM silica gel 40 (70-230 mesh) and eluted using chloroform. The methanol extract combined with silica gel is subsequently introduced into the column. Elution was performed utilizing a chloroform and methanol combination with a progressively increasing polarity ratio, commencing from 90:10 (v/v) to 60:40 (v/v). Each collected fraction was further analyzed for purity using thin-layer chromatography (TLC). Fractions exhibiting same Rf values were further analyzed using 5% $FeCl_3$/reagent to verify the presence of flavonoid components. The purified fractions produced post-solvent evaporation were subsequently examined via UV-Vis spectrophotometry for spectral profiling, FTIR spectroscopy for functional group identification, and 1H NMR spectroscopy for structural elucidation of the compounds [7].

3. Results and Discussion

3.1 Result

The fractions 116-150 were separated again by preparative thin layer chromatography with chloroform: ethyl acetate eluent 40:60 (v/v). The compound was obtained as a yellow paste, weighing 19.6 mg, and Rf value = 0.73. UV-visible spectrum using methanol solvent is shown in Figure 1.

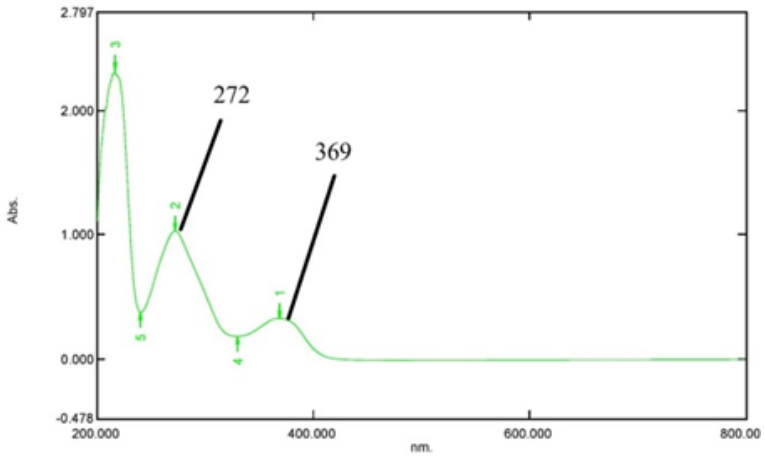

Figure 1. UV-Visible Spectrum of Isolated Compounds

Table 1. Ultraviolet-Visible (UV-Vis) wavelength absorption of isolated compounds

No	Wavelength (nm)	Absorbance
1	369	0.328
2	272	1.032

Figure 2 below displays the results of the FT-IR spectrophotometer analysis of the isolated compounds using KBr pellets.

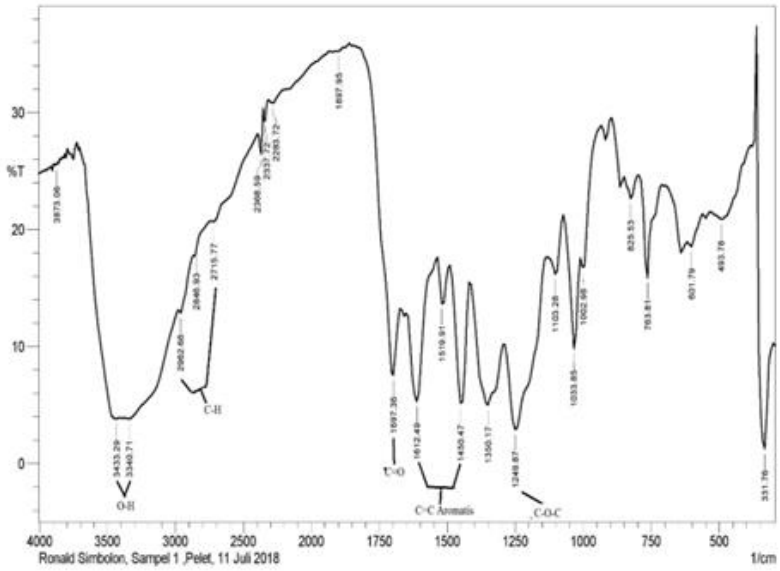

Figure 2. FT-IR spectra of isolated compound

Table 2. Isolated compounds interpretation based on Figure 2

Wavenumber (cm^{-1})	Intensity	Functional Group
3433.29-3340.71	Sharp	-OH stretching vibration
2962.66	Low	Aromatic C-H stretching vibration
1697.36	Medium	C=O stretching vibration
1612.49-1450.47	Medium	Aromatic C=C stretching vibration
1249.87	Medium	C-O-C stretching vibration

The results of the analysis of proton core magnetic resonance spectrophotometry (1H-NMR) of isolated compounds, using Methanol-d4 solvent and TMS as a standard for isolated compounds, are listed below:

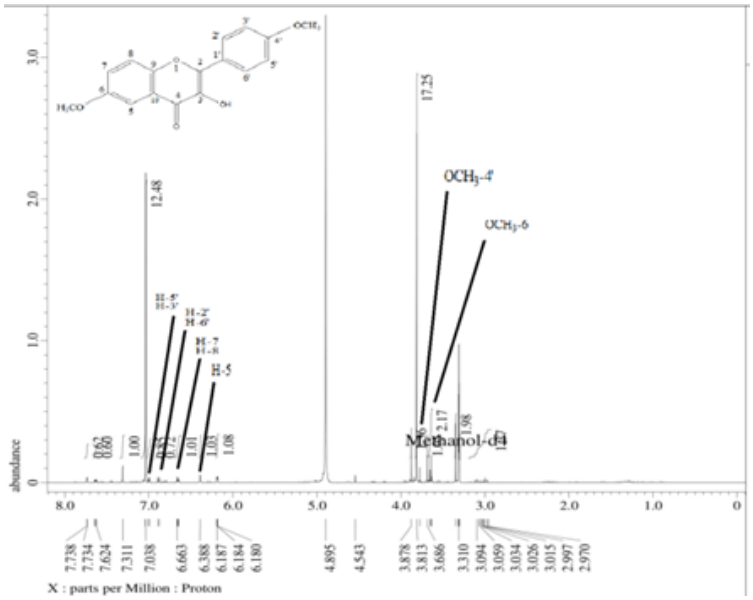

Figure 3. [1]H-NMR result of the isolated

Table 3. Chemical Shift of Isolated Compound based on Figure 3

H atoms	Intensity
H-3' & H-5'	7.007-6.989 (d)
H-2' & H-6'	6.892-6.875 (d)
H-7 & H-8	6.660-6.654 (d)
H-5	6.388(S)
H dari OCH3 -4'	3.878 (s)
H dari OCH3 -6	3.686 (s)

3.2 Discussion

UV-Vis spectrum analysis showed the presence of two main absorption bands in the isolated compound, namely at wavelengths of 369 nm (band I) and 272 nm (band II). This absorption pattern is typical for flavonoid compounds of the flavonol group, where band I generally appears in the 350-385 nm range associated with the π→π* transition in the C=O and benzene conjugation systems, while band II in the 250-280 nm range is associated with the π→π* transition in the benzene ring B.

The infrared spectrum analysis revealed a range of 1612.49-1450.47 cm^{-1}, indicating the presence of carbon-carbon double bond (C=C) stretching within the aromatic ring structure of flavonoid compounds. Additionally, an absorption band at 2962.66 cm^{-1} signifies the region of carbon-hydrogen (C-H) bond stretching vibrations within the aromatic context. The [1]H-NMR spectrometry data corroborated the existence of methoxy groups in rings A and B of the flavonoid compound, as evidenced by a singlet signal at δ 3.878 ppm corresponding to a methoxy proton at position 4' of ring B, and a singlet signal at δ 3.686 ppm corresponding to a methoxy proton at position 6 of ring A [8].

Analysis of the [1]H-NMR spectrum showed the presence of several aromatic proton signals. A doublet signal at δ 7.007-7.989 ppm indicated the presence of two protons at the 3' and 5' positions of ring B of the flavonoid compound. Another doublet signal at δ 6.892-6.875 ppm indicates the presence of two protons at positions 2' and 6' of ring B. In addition, the doublet signal at δ 6.660-6.654 ppm indicates the presence of two protons at positions 7 and 8, and the singlet signal at δ 6.388 ppm indicates the presence of protons at position 5 in ring A [9].

A full study of all three spectroscopies on the compound that was taken from the kareumbi fruit (H. populneus) shows that it matches the spectral features of flavonoid compounds, especially the flavonol group. Figure 4 proposes the compound structure based on the interpretation of the spectroscopy data.

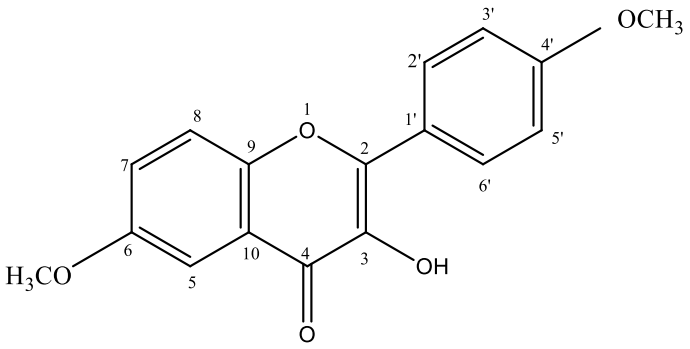

Figure 4. Flavonoid structure of isolated compound

4. Conclusion

The isolation output from 1500 grams of Kareumbi fruit (*H. populneus*) revealed the presence of flavonoid compounds as a yellowish orange paste with a mass of 19.6 mg and an Rf value of 0.73. Using UV-Vis, FT-IR, and ^{1}H-NMR spectrophotometry to learn more about the compound showed that it is in the flavonol group. This was shown by the fact that it absorbs light normally in the UV-Vis spectrum and has aromatic proton signal patterns in the ^{1}H-NMR spectrum.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

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