JCNaR
Journal of Chemical Natural Resources
Journal homepage: https://jcnar.usu.ac.id

Isolation of Flavonoids Compounds from Akalifa (Acalypha wilkesiana Muell. Arc.) Plant Leaves

Siska NA Simangunsong, Sovia Lenny*, Lamek Marpaung
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia
*Corresponding Author: sofia1@usu.ac.id

ARTICLE INFO
Article history:
Received 17 December 2022
Revised 22 January 2023
Accepted 15 February 2023
Available online 18 May 2023

E-ISSN: 2656-1492

How to cite:
Siska NA Simangunsong, Sovia Lenny, Lamek Marpaung. Isolation of Flavonoids Compounds from Akalifa (*Acalypha wilkesiana Muell*. Arc.) Plant Leaves. Journal of Chemical Natural Resources. 2023. 5(1):40-45.

https://doi.org/10.32734/jcnar.v5i1.11990

ABSTRACT
Flavonoid compounds from the leaves of Akalifa (Acalypha wilkesiana Muell. Arc.) have been extracted with maceration by methanol solvent, then added with ethyl acetate and partition extracted with n-hexane. 6% HCl acidifies the concentrated methanol extract, then partition extracted with chloroform. The concentrated chloroform extract was separated by column chromatography with silica gel 40 as the stationary phase and n-hexane: ethyl acetate 90:10; 80:20; 70:30; 60:40; 50:50 (v/v) as the mobile phase. The compounds were purified with TLC preparative yielding tawny paste weighing 4 mg with Rf= 0.42 with eluent n-hexane: ethyl acetate 60:40 (v/v). The identified analysis by using Ultraviolet-Visible (UV-Vis) spectroscopy, Fourier Transform Infra Red Spectroscopy (FT-IR), and Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy was estimated as a flavonoid is an isoflavone.

Keywords: Akalifa, Flavonoids, Isoflavones, Isolation

ABSTRAK
Ekstraksi senyawa flavonoid dari daun akalifa (Acalypha wilkesiana Muell. Arc.) dilakukan dengan maserasi dengan pelarut 40soflavo, kemudian ditambahkan etil asetat dan partisi diekstraksi dengan n-heksana. Ekstrak pekat 40soflavo diasamkan dengan HCl 6%, kemudian dipartisi dengan kloroform. Ekstrak pekat kloroform dipisahkan dengan kromatografi kolom dengan fase diam silika gel 40 dan nheksana : etil asetat 90:10; 80:20; 70:30; 60:40; 50:50 (v/v) sebagai fase gerak. Senyawa dimurnikan dengan 40soflavone40 KLT menghasilkan pasta berwarna kuning kecoklatan dengan berat 4 mg dengan Rf= 0,42 dengan eluen n-heksana:etil asetat 60:40 (v/v). Analisis yang diidentifikasi dengan menggunakan spektroskopi ultraviolet tampak (UV-Vis), Fourier Transform Infra Red Spectroscopy (FT-IR) dan Spektroskopi Resonansi Magnetik Nuklir Proton ($_1$H-NMR) diperkirakan sebagai flavonoid adalah isoflavone.

Kata Kunci: Akalifa, Flavonoid, Isoflavone, Isolasi

1. Introduction

Flavonoid compounds are polyphenol compounds with 15 carbon atoms, consisting of two benzene rings linked together by a linear chain of three carbon atoms [1]. Flavonoids are one of the largest groups of natural phenols. About 2% of all carbon photosynthesized by plants is estimated to be converted into flavonoids or compounds closely related to them [2]. Plant flavonoids can protect the human body from free radicals and reduce the risk of cancer and inflammation [3]. *Acalypha wilkesiana* is a large genus of plants and shrubs native to tropical and subtropical regions. It is generally found worldwide, especially in the tropics of Africa, America, and Asia [4]. This plant is usually found as an ornamental plant in housing [5]. Some plant species have been used as primary ingredients in traditional medicine, namely indigestion, fungal, and skin infections that reduce infant fever [4].

Previous research conducted on Akalifa plants, among others, by Madziga, et al., 2010 proved that Akalifa leaf water extract contains carbohydrate compounds, tannins, flavonoids, saponins, alkaloids, cardiac glycosides, terpenes, and steroids [6]. Gotep, JG et al. 2010 examined the antibacterial activity of ethanol extracts *Acalypha wilkesiana Muell. Arc* [7]. Furthermore, research on cytotoxic activity by combining ethyl acetate extract from the leaves *Acalypha wilkesiana Muell*. Arc. with α- tocopherol by Lim et al. and Ikewuchi

et al. in 2009. The preliminary test the researchers do, namely with a phytochemical screening test with reagents Mg-HCl, $FeCl_3$ 5%, 10% NaOH, and H_2SO_4, shows that the extract methanol and ethyl acetate of Akalifa plant leaves (*A. Wilkesiana Muell*. Arc.) contains flavonoid compounds. From the description above and some research literature that has been carried out on Akalifa plants, the researcher is interested in examining the *Acalypha wilkesiana Muell*. Arc leaves are one of the Akalifa Genus species, especially regarding the flavonoid compounds contained in this plant [8,9].

2. Materials and Methods

The tools used in this study were a measuring cup, beaker glass, Erlenmeyer glass, glass funnel, separating funnel, extractor, test tube, dropper pipette, capillary tube, spatula, Buchi B-480 rotary evaporator, distillation apparatus, bottom flask, column chromatography, UV lamp 254/356 nm, analytical balance, water bath, vials, chamber, stands and clamps, stir bar, UV-Visible spectrophotometer, IR spectrophotometer, and spectrometer 1H-NMR. The materials used are plant leaves, methanol, n-hexane, ethyl acetate, aquadest, silica gel 40, $FeCl_3$ 5%, 10% NaOH, Mg powder, $HCl_{(P)}$, H_2SO_4, HCl 6%, chloroform, cotton, TLC plate Silica gel 60 F_{254}, 60F preparative TLC plate *254*, benzene, ether, acetone, and Benedict's reagent.

2.1. Provision of Samples

Akalifa leaf was taken from around the Universitas Sumatera Utara campus, Medan. Akalifa leaves are dried in the open air, then pulverized until 700 g of Akalifa leaf powder is obtained.

2.2. Preliminary Test on Akalifa Plant Leaf Extract

Akalifa leaf powder was identified using a phytochemical screening method. A preliminary qualitative test was conducted to prove the presence of flavonoid compounds in Akalifa leaves. Ten grams of dried Akalifa leaves powder were placed into two Erlenmeyer. They added 100 mL of methanol to the Erlenmeyer I glass and 100 mL of ethyl acetate to the Erlenmeyer II glass. They were left for one night, filtered, divided each sample extract into 4 test tubes, and added each reagent.

- For sample methanol extract

a. Tube I: with $FeCl_3$5% produces a black solution positive for flavonoids

b. Tube II: with Mg powder, and $HCl_{(p)}$ produce a positive pink solution of flavonoids

c. Tube III: with 10% NaOH produces a positive greenish blue solution of flavonoids

d. IV tube: with $H_2SO_{4(p)}$produce a yellowish orange solution negative for flavonoids

- For sample ethyl acetate extract

a. Tube I: with $FeCl_3$5% produces a black solution positive for flavonoids

b. Tube II: with Mg powder, and $HCl_{(p)}$ produce a positive pink solution of flavonoids

c. Tube III: with 10% NaOH produces a greenish-blue solution positive for flavonoids

d. IV tube: with $H_2SO_{4(p)}$, produce a yellowish orange solution negative for flavonoids. Glass, leave for one night, filter, divide each sample extract into 4 test tubes, and add each reagent.

2.3. Extraction and Fractionation of Akalifa Plant Leaves

As much as 700 g of Akalifa leaf powder was weighed and then macerated with ± 6 L of methanol until all samples were submerged and left for 48 hours. The macerate is collected and concentrated using a rotary evaporator to obtain a concentrated methanol extract. Then evaporated until all the methanol solvent evaporated. Then the tannins were separated by dissolving the concentrated methanol fraction with ethyl acetate and filtered. The filtrate is then put in the rotary evaporator and then evaporated until all the ethyl acetate solvent has evaporated. Then the concentrated ethyl acetate fraction was dissolved in methanol and repeatedly partitioned with n-hexane until the n-hexane layer was almost transparent. The methanol layer was separated from the n-hexane layer, then concentrated with a rotary evaporator and evaporated again to obtain a concentrated extract of the methanol layer. The methanol fraction was tested for sugar content with Benedict's

reagent, then hydrolyzed using 6% HCl while heating over a water bath for ± 1 hour. Then it was filtered, and the filtrate obtained was partitioned with chloroform three times. The chloroform extract was concentrated using a rotary evaporator and re-evaporated to obtain 1.52 g of concentrated chloroform extract.

2.4. Thin Layer Chromatography Analysis

As much as 250 g of candlenut seeds were put into a 1000 mL beaker glass, then 500 mL of n-hexane solvent was added and then mashed with a blender. Then the crushed candlenut seeds and the solvent were put into a plastic bottle and tightly closed. The bottles were then shaken in a shaker incubator for 48 hours. Then filtered candlenut seed oil extract. The oil was separated from the solvent using a rotary evaporator. Then the oil obtained was characterized by HPLC.

2.5. Adsorption and Desorption of Vitamin E from Candlenut Oil with a Mass Ratio of Adsorbent: Candlenut Oil (1:1)

Thin Layer Chromatography analysis was performed on chloroform extract using silica gel $60F_{254}$ (Merck) as stationary phase. This analysis is intended to find the appropriate solvent system and ratio for column chromatography. The mobile phase used was n-hexane: ethyl acetate with a ratio of 90:10, 80:20, 70:30, 60:40, and 50:50 (v/v).

10 ml of n-hexane: ethyl acetate 90:10 (v/v) mixed mobile phase solution was saturated in the chromatography vessel. Dot the concentrated chloroform extract on the activated TLC plate. The plate is inserted into a saturated solvent mixture vessel, then closed and eluted. The dish that has been eluted is removed from the vessel, then dried. The stains formed were observed under UV light, then fixed with FeCl3 5%. Observe the color of the spots that appear and calculate the Rf values obtained. The same treatment was carried out for the ratio of n-hexane: ethyl acetate with a ratio of 80:20, 70:30, 60:40, and 50:50 (v/v).

2.6 Identification of Isolated Compounds

2.6.1. Identification with a UV-Visible Spectrophotometer

Analysis with a UV-Visible Spectrophotometer was obtained from the USU Pharmaceutical Research Laboratory in Medan using methanol as a solvent.

2.6.2. Identification with Infrared Spectrophotometer (FT-IR)

Analysis with the FT-IR Spectrophotometer was obtained from the Chemical Research Center Laboratory - LIPI, PUSPITEK Serpong Area, Tangerang using KBr as a solvent. A UV-Visible Spectrophotometer analysis was obtained from the USU Pharmaceutical Research Laboratory in Medan using methanol as a solvent.

2.6.3. Identification with a Proton Nuclear Magnetic Resonance Spectrometer (^{1}H-NMR)

Analysis with a spectrometer ^{1}H-NMR was obtained from the Chemical Research Center Laboratory - LIPI, Serpong PUSPITEK Area, Tangerang, using acetone as a solvent.

3. Results and Discussion

Preliminary screening results on methanol and ethyl acetate extracts from the leaves of the Akalifa plant (*A. Wilkesiana Muell*. Arc.) using a flavonoid reagent showed that the positive sample contained flavonoids. The elution results from n-hexane: ethyl acetate 60:40 (v/v) ratio to fraction 71-93. Preparative TLC was performed with n-hexane: ethyl acetate 60:40 (v/v) to obtain a pure compound. In order to get a pure compound in the form of a brownish-yellow paste weighing 4 mg and an Rf value = 0.42. The results of UV-Visible spectrophotometer analysis on the isolated paste with methanol solvent gave a maximum wavelength of 272.0 nm for band II can be seen in Figure 1:

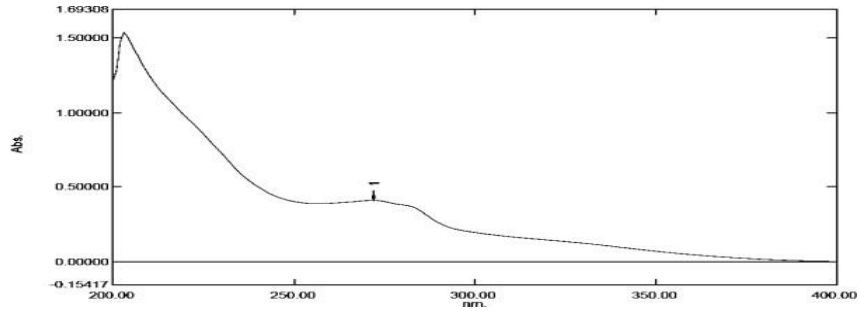

Figure 1. The UV-Vis spectrum of the compound isolated

The results of the FT-IR spectrophotometer analysis of the isolated paste produce absorption bands in the wave number region, which can be seen in Figure 2 with the following explanation:

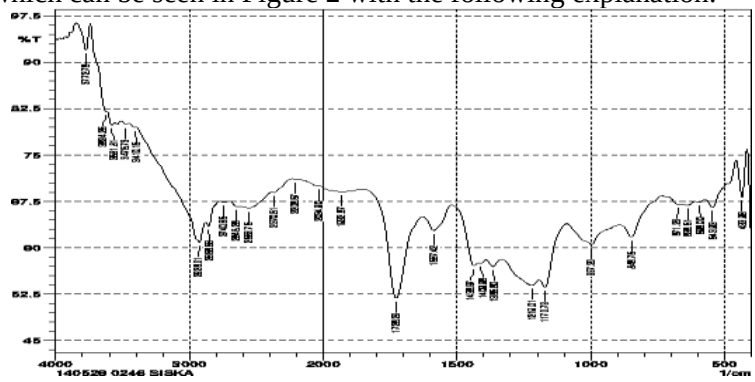

Figure 2. Infrared Spectrum (FT-IR) Isolated Compound Results

1. At wave number 3419.15 – 3475.73 cm-1, moderate peaks indicate the presence of OH stretching vibrations
2. At wave number 2926.01 - 2856.58 $cm^{-1,}$ Sharp peaks indicate the presence of aliphatic –CH stretching vibrations
3. At a wave number of 1726.29 $cm^{-1,}$ moderate peaks indicate the presence of C=O stretching vibrations of ketones
4. At wave number 1587.42 $cm^{-1,}$ moderate peaks indicate the presence of C=C stretching vibrations of the aromatic system
5. At wave number 1365.60 $cm^{-1,}$ moderate peaks indicate the presence of –CH bending vibrations3
6. At wave number 1170.79 $cm^{-1,}$ moderate peaks indicate the existence of stretching vibrations of asymmetric COC
7. At wave number 997.20 cm^{-1} moderate peaks indicate the presence of aromatic =CH bending stretching vibrations

Results of the analysis of Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR) of isolated compounds using d-acetone solvent6 and TMS as a standard can be seen in Figure 3:

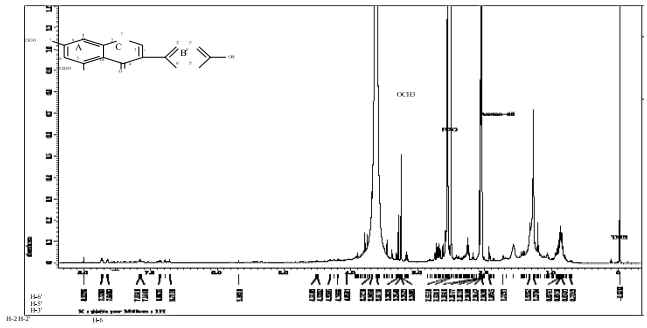

Figure 3. ^{1}H-NMR spectrum of compound results

1. The chemical shift in the δ region = 3.61-3.62 ppm singlet peak indicates a proton from –OCH3.
2. The chemical shift in the region δ = 6.71-6.72 ppm of the doublet peak indicates the proton from H-6 in the A ring.
3. Chemical shift in the region δ = 6.7805- The 6.7869 ppm doublet peak indicates a proton from H-8 in the A ring.
4. The chemical shift in the δ= 7.63-7.64 ppm peak doublet indicates the H-2' and H-6' protons in ring B.
5. The chemical shift in the δ= 7.71-7.72 ppm peak doublet indicates the H-3' and H-5' protons in ring B.
6. The chemical shift in the δ = 8.0036 ppm singlet peak indicates the proton from H-2 in the C ring.

Based on analysis data And interpretation carried out on the UV-Visible Spectrum, Infrared Spectrum (FT-IR), Spectrum1 H-NMR concluded that it was likely that the paste isolated from the leaves of the Akalifa plant (*A. wilkesiana Muell*. Arc.) is a flavonoid compound of the isoflavone group. It is evidenced by the presence of a characteristic chemical shift in the region of δ = 8.0036 ppm, with the singlet peak showing protons from H-2 in the C ring of the isoflavone group of flavonoid compounds and supported by a chemical shift of the H-2 proton type of the isoflavone group being the singlet peak in the isoflavone group chemical 7.5-8.0 ppm [7].

Based on the results of phytochemical screening, FT-IR spectrum, UV-Visible spectrum, and compared to ^{1}H-NMR data of isolated compounds. From the ^{1}H-NMR comparison data of isoflavone compounds, it can be concluded that it is most likely that the paste isolated from the leaves of the Akalifa plant is a flavonoid compound of the Isoflavone group. The following is the structure of the isoflavones that are suspected from the isolated compounds:

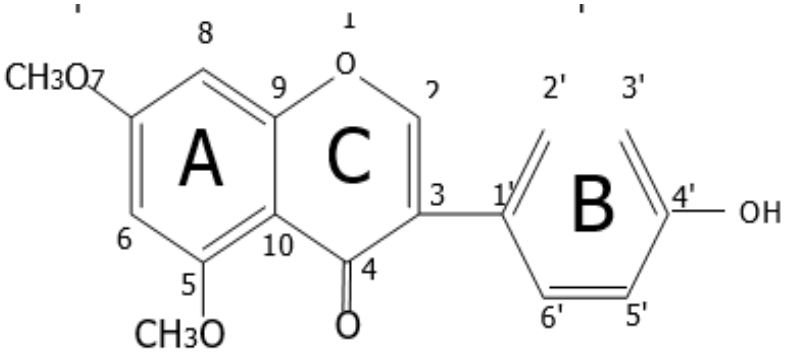

Figure 4. Isoflavone structure isolated from Akalifa plant leaves

4. Conclusion

The isolation results obtained from 700 g of Akalifa plant leaves (*A. wilkesiana Muell.* Arc.) is a brownish yellow paste, obtained as much as 4 mg, Rf = 0.42 with n-hexane: ethyl acetate 60:40 (v/v) eluent, positive for flavonoid compounds, and from the results of the analysis with UV-Visible Spectrophotometry, Infrared Spectrophotometry (FT-IR) and Proton Nuclear Magnetic Resonance Spectrometry (1H-NMR) showed that the compound isolated from the leaves of the Akalifa plant (*A. wilkesiana Muell*. Arc.) is suspected to be a flavonoid compound of the isoflavone group.

5. Acknowledgements

The authors thank the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, for providing the facilities.

6. Conflict of Interest

Authors declare no conflicts of interest

References

[1] Manito, P. 1981. Biosintesis Produk Alami. Terjemahan Koensoemardiyah. IKIP Semarang Press. Semarang.
[2] Markham, KR 1988. How to Identify Flavonoids. Translation of Kosasi Padmawinata. ITB Press. Bandung.

[3] Nessa, F. 2003. Free Radical-Scavenging Activity of Organic Extracts and Pure Flavonoids of Blumea balsamifera DC Leaves. Food Chemistry.
[4] Sule, OJ, Elekwa, I., Ayalogu, EO 2012. The Protective Nature of Acalypha wilkesiana Muell Arg. Leaves. Journal of Pharmaceutical and Biomedical Sciences. Nigeria.
[5] Semangun, H. 2004. Diseases Horticultural Plants in Indonesia. Gadjah Mada UniversityPress. Yogyakarta.
[6] Madzinga, Sanni S., Sandabe UK 2010. Phytochemical and Element Analysis of Acalypha wilkesiana Leaf. Journal of American Science. Nigeria.
[7] Gotep, JG, Agada, GOA, Gbsise, DS, Chollom, S. 2010. Antibacterial activity of ethanolic extract of Acalypha wilkesiana leaves growing in Jos. Malaysian Journal of Microbiology. Malaysia.
[8] Lim, SW, Loh, HS, Ting, KN, Bradshaw, TD, Zeenathul, NA 2013. *Acalypha wilkesiana* Ethyl Acetate Extract Enhances the in Vitro Cytotoxic of α- Tocopherol in Human Brain and Lung Cancer Cells-International Journal of Bioscience, Biochemistry, and Bioinformatics.
[9] Ikewuchi, JC 2009. Changes in the weight, plasma lipid profile, and atherogenic indices of salt Loaded Rats. University of Port Harcourt. Nigeria.