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Isolation and Structural Elucidation of Flavonoid Compounds from Ciplukan (Physalis angulata L.) Leaf Extract

Cucu Arum Dwi Cahya[1*], Jhon Patar Sinurat[2], Nur Ulina M. Br Turnip[3], Reh Malem Br Karo[4]

[1,2,3] *Institut Kesehatan Medistra Lubuk Pakam, Deli Serdang– Indonesia, 20512 Email: cucuarumm22@gmail.com*
[4] *Universitas Prima Indonesia, Medan, Indonesia)*

Abstract

The exploration and utilization of herbal medicines continue to expand due to the presence of plant-derived secondary metabolites, which have been proven to prevent and treat various diseases with minimal side effects. One plant widely recognized for its medicinal properties is Physalis angulata L. (commonly known as ciplukan). Despite its frequent use in traditional medicine, particularly its leaves, limited studies have focused on the isolation of flavonoid compounds from this part of the plant. The isolation and structural elucidation of these flavonoids are essential to identify the bioactive compounds responsible for the plant's pharmacological effects. This study aimed to isolate and elucidate the chemical structure of flavonoid compounds from P. angulata leaf extract. The powdered leaves were subjected to maceration, solvent partitioning, phytochemical screening, thin-layer chromatography (TLC), and compound isolation using column chromatography. The isolated compounds were then characterized using UV-Visible (UV-Vis) spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS). The flavonoid compound isolated in this study was identified as quercetin ($C_{15}H_{10}O_7$), a member of the flavonol subclass. It exhibited a molecular mass of 302 g/mol and showed maximum absorbance at wavelengths of 372.5 nm and 305.5 nm. Spectroscopic analyses revealed the presence of functional groups including C–O, C=C, C=O, CH, and OH, as well as 10 hydrogen and 15 carbon atoms in the NMR spectra. Based on these data, the isolated compound was confirmed to be 3,3',4',5,7-pentahydroxyflavone, commonly known as quercetin, with the molecular formula $C_{15}H_{10}O_7$.

Keywords: *Ciplukan Leaves; Elucidation; Isolation; Flavonoid Compounds*

1. INTRODUCTION

The interest in and utilization of herbal medicines have significantly increased over time. Plant-derived secondary metabolites have been shown to effectively prevent and treat various diseases, often with fewer adverse side effects compared to synthetic drugs. According to the World Health Organization (WHO), approximately 70–80% of the global population relies on traditional medicine for primary healthcare needs. The therapeutic potential of medicinal plants can be better harnessed through the isolation and characterization of their bioactive compounds [1].

One such medicinal plant is *Physalis angulata L.*, commonly known as ciplukan. Although the fruit of *P. angulata* is widely consumed due to its sweet flavor, the leaves remain underutilized despite their reported medicinal properties. Previous studies have indicated that *P. angulata* leaves contain a variety of bioactive compounds, including flavonoids, phenolics, alkaloids, saponins, tannins, and terpenoids [2].

elucidation of isolated compounds may lead to the discovery of novel therapeutic agents. If proven to possess antioxidant properties, the isolated flavonoids could contribute to the neutralization of free radicals, which are

* Corresponding Author: Cucu Arum Dwi Cahya, Institut Kesehatan Medistra Lubuk Pakam
E-mail : cucuarumm22@gmail.com
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implicated in the pathogenesis of numerous diseases. Given the growing need to improve public health through natural remedies, research on the isolation and characterization of these compounds is both timely and relevant [3].

The successful isolation of flavonoid compounds from *P. angulata* leaves holds promise for the development of more effective treatments, as these compounds exhibit a broad range of bioactivities. Moreover, the structural

Flavonoids are a diverse group of secondary metabolites that belong to the polyphenol family and are widely distributed in plants. Chemically, flavonoids are composed of two aromatic rings connected by a three-carbon bridge that forms a heterocyclic ring containing oxygen. The general backbone structure of flavonoids is represented as C6–C3–C6 [4].

Structural elucidation is a crucial step in the identification of molecular structures, involving the determination of atomic arrangements and bonding relationships within a molecule. This process typically employs spectroscopic techniques such as ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). These tools provide critical insights into the three-dimensional configuration and functional groups of bioactive compounds. The elucidation of flavonoid structures from plant extracts not only enhances our understanding of their pharmacological potential but also aids in drug discovery and efficacy evaluation [5].

Several studies have demonstrated the bioactive potential of *P. angulata*. Ton Nu Lien Huong et al. (2016) successfully isolated a flavonoid glycoside, quercetin 3-O-rutinoside, from the plant's stem [6]. Kim-Ngan Huynh Nguyen et al. (2021) reported that the leaves of *P. angulata* contain a high total flavonoid content, ranging from 701.9 to 6920 mg/kg [7]. Additionally, research by Jayachithra Ramakrishna Pillai et al. (2022) revealed that *P. angulata* leaf extract exhibited strong antioxidant activity, with a DPPH free radical scavenging ability of 85%, compared to 98% for standard vitamin C [8].

Despite these findings, the isolation and structural elucidation of flavonoids specifically from *P. angulata* leaves remain limited. Considering the frequent use of P. angulata in traditional medicine and the bioactivity of its phytochemical constituents, further investigation is warranted.

This study aims to isolate flavonoid compounds from the leaves of *Physalis angulata L.* and to determine their chemical structures through spectroscopic methods. The isolated compounds are expected to undergo future bioactivity assays to explore their pharmacological properties, thereby contributing to the development of natural therapeutic agents and providing a scientific basis for traditional medicine applications.

2. METHODS

Apparatus and Materials

The apparatus used in this study included UV-visible spectrophotometer (Shimadzu), Infrared (Shimadzu), NMR Spectrometer, ESI-HRMS Spectrometer, Rotary Evaporator (Büchi R-114), Waterbath (Memmert), Incubator (Memmert), Hot plate (Velp), Macerator (Schott), Separatory Funnel (Duran), Column Chromatography (Pyrex), Chamber, TLC Plate, UV Lamp, Blender, sieve, and Glassware. The materials employed in the research comprised ciplukan leaves, methanol (Merck KgaA), ethanol (Merck KgaA), ethyl acetate (Baker Analyzed), n-Hexane (Merck KgaA), Chloroform (Merck KgaA), Ferri (III) Chloride (Sigma Aldrich), Magnesium trace (Sigma Aldrich), HCl (Merck KgaA), NaOH (Sigma Aldrich), H2SO4 (Merck KgaA), ammonia (Merck KgaA), silica gel (Baker Analyzed) and water.

Procedural

1. Preparation

A sample of 5 kg of ciplukan leaves powder was washed and cut into small pieces. Then the sample was dried and blended, and sieved until powder was obtained.

2. Maceration

Ciplukan leaves powder is macerated using 5 liters of methanol and repeated until an extract is obtained. For the extract to become concentrated, the solvent is evaporated in a rotary evaporator until a dry extract is obtained.

3. Flavonoid Screening
   a. Iron (III) Chloride: Ciplukan leaves powder is soaked in ethyl acetate, then filtered and the filtrate is added with 5% iron (III) chloride until a black solution appears.
   b. Ammonia: Ciplukan leaves extract is spotted on a TLC plate. Then exposed to ammonia vapor for a few moments until a yellow spot is obtained. [9].
   c. Sodium Hydroxide and Sulfuric Acid: The extract of ciplukan leaves is given 10% NaOH until a yellow color is produced. Then concentrated sulfuric acid is added until a brown solution is obtained. [10]
   d. Lead Acetate: The extract is dripped with 1% lead acetate until a cream-colored solution is formed.
   e. Magnesium and Hydrochloric Acid: The extract is dissolved in methanol and magnesium powder and concentrated hydrochloric acid are added until a red color appears. [11]

4. Partition

The sample was dissolved in distilled water and partitioned using ethyl acetate. The ethyl acetate filtrate was evaporated to dryness and dissolved in methanol. The methanol filtrate was partitioned again using n-hexane to obtain the total flavonoid dry fraction. [12]

5. TLC Analysis

TLC analysis used a stationary phase of silica gel $60F_{254}$ and a mobile phase in the form of eluent (chloroform: methanol; chloroform: ethyl acetate; and n-Hexane: ethyl acetate) with a ratio of 90:10, 80:20, 70:30, and 60:40 v/v.

6. Isolation

The column was filled with silica gel mixed with chloroform, followed by the sample dissolved in methanol. Elution was carried out and all fractions were collected into vials, while the height of the spots was checked using TLC. The pure fractions obtained will be analyzed using Preparative TLC.

7. Chemical Structure Elucidation

Structure elucidation was carried out using a UV-Vis spectrophotometer to determine the maximum wavelength, FT-IR for functional groups, NMR to determine the positions of hydrogen and carbon, and fragmentation of flavonoid compounds was also measured using ESI-HRMS.

3. RESULTS

3.1 Extraction and Screening

The extraction of ciplukan leaves was carried out through maceration until 160 g of dry macerate was obtained. This macerate was re-extracted using the partition process until 23.18 g of partition extract was obtained. Screening of the Ciplukan leaves extract proved that the sample contained flavonoid compounds, as proven using chemical reagents shown in Table 1.

Table 1. Flavonoid Screening

No	Test	Positive Result
1	Iron (III) Chloride 5%	Black
2	Ammonia	Yellow
3	Sodium Hydroxide 10% + Conc. Sulfuric Acid	Brown
4	Lead (II) Acetate 1%	Cream
5	Magnesium+ Chloride Acid	Red

3.2 TLC Analysis and Isolation of Flavonoid

Based on the TLC results, it can be stated that the most appropriate eluent for isolating flavonoid compounds is chloroform: methanol (70:30 v/v) eluent, which shows good spot separation with an Rf of 0.58. A sample of 2.5

g was inserted into the chromatography column and then eluted using chloroform: methanol eluent. The fractions were collected in a vial bottle, and a total of 146 fractions were obtained.

After column chromatography, the fractions were combined using chloroform: methanol 80:20 v/v to obtain 3 fractions with a total weight of 0.6820 g. Based on the spots produced, fraction I was subjected to preparative TLC using chloroform: ethyl acetate (90:10 v/v) eluent to obtain a single fraction found on the surface of the preparative glass plate with the assistance of a UV lamp.

The pure fraction on the glass plate was ground and eluted using methanol: ethyl acetate (1:1). Then evaporated until a mass of 5 mg was obtained. Purification was carried out by dissolving a mass of 5 mg in acetone and adding n-hexane until a precipitate appeared. Then the filtrate was decanted and evaporated until the isolated compound was obtained. The purity of the isolation results was proven by performing TLC using chloroform: ethyl acetate and n-hexane: ethanol (70:30 v/v) eluents, observed under UV light.

3.3 Elucidation of Flavonoid Compound Structure

a. UV-Vis Spectrum

The isolation compound was determined for its maximum lambda using a UV-Visible spectrophotometer in the range of 300-600 nm. The analysis results showed that the compound had 2 maximum wavelengths at 372.5 and 305.5 nm, as shown in Figure 1.

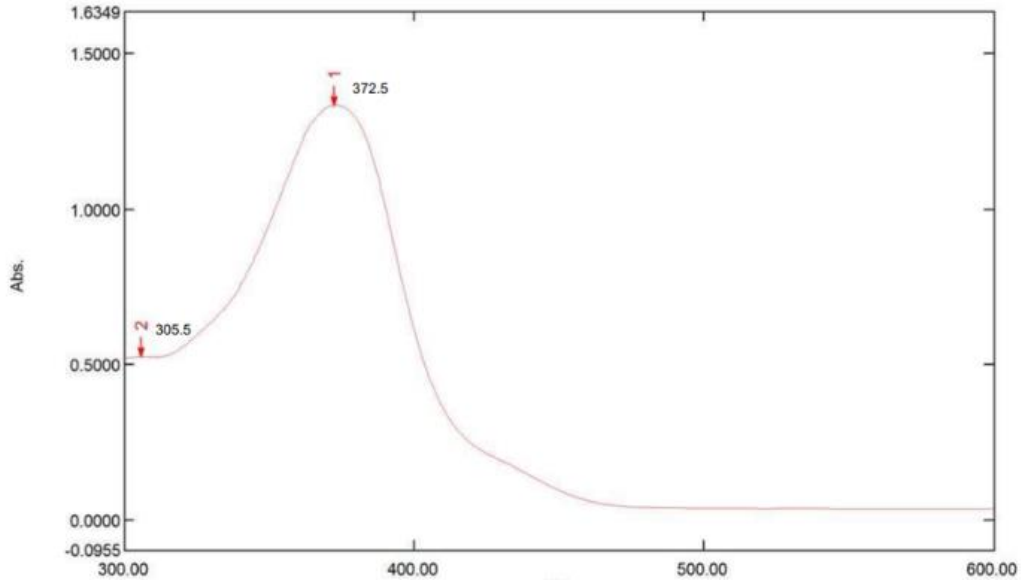

Figure 1. UV-Vis Spectrum

Generally, flavonoid compounds have two maximum wavelengths which are at 255-285 nm (band II) and 300-550 nm (band I). The maximum lambda in the results of this analysis experienced a bathochromic shift in band II, namely from 285 to 305 (Band II). This occurs because there is the addition of NaOH and $AlCl_3$ reagents [13]

b. Infrared Spectrum

The isolated compounds were analyzed for their functional groups using FT-IR in the wave number range of 400-4000 cm-1. The results showed that there were many typical transmittance peaks at certain wave numbers along with their intensities, as shown in Figure 2.

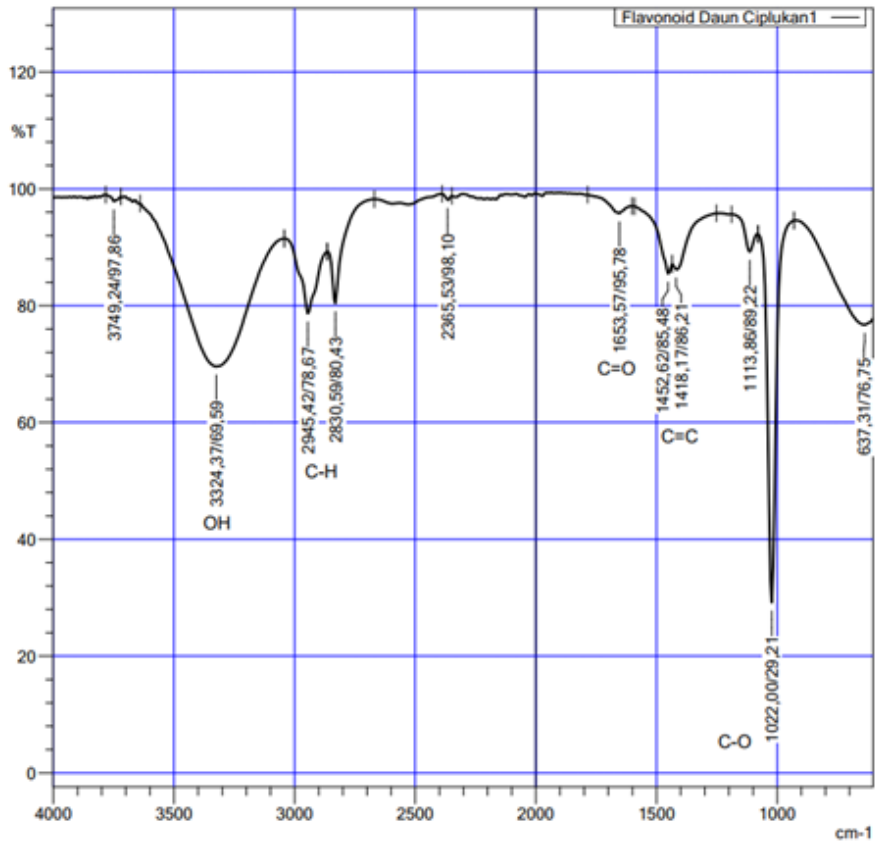

Figure 2. FT-IR Spectrum

Figure 2 shows that the isolated flavonoid compounds contain functional groups such as C-O Aromatic Ether (1022.00); C=C Aromatic (1452.62 and 1418.17); C=O Aromatic Ketone (1653.57); CH Aromatic (2945.42 and 2830.59); and OH Aromatic broadened (3324.37) [14]

c. NMR Spectrum

The isolated compound was determined for its hydrogen and carbon composition using a Nuclear Magnetic Resonance (H-NMR and C-NMR) spectrometer, where in this measurement using methanol solvent (CD_3OD). The results of H-NMR and C-NMR analysis are shown in Figure 3 and 4.

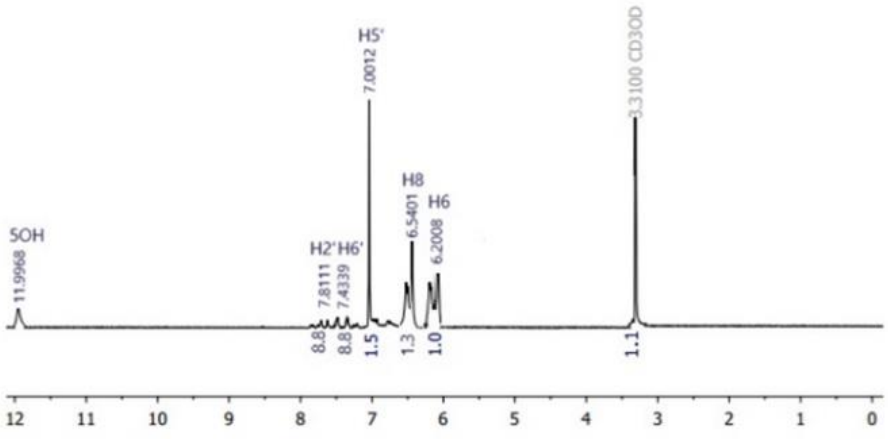

Figure 3. H-NMR Spectrum

Based on Figure 3, it is obtained information that flavonoid compounds have 10 Hydrogens (Protons) that appear at chemical shifts of 6.2008 (H6); 6.5401 (H8); 7.0012 (H5'); 7.4339 (H6'); 7.8111 (H2'); and 11.9968 (5-OH).

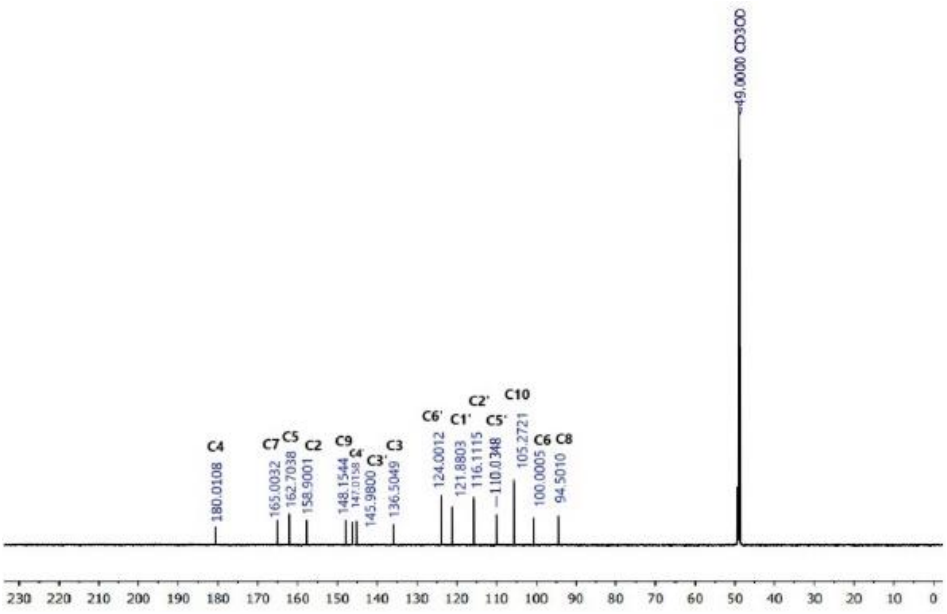

Figure 4. C-NMR

Meanwhile, Figure 4 explains that flavonoid compounds have 15 carbons, including 14 carbons originating from the aromatic group, namely chemical shifts at 94.5010 (C8); 100.0005 (C6); 105.2721 (C10); 110.0348 (C5'); 116.1115 (C2'); 121.8803 (C1'); 124.0012 (C6'); 136.5049 (C3); 145.9800 (C3'); 147.0158 (C4'); 148.1544 (C9); 158.9001 (C2); 162.7038 (C5); and 165.0032 (C7), while there is one carbon atom from the carbonyl group, namely at a chemical shift of 180.0108 (C4) [15]

d. Mass Spectrum

The mass spectrum results operated in negative mode showed the emergence of ions m/z, namely 302.0012, followed by its fragmentation, including 284.0133; 273.0205; 165.0244; 153.0241; and 125.1132 as shown in Figure 5.

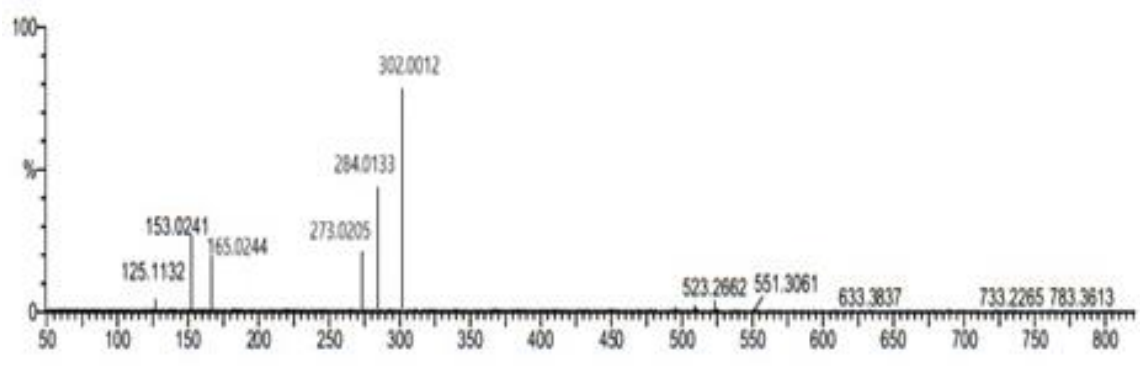

Figure 5. Mass Spectrum

4. DISCUSSIONS

Based on Figure 1, flavonoid compounds exhibit two characteristic absorption bands in their UV-Visible spectra, referred to as Band I and Band II. Band II typically appears within the wavelength range of 255–285 nm and is associated with the A-ring of the benzopyran system. In contrast, Band I is observed in the range of 300–550 nm and corresponds to the B-ring, particularly when substituted. Variations in the position and intensity of these

absorption bands provide valuable information regarding the structural features and the nature of substituents present in the flavonoid molecule [16].

Based on Figure 3, flavonoid compounds typically exhibit aromatic proton signals (from rings A and B) in the chemical shift range of 6.0–8.0 ppm in the proton NMR spectrum. Hydroxyl protons are also commonly observed in the range of 9.0–12.0 ppm. Although aliphatic proton signals may occasionally be present, they are generally less prominent in the 1H NMR spectra of flavonoids [17]. Meanwhile, as shown in Figure 4, the 13C NMR spectrum of flavonoid compounds typically exhibits several characteristic peaks. Carbonyl carbon signals are generally observed in the chemical shift range of 180–190 ppm. Aromatic carbons from rings A and B appear between 90–160 ppm, while signals corresponding to sp^3-hybridized carbons are found in the range of 10–50 ppm. [18].

As shown in Figure 5, the mass spectrum exhibits a molecular ion peak at approximately m/z 302, which corresponds to the molecular weight of the flavonoid compound. A fragment ion commonly observed at m/z 287 results from the loss of a hydroxyl group (–OH). Further fragmentation may occur through cleavage of the flavonoid ring system, producing fragment ions in the range of m/z 153–179. Additionally, smaller fragments originating from the benzopyran core and other aromatic ring systems are typically observed in the m/z range of 100–150. [19].

The results of the spectroscopic analyses—comprising UV-Vis, FT-IR, NMR, and mass spectrometry—confirmed that the compound isolated from Physalis angulata L. (ciplukan) leaves is a flavonoid belonging to the flavonol subclass. The compound was identified as 3,3’,4’,5,7-pentahydroxyflavone, commonly known as quercetin, with the molecular formula $C_{15}H_{10}O_7$ [20]. Quercetin is a well-known flavonoid compound that exhibits a wide range of bioactivities, including antioxidant, anti-inflammatory, antibacterial, and anticancer properties [21]. The molecular structure of quercetin is presented in Figure 6.

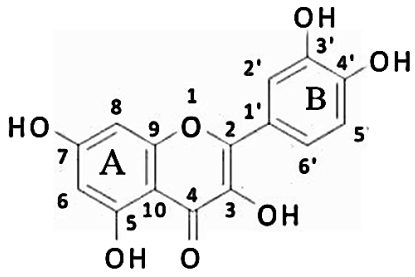

Figure 6. Structure of the Isolated Compound (Quercetin)

5. CONCLUSIONS

The isolated compound from Physalis angulata L. (ciplukan) leaves is a flavonoid belonging to the flavonol group, identified as 3,3’,4’,5,7-pentahydroxyflavone, commonly known as quercetin. The compound has the molecular formula $C_{15}H_{10}O_7$, with a molecular mass of 302. It exhibits maximum absorbance at wavelengths of 372.5 nm and 305.5 nm in the UV-Vis spectrum. The compound contains functional groups such as carbonyl (C=O), conjugated double bonds (C=C), hydroxyl (OH), and methylene (CH) groups. The NMR data reveal the presence of 10 hydrogen atoms and 15 carbon atoms in the structure.

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