

STRUCTURE ELUCIDATION OF FLAVONOID COMPOUND FROM THE LEAVES OF *COLEUS ATROPURPUREUS* BENTH USING 1D- AND 2D-NMR TECHNIQUES

(Elusidasi Struktur Sebatian Flavonoid Dari Daun *Coleus Atropurpureus* Benth Menggunakan
Teknik 1D- Dan 2D-NMR)

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Abstract

Isolation of flavonoid compound from ethylacetate extract of the leaves of *Coleus atropurpureus* Benth using column chromatography have been carried out. Structure elucidation of the isolated compounds was done by one-and two-dimensional NMR (¹H, ¹³C, DEPT, COSY, HMQC and HMBC). Analysis of 1D-NMR spectra (¹H-NMR showed signals at δ 6-8 ppm for the aromatic region of the flavonoid aglycone and ¹³C-NMR showed signals for three carbon atoms of the flavonoid ring C at δ 182.8 ppm (C-4), 103.9 ppm (C-3), 166.4 ppm (C-2) and DEPT showed the presence of CH and CH₂ group). Analysis of 2D-NMR spectra (COSY showed correlation of proton at δ 7.86 and 6.92 ppm and HMBC showed correlation between proton at δ 6.61 with 166.4 ppm and 6.92 with 123.3 ppm).

Keywords: *Coleus atropurpureus* Benth, column chromatography, flavonoid, structure elucidation, 1D- and 2D-NMR techniques

Abstrak

Pengasingan flavonoid dari ekstrak etilasetat daun *Coleus atropurpureus* Benth menggunakan kromatografi turus telah dijalankan. Elusidasi struktur sebatian yang telah diasingkan dilakukan dengan teknik 1D- and 2D-NMR (¹H, ¹³C, DEPT, COSY, HMQC and HMBC). Analisis spektrum 1D-NMR (¹H-NMR menunjukkan isyarat δ 6-8 ppm untuk kumpulan aromatik dari aglikon flavonoid dan ¹³C-NMR menunjukkan adanya isyarat tiga atom karbon dari cincin C flavonoid pada δ 182.8 ppm (C-4), 103.9 ppm (C-3), 166.4 ppm (C-2) and DEPT menunjukkan adanya kumpulan berfungsi CH and CH₂). Analisis spektrum 2D- NMR (COSY menunjukkan korelasi proton pada δ 7,86 and 6,92 ppm and HMBC menunjukkan korelasi antara proton pada δ 6.61 dengan 166.4 ppm dan 6.92 dengan 123.3 ppm).

Kata kunci: *Coleus atropurpureus* Benth, kromatografi turus, flavonoid, elusidasi struktur, teknik 1D- and 2D-NMR

Introduction

Iler (*Coleus atropurpureus* Benth) is a species of the genus *Coleus* in family Lamiaceae. Typical secondary metabolites of Lamiaceae include various terpenoids, especially mono-, sesqui- di- and tri-terpenes. Also various phenolic compounds, especially phenolic acids, such as rosmarinic acid and flavonoids are abundant. Nitrogen containing secondary metabolite play a minor role, such as stachydrine and other simple alkaloids [1]. Exudate flavonoids in the Lamiaceae concern flavones with a strong tendency towards 6-substitution and/or 8-substitution [2,3].

Flavonoid compounds have a variety of structures with interesting biological activity and is a major component of the family Lamiaceae. The systematic and phylogenetic analysis of plants was traditionally based on macroscopic

and microscopic morphological characters. Since secondary metabolites are often similar within members of a clade, their occurrence or absence might be taken as an indication of common descent and thus relatedness[1].

Study of the molecular structure of flavonoid compounds using ^1H -NMR and ^{13}C -NMR has become the method of choice for structure elucidation of flavonoid compounds. Chemical shifts and signal multiplicity of specific atoms and clutch with other atoms in the molecule allows for the identification of aglycone structure, glycosylation pattern and identification of the sugar cluster [4].

NMR spectroscopy in chemistry is not based on the ability to distinguish the elements in the compound, but it is based on the ability to learn certain core with respect to their chemical environment in the molecule would produce a signal expressed in resonance chemical shift. Based on the chemical shifts, the NMR spectroscopy yield important data that can be used to determine the molecular structure of a compound [5].

The purpose of a standard ^1H -NMR experiment is to record chemical shift, spin-spin coupling, and integration data, thus providing information about the relative number of hydrogen atoms. Applied to a flavonoid, this information may help in identifying the aglycone and acyl groups, the number of monosaccharides, and the anomeric configuration of the monosaccharides. However, for most flavonoids the information provided by a standard ^1H -NMR experiment is insufficient for complete structural elucidation. Thus, ^{13}C -NMR experiments combined with various 2D NMR experiments, especially those using gradient techniques that imply increased sensitivity, have to be used for assignments of all ^1H and ^{13}C -NMR signals. Two-dimensional NMR spectra are mainly produced as contour maps. These maps may be best imagined as looking down on a forest where all the trees (representing peaks in the spectrum) have been chopped off at the same fixed height. Two-dimensional NMR spectra are produced by homonuclear and heteronuclear experiments [4, 6].

NMR spectroscopy is an important method for the elucidation of the complete structure of flavonoid compounds based on the chemical shift (δ) and coupling constants (J) that they have with the ^1H -NMR and ^{13}C -NMR. 1D and 2D NMR spectroscopy supported by homo-and heteronuclear correlation crosspeaks. NMR spectroscopic method development has been made in determining the structure of the natural compound material which techniques such as DEPT 1D and 2D techniques such as COSY, HMBC and HMQC. 2D-NMR spectrum provide more information on the molecular 1D-NMR spectrum and is useful in determining the molecular structure [7]. Although the ^{13}C -NMR is usually not the method of choice to differentiate the flavonoid compounds, but can be useful in differentiating types of aglycone by aromatic carbon resonance chemical shift and three carbon atoms in the ring C are typical [8].

Experimental

Air-dried and powdered of the leaves of *Coleus atropurpureus* Benth (1.2 kg) were extracted at room temperature with methanol as solvent. Further partition with *n*-hexane and ethylacetate. The ethylacetate extract (9.6 g) was subjected to column chromatography (CC) over silica gel as stationary phase using mixtures of *n*-hexane-ethylacetate and ethylacetate as mobile phase based of gradient elution system or an increase in the polarity rise. Fractions with similar TLC patterns are combined and the solvent evaporated.

Fractions which showed positive results containing flavonoids then performed purification using preparative TLC with eluent *n*-hexane: ethylacetate (6:4 v/v). The pure isolate were determined by spectral analysis, i.e. 1D- and 2D-NMR techniques using a spectrometer JEOL 500 MHz to 1D-NMR (^1H -NMR, ^{13}C -NMR, DEPT) and 2D-NMR spectra (COSY, HMQC, HMBC).

Results and Discussion

The ethylacetate extract of the leaves of *Coleus atropurpureus* Benth were separated into its components using column chromatography with silica gel 60 as stationary phase and *n*-hexane-ethylacetate (6:4 v/v) as mobile phase. A pure isolate was obtained as an amorphous yellowish solid (37.5 mg). The structure of the pure isolate were determined by spectral 1D- and 2D-NMR experiments.

The ^1H -NMR spectrum of the isolated compound showed signals at chemical shifts of aromatic region δ 6-8 ppm as shown in Figure 1. The ^1H -NMR spectrum of the isolated compound displayed two meta-coupled doublets at δ 6.46

ppm (d, $J = 2.0$ Hz, 1H) and δ 6.21 ppm (d, $J = 2.0$ Hz, 1H) respectively for aromatic protons H-6 and H-8 on the ring A. The position of H-6 and H-8 are meta to each other. This is evidenced by the coupling constant values (J) 2.0 Hz, which is *J*_{meta} 1-3 Hz [9]. This also proves the ring A contain two substituents at position C-5 and C-7.

Two doublet signals at δ 6.92 ppm (d, $J = 9.1$ Hz, 1H) and δ 7.86 ppm (d, $J = 9.1$ Hz, 1H) showed the orientation of the ortho proton signal for the proton H-3', 5' and H-2', 6' ring B with the substituents at position C-4'. A singlet signal at δ 6.61 ppm (1H, s) showed proton H-3 in ring C has isolated proton. The ^1H -NMR spectrum exhibited proton signals characteristic of flavone nucleus [2, 3].

Based on the chemical shifts of the aromatic rings indicated that the isolated compound is a flavone compound with substituents at position C-5, C-7 and C-4' (Figure 2). It is also based on the phytochemical of Lamiaceae family that the main flavonoids found in these families were flavone compound.

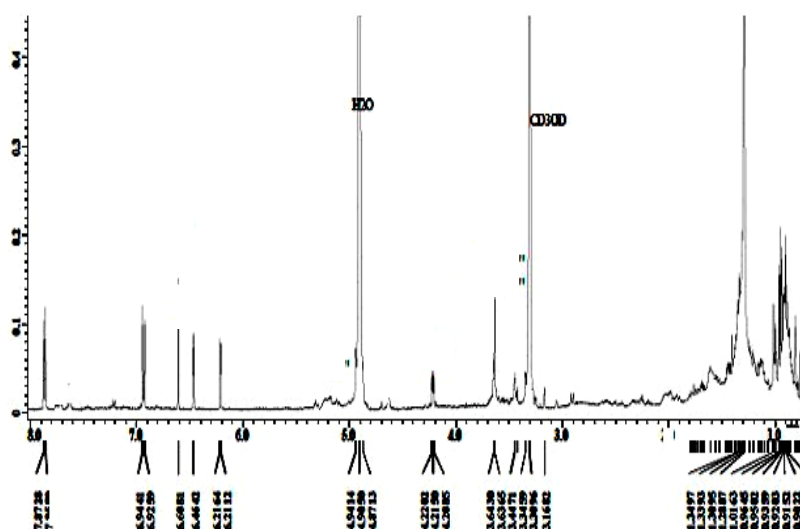


Figure 1. ^1H -NMR spectrum

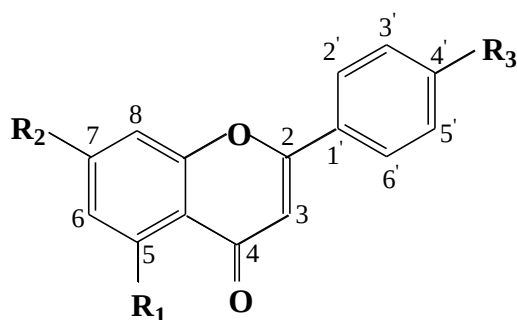


Figure 2. The isolated compound

The ^{13}C -NMR spectrum of the isolated compounds as shown in Figure 3. The signal for C-4 ' appears a singlet at δ 162.9 ppm for 4'-oxygenated ring B and the chemical shifts for the ortho carbon (C-3', 5') at 117.1 ppm, meta carbon (C-2', 6')) at δ 129.6 ppm and para carbon (C-1') at δ 123.3 ppm. It is closely related to the spectrum of 5-hydroxyflavone which the changes occur primarily to the carbon atoms in the ring B.

The chemical shift for the carbon of ring A appears on chemical shift 100.2 ppm (C-6) and 95.1 ppm (C-8). This is because of oxygenation at C-7 which will cause the signal of the ortho positions C-6 and C-8 shift towards upfield about 13 ppm of the chemical shifts of compounds 5,4'-dihydroxyflavone 111.43 ppm for C-6 and 95.6 ppm for C-8. It is also influenced by the effects of the ortho and para hydrogen bonds OH at C-5 to C-6 carbon and C-8. Thus, C-6 is represented by signals at higher chemical shift region (downfield) than the C-8 atom. In the isolated compound C-3 signal appears at δ 103.9 and 105.6 ppm for C-10 similar to the signal for the compound 5,7,4'-trihydroxyflavone at 104.3 ppm ascribed to C-3 and signal 105.1 ppm for C-10.

The chemical shifts for the signals of the carbon C-5 and C-9 shifted to downfield to 158.9 ppm and 163.2 ppm from 158.7 ppm and 162.0 ppm due to the influence meta position of hydroxyl group on the C-5 to the C-9 signal shift towards downfield [10].

The chemical shifts for the ring C of C-2 signal at δ 166.4 ppm, while the core compound flavone C-2 signal appears at 163.2 ppm, this is caused by the presence of 5-hydroxyl group so the C-2 downfield shifted signal. Atom C-3 signal appear at δ 103.9 ppm (104.3 ppm). The carbonyl carbon C-4 appeared as a less intense peak or very low in the region 182.8 ppm. Chemical shift downfield region only remaining 163.4 ppm signal is thus ascribed to C-7 [10].

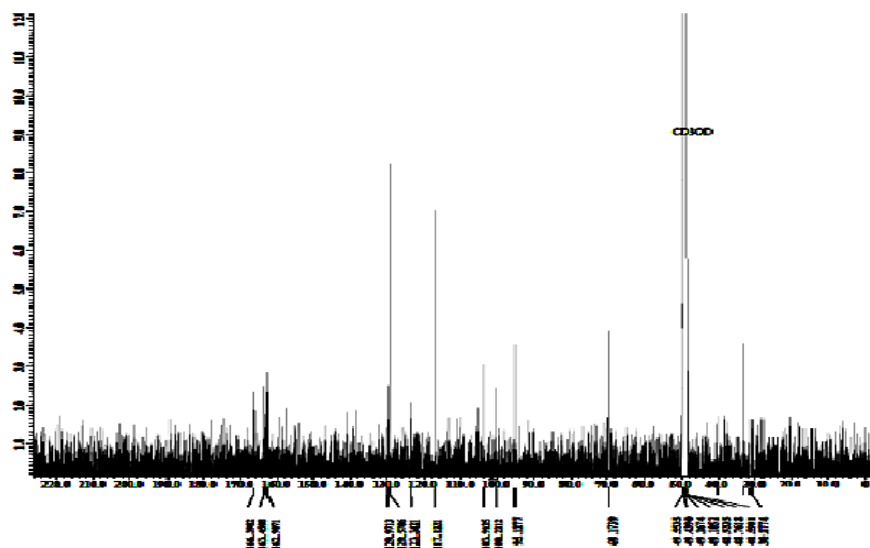


Figure 3. ^{13}C -NMR spectrum

DEPT spectrum of the isolated compound shows the number of CH_2 groups and CH/CH_3 groups. DEPT spectrum showed the presence one of methylene carbon (CH_2) at δ 69.2 ppm, twelve of methin carbon (CH) at δ 129.6 ppm (C-2',6'), 117.1 ppm (C-3',5'), 103.9 ppm (C-3), 100.2 ppm (C-6,1"), 95.1 ppm (C-8), 71.4 ppm (C-2",3",5") and 69.2 ppm (C-4") with peaks up and eight quaternary carbon as shown in Figure 4.

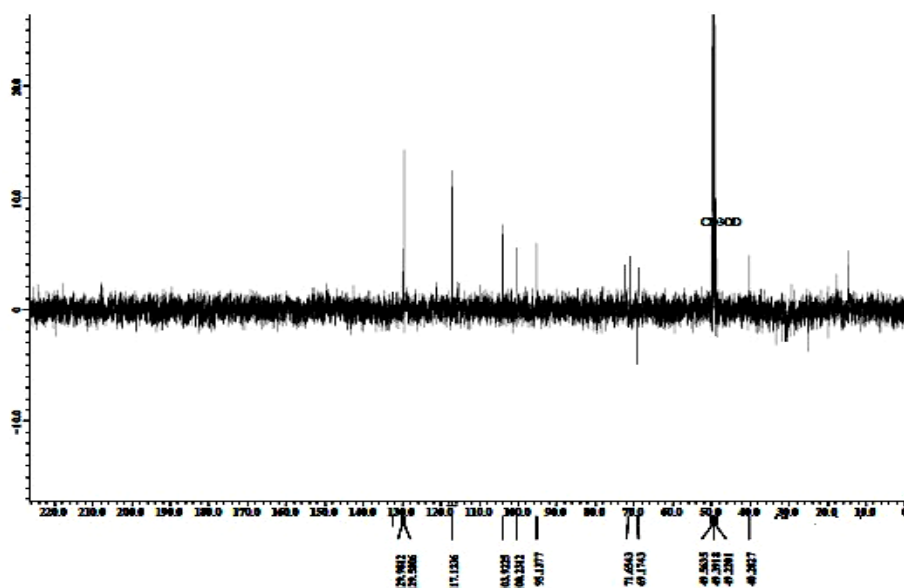


Figure 4. DEPT spectrum

^1H -NMR spectrum of the isolated compound can be explained in detail by using two-dimensional NMR spectrum (2D) correlation spectrum are homonuklir (^1H - ^1H COSY) as shown in Figure 5. Figure 5 shows the correlations between proton H-2' and H-6' at δ 7.86 ppm with proton H-3' and H-5' at δ 6.92 ppm doublet which generates a signal which describes a system of aromatic monosubstitution on ring B. Correlation proton to the isolated compound just described for the proton H-2', 6' with proton H-3', 5'. ^1H - ^1H COSY spectrum shows the correlations between the four protons in the form of vicinal protons.

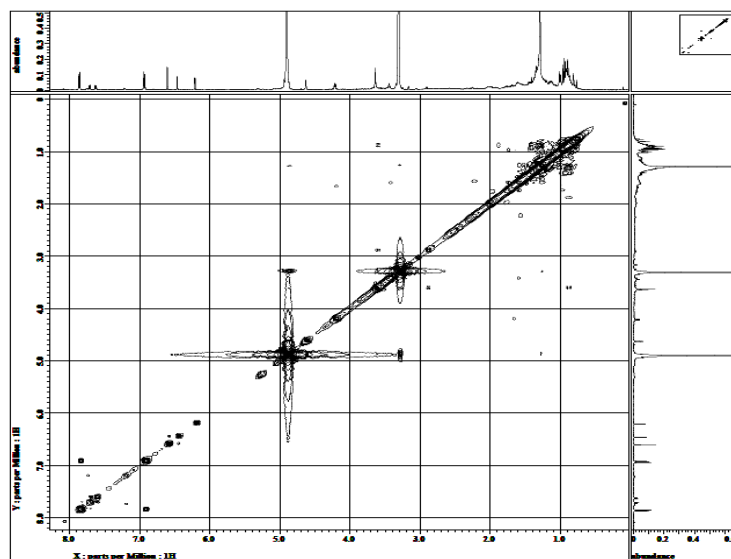


Figure 5. COSY spectrum

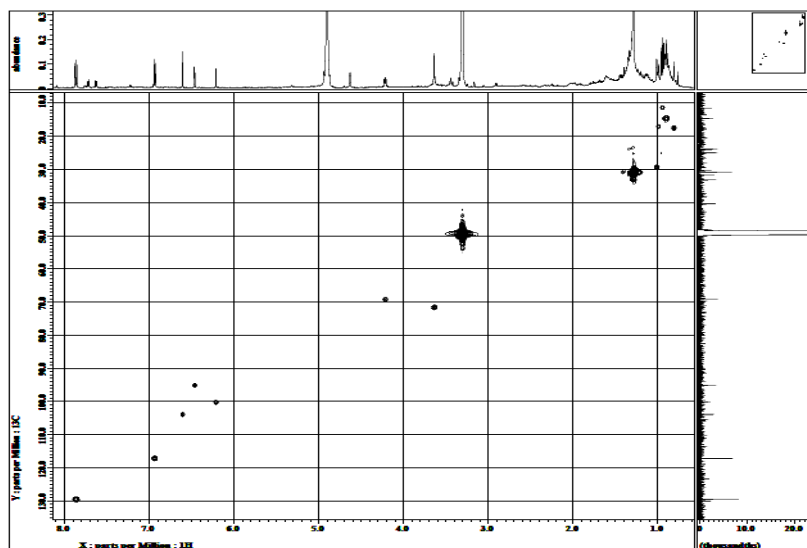


Figure 6. HMQC spectrum

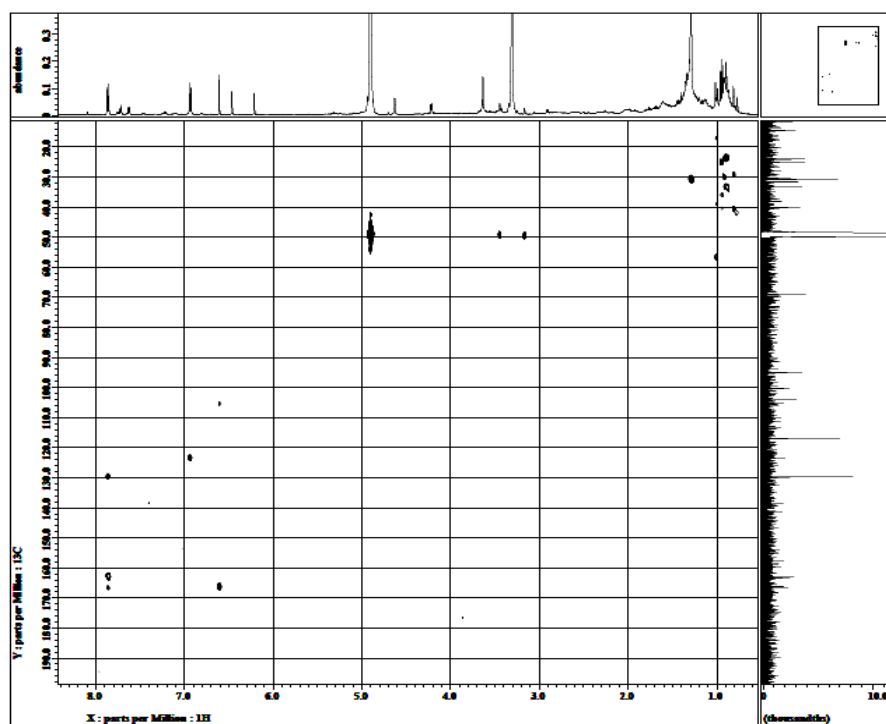


Figure 7. HMBC spectrum

HMQC spectrum (Figure 6) shown correlation between carbon and protons of skeleton of flavone compounds from the isolated compound. Characteristic of flavone compounds is the presence of a proton at position C-3 of ring C are indicated by the correlations between proton at δ 6.61 ppm (H-3) with δ 103.9 (C-3). The chemical shifts of aromatic protons in ring B at δ 7.86 (H-2', 6') correlated with a carbon at δ 129.6 ppm (C-2', 6') and proton at 6.92 ppm (H-3', 5') correlated with a carbon at δ 117.1 ppm (C-3',5').

The chemical shifts of the proton at ring A showed a correlation between proton at δ 6.21 ppm (H-6) with a carbon at δ 100.2 ppm (C-6) and proton at δ 6.46 ppm (H-8) with a carbon at δ 95.1 ppm (C-8). Based on the correlations between proton and carbon of the isolated compound indicated that structure of the compound has only a hydrogen atom at position C-3, 6, 8, 2', 3', 5' and 6' while the atom C-5, 7 and 4' are substituent. Further evidence regarding the skeleton and substitution pattern of isolated compounds obtained from HMBC data analysis as shown in Figure 7.

Figure 7 shows long-range correlations between the singlet at δ 6.61 ppm (H-3) with a carbon at δ 166.4 ppm (C-2). The aromatic proton doublet signal at δ 7.86 ppm (H-2' and H-6') showed long-range correlations with two quaternary carbon atoms at δ 162.9 ppm (C-4') and δ 166.4 ppm (C-2) and correlated well with the carbon atom δ 129.6 ppm (C-6' and C-2'). This suggests that the presence of substituents on the atom C-4'. The HMBC spectrum also showed a correlation between aromatic protons at δ 6.92 ppm (H-3' and H-5') with a quaternary carbon at δ 123.3 ppm (C-1'). From the data HMBC shows the structure of flavone ring B substituted at C-4'.

Conclusion

On the basis of $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, DEPT, COSY, HMQC and HMBC data the isolated compound were identified as flavone compound with substituents at C-5, C-7 and C-4'.

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