Flavonoid Derivatives from The Leaves of *Muntingia calabura* L.

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**Abstract.** The Isolation and Purification of ethyl acetate fraction from *Muntingia calabura* leaves have resulted three flavonoid derivatives. UV-Vis, ^1^H-NMR dan ^13^C-NMR data showed that isolate compounds are 2',4'-dihydroxychalcone (1), 3,7-dimetoxy-5-hydroxyflavone (2), and 3,5,7-trihydroxy-8-methoxyflavone (3). The antioxidant evaluation against 2,2'-diphenyl-1-picrylhydrazyl (DPPH), compound (3), showed to be a very high activity (IC$_{50}$ 3.7 x10$^9$ ppm). The isolated compounds were also evaluated for their cytotoxic activities against murine leukemia P-388 cells resulted that all isolated compounds were inactive.

1. Introduction
*Muntingia calabura* L. or kersen (Elaeocarpaceae) is a plant with a shape like a lush tree. This plant has been widely cultivated and found in India and Southeast Asia, such as Malaysia, Indonesia, and the Philippines[1]. Preliminary work on this species also showed that many of the phenolic constituents isolated from this species investigated were significantly bioactive[2][3][4][5]. This information throws a vivid light on *Muntingia calabura* Las medicinal plants. This paper reports the isolation, structure determination, and cytotoxic evaluation of three flavonoid derivative compounds: 2',4'-dihydroxychalcone (1), 3,7-dimetoxy-5-hydroxyflavone (2) and 3,5,7-trihydroxy-8-methoxyflavone (3) from the ethyl acetate fraction of the leaves of this plant.

2. Research Methods

2.1. Plant materials
Samples of the leaves of *Muntingia calabura* L were collected in 2016 in Sukabumi, Jawa Barat, Indonesia. General experimental procedures: UV spectra were measured with Shimadzu, UV Biospec-1601. ^1^H and ^13^C NMR spectra were recorded with a JEOL ECA 500 spectrometer operating at 500 (^1^H) and 125 (^13^C) MHz, using residual and deuterated solvent peaks as reference standards. Vacuum column liquid chromatography (VLC) and centrifugal planar chromatography (ChromatotronTM, Harrison Research, USA) were carried out using Si gel 60 G and Si gel GF254, respectively, and, for TLC analysis, precoated Si gel plates (Merck Kieselgel 60 GF254, 0.25 mm) were used.
2.2. Extraction and isolation methods

The dried powdered leaves of *Muntingia calabura* L. (2 Kg) were macerated with MeOH. The dried MeOH extract (30 g) was dissolved in MeOH, partitioned using EtOAC (3x), and evaporated to yield EtOAc extract (12 g). The EtOAc extract was fractionated using VLC (Si gel, n-hexane, n-hexane-EtOAc of increasing polarity) into eight major fractions, A–H. The E fraction (8.6 g) was separated by radial chromatography with silica as stationary phase (n-hexane:CHCl$_3$= 8:2) to produce seven fractions, namely E1–E7. The E6 fraction was then purified by repeated radial chromatography with silica as stationary phase (n-hexane: CHCl$_3$=7:3) to yield 3,7-dimethoxy-5-hydroxyflavone (2) (20 mg). The F fraction was further fractionated by VLC (n-hexane: EtOAc = 80:20 to 0:100) resulting in 8 fractions, namely fraction F1–F8. The F7 fraction was separated and purified using radial chromatography (n-hexane: acetone = 8.5:1.5) to produce 2',4'-dihydroxychalcone (1) (8.1 mg). Meanwhile, G fraction (2 g) was separated using VLC (Si gel, n-hexane, n-hexane-EtOAc of increasing polarity) into ten major fractions G1–G10. The G3 fraction was purified using radial chromatography obtained 3,5,7-trihydroxy-8-methoxyflavone (3) (6.3 mg).

2.3. Biological evaluation

Cytoxic properties of the isolated compounds against murine leukemia P-388 cells was evaluated according to the method of MTT assays previously described [6] while DPPH free radical scavenging assays was evaluated according to the described method[1].

3. Result and Discussion

Compound 1 was obtained in the form of yellow powdered. The UV spectrum of this compound showed the maximum absorption for chalcone derivative compounds ($\lambda_{max}$ 211, 304.2, and 315.6 nm). The H NMR data of 1 (Table 1) also showed a characteristic signal of a chalcone structure by the presence of a pair of doublets at $\delta_H$ 7.57 and 7.88 ppm with a trans coupling constant ($J$ = 15.5Hz). The phenolic –OH group was determined to be at C-2' by the observation in the 1H NMR spectrum of a chelated –OH signal at $\delta_H$ 13.35 ppm and three aromatic signals that appeared as ABX systems at $\delta_H$ 7.83 ppm (d, $J$ = 8.7Hz), 6.45 (dd, $J$ = 8.7 and 2.3 Hz) and 6.44 (d, $J$ = 2.3 Hz) ppm which typical for a 1,2,4-trisubstituted benzene. Consequently, the ring B in 1 was an unsubstituted phenyl group $\delta_H$ (7.65, 3H and 7.43, 2H) ppm. In the 13C NMR spectrum (APT, attached proton test) (Table 1), 1 showed 15 carbon signals, all of them having chemical shifts of sp² carbon, in which three of the signals were assignable to a conjugated carbonyl ($\delta_H$ 192.07) and two oxyaryl ($\delta_H$ 166.63 and 163.21) carbon atoms.

Compound 1, therefore, was assigned as 2',4'-dihydroxychalcone. Further support for the structure 1 was obtained from the one- and two/three-bond 1H-13C correlations found in the heteronuclear multiple bond connectivity (HMBC) spectra of 1, as shown in Table 1 and figure 1. Other supporting data were the results of comparison of NMR data of compound 1 with the same data from 2',4'-dihydroxychalcone reported by Yusof [7]show high suitability. Thus, compound 1 is defined as 2',4'-dihydroxychalcone (1).

| Position | $\delta_H$ (multiplicity, $J(\text{Hz})$) | $\delta_C$ | HMBC |
|----------|---------------------------------|-----------|------|
| 1        | -                               | 134,92    | $\alpha$-H, $\beta$-H, |
| 2,6      | 7,65 (m)                        | 128,69    | $\beta$, 3, 4, 5 |
| 3,5      | 7,43 (m)                        | 129,02    | 4    |
| 4        |                                 | 130,83    | 2, 6, |
| 1'       | -                               | 114,52    | 3', OH-2 |
| 2'       | -                               | 166,63    | OH-2 |

Table 1. 1H and 13C NMR data of 2',4'-dihydroxychalcone (1)
The UV spectrum of 2 showed maxima λ (max) 210, 289 nm typical for the chromophore of benzoil grouping. \(^1\)H-NMR Data of compound 2 disclosed the presence of a singlet proton signal at \(\delta_H 12.58 \text{ ppm}\), which indicates a chelated hydroxy group (-OH), i.e., the hydroxy group at C5, twomethoxyl signals at \(\delta_H 3.88 \text{ (2 x OMe) ppm}\), and a pair of meta-coupled doublets at \(\delta_H 6.46 \text{ and } 6.37 \text{ ppm (J = 2.2 Hz)}\) this corresponds to H-6 and H-8 in ring A. Also, the \(^1\)H NMR spectrum also showed the presence of an unsubstituted phenyl group (ring B) \(\delta_H 7.52, 3H, \text{ and } 8.07, 2H \text{ ppm}\). Meanwhile, \(^{13}\)C NMR spectrum indicated the presence of two methoxy carbon at \(\delta 60.36 \text{ and } 55.08 \text{ ppm}\), one carbonyl carbon at \(\delta 178.92 \text{ ppm}\), and five oxyaryl carbons at \(\delta 155.8; 139.6; 161.99; 155.8; \text{ and } 156.8 \text{ ppm}\), seven methine carbons at \(\delta 95.5; 128.5 \text{ (2C)}; 128.7 \text{ (2C)} \text{ and } 130.9 \text{ ppm}\). Further evidence came from a comparison of the spectroscopic data of 2 to that reported in the literature (table 2)[8].

**Figure 1.** Structures of 2’,4’-dihydroxychalcone (1) and HMBC Correlation

| Position | \(\delta_H \text{ (multiplicity, J in Hz)}\) | \(\delta_C\) |
|----------|------------------------------------------|-------------|
|          | \(\delta_H \text{ (multiplicity, J in Hz)}\) | \(\delta_C\) |
| 2        | -                                        | 155.9       |
| 3        | -                                        | 139.6       |
| 7        | -                                        | 165.5       |
| 4        | -                                        | 178.9       |
| 5        | -                                        | 161.9       |
| 6        | 6.44 \(d, 2, 3\)                          | 97.9        |
| 8        | 6.35 \(d, 2, 3\)                          | 92.2        |
| 9        | -                                        | 156.8       |
| 10       | -                                        | 106.1       |
| 1’       | -                                        | 130.9       |
| 3’ & 5’  | 7.51 \(3H, m\)                           | 128.7       |
| 2’ & 6’  | 8.08 \(2H, m\)                           | 128.5       |
| OH-2’    | 13.35 \(s\)                              | 130.4       |
Compound 3 was obtained in the form of orange powdered. The UV spectrum of this compound shows the maximum absorption for flavone derivative compounds ($\lambda_{\text{max}}$ 210 and 280 nm). $^{13}$C-NMR data shows the presence of 18 carbon atoms, including 8 quaternary carbons, including one carbonyl carbon at δ 179.2 ppm and six oxyaryl carbons at δ 158.5; 139.4; 148.6; 155.8; 128.9; and 157.9 ppm, five methine carbons at δ 95.5; 128.4 and 128.7 ppm, and three methoxy carbon at δ 61.6; 60.3 and 56.9 ppm. While from the $^1$H NMR spectrum (Table 3) there were identified one phenyl groups or B ring in 3 (5 H in the range δH 7.53-8.16 ppm), a singlet proton signal at δ12.42 ppm, which indicates a chelated hydroxy group (-OH), i.e., the hydroxy group at C5, and one singlet proton signal at δ6.45 ppm which typical for a tetrasubstituted benzene. Compound 3, therefore, was assigned as 3,5,7-trihydroxy-8-methoxyflavone (3). Further support for the structure 3 was obtained from the one- and two/three-bond $^1$H–$^{13}$C correlations found in the heteronuclear multiple bond connectivity (HMBC) spectra of 3 as shown in Table 3. Other supporting data were the results of comparison of NMR data of compound 3 with the same data from 3,5,7-trihydroxy-8-methoxyflavone reported by Sufian[8] show high suitability. Thus, compound 3 is defined as 3,5,7-trihydroxy-8-methoxyflavone (3) (figure 3).
Table 4. Data $^1$H-NMR dan $^{13}$C-NMR of 3,5,7-trihydroxy-8-methoxyflavone (3)

| Position | $\delta_H$ (multiplicity, J in Hz) | $\delta_C$ |
|----------|-----------------------------------|-----------|
|          |                                   | 3         | 3* |
| 2        | -                                 | 158,6     | 158,5 |
| 3        | -                                 | 139,6     | 139,4 |
| 4        | -                                 | 179,3     | 179,2 |
| 5        | -                                 | 148,7     | 148,6 |
| 6        | 6,45 (s)                          | 95,66     | 162,0 |
| 7        | -                                 | 155,9     | 97,9  |
| 8        | -                                 | 129,0     | 92,2  |
| 9        | -                                 | 157,5     | 156,8 |
| 10       | -                                 | 105,6     | 106,1 |
| 1'       | -                                 | 130,7     | 130,6 |
| 2' & 6'  | 8.16 (2H, m)                      | 128,5     | 128,4 |
| 3' & 5'  | 7,53 (3H, m)                      | 128,8     | 128,7 |
| 4'       | 7,53 (3H, m)                      | 131,1     | 131,1 |
| 3-OCH$_3$| 3,95 (3H, s)                      | 56,5      | 56,9  |
| 7-OCH$_3$| 3,91 (3H, s)                      | 61,8      | 61,6  |
| 8- OCH$_3$| 3,88 (3H, s)                    | 60,5      | 60,3  |
| 5-OH     | 12,42 (1H, s)                     | 12,62 (1H, s) | -  |

Figure 3. Structures of 3,5,7-trihydroxy-8-methoxyflavone (3)

4. Conclusion

Three flavonoid derivative compounds: 2',4'-dihydroxychalcone (1), 3,7-dimetoxy-5-hydroxyflavone (2), and 3,5,7-trihydroxy-8-methoxyflavone (3) were isolated from the leaves of Muntingia calabura L. Compound 3 exhibited high activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH). While 1-3 were inactive against murine Leukemia P388 cells.

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