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Methyl β-(p-hydroxyphenyl) acrylic compound from Melochia umbellata (Houtt) Stapf var. degrabata and their antitumor activity

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Abstract : Potential biologically active methyl *β*-(*p*-hydroxyphenyl) acrylic (**1**) was isolated from root bark of *Melochia umbellata* (Houtt) Stapf var. degrabata (Malvaceae). The structure of compound was elucidated using spectroscopic data. The structure assignment is based on Infrared spectrum and two dimensional (2D) NMR techniquest including HSQC and HMBC xperiments. Compound **1** was evaluated for antitumor activity using P-388 murine leukemia cell showed high activity with value IC_{50} 5,35 µg/mL.

Keywords : methyl *β*-(*p*-hydroxyphenyl)acrilic, *Melochia umbellata* (Houtt) Stapf var. degrabrata, antitumor.

Introduction

Melochia is one of genus from Malvaceae family. Plant from this genus has been used as traditional medicine such as an anti-inflammatory, hepatitis , cholesterol and antitumor in eastern Indonesia.

The classes of secondary metabolite from Melochia have been isolated including alkaloids, flavonoids and phenolic compound, steroids, triterpenes, etc[1,2,2]. *Melochia umbellata* is one of species of *Melochia* well known in South Sulawesi as Paliasa, and the people used to treat as hepatitis, hypercholesterolemia, diabetes, and hypertension[4].

Previous research have isolated a compound waltherione C from chloroform extract of heartwood of *Melochia umbellata*. This compound was active in the brine shrimp (*Artemia salina* Leach) assay (*(*LC_{50} 0,29 µg/mL) and showed significant cytotoxicity against P-388 murine leukemia cell (IC_{50} 0,26 µg/mL)[2] while IC_{50} value of 9,10-epoksi-melochinon is 0,83 µg/mL.

Herein we report the isolation of compound from root bark of *Melochia umbellata* (Houtt) Stapf var. degrabrata, methyl *β*-(*p*-hydroxyphenyl) acrylic (Fig 2) and the activity antitumor. This report continues of previous study of *Melochia umbellata* (Houtt) Stapf var. degrabrata.

Experimental

2.1. General Information

All chemicals used in this research is n-hexane, chloroform p.a, ethyl acetate, methanol, dan acetone, silica gel 60 (Merck, catalog number 7730) for vacumn colomn chromatography, silica gel 60 (Merck, catalog

number 7733) for flash column chromatography, silica gel 60 (Merck, catalog number 7734) for gravitation column chromatography, TLC plates (Merck Kieselgel 60 F254 0,25 mm) for TLC analysis. Bioactivity test using brine shrimp (*Artemia salina* Leach) and P-388 murine leukemia cell. Spectrophotometry analysis using varian FTIR 8501 Shimadzu. Both ^{1}H NMR and ^{13}C NMR using JEOL JMN A 5000 operating at 500,0 MHz and 125,65 MHz respectively.

2.2. Extract Preparation

The root bark of *Melochia umbellata* (Houtt) Stapf var. degrabrata (1,7 kg) maserated in methanol for 4 times 24 hours. The extract was concentrated under vacuum to yield 76,84 g of the extract and extracted with n-hexane, chloroform, and ethyl acetate to produce 5,2624 g, 11,3271 g dan 4,7541 g respectively.

2.3 Isolation of the compound from chloroform extract

Chloroform extract (11,3271 g) fractionated using vacuum column chromatography with the following eluent n-hexane, n-hexane: ethyl acetate, ethyl acetate, acetone dan methanol to yield 8 main fractions, i.e A, B, C, D, F, G, and H fractions. D fractions (303,2 mg) fractionated using flash column chromatography with eluen n-hexane, n-hexane:chloroform (3:7) to get 6 main fractions, i.e. D_1 , D_2 , D_3 , D_4 , D_5, D_6. All fractions were examined by TLC. Farction D_6 were crystalized and recrystalized using two solvent system, n-hexane and chloroform, yielding compound **1,** white crystal (21 mg) and the melting point 134-135 ^{o}C.

3. Spectra data

Compound **1** light under UV lamp short wave. IR (KBr) ν_{maks}: 3379,29, 3045,60, 3008,95, 2949,16, 2926,01, 2850,79, 1687,71, 1633,71, 1600,92, 1452,40, 1355,96, 1197,7, 1172,72, and 833,25 cm^{-1} . ^{1}H NMR ($CDCl_3$, 500,0 MHz) δ ppm (7,6 (1H, *d*, *J* = 15 Hz), 7,4 (2H, *d*, *J* = 8,5 Hz), 6,8 (2H, *d*, *J* = 8,5 Hz), 6,3 (1H, *d*, *J* = 15 Hz), 5,3 (1H, brs), 3,7(3H, s); ^{13}C NMR ($CDCl_3$, 125,65 MHz) δ ppm 167,98 (1C), 157,76 (1C), 144,65 (1C), 130,11 (2C), 127,46 (1C), 116,01 (2C), 115,48 (1C) dan 51,78 (1C).

4. Bioactivity test

4.1 Brine Shrimp Lethality Test

All of extract (n-hexane, chloroform, and ethyl acetate, and methanol) were tested bioactivity against *Artemia salina* Leach using *Brine Shrimp Lethality Test* methode[5].

4.2 Cyotoxycity test

Compound **1** was tested the cytotoxycity against P-388 murine leukemia cell[6].

Results and Discussion

Compound 1, was obtained as a light under UV lamp short wave. It was indicated the presence of an extended aromatic conjugated system.

Its IR spectrum showed characteristic absorption band for hydroxyl groups (OH) (3379,29 cm^{-1}), carbonyl (C=O) (ester function) (1687,71, 1197,79, and 1172,72 cm^{-1}), aromatic ring (3045,6, 3008,95, and 1600,92 cm$^{-1)}$, substitution pattern para aromatic ring (833,25 cm^{-1}), alifatic C-H ((2949,16, 2926,01 and 2850,79 cm^{-1}) supported by CH_3 and CH_2 (1355,96 and 1452,40 cm^{-1}) and C=C olefin (1633,71 cm^{-1}).

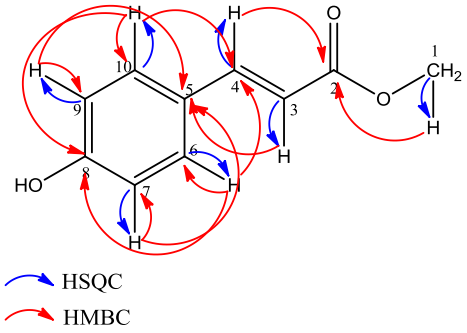
The ^{13}C-NMR spectrum of compound **1** shows 8 signals for 10 carbon. These signals consist of one carbon sp^3 at δ 51,78 ppm, 8 carbons of alkena (115,48, C-3 and 144,65 ppm, C-4), and 6 aromatis carbons (116,01 (2C); 127,46; 130,11 (2C); dan 157,76 ppm). Signals at δ 116,01 ppm (C-7 & C-9) dan δ 130,11 ppm (C-6 & C-10) show that the intensitas signal higher than others. Its indicated that there are two equivalen carbons in the ring system phenyl *di*-subtituted and a carbon carbonyl (-CO) at δ 167,98 ppm.

The ^{1}H-NMR spectrum of compound **1** show that δ 3,7 ppm (3H, *s*, H-1) indicated proton methyl (-CH_3) from oxycarbon (-OCH_3). A pair of signals at δ_H 6,8 ppm (2H, *d*, *J* = 8,5 Hz, H-7 and H-9) and δ 7,4 ppm (2H, *d*, *J* = 8,5 Hz, H-6 dan H-10) are consistent with pair of equivalent proton and mutual coupling ortho in the

phenyl *di*-substituted system. A pair of signals at δ_H 6,3 ppm (1H, *d*, *J* = 15 Hz, H-3) dan δ 7,6 ppm (1H, *d*, *J* = 15, H-4) show that the position of proton at the trans geometry in the olefin system. The presence of signal at δ_H 5,3 ppm show that the compound have hydroxyl group (-OH).

HSQC spectrum of compound **1** show that correlation between proton and carbon at δ_H 3,79 ppm (H-1) and δ_C 51,78 ppm (C-1), at δ_H 6,31 ppm (H-3) and δ_C 115,48 ppm (C-3), δ_H 6,85 ppm (H-7 and H-9) and δ_C 116,01 ppm (C-7 and C-9), δ_H 7,43 ppm (H-6 and H-10) and δ_C 130,11 ppm (C-6 and C-10), δ_H 7,65 ppm (H-4) and δ_C 144,65 ppm (C-4).

HMBC correlation of compound **1** between proton δ_H 6,31 ppm (H-3) and δ_C 127,46 ppm (C-5), δ_H 7,43 ppm (H-6 and H-10) and δ_C 144,65 ppm (C-4). The long range correlation between H-3 and C-5, H-6, H-10 and C-4 proved that C-4 substituted at C-5 in the aromatic ring.



Gambar 1. Korelasi HSQC dan HMBC Senyawa 1

Based on the FTIR, ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC data show that compound 1 is methyl *β*-(*p*-hydroxyphenyl) acrylic as Fig. 2. Methyl *β*-(*p*-hydroxyphenyl) acrylic have been isolated from root bark of *Melochia umbellata* (Houtt) Stapf var. degrabrata for the first time.

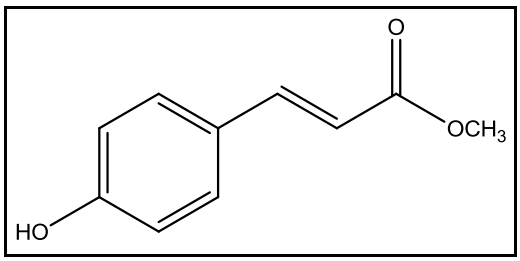


Fig 2. Methyl *β*-(*p*-hydroxyphenyl) acrylic

Biological activity

The toxycity of n-heksan, chloroform, ethyl acetate, dan methanol extract against *Artemia salina* show that LC_{50} value were 2,64, 11,54, 72,51 and 101,76 µg/ml respectively, while the IC_{50} value of methyl *β*-(*p*-hydroxyphenyl) acrylic is 5,351 µg/mL significant cytotoxicity against P-388 murine leukemia ce

Conclusion

Methyl *β*-(*p*-hydroxyphenyl) acrylic have been isolated from *Melochia umbellata* (Houtt) Stapf var. degrabrata for the first time and showed significant cytotoxicity against P-388 murine leukemia cell (IC_{50} 5,351 µg/mL).

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